OBSERVATIONS ON THE GROWTH OF *ESCHERICHIA COLI* IN MEDIA CONTAINING AMINO ACIDS AS THE SOLE SOURCE OF NITROGEN

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The deaminase activity of *Escherichia coli* for various amino acids has been investigated by many workers, but cells were almost invariably grown in complex media. Lodge and Hinshelwood (1943) have described the growth of *Aerobacter aerogenes* in media containing various amino acids, but the findings were not related to any biochemical aspects of amino acid metabolism except that the production of toxic products was postulated in unaerated cultures. The present paper describes a study of the growth constants and characteristics of *E. coli* in glucose-phosphate medium containing single amino acids as the sole nitrogen source. Such growth is a measure of the production of requisite deaminase systems which make available the nitrogen required for cell division, and, therefore, this method of growth is capable of application to the investigation of various other aspects of the organism's amino acid metabolism. Applications of the method are embodied in this communication, and they afford further examples of the value, which has been stressed by Monod (1949), of bacterial growth constant measurements.

EXPERIMENTAL METHODS

The organism used throughout this work was *Escherichia coli*, NCTC strain no. 5928. Growth curves were constructed by the methods described in earlier work (Dagley, Dawes, and Morrison, 1950a). Bacterial populations were determined turbidimetrically by a Spekker photoelectric absorptiometer using microcells and Ilford filters (neutral H508 and blue OB2). The instrument was calibrated to convert turbidities into bacterial counts as obtained by hemocytometer. Early stages of growth, where the culture is not turbid, were followed by plating out 0.1 ml of a suitable dilution on nutrient agar in a petri dish.

The following solutions were used: (A) 9 g KH_2PO_4 per liter of glass distilled water, pH adjusted to 7.1; (B) as for (A) with the addition of 2 g $(\text{NH}_4)_2\text{SO}_4$ per liter; (C) 30 g glucose and 1 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ per liter; (D) 10 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ per liter. Amino acids, which were all DL-forms unless otherwise stated, were made up in 0.3 per cent (w/v) solution, pH 7.1. All solutions were sterilized separately, and growth media were made up by dispensing aseptically into sterile boiling tubes the solutions required to make up 25 ml medium. Where necessary, cultures were aerated by a gentle stream of sterile air. Bacteria for any series of experiments were prepared by 3 serial subcultures in the required medium and 0.1 ml of the third culture taken at the onset of the stationary phase and used as inoculum. At this point in the growth cycle lag is minimal. All growth curves were constructed at 37 C.

Pyruvic acid was determined by the toluene extraction method of Friedemann and Haugen (1943) using Ilford filters H508 and green OG1 in the Spekker absorptiometer. Sodium pyruvate was prepared by the method of Robertson (1942). Ammonia was determined by nesslerization using Ilford filters H508 and blue OB1 in the Spekker. Oxygen consumption was measured in a conventional Warburg apparatus at 37 C with air as the gas phase.

RESULTS

Effect of aeration on growth with amino acids as sole nitrogen source. Cells which have been growing in media containing ammonia as the sole nitrogen source will display lag periods corresponding to the ease of production of the

Figure 1. Growth of *Escherichia coli* in unaerated and aerated glucose- KH_2PO_4 media containing single amino acids as sole nitrogen source. 1, $(\text{NH}_4)_2\text{SO}_4$ control; 2, aspartic acid; 3, serine; 4, glutamic acid; 5, alanine.

requisite deaminase when inoculated into media containing single amino acids as sole nitrogen source. This behavior in aerated and unaerated media has been followed. The medium consisted of 12 ml of solution A, 8 ml of C, and 5 ml of the amino acid under investigation. Control tubes contained 15 ml of B and 10 ml of C. The inoculum for the aerated series was subcultured in aerated medium. Results are shown in figure 1 for amino acids supporting reasonable growth; poor growth occurred in both aerated and unaerated media with valine, tryptophan, phenylalanine, tyrosine, threonine, methionine, and *iso*-leucine. In

unaerated culture aspartic acid displayed behavior similar to that previously noted with *A. aerogenes* (Dagley, Dawes, and Morrison, 1950a) in growing ahead of the control, presumably by promoting growth rate in the early stages of growth. Glutamic acid in both aerated and unaerated cultures shows the same effect of supporting growth at normal rate until reaching a population of approximately 10^8 cells per ml. Then, growth continues at a greatly reduced rate.

Figure 2. Relationship between stationary population of *Escherichia coli* and concentration of alanine and serine as sole nitrogen source in glucose-KH₂PO₄ medium. Blacked-in symbols indicate aerated culture, open symbols unaerated.

Comparison of growth on alanine and serine. The relation between stationary population and alanine and serine concentration for aerated and unaerated cultures is shown in figure 2. The medium consisted of 12 ml of double strength solution A, 8 ml of 4 per cent (w/v) glucose solution, 0.1 ml of D to which increasing amounts of the amino acids were added (total volume 25 ml). Aeration of alanine medium increases the stationary population to a marked degree, but growth in unaerated culture is poor and usually haphazard, although subsequent aeration rapidly increases the population. This is in accordance with the observation of Gale and Stephenson (1937) that alanine deaminase is an aerobic enzyme and is formed best during aerobic growth. Aeration of serine shows the

same effect, however, and this is surprising since serine deaminase is an anaerobic enzyme, the production of which in peptone medium is inhibited by aerobic conditions (Gale and Stephenson, 1938). Similar results were obtained with aspartic acid which is also an anaerobic enzyme.

Lodge and Hinshelwood (1943), using *A. aerogenes*, found that with certain amino acids as sole nitrogen source the stationary population was greatly increased by aeration of the culture, and they suggested that the maximum population is in part controlled by the formation of an inhibiting substance which is removed by cellular oxidation. Their results are compatible with an explanation in terms of the type of deaminase involved, i.e., whether aerobic or anaerobic. Therefore, experiments were conducted with *E. coli* to test whether or not toxic products were obtained in unaerated medium. Cells were grown in media containing alanine, serine, aspartic and glutamic acids as sole nitrogen source (as for figure 1), and when no further growth occurred, sterile filtrates of each culture were prepared by centrifugation and Seitz filtration. Media were then made up as follows: 7.5 ml of solution B, 5 ml of C, and 12.5 ml of culture filtrate; and these were inoculated with 0.1 ml of a culture displaying early lag (i.e., a culture in its early logarithmic phase of growth) together with control tubes containing 15 ml of B and 10 ml of C. It was found that all the tubes containing filtrates grew well ahead of the controls in the order glutamate, aspartate, serine, and alanine. Results resembled those obtained with culture filtrates from glucose-ammonium salt medium (Dagley, Dawes, and Morrison, 1950a). This growth promoting effect of filtrates from unaerated cultures precludes the possibility that inhibitory compounds are formed in the medium under such conditions.

The primary product of deamination of both serine and alanine is pyruvic acid, a compound readily utilized by *E. coli* as a carbon source and which plays a key role in growth processes of both *E. coli* and *A. aerogenes* (Dagley, Dawes, and Morrison, 1950b, 1951). Thus, in cultures where carbon exhaustion is the factor limiting growth and nitrogen is provided to the same concentration in the form of ammonium sulfate and either serine or alanine, growth in the latter culture should reach a higher stationary population since the pyruvic acid produced by the deamination would be an available carbon source for growth. Serine was used as it supports good growth in unaerated culture. A fairly low glucose concentration (2.65×10^{-3} M) in solution A formed the basal medium to which increasing amounts of $(\text{NH}_4)_2\text{SO}_4$ and serine were added. Figure 3 shows that in the region of growth limited by glucose exhaustion serine supports a higher crop of organisms than does the corresponding amount of ammonium nitrogen. With aerated cultures the effect is greatly enhanced due to increased utilization of pyruvic acid under such conditions as shown by determination of the amounts of pyruvate present during growth. At the beginning of the stationary phase in unaerated culture, $(\text{NH}_4)_2\text{SO}_4$ medium contained 11 mg per liter and serine medium 13.7 mg per liter pyruvate, whereas in aerated culture both showed the same low value of 0.43 mg pyruvate per liter. In this experiment the ratio of pyruvate present in serine and $(\text{NH}_4)_2\text{SO}_4$ medium at the

onset of the stationary phase was 1.24:1. Subsequent aeration of these cultures resulted in an increase in stationary population in the ratio of 1.31:1, a fairly reasonable correlation.

Growth with pyruvic acid as sole carbon source in ammonium salt medium. The relationship between pyruvic acid concentration and stationary population for aerated and unaerated cultures was established in view of the previous findings. Table 1 shows that aeration greatly facilitates pyruvate utilization.

Figure 3. Relationship between stationary population of *Escherichia coli* and serine and $(NH_4)_2SO_4$ concentration in glucose- KH_2PO_4 medium. $(NH_4)_2SO_4$, O; serine, □. Blacked-in symbols indicate aerated culture, open symbols unaerated.

Growth with amino acids as sole carbon and nitrogen sources. Tubes contained 20 ml of solution A, 0.1 ml of D, and 5 ml of a 1 per cent (w/v) solution of the amino acids used, viz., alanine, serine, glutamic and aspartic acids. These amino acids were selected since in each case deamination yields a carbon skeleton which is of metabolic importance and readily available as an energy source for growth. Growth was poor in unaerated culture with all four amino acids although serine supported a higher population than the others (205×10^6 cells per ml). All supported good growth in aerated medium, but the mean generation time was greater than in the corresponding medium containing glucose as additional carbon source. A direct comparison of growth rates in serine medium and medium containing pyruvate and ammonium sulfate to the equivalent

carbon and nitrogen concentrations revealed mean generation times of 92 and 57 minutes, respectively, suggesting that either deamination or poor energy availability due to low pyruvate concentration is the rate-determining step. Later findings indicate the latter.

Effect of glucose on deamination. The inhibition by glucose of enzymes concerned with the breakdown of proteins and amino acids in bacteria growing in complex media is a classical microbiological problem and has been investigated by many workers (Gale, 1943). Serine deaminase formation was shown to be inhibited by glucose (Gale and Stephenson, 1938). Although it has been stated that the effect is not so marked in synthetic medium, it seemed that under conditions where serine is the nitrogen source an effect might be determined easily since serine deaminase production would be the factor controlling growth, and either the growth rate or stationary population or both would be affected if production of the enzyme were inhibited.

TABLE 1
Relationship between stationary population and pyruvic acid concentration in $(\text{NH}_4)_2\text{SO}_4\text{-KH}_2\text{PO}_4$ medium

PYRUVIC ACID CONCENTRATION $\text{M} \times 10^{-3}$	STATIONARY POPULATION (MILLIONS PER ML)	
	Un aerated	Aerated
1.7	91	175
3.4	103	210
6.8	148	304
10.2	160	423
17.0	225	720
26.3	275	1090
34.0	323	1400

The relationship between stationary population and serine concentration for a limiting amount of glucose ($3.98 \times 10^{-3} \text{ M}$) was investigated and the concentration of serine corresponding to exhaustion of the glucose in un aerated medium determined. This amount (0.24 g per liter) was added to series of tubes containing solution A, and increasing amounts of glucose over the range 3.98 to $66.3 \times 10^{-3} \text{ M}$ were added, the total volume in each case being 25 ml. Both serine and ammonium-grown cells were used as inocula and growth curves constructed for each. There was no effect on either growth rate or stationary population, and glucose caused no inhibition of serine deaminase which could be detected under these conditions. Furthermore, serine deaminase activity of washed cell suspensions prepared from serine-glucose medium could not be increased by addition of the recognized coenzymes adenosine-5-phosphate, glutathione, and biotin (Wood and Gunsalus, 1949; Lichstein and Christman, 1949). This seems to rule out any marked inhibition by glucose of the production or the destruction of the coenzymes.

Serine and threonine deaminases. Wood and Gunsalus (1949) carried out the resolution and partial purification of serine and threonine deaminases of *E. coli*

and suggested on the basis of their findings that these enzymes may be one and the same deaminase. Confirmation of this suggestion seemed possible by a study of the growth of *E. coli* in media containing serine and threonine as sole nitrogen source. Thus, an organism trained to grow without lag in medium containing serine as sole nitrogen source should also grow without lag in threonine medium and at approximately the same rate since the enzyme-substrate affinity for each substrate is very nearly identical. Similarly, threonine-trained cells should grow without lag in serine medium. However, whereas growth in serine began after a short lag period (see figure 1), growth in threonine lagged indefinitely and was very slight in unaerated cultures after several days. It was slightly higher, but still poor, in aerated culture. No improvement in growth was obtained with serine-grown inocula, and in numerous experiments good growth in threonine medium could not be achieved.

Umbreit (1949), using *E. coli*, showed that oxidation of fumarate previous to incubation with DL-threonine increased the rate of oxidation of the latter. The possibility that growth on threonine could be stimulated likewise was investigated by growing the organism with aeration in media containing fumarate as sole carbon source (15 ml of solution B, 10 ml of 1 per cent sodium fumarate solution, and 0.1 ml of D) and using this as inocula for tubes containing 9 mg DL-threonine with glucose and fumarate as carbon sources. Poor growth resulted, the stationary populations being 140×10^6 and 101×10^6 cells per ml, respectively, and fumarate exerted no stimulatory effect. Possible growth inhibition by the D-isomer was disproved by using L-threonine as sole nitrogen source.

Effect of threonine on growth in serine and ammonium sulfate media. DL-Threonine was added to glucose-ammonium salt medium (15 ml of solution B and 10 ml of C) to give concentrations of 1, 2, 3, 4, and 5×10^{-3} M. Growth curves for each medium are shown in figure 4. Both mean generation time and stationary population are affected by the addition. Furthermore, extrapolation of the threonine curves to zero time, i.e., inoculum time, does not result in interception of the inoculum population; a higher figure is obtained. This indicates that a change in growth rate has occurred and that in the early stages before visible turbidity is reached the growth rate is either equal to or slightly greater than the control culture containing no threonine. The early stages of growth were studied by viable count experiments in ammonium sulfate medium to which increasing amounts of threonine were added, and it was found that all the cultures grew together initially. In tubes containing threonine a transition to a slower rate of growth occurred.

This transition in growth rate is compatible with the formation of an inhibitory substance which retards growth when a certain threshold concentration is reached. Similar results were obtained with serine as the nitrogen source, and the effect of threonine on growth rate and stationary population is recorded in table 2.

Deamination of threonine produces α -ketobutyric acid, a compound not readily oxidized by washed suspensions of *E. coli* which Umbreit (1949) explained

by nonpermeability to the bacterial cell. Further oxidation of α -ketobutyric acid may produce propionic acid (Long and Peters, 1939; Umbreit, 1949).

Figure 4. Effect of threonine on growth and stationary population of *Escherichia coli* in glucose- $(\text{NH}_4)_2\text{SO}_4$ medium. DL-Threonine concentrations ($\times 10^{-3}$ M): 1, nil; 2, 1.0; 3, 2.0; 4, 3.0; 5, 4.0; 6, 5.0.

TABLE 2

Effect of DL-threonine on growth in glucose- KH_2PO_4 medium containing DL-serine as sole source of nitrogen

DL-THREONINE CONCENTRATION	MEAN GENERATION TIME	STATIONARY POPULATION
$\text{M} \times 10^{-3}$	Minutes	Millions per ml
0	60	725
0.5	69	718
1.0	80	676
1.5	85	676
2.0	92	645
2.5	103	625

Since either of these compounds might produce the observed effect, their action on growth in glucose-ammonium salt medium was investigated.

Effect of propionic acid on growth. Table 3 shows that sodium propionate has

no effect on growth rate or stationary population in glucose-(NH₄)₂SO₄ medium, but increasing concentration increases the length of the lag phase.

Effect of α -ketobutyric acid on growth. A direct comparison was made of growth in media containing: (1) threonine as sole nitrogen source; (2) α -ketobutyrate plus (NH₄)₂SO₄; and (3) propionate plus (NH₄)₂SO₄. The carbon and nitrogen levels in all three media were equivalent to 3×10^{-3} M threonine. Also, the effect of the addition of these three compounds to glucose-(NH₄)₂SO₄ medium was directly compared. From figure 5 it is seen when threonine is present as sole nitrogen source that growth is very much less than in the equivalent amount of α -ketobutyrate and (NH₄)₂SO₄. This in its turn displays reduced growth rate and stationary population as compared with propionate and (NH₄)₂SO₄. When additions are made to glucose-(NH₄)₂SO₄ medium, α -ketobutyrate has a greater inhibitory effect than threonine while propionate behaves as previously noted. When threonine is the sole source of nitrogen, it is not possible to explain completely poor growth on the basis of the inhibitory α -ketobutyrate produced by

TABLE 3
Effect of propionic acid on growth in glucose-ammonium sulfate medium

CONCENTRATION OF PROPIONIC ACID	LENGTH OF LAG PHASE	MEAN GENERATION TIME	STATIONARY POPULATION
(M $\times 10^{-3}$)	Minutes	Minutes	Millions per ml
0	0	61	545
1.08	35	61	540
3.24	54	60	550
4.32	79	61	540
5.40	103	61	540

deamination. Rather it would appear that an additional factor is the rate of deamination of threonine and hence nitrogen availability for growth.

Serine and threonine deaminase activity of cells in synthetic medium. A comparison of the serine and threonine activity of cells grown in glucose-(NH₄)₂SO₄ and glucose-serine media was made. Cells were harvested from overnight growth and washed cell suspensions prepared, adjusted to the same bacterial density, and 1 ml quantities tested against 1.5 mg of each substrate in 2 ml buffer, pH 7.6. Ammonia production was determined at intervals, and results are summarized in table 4. With both (NH₄)₂SO₄ and serine as nitrogen source the deaminase activity for serine is greater than that for threonine although growth in serine does stimulate threonine deaminase activity markedly. Free ammonia present in the growth medium was determined at intervals after the inoculation of media containing serine and threonine as sole nitrogen sources. Very little occurred in the threonine tube while good growth was proceeding in the serine medium, indicating that ammonia is produced from serine in excess of immediate growth requirements.

Oxidation of serine, threonine, and alanine by cell suspensions grown in synthetic medium. The ability of *E. coli* to oxidize threonine was of interest in view

of the poor growth on this amino acid. The Gratia strain used by Umbreit (1949) oxidized threonine rapidly when harvested from complex medium. Cells were harvested from glucose- $(\text{NH}_4)_2\text{SO}_4$, serine, and threonine media and the oxida-

Figure 5. Comparison of growth of *Escherichia coli* (I) in glucose- KH_2PO_4 medium containing DL-threonine as sole nitrogen source, α -ketobutyrate + $(\text{NH}_4)_2\text{SO}_4$ and propionate + $(\text{NH}_4)_2\text{SO}_4$, all equivalent to 3×10^{-3} M threonine, and (II) in glucose- $(\text{NH}_4)_2\text{SO}_4$ medium with additions as in (I). Growth curves: 1, $(\text{NH}_4)_2\text{SO}_4$ control; 2, propionate + $(\text{NH}_4)_2\text{SO}_4$; 3, α -ketobutyrate + $(\text{NH}_4)_2\text{SO}_4$; 4, DL-threonine.

TABLE 4

Comparison of serine and threonine deaminase activity of cells grown in synthetic media; washed cell suspensions adjusted to same bacterial density

NITROGEN SOURCE IN GROWTH MEDIUM	DEAMINASE ACTIVITY ($\mu\text{MOL NH}_3/\text{LITER}/\text{HOUR}$)		RATIO OF ACTIVITIES SERINE/THREONINE
	Serine	Threonine	
$(\text{NH}_4)_2\text{SO}_4$	855	40	21:1
Serine	2205	400	5.5:1

tion rates of serine, threonine, and alanine measured. To secure sufficient cells from threonine medium it was necessary to give a large bulk of medium a fairly large inoculum. In all cases it was found that threonine was not readily oxidized even by the cells grown in threonine. Serine and alanine were rapidly oxidized

but threonine reduced the oxygen uptake with serine when present simultaneously. Results are shown in figure 6 for threonine-grown cells.

Effect of methionine, valine, and iso-leucine on growth in threonine medium. Threonine, methionine, valine, and iso-leucine individually support little growth when present as the sole nitrogen source, and when threonine is added to media

Figure 6. Oxidation of various amino acids by washed cell suspensions of *Escherichia coli* grown in glucose- KH_2PO_4 medium containing DL-threonine as sole nitrogen source. Endogenous respiration, O; DL-threonine, Δ ; DL-serine, $\bullet$; DL-alanine, $\square$; DL-serine + DL-threonine, ∇ .

containing any one of the other three amino acids, growth after 48 hours is the summation of the individual growths. However, growth at 24 hours is greater than the summation of the growth on the single amino acids, indicating that growth rate is increased by the simultaneous presence of threonine.

DISCUSSION

Attention has been confined to amino acids believed, from previous work, to be of potential importance. Of these only serine, alanine, glutamic and aspartic

acids supported good growth. In every case the stationary population supported by a given amount of amino acid was increased by aeration of the culture. It is noteworthy that the recognized deaminases of *E. coli* all yield α -keto acids of prime metabolic significance; they can be designated aerobic or anaerobic according to whether oxygen is involved in the deamination reaction. Thus, alanine and glutamic acid deaminases are aerobic enzymes while serine and aspartic acid deaminases are anaerobic. In the course of studies with *E. coli* grown in complex media Gale and Stephenson (1937, 1938) concluded that the production of aerobic deaminases was stimulated in aerated culture while anaerobic deaminase production was stimulated by anaerobic conditions. The former observation has been confirmed with synthetic media. With either serine or aspartic acid as sole nitrogen source aeration stimulates the deamination process, and thus a given amount of serine or aspartate supports a higher stationary population in aerated than in unaerated medium. That this effect is not due to more efficient utilization of the carbon source under aerated conditions is shown by the fact that the marked increase occurs over the range where nitrogen exhaustion is the factor limiting growth. The possibility that poorer growth in unaerated culture is due to the production of inhibitory substances, as suggested by Lodge and Hinshelwood (1943), has been disproved by showing that sterile filtrates from the unaerated stationary phase actually promote growth of cultures displaying lag in glucose-ammonium salt medium. It would seem that an explanation in terms of the type of deaminase involved is the better one.

Where growth is limited by glucose exhaustion, the provision of nitrogen in the form of serine or alanine enables a higher stationary population to be reached than with the equivalent amount of ammonium nitrogen since the pyruvic acid formed by deamination is an available carbon source for further growth. The effect is most pronounced in aerated media. Good growth is also obtained with alanine, serine, glutamic and aspartic acids as the sole source of carbon and nitrogen, but here aeration is essential for the adequate utilization of the keto acids produced by deamination which are required as energy sources for growth.

The well known effect of glucose in inhibiting the production of deaminase enzymes in complex media could not be demonstrated in synthetic serine media. Over a wide range of glucose concentration no effect on deaminase activity was found as evidenced by growth rate and stationary population. As most of the information on this effect stems from work with complex media where alternative sources of nitrogen are available for the bacteria, it is interesting to note that Hills (1940) was unable to demonstrate any effect of glucose on arginine dihydrolase production in pathogenic cocci, especially as this enzyme is believed to be the only mechanism used by certain cocci to liberate ammonia from amino acids. Addition of the recognized coenzymes of serine deaminase did not increase the activity of washed cell suspensions prepared from synthetic $(\text{NH}_4)_2\text{SO}_4$ or serine media, or from complex Lab-Lemco medium. The glucose inhibition of serine deaminase activity in the latter medium appears, therefore, to be due to an effect on apoenzyme formation as was shown for tryptophanase by Dawes and Happold (1949).

An attempt was made to verify by growth studies the suggestion put forward by Wood and Gunsalus (1949) that serine and threonine deaminases may be one and the same enzyme. Under all conditions tested, however, neither L- nor DL-threonine supports reasonable growth, and their addition to either glucose-(NH₄)₂SO₄ or glucose-serine medium causes a reduction of growth rate and stationary population. A transition in growth rate occurs, compatible with production of a threshold concentration of an inhibitory compound. This compound is α -ketobutyric acid, the primary product of threonine deamination, and it affects both growth rate and stationary population whereas propionic acid, which may arise from it, affects only the length of the lag phase. Poor growth on threonine as sole nitrogen source cannot be explained by the inhibitory effect of α -ketobutyric acid produced by deamination. However, it seems that the rate of threonine deamination is also a controlling factor. A comparison of serine and threonine deaminase activities of cells grown in synthetic media showed that the serine deaminase is much more active. Furthermore, threonine is not readily oxidized by cells grown in synthetic media, in contradistinction to both serine and alanine, but it decreases the rate of oxidation of serine when present simultaneously.

Although these findings do not appear to support the identity of serine with threonine deaminase, it should be noted that they could be reconciled with this suggestion if the bacterial cell is not readily permeable to threonine. Support is given to this interpretation by the fact that growth in serine medium does stimulate threonine deaminase activity. In this respect it is interesting to note that the Gratia strain of *E. coli* used by Umbreit (1949) readily oxidized threonine yet was without action on α -ketobutyric acid, and here a permeability effect was postulated.

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SUMMARY

The growth constants of *Escherichia coli* in glucose-phosphate medium containing single amino acids as sole nitrogen source have been determined and this method of growth applied to various aspects of the organism's amino acid metabolism.

In such synthetic media aeration has a stimulatory effect on growth on amino acids, including those which are deaminated by anaerobic mechanisms (serine and aspartic acid), but the effect is not due to removal of toxic compounds by the air stream. The inhibitory effect of glucose on deaminase production, well known in complex media, is not detectable in synthetic serine medium.

The poor growth of *E. coli* in threonine medium, as compared with serine, is caused by a combination of slow rate of deamination and the inhibitory effect of α -ketobutyric acid produced by deamination. The bearing of these findings on the suggested identity of serine with threonine deaminase is discussed.

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