87. *Nitrogen Utilisation and Growth of Coliform Bacteria. Part II. Relation between Growth and Deaminase Activity.*

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The development of lag in ageing cultures of *Bact. coli* is not necessarily associated with decay of deaminase activity; nor does recovery of decayed deaminase activity on sub-culture occur until active growth sets in.

Optimum deaminase activity towards a given amino-acid is in general only shown by strains which have been specifically trained by serial sub-culture in its presence, but growth in a given amino-acid usually occurs at a greater rate than ammonia can be furnished by deamination. The amino-acid has two functions the relative importance of which varies from case to case, namely, to provide ammonia and to be utilised directly.

There is a well-defined correlation between growth of *Bact. lactis ærogenes* with various carbon sources and its dehydrogenase activity towards them (Davies and Hinshelwood, *Trans. Faraday Soc.*, 1947, **43**, 257; Cooke and Hinshelwood, *ibid.*, p. 733).

This section of the present work explores the connexion between utilisation of various amino-acids and the deaminase activity of *Bact. coli* towards them.

The strain used was derived from M referred to in Part I and was trained to asparagine and ammonia by repeated sub-culture in a glucose–phosphate–magnesium sulphate medium containing the former.

Deaminase activity and growth characteristics were then determined for this strain and for derived strains trained to various other amino-acids.

Method.—Growth rates, lags and total populations were determined in the usual way at 40·0°.

Deaminase activities were measured with preparations of cells centrifuged from the appropriate cultures, washed with isotonic saline, suspended in a little water and stirred by aeration. The heavy suspension thus obtained was added to a medium containing all the normal constituents for growth except glucose (phosphate, magnesium sulphate, amino-acid under test). The count was adjusted to a value suitable for the determination (100—500 million per ml. being convenient according to circumstances).

Samples were taken as soon as possible after the addition of the cells and at intervals of about 15 minutes, the ammonia formed being estimated by Conway and O'Malley's method (*Biochem. J.*, 1942, **36**, 655). 1 Ml. of the sample was immediately pipetted into the outer compartment of the Conway dish and 1 ml. of the saturated potassium metaborate added, the determination being then completed in the standard manner. Blank tests gave values ranging from 0·0 to 1·5 mg./l. of ammonia. The results on deamination rate are conveniently expressed in mg./l. of ammonia produced per minute for each 1·25 million cells per ml. (corresponding to unit

hæmocytometer count). Some typical measurements are given in Fig. 1, and Table I shows that the activity so measured is independent of the actual cell count used in the experiment.

TABLE I.

Deaminase activity and cell count.

Count during test in 10^6 per ml.	162	250	625
$A \times 10^4$	8·8	8·9	8·8

Training and Deaminase Activity.—The strain was trained by serial sub-culture in the appropriate media.

Table II shows the deaminase activities of the various trained strains towards the different amino-acids.

TABLE II.

Deaminase activity of trained sub-strains.

	Deaminase activity $\times 10^4$.			
	Asparagine.	Aspartic acid.	Alanine.	Glycine.
Strain trained to asparagine only	8·9	1·6	1·6	0·5
Strains trained to asparagine and aspartic acid ...	26·0	15·0	8·4	4·4
,, ,, ,, alanine	18·3	6·1	13·3	2·7
,, ,, ,, glycine	13·5	4·0	4·7	8·8

From Table II emerge the conclusions (*a*) that the activity towards asparagine can be still further increased by additional training especially in aspartic acid, (*b*) that in general *some* increase in activity towards all the amino-acids is brought about by training to any one of them, but that (*c*) *optimal* activity towards aspartic acid, alanine or glycine is only reached by strains which have been previously trained by serial sub-culture in the presence of that particular nitrogen source. The specificity, though considerable, is on the whole less marked than that appearing in the dehydrogenase activity of strains trained to various carbon sources.

Growth Characteristics.—The growth characteristics of the various trained sub-strains are recorded in Table III.

TABLE III.

Growth characteristics of trained sub-strains.

ΔL = lag in test medium minus lag in asparagine (minutes); m.g.t. = mean generation time (minutes); n_s = total bacterial population at end of growth ($n = 1$ corresponds to 1·25 million per ml.).

	Tested in											
	Asparagine.			Aspartic acid.			Alanine.			Glycine.		
	ΔL.	M.g.t.	n_s.	ΔL.	M.g.t.	n_s.	ΔL.	M.g.t.	n_s.	ΔL.	M.g.t.	n_s.
Trained to asparagine only	0	29	2700	10	73	2000	60	31	2200	10	(113)	570 (1000)
Trained to asparagine plus aspartic acid	0	25	2400	0	38	2000	110	35	2100	100	(61)	580 (1000)
Trained to asparagine plus alanine	0	30	2700	25	73	2000	55	30	2200	35	(153)	650 (1000)
Trained to asparagine plus glycine	0	33	2700	10	70	2000	90	33	2000	55	52·5	2100

Of the results in Table III the following may be said.

(*a*) The changes in lag are not important, though there is a small tendency for the lags in alanine and glycine to be longer than those in aspartic acid and asparagine.

(*b*) The mean generation time for all strains approaches the optimal in alanine whether or not they have been specifically trained. On the other hand, in glycine and aspartic acid the growth of any but the specifically trained strains is much slower. In glycine, moreover, the phenomenon of broken growth curves appears (Lodge and Hinshelwood, *Trans. Faraday Soc.*, **1944, 40**, 571), the logarithmic form only being developed after considerable training.

(*c*) As far as total population is concerned the differences recorded in n_s are hardly significant. The only specificity is that observed in connexion with the step-wise growth in glycine.

The parallelism between growth rate and deaminase activity is only partial. With glycine and aspartic acid, optimal growth is correlated with optimal deaminase activity, but with

alanine the former seems attainable without the latter, as appears from a comparison of Tables II and III.

Nitrogen Consumption and Cell Count.—It is important for many purposes to know the total increase in bacterial population corresponding to a given utilisation of asparagine or ammonia. Very small inocula of *Bact. coli* (M) were given to media containing varying amounts of asparagine or ammonium sulphate (which have the same molecular weight and the same nitrogen content), and the total populations counted 24 hours after growth had ceased. The results are shown in Fig. 2. From the slope of the curve it is calculated that 1·25 million cells per ml. (hæmocytometer counts of unity) are equivalent to 0·0732 mg./l. of ammonia.

FIG. 1.

Deaminase activity of Bact. coli (M) *towards various substrates.*

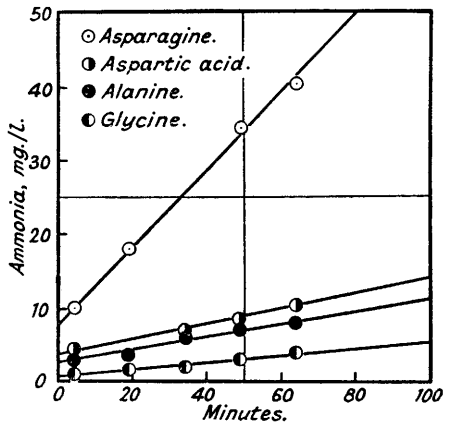
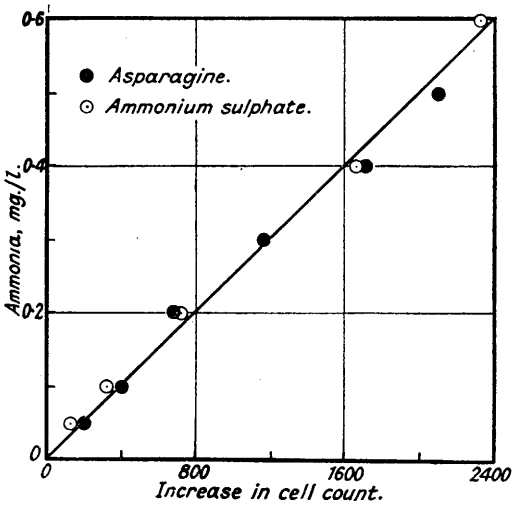


FIG. 2.

Nitrogen utilisation (expressed in terms of NH_3*) for given increases of cell population.*



Decay and Recovery of Deaminase Activity.—The next problem to be considered was the variation of deaminase activity towards asparagine as the cells aged and passed into the "resting" state. In this phase there is a progressive increase in the lag which must occur before growth takes place on inoculation into fresh medium. Table IV shows that the development of lag is *not* normally correlated with loss of deaminase activity, a lag of 49 hours being reached with only a relatively small change in this activity.

TABLE IV.

Variation of deaminase activity with age.

(All measurements made at 40·0°.)

Age of culture, days.	Lag, hours.	Deaminase activity × 10^4. Glucose absent.	Glucose present.
0·9	3·5	8·6—9·2	—
3·9	—	7·8—9·2	6·9—9·0
5·9	16	8·0—8·9	7·2—7·5
7·9	19	8·2—8·7	6·3—7·8
11·9	29	7·1—8·5	6·5—8·1
17·9	49	5·7—7·6	5·7—8·7
26·9	—	4·6—5·1	5·1—6·4

Only after a period of ageing considerably longer than that shown in Table IV does the deaminase activity drop towards zero. At this stage the lag rises rapidly to an infinite value, and, since no sub-cultures are possible, there is good reason to believe that most of the cells are dead. These results are generally similar to those found in the study of dehydrogenase activity (James and Hinshelwood, *Trans. Faraday Soc.*, 1948, **44**, 967).

The recovery of activity of a 78-day old culture with deaminase activity 1·6 × 10^{-4} units was next studied. A suspension of 250 million cells/ml. was made in the full asparagine growth medium, the ammonia concentration and the bacterial count being determined at intervals.

From the results giving the ammonia utilised for a given increase in n (Fig. 2), the ammonia concentration could be corrected for that which might have been formed and subsequently used in growth. Maximum ammonia production could then be plotted against time, and from the tangent to this curve the maximum possible value of the deaminase activity $\frac{1}{n}\frac{d[NH_3]}{dt}$ could be calculated. Some results are given in the first three columns of Table V and show that there is no appreciable recovery before growth sets in.

TABLE V.

Recovery of deaminase activity.

Time (minutes).	Total count, n.	Deaminase activity, $A \times 10^4$.	Viable count, n'.	$\frac{An}{n'} \times 10^4$.
0—150 (6 readings)	178	1·7	38	8·0
200	190	2·5	50	9·5
304	363	6·0	223	9·8
375	660	6·8	520	8·6
436	1520	8·1	1380	8·8

An estimate of the viable count of the initial 78-day old culture by a method described previously (Lodge and Hinshelwood, *J.*, 1943, 213) gave the result that 140 out of 178 cells were dead. The fourth column of the Table gives the viable count, and the last the deaminase activity related to the number of living cells rather than to the total number. The approximate constancy of these results supports the view that the deaminase activity is only seriously lost with the death of the cell and that the recovery is a measure of the production of fresh viable cells.

Deaminase Activity and Growth Rate.—The question now arises as to whether growth can follow the route: amino-acid ⟶ ammonia ⟶ synthesis, or whether direct utilisation of the amino-acid by trans-amination and other reactions occurs.

The relation between the increase of bacterial count and the ammonia consumption is already known, and is expressed by the relation

$$-\frac{d[NH_3]}{dn} = a$$

From this it follows that

$$-\frac{1}{n}\cdot\frac{d[NH_3]}{dt} = \frac{a}{n}\frac{dn}{dt} = ak$$

but $k = 0{\cdot}693/T$, where T = mean generation time. Therefore

$$-\frac{1}{n}\cdot\frac{d[NH_3]}{dt} = \frac{0{\cdot}693a}{T}$$

If this rate of ammonia consumption were provided by the deamination reaction, $-\frac{1}{n}\frac{d[NH_3]}{dt}$ would equal A, the deaminase activity. Thus $A_0 = 0{\cdot}693a/T$, where A_0 is the deaminase activity just able to furnish ammonia at the rate required when the mean generation time is T.

$a = 0{\cdot}0732$, when $[NH_3]$ is expressed in mg./l. and n in units of 1·25 millions/ml. (unit hæmocytometer count).

TABLE VI.

Nitrogen utilisation and deaminase activity.

(A = actual deaminase activity; A_0 = deaminase activity required to supply ammonia fast enough to account for observed growth rate.)

	Growth in:							
	Asparagine.		Aspartic acid.		Alanine.		Glycine.	
	$A \times 10^4$.	$A_0 \times 10^4$.	$A \times 10^4$.	$A_0 \times 10^4$.	$A \times 10^4$.	$A_0 \times 10^4$.	$A \times 10^4$.	$A_0 \times 10^4$.
Strain trained to asparagine only ...	8·9	17·5	1·6	7·0	1·6	16·4	0·5	4·5
Trained to asparagine and aspartic acid...	26·0	20·3	*15·0*	*13·4*	8·4	14·5	4·4	8·6
Trained to asparagine and alanine	18·3	16·9	6·1	7·0	13·3	16·9	3·7	3·3
Trained to asparagine and glycine	13·5	15·5	4·0	7·2	4·7	15·5	*8·8*	*9·7*

From the values of T in Table III and the values of A in Table II the comparisons in Table VI may be worked out.

Consideration of Table VI reveals the following facts.

(*a*) The strain trained to asparagine only cannot deaminate any of the amino-acids rapidly enough to account for the growth rate if growth is supposed to occur by way of ammonia liberation. Some more direct mode of utilisation is therefore operative, probably one involving a trans-amination mechanism.

(*b*) In the sub-strains trained to aspartic acid, alanine or glycine the activity approaches or exceeds that required to account for growth *via* ammonia. With alanine as substrate the direct utilisation appears to be very efficient and no great advantage accrues from the addition or substitution of the deamination mechanism. With glycine, however, the whole character of the growth curves changes when the new mechanism is developed. The present results provide corroborative evidence for the idea of alternative growth mechanisms already advanced on other grounds (Lodge and Hinshelwood, *loc. cit.*, 1944).

(*c*) The behaviour in the asparagine medium requires further consideration. The important point to note is that subsequent training to other amino-acids can improve the deaminase activity towards asparagine itself without appreciably increasing the corresponding growth rate. This is considered further in the following paragraph.

Deamidation and Deamination of Asparagine.—The two nitrogen atoms in asparagine, $\begin{matrix}CH(NH_2)\cdot CO_2H\\ CH_2\cdot CO\cdot NH_2\end{matrix}$, may obviously be used in different ways.

Application of the Martin and Synge method of partition chromatography, in a way to be described later, showed that, during growth in asparagine, aspartic acid first accumulated and was then consumed again. An estimate could be made of the rate of deamidation of asparagine to aspartic acid and yielded the approximate value of $7{\cdot}0 \times 10^{-4}$ units (on the scale employed for the determination of deaminase activity). If, as a rough approximation, this value for the deamidation rate of asparagine is assumed to remain constant, and the changes accompanying the training to other media are ascribed solely to improvements in the deamination of the aspartic acid part of the system, we should expect the relation: *Total activity towards asparagine in given medium* $= 7{\cdot}0 +$ *activity towards aspartic acid in that medium.* Table VII shows that this possibility is approximately realised.

TABLE VII.

	$A \times 10^4$ for aspartic acid $= B$.	$B + 7{\cdot}0$.	Observed total activity ($\times 10^4$) to asparagine.
Strain trained to asparagine	1·6	8·6	8·9
Trained to asparagine and aspartic acid ...	15·0	22·0	26·0
Trained to asparagine and alanine	6·1	13·1	18·3
Trained to asparagine and glycine	4·0	11·0	13·5

Conclusion.—This part of the work shows clearly that amino-acids in general may be first deaminated by *Bact. coli* to ammonia which is then used in synthesis, or that, alternatively, they may be more directly utilised by trans-amination and other processes. Optimum growth rate of the *Bact. coli* does not necessarily involve optimum deaminase activity. Maximum deaminase activity is only reached after training to the specific substrate. With some amino-acids, *e.g.*, glycine, this is true also of growth rate; with others, however, *e.g.*, with alanine, the optimal growth rate is attained without any training of the deaminase activity. Possibly the three-carbon molecule of alanine is more suitable for direct conversion into essential intermediates than the two-carbon molecule of glycine, whose best rôle in the bacterial economy is the simple provision of nitrogen for reaction with other compounds from the carbon source. That alanine may be able to transfer its amino-nitrogen more easily than glycine is a possibility which must also be borne in mind. Asparagine and aspartic acid (and also glutamic acid) are specially suitable for direct use (see Part III).

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