69. The Effect of the *p*H of the Medium during Growth on the Enzymic Activities of Bacteria (*Escherichia coli* and *Micrococcus lysodeikticus*) and the Biological Significance of the Changes Produced

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Researches of recent years on the enzymic activities of micro-organisms have shown that the bacterial cell possesses a very variable enzymic constitution. Karström [1938] pointed out that bacterial enzymes can be divided into two main classes: adaptive enzymes which are formed by the cell in response to the presence of the specific substrate, and constitutive enzymes which are formed whether the corresponding substrate is present during growth or not. This covers only one aspect of enzyme variation as both adaptive and constitutive enzymes undergo considerable variation with the physical conditions holding during growth. Thus the activities of the cells may alter with the temperature and degree of anaerobiosis during growth, the age of the culture and the presence in the medium of substances which are not related to the enzyme substrate, etc. The present communication deals with the alterations in enzyme activities of two organisms, *E. coli* and *M. lysodeikticus*, with the *p*H of the medium in which they are grown.

Gale [1940*a*; 1941] has shown that certain bacteria are able to produce amines by the decarboxylation of amino-acids by specific decarboxylases. These enzymes, studied in suspensions of washed intact organisms, have *p*H optima at unusually low values lying between 2·5 and 5·5, and are only formed by the organisms when growth occurs in acid media, the lower the *p*H the greater the resulting decarboxylase activity within physiological limits. Silverman & Werkman [1941] have shown that *A. aerogenes* can decompose pyruvic acid in two ways: a hydroclastic breakdown to acetic and formic acids and a second mechanism involving the condensation of two pyruvic acid molecules to acetylmethylcarbinol. The carbinol system has an optimal activity in the vicinity of *p*H 5 and is formed only in response to an acid growth environment, so that if the organism is grown in a medium maintained at an alkaline reaction, then the formation of the carbinol system is completely suppressed. The results are due to a true variation in the enzyme content of the cells as they are confirmed by experiments carried out with enzyme preparations obtained by disintegration of the cells with powdered pyrex glass. Earlier observations by Kocholaty & Hoogerheide [1938] on certain dehydrogenation reactions obtained with *Cl. sporogenes* suggested that the optimum activity *p*H of certain dehydrogenases, e.g. alanine and pyruvic dehydrogenases, can shift with alteration of the growth *p*H. A similar result is claimed by Kocholaty & Weil [1938] for the proteases of *Cl. histolyticum* where the *p*H optimum of the excreted protease is stated to shift from 7 when growth occurs in casein broth, to 6 when growth occurs in the presence of glucose. It is not possible to tell from the results in this case whether more than one protease is involved and, as it appears that glucose can alter the enzymic constitution of bacteria apart from any *p*H effect involved in its fermentation [Epps & Gale, 1942], these results

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require further elucidation. In the organisms studied, we have been unable to show any significant shift of the optimum *p*H of any enzyme with alteration of the growth *p*H, and it seems that this factor is a characteristic of the enzyme rather than of the culture in which it is produced.

Methods

The results described in this paper were obtained with the 'original *Escherichia coli*' National Collection of Type Cultures no. 86, and with *Micrococcus lysodeikticus* no. 2665. The basal medium used throughout was tryptic digest of casein with the addition of 0·5 % NaCl and, in the case of *M. lysodeikticus*, 1 % marmite. For the greater part of the work, growth took place in flasks incubated at 27° but *M. lysodeikticus* is difficult to grow in liquid media and in order to get sufficient material this organism was grown in Roux bottles containing 70 ml. medium, lying on their sides and incubated at 37°. *E. coli* will grow under these conditions at any *p*H between 4·5 and 9 although the yield of organism towards the limits of this range is small and it was sometimes impossible to harvest cultures grown at *p*H 4·5. The media were adjusted to the desired *p*H before growth by the use of a glass electrode and the *p*H was again checked when growth had finished. The metabolic activities of the organisms tend to alter the *p*H of the medium so that there is a drift towards neutrality from either side. This drift may amount to as much as 1·5 *p*H units at the end of the range and for the purpose of this work it was necessary to prevent the drift as far as possible. The best way to accomplish this was found to be the incorporation of buffer in the medium and the following final concentrations of buffer were used: for media adjusted to *p*H 4·5 and 5, *M*/60 phthalate; for *p*H 6–8, *M*/45 phosphate, and for *p*H 8·5 and 9, *M*/60 borate. These additions do not interfere with the growth of the organisms and we have satisfied ourselves that the results to be presented are not due to, or affected by, the presence of the buffers. This point has been checked in all possible cases by repetition of the estimations as far as possible with cultures grown in media adjusted without buffers and by checking the activity of the *p*H 7 culture with that of a culture grown at *p*H 7 in the presence of *M*/60 phthalate and borate; in no case has there been any significant effect due to the presence of buffers as such during growth. Even with the addition of these amounts of buffer to the media, there is still a small drift in *p*H during growth and the extent of this drift is shown for a typical example in Table 1. The organisms were harvested at the end of growth, washed

Table 1. *Variation of* p*H of media during growth of* E. coli

Buffer	Initial *p*H	Final *p*H	Mean *p*H	Buffer	Initial *p*H	Final *p*H	Mean *p*H
M/60 phthalate	4·55	4·71	4·63	*M*/45 phosphate	8·21	7·82	8·01
M/60 phthalate	4·95	5·58	5·26	*M*/60 borate	8·82	8·70	8·75
M/45 phosphate	6·16	6·58	6·37	*M*/60 borate	9·10	9·10	9·10
M/45 phosphate	7·01	7·10	7·05				

in water and made up into washed suspensions in water. The dry weight of the suspensions was determined by the use of a photoelectric turbidimeter previously calibrated against the organisms concerned.

The methods used for the estimation of enzymic activity differ with the system concerned and so will be described in the main text. It is desirable in work of this nature to restrict the investigation to single enzymes so that complication of the results does not arise owing to different types of variation of parts of an enzyme system, and so it is necessary to choose the estimation technique in each case so that the results obtained may be ascribed with reasonable certainty to the action of a single enzyme.

Range of p*H of medium in which* E. coli *will grow*

Fig. 1 shows the variation of the generation time of *E. coli* with the *p*H of the medium during growth. The generation time was estimated by the use of the photoelectric turbidimeter, the amount of organism present being estimated every 20 min. from the first appearance of turbidity to the end of the logarithmic phase of growth, and thus represents the time taken for the dry weight of organism to double itself within the logarithmic phase. This method involves certain errors due to variation in size of the

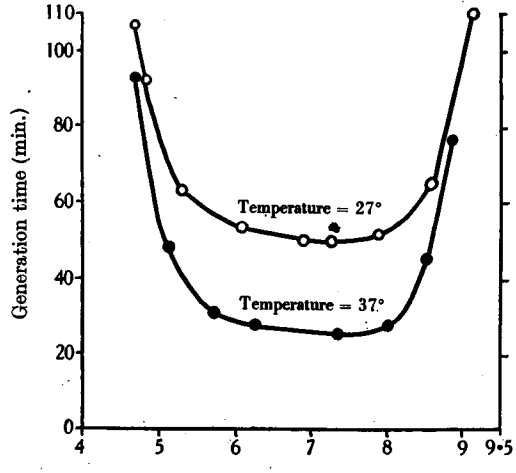

Fig. 1. Variation of generation time with growth *p*H (*E. coli*).

individual organisms in the early stage of growth but is sufficiently accurate for the purpose required. *E. coli* is able to grow at any *p*H between 4·5 and 9, the generation time outside these limits being so long that growth is insignificant. The generation time is unaffected by growth *p*H as long as the latter falls between the approximate limits 5·8–8·0. Fig. 1 also shows the effect of growth temperature on the generation time at all growth *p*H values.

Maintenance of cultures

At first it was thought that it might be possible to adapt the organism to various growth *p*H values. Accordingly cultures were grown at various values of *p*H within the growth range; each was subcultivated daily for one month into media of the same *p*H, and then used for inoculation of the medium at the same *p*H used for bulk experiments. The activities of such cultures were compared with those inoculated from neutral media, with results shown in Table 2. It can be seen that cultures inoculated from organisms 'trained' at *p*H 5·3 and 8·9 and grown at those *p*H values have substantially the same activities as cultures grown at *p*H 5·3 and 8·9 but inoculated from organisms maintained

Table 2. *Effect of maintenance* p*H of culture on activities*

*p*H of medium ...	5·3	8·9	5·3	8·9
Inoculum grown at *p*H ...	7·0	7·0	5·0	9·0
Enzyme:				
Arginine decarboxylase	264	0	238	0
Ornithine decarboxylase	374	0	320	0
Lysine decarboxylase	233	0	242	0
Histidine decarboxylase	22	0	16	0
Glutamic acid deaminase	5	17	5	14
Formic dehydrogenase	138	319	147	272

in neutral media. Likewise we have found that it is not possible to extend the growth *p*H range by 'training' at the extreme values. Accordingly this method of maintaining the cultures was abandoned and media were inoculated from cultures in normal neutral medium.

To avoid complications due to age of culture effects, it has been our custom to inoculate the media at the ends of the growth range a few hours earlier than the remainder and all cultures have been harvested as nearly as possible at the end of the period of active cell division, when activities are maximum for the culture. In order that activities determined for cultures grown at various *p*H values might be comparable, it has also been the custom to deal with one enzyme at a time and to grow cultures over the full *p*H range simultaneously. To avoid dilution effects, suspensions prepared from each day's set of cultures have been adjusted to approximately the same dry weight of organism per ml.

The results set out below for *E. coli* have all been obtained with suspensions of intact organism and consequently all activities are referred to the enzymes concerned as estimated in the intact organism and *p*H values refer to those holding in the external environment.

Enzymes concerned in the breakdown of amino-acids

The strain of *E. coli* used in this work can form, under suitable growth conditions, specific enzymes bringing about the decarboxylation of *l*(+)-arginine, *l*(+)-ornithine, *l*(+)-lysine and *l*-(−)histidine with the formation of agmatine, putrescine, cadaverine and histamine respectively. When investigated in the intact organism, the arginine decarboxylase has an optimum *p*H lying between 4·0 and 4·5; ornithine decarboxylase at 5·0; lysine decarboxylase between 4·5 and 5·0, and histidine decarboxylase between 4·0 and 4·5. The conditions of growth under which these enzymes are formed have been studied in detail [Gale, 1940*a*]: when growth occurs at *p*H 7 or higher the resulting organisms possess little or no decarboxylase activities, the enzymes appearing as the growth *p*H falls and reaching very high activities when the growth *p*H is of the order of 5 or less (Q_{CO_2} ornithine = 500; arginine = 330; lysine = 200; histidine = 30). The presence of the specific substrate during growth at an acid reaction is probably necessary for the formation of these enzymes. This result has now been obtained with many organisms [Gale, 1940*a*; 1941] and it appears certain that amino-acid decarboxylases are formed by bacteria only in response to an acid growth environment. Accordingly we began this investigation by studying the variation with growth *p*H of the amino-acid deaminases of *E. coli*. As the decarboxylases of this organism are inhibited by high growth temperatures, we have grown all cultures at 27° so that the results should be comparable with those previously obtained with the decarboxylases. The experimental temperature used, except where otherwise stated, was 30°.

dl-*Alanine deaminase.* *E. coli* possesses an enzyme which deaminates *dl*-alanine with the formation of pyruvic acid and NH_3 [Gale, 1940*b*]. The enzyme formation is inhibited by anaerobic growth conditions so that cultures were grown in this case in liquid media in Roux bottles lying on their sides. The activity of the washed suspensions was determined by shaking aerobically with *dl*-alanine solution and buffer, taking samples at intervals for NH_3 determination by the method of Parnas; details as described by Stephenson & Gale [1937*a*]. Activities are expressed as $Q_{NH_3} = \mu$l. ammonia-N liberated per hr. per mg. dry weight of organism. In the first place it was necessary to determine the optimum activity *p*H which has been recorded previously as 7·5 [Stephenson & Gale, 1937*a*]. However it was found that the optimum activity *p*H varies with the nature of the buffer in which the experiment is carried out. Fig. 2*b* shows that the optimum *p*H lies between 7·0 and 7·5 if determinations are carried out in the presence of phosphate, but if borate or veronal buffers are used, then the optimum *p*H lies near 9·5, while the activity at *p*H 7 is less in veronal than in phosphate. The figures quoted were obtained with a

culture grown at *p*H 7 but the curves obtained with cultures grown at other *p*H values differ only in magnitude. Since the enzyme thus exhibits two *p*H optima, estimations of the activities of cultures have been made in every case at *p*H 7 in phosphate and at *p*H 9·5 in borate. Fig. 2*a* shows the variation in Q_{NH_3} so determined for cultures grown in

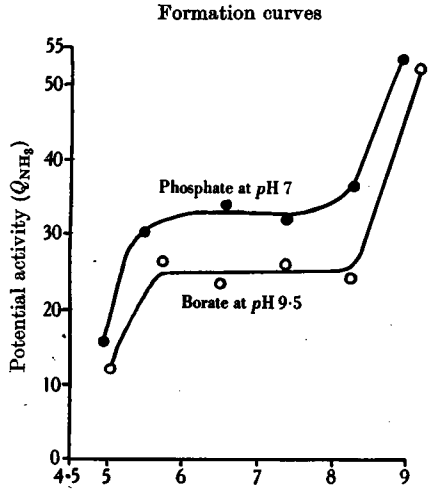

Fig. 2*a*.

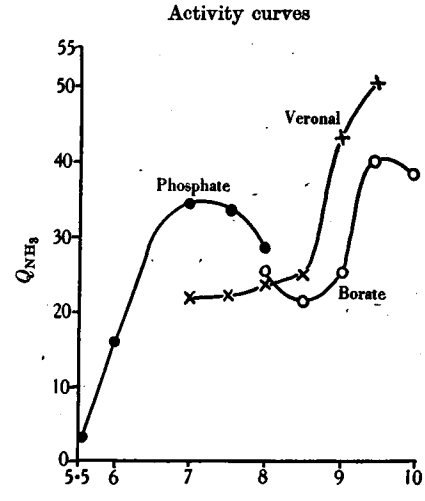

Fig. 2*b*.

Fig. 2. Alanine deaminase. (*a*) Variation of activity (Q_{NH_3}) with growth *p*H. (*b*) Activity-*p*H curves in various buffers: culture grown at *p*H 7.

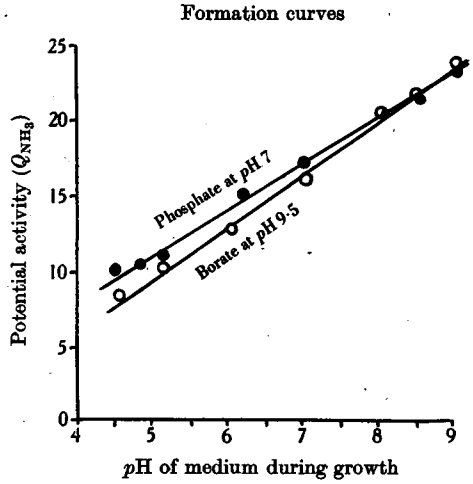

Fig. 3*a*.

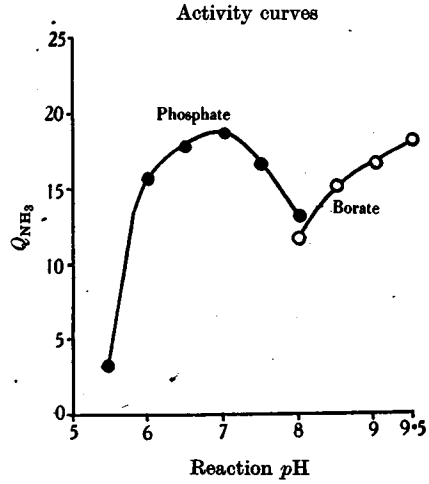

Fig. 3*b*.

Fig. 3. Glutamic acid deaminase. (*a*) Variation of activity (Q_{NH_3}) with growth *p*H. (*b*) Activity-*p*H curves in phosphate and borate: culture grown at *p*H 7.

media adjusted to *p*H values between 4·5 and 9; the growth *p*H values recorded are the average of the initial and final *p*H of the medium in each case (Table 1). Whichever buffer is used we get a curve of the same shape showing little alanine deaminase formation in acid growth conditions, a plateau over the centre of the range and increasing enzyme formation as the *p*H moves from 8 to the alkaline limit of growth. Determination of the

optimum activity *p*H for cultures grown at *p*H 5 or 9 shows that this has not shifted with growth *p*H whether estimated in phosphate or borate.

l(+)-*Glutamic acid deaminase.* The deamination of glutamic acid by *E. coli* is carried out by a reversible dehydrogenase-coenzyme system similar to that found in animal and

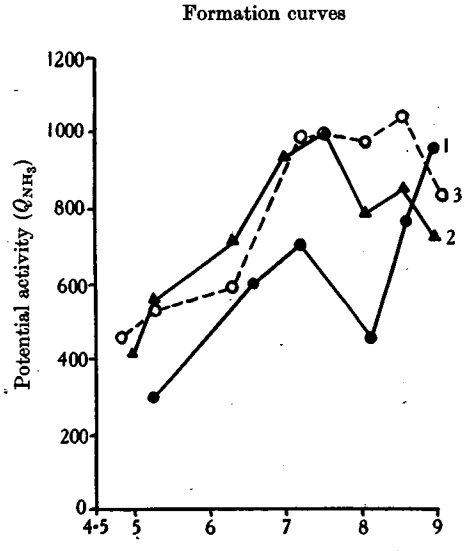

Fig. 4 *a*.

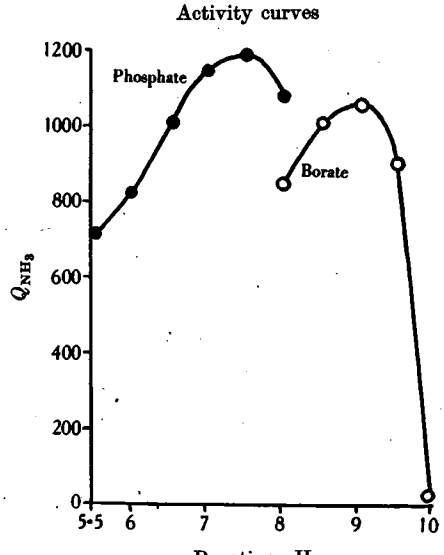

Fig. 4 *b*.

Fig. 4: Serine deaminase. (*a*) Variation of activity (Q_{NH_3}) with growth *p*H. 1, 14 hr. culture: estimations in phosphate at *p*H 7·5. 2, 16 hr. culture: estimations in phosphate at *p*H 7·5. 3, 16 hr. culture: estimations in borate at *p*H 9. (*b*) Activity-*p*H curves in phosphate and borate for culture grown at *p*H 7·5.

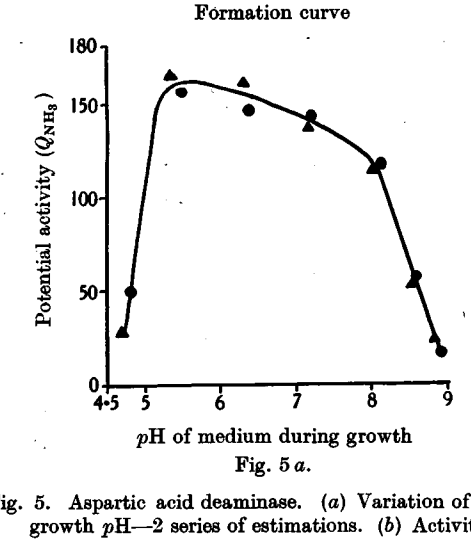

Fig. 5 *a*.

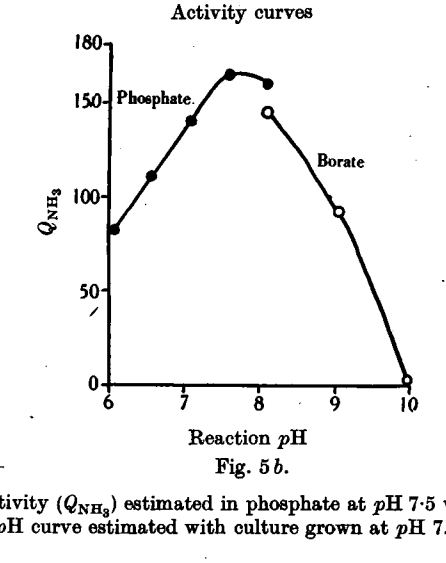

Fig. 5 *b*.

Fig. 5. Aspartic acid deaminase. (*a*) Variation of activity (Q_{NH_3}) estimated in phosphate at *p*H 7·5 with growth *p*H—2 series of estimations. (*b*) Activity-*p*H curve estimated with culture grown at *p*H 7.

plant cells [Adler, Hellstrom, Gunther & Euler, 1938]. Estimations of activity were carried out in a manner similar to that used for alanine deaminase except that cultures were grown in flasks, as semi-anaerobic conditions do not inhibit enzyme formation in this case [Stephenson & Gale, 1937*a*]. Fig. 3*b* shows that the value of the optimum

activity *p*H for a culture grown at *p*H 7·5 again varies with the nature of the buffer used. Accordingly estimations have been made for all cultures at *p*H 7 in phosphate and at *p*H 9·5 in borate. Fig. 3*a* shows the variation of the activity, so determined, of cultures grown at various *p*H values. In this case we get a linear increase in enzyme formation as the growth *p*H moves from 5 to 9, whether the activities are determined in phosphate or borate.

dl-*Serine deaminase.* Gale & Stephenson [1938] showed that *E. coli* can deaminate *dl*-serine anaerobically, the formation of the deaminase being favoured by anaerobic growth conditions. Estimations of the activity in this case are complicated by the fact that the activity of a culture falls rapidly once the cells are made up into washed suspension. This loss of activity can be checked if the cells are washed and kept in the presence of *M*/100 phosphate and *M*/200 formate, which acts as a reducing agent. In the present work, cultures have been grown for 14 or 16 hr. at 27°, centrifuged out of

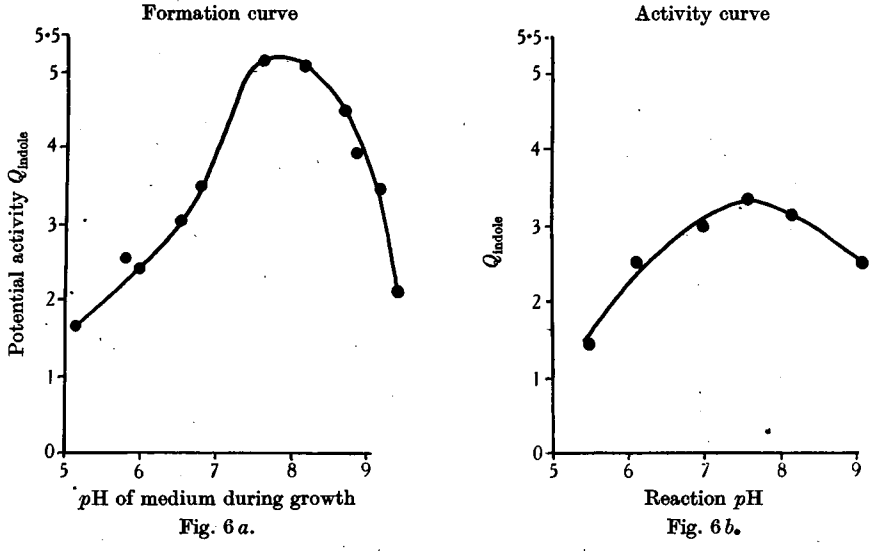

Fig. 6*a*. Fig. 6*b*.

Fig. 6. Tryptophanase. (*a*) Variation of activity (Q_{Indole}) estimated at *p*H 7·5 with growth *p*H. (*b*) Activity-*p*H curve estimated with culture grown at *p*H 7.

culture, washed once in *M*/100 phosphate *p*H 7·5 and *M*/200 formate, made up in *M*/100 phosphate and the activities estimated as rapidly as possible after preparation of the suspensions. In this way decay of the activities should have been minimized. Fig. 4*b* shows that the *p*H optimum again shows a variation with the nature of the buffer used but in this case the enzyme is almost inactive at an external *p*H of 10 in borate, and shows two marked optima at *p*H 7·5 in phosphate and at *p*H 9 in borate. The formation curve varies slightly according to the age of the culture but exhibits two optima, one at a growth *p*H of 7·5 and another at a growth *p*H of 8·5 or 9 according to the age of the culture. The shape of the curves is the same whether they are determined in phosphate or borate and resembles that which would be obtained by combination of the two activity curves. The significance of these curves is discussed later.

l(−)-*Aspartic acid.* Quastel & Woolf [1926] found a reversible enzyme which deaminates aspartic acid anaerobically to fumaric acid in *E. coli*. This enzyme is not affected by the presence of toluene while Gale [1938] showed that *E. coli* contains at least two enzymes capable of the anaerobic deamination of aspartic acid, one being stable to toluene treatment and the other being completely inhibited under such conditions. The unstable aspartase II fraction requires the presence of a coenzyme replaceable *in vitro* by adenosine: if the activity of washed suspensions is measured aerobically

a linear rate of NH_3 evolution is obtained, but anaerobically the rate falls off rapidly with time. For the present estimations, suspensions were shaken aerobically with *l*(−)-aspartate and NH_3 estimated as usual [Gale, 1938]. The results given in Figs. 5*a* and 5*b* represent the total activities of the suspensions towards aspartic acid under such conditions. Attempts were made to separate the enzymes involved by toluene treatment but it was found that the response varied with the growth *p*H so that the activity of suspensions grown at *p*H 7–9 was greatly decreased by toluene but that of organisms grown at *p*H 5–7 was actually increased. It is obvious that such a method cannot be used to differentiate the enzymes in intact suspensions for this type of work. 'Aspartase' gives activity and formation curves of quite different shape from those obtained with the other deaminases so far studied: in this case the activity curve, Fig. 5*b*, exhibits a single optimum at *p*H 7 with no significant difference at *p*H 8 whether the activity at that *p*H is determined in phosphate or borate. The formation curve, Fig. 5*a*, shows that 'aspartase' activity is inhibited by growth on the alkaline side of *p*H 8 or at very acid values less than 5. Over the growth *p*H range 5–8, maximum formation of the enzyme takes place at the acid end of the range, the curve then showing a linear decrease in formation until the rapid fall sets in beyond *p*H 8. The formation of this deamination system is thus favoured by moderately acid growth conditions.

l(−)-*Tryptophan.* Tryptophan is degraded to indole under strongly aerobic conditions by *E. coli* and the enzyme system involved has been named 'tryptophanase' although it is realized that this is not a single enzyme [Happold & Hoyle, 1935; Woods, 1935]. The first step in the degradation has not yet been identified although Krebs, Hafez & Eggleston [1942] have recently produced evidence that *o*-aminophenylethyl alcohol is an intermediate in the production of indole. For the purpose of this work; 'tryptophanase' activity has been estimated by the simplified technique described by Fildes [1938] and activity is expressed as $Q_{\text{indole}} = \mu$l. indole produced/hr./mg. dry weight of organism. The activity—*p*H curve (Fig. 6*b*) is fairly flat as might be expected for a complex system, and has an optimum at 7·5, which *p*H has been used for the determination of the activities of all cultures. The formation of the enzyme system is greatest when the growth *p*H lies between 7·5 and 8·0 (Fig. 6*a*) and is markedly inhibited by acid or strongly alkaline growth conditions.

It is a reasonable assumption that enzymes acting upon amphoteric substrates are themselves of an amphoteric nature and it might be expected that such substances should vary in activity with the *p*H at which they are formed and so we next turned our attention to certain enzymes acting upon substrates of a non-amphoteric nature.

Enzymes concerned in the degradation of non-amphoteric substrates

The technique employed throughout this section of the work was (1) to determine the optimum activity *p*H for the enzyme concerned using a culture grown at *p*H 7, (2) using this optimum activity *p*H, to determine the activities of cultures grown at *p*H values throughout the range 4·5–9·0, (3) to redetermine the optimum activity *p*H with cultures grown at the ends of the range and at any other growth *p*H of obvious interest, and (4) in selected cases, to determine the activities of the range of cultures each at the *p*H at which it was grown instead of at the optimum activity *p*H. In the description of the work, the following terms will be employed:

Optimum activity p*H*: that *p*H at which the enzyme activity in any one culture is an optimum. This we have found experimentally does not vary with growth *p*H.

Potential activity: the activity of a culture determined at the optimum activity *p*H for the enzyme concerned.

Effective activity: the activity of a culture determined at the *p*H at which growth occurred as distinct from the optimum activity *p*H, and representing that portion of the potential activity which would actually be effective during growth in culture.

In the determination of the optimum activity *p*H, tests were made over the *p*H range 4·0–10·0 using *M*/10 phthalate, *M*/15 phosphate and *M*/20 borate buffers; to check the possibility of activity varying with the nature of the buffer, these were made to overlap; phthalate and phosphate at 5·5, phosphate and borate at 8·0. In no case described below was there any evidence of the optimum activity *p*H varying with the nature of the buffer employed.

The enzymes studied fall into two groups according to the type of variation which their formation in the cell displays with growth *p*H: in group I the loss in effective activity of the enzyme as the growth *p*H deviates from the optimum activity *p*H is compensated by an increased formation of enzyme per cell, so that the effective activity in culture is constant whatever the growth *p*H; while in group II this compensatory formation does not occur, or only to a restricted degree, while the growth *p*H is in the vicinity of the optimum activity *p*H and the enzyme is formed best when the growth *p*H approaches the value of the optimum activity *p*H.

Group I

Formic dehydrogenase. This enzyme is one of the most active of the dehydrogenases of *E. coli* and has an exceptionally high affinity for its substrate [Stickland, 1929]. The dehydrogenase reacts directly with methylene blue without the intervention of coenzyme I or II and reacts with O_2 through the cytochrome system of the bacterial cell [Gale, 1939]. Fig. 7*b* shows that the optimum activity *p*H is approximately 6·0 whether the activity is determined by the methylene blue or O_2 uptake method, and is independent of the growth *p*H. When the potential activity of cultures grown at various *p*H values is determined, we get a curve of the shape shown in Fig. 7*a* whether the estimations are made with methylene blue reduction or O_2 consumption in presence of the substrate; this shows that the formation of the enzyme is minimal when the growth *p*H coincides with the optimum activity *p*H. As the growth *p*H deviates from the value of the optimum activity *p*H, a given quantity of enzyme loses efficiency owing to that deviation, but in the cell this loss of efficiency is compensated by an increased formation of enzyme. Consequently if we estimate the effective activity of the cultures, we find that this is roughly constant throughout the growth range.

Alcohol dehydrogenase. Still [1940] has shown that *E. coli* can oxidize ethyl alcohol by means of alcohol dehydrogenase which reacts with methylene blue in the presence of coenzyme I. The enzyme can be studied as a single system if semicarbazide is present to trap the acetaldehyde and so prevent further oxidation. Activities were accordingly estimated by the methylene blue technique using Thunberg tubes with 1 ml. buffer, 1 ml. bacterial suspension, 0·5 ml. *M*/10 semicarbazide in the main compartment and 0·1 ml. 0·5% methylene blue with 0·2 ml. 50% ethyl alcohol (water in controls) in the hollow stopper. After equilibration, the contents of the stopper were tipped into the main tube and the time noted for the blue colour to disappear. Activities are expressed as $Q_{MB} = \mu$l. O_2 equivalent to amount of methylene blue reduced/hr./mg. dry weight of organism and figures are corrected for the values of the blank controls. Still [1940] noted that the addition of coenzyme to suspensions of intact cells did not significantly affect the activities, indicating that the enzyme within the cell is fully saturated with coenzyme; we confirmed this finding. Fig. 8*b* shows that the optimum activity *p*H falls at approximately 8 whatever the growth *p*H of the culture used, while the formation curve (Fig. 8*a*) has a minimum position when the growth *p*H coincides with the optimum activity *p*H. The potential activity thus shows full compensation for the deviation of the growth *p*H from the optimum activity *p*H, with the result that the effective activity is once more constant throughout the growth range.

Catalase. The catalase activity of suspensions of *E. coli* is so high that special measures have to be adopted for its measurement to attain any degree of accuracy and we are

indebted to Dr Hartree of the Molteno Institute for advice in this matter. The activity has been measured by the use of Warburg manometers containing 1 ml. bacterial suspension and 1·8 ml. buffer in the main compartment, and 0·2 ml. 3 vol. H_2O_2 in the side bulb. The amount of organism found to be satisfactory is 0·1–0·15 mg./ml. suspension.

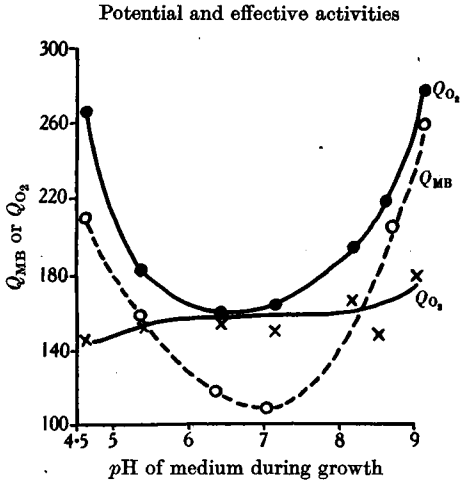

Fig. 7 *a*.

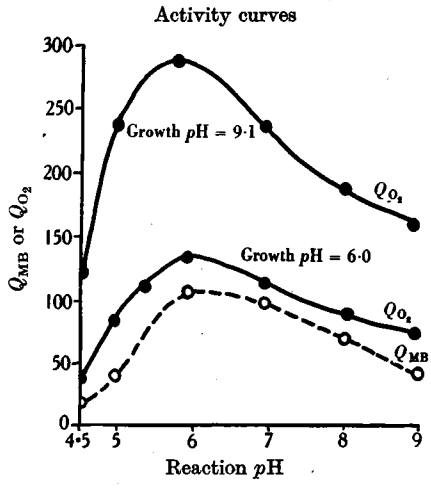

Fig. 7 *b*.

Fig. 7. Formic dehydrogenase. (*a*) Variation of activity with growth *p*H. •—• Potential activity (Q_{O_2}); ×—× effective activity (Q_{O_2}); o- - -o potential activity (Q_{MB}). (*b*) Activity-*p*H curves for cultures grown at *p*H 6·0 and 9·1.

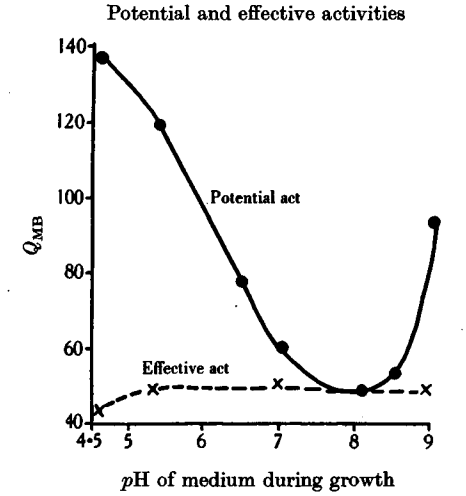

Fig. 8 *a*.

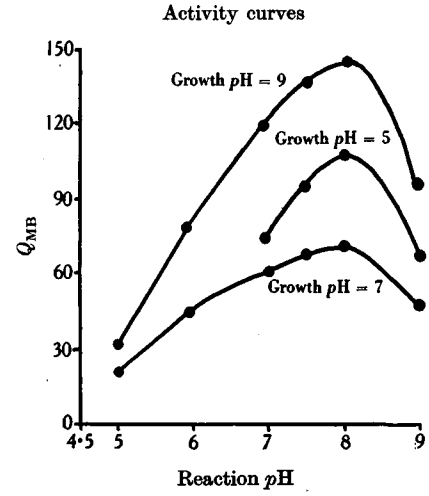

Fig. 8 *b*.

Fig. 8. Alcohol dehydrogenase. (*a*) Variation of potential activity (•—•) and effective activity (×- - -×) with growth *p*H. (*b*) Activity *p*H curves for cultures grown at *p*H 5, 7 and 9.

The manometers are shaken in a bath at room temperature (17°); the substrate is then tipped in after equilibration and readings are taken every 2 min. without stopping the shaker. The manometers are adjusted constantly by one person who takes the readings whilst another notès the times and figures. Under such conditions the enzyme is poisoned in about 20 min. but a linear rate of O_2 evolution can be followed for the first three or four 2 min. periods. Activities are given as Q_{O_2} = rate of O_2 evolution during this linear

period expressed as μl. O_2 evolved/hr./mg. dry weight organism. Fig. 9*b* shows that the optimum activity *p*H falls at about 6·5 though the variation of activity with *p*H is not very great in that region. H_2O_2 tends to decompose spontaneously in alkali but control experiments without organism showed that this effect does not vitiate the results under the experimental conditions described. The formation curve (Fig. 9*a*) is similar to those

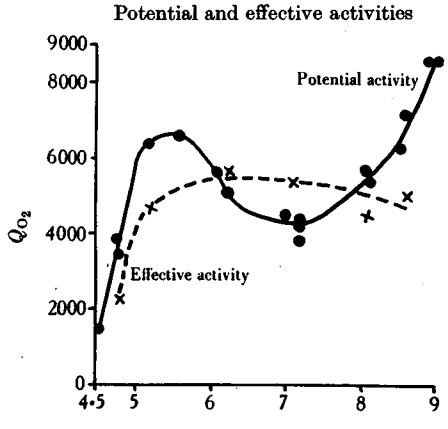

Fig. 9*a*.

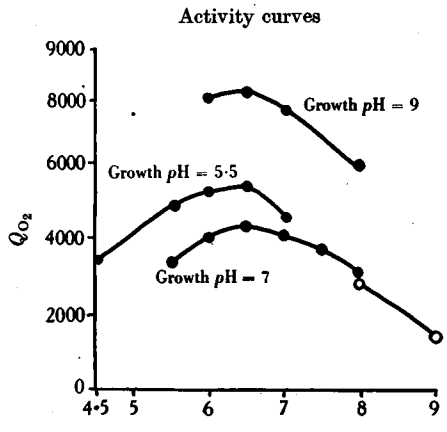

Fig. 9*b*.

Fig. 9. Catalase. (*a*) Variation of potential activity (•—•) and effective activity (×- - -×) with growth *p*H. (*b*) Activity-*p*H curves for cultures grown at *p*H 5·5, 7 and 9.

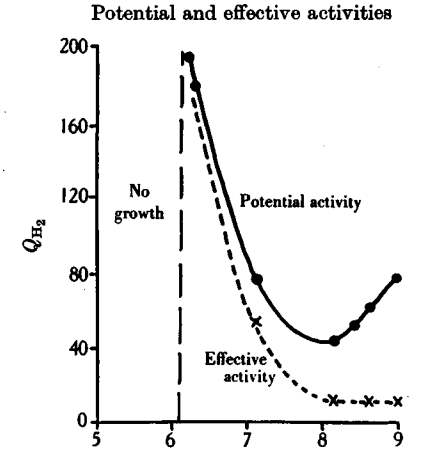

Fig. 10*a*.

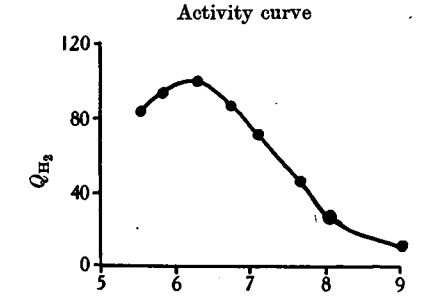

Fig. 10*b*.

Fig. 10. Formic hydrogenlyase. (*a*) Variation of potential activity (•—•) and effective activity (×- - -×) with growth *p*H. (*b*) Activity-*p*H curve for culture grown at *p*H 7.

obtained with formic and alcohol dehydrogenases over the greater part of the growth range but the compensatory formation of the enzyme breaks down when growth takes place at *p*H values below 5·5. Consequently the effective activity is roughly constant when growth occurs from *p*H 6 to 9 but below 6 the effective activity falls rapidly.

Formic hydrogenlyase. Stephenson & Stickland [1932; 1933] found that *E. coli* grown anaerobically in the presence of formate develops an adaptive enzyme decomposing

formic acid to CO_2 and H_2. Woods [1936] showed that the action of this formic hydrogenlyase is reversible. To study the variation of the enzyme with growth *p*H, cultures were grown in the usual media to which 0·5 % sodium formate was added. The addition of formate results in a decreased crop of organism and at medium *p*H values $<6{\cdot}2$ no growth will take place in its presence even when the concentration is reduced to 0·2 %. The activity of the enzyme was estimated in Warburg manometers containing 1 ml. bacterial suspension 3–4 mg. dry weight, 1·5 ml. buffer in the main compartment and 0·5 ml. *M*/30 Na formate in the side bulb (water in controls) with 0·2 ml. 10 % NaOH in the centre pot. The manometers were filled with N_2 gas freed from traces of O_2 by passage over heated copper. After equilibration, the formate was tipped in and the steady rate of H_2 evolution measured; activities are expressed as Q_{H_2}. The optimum activity *p*H is approximately 6·3 (Fig. 10*b*) and the variation of the potential activity with growth *p*H gives a unique curve (Fig. 10*a*). In this case we get minimal formation of the enzyme at a growth *p*H of approximately 8 and a rapidly increasing formation of the enzyme as the growth *p*H falls. The potential activity is greatest at the edge of the growth range where the formate becomes too inhibitory for growth to occur. The effective activity shows a similar type of variation reaching a constant low level when the growth *p*H falls below 8. It appears as though the organism attempts to remove the inhibitory substance by increased enzyme formation where the substrate is most toxic. This variation is a special case in which the formation curve bears no relation to the activity curve.

Group II

Hydrogenase. Suspensions of *E. coli* can activate molecular H_2 to reduce methylene blue by the enzyme hydrogenase [Stephenson & Stickland, 1931]. To investigate this activity, 1 ml. bacterial suspension, 3–4 mg. dry weight, and 1 ml. buffer are shaken with 0·1 ml. 0·5 % methylene blue in Thunberg tubes filled with H_2 and the time noted for the dye to be completely reduced. Fig. 11*b* shows that the optimum activity *p*H is approximately 6 but the curve obtained for the variation of potential activity with growth *p*H is completely different from those obtained with the group I enzymes. In this case the potential activity is least at the ends of the growth range and greatest at a growth *p*H of 8. Thus the optimum formation *p*H does not coincide with the optimum activity *p*H. If we determine the effective activity (Fig. 11*a*) we see that the potential activity is such that the loss of enzyme efficiency as the growth *p*H deviates from the optimum activity *p*H is compensated to some degree over the central part of the growth range only, with the result that the effective activity is roughly constant over a growth *p*H range of 6–7 but falls rapidly outside that range and is almost insignificant at the ends of the complete growth range.

Succinic dehydrogenase. Figs. 11*a* and 11*b* include the curves for succinic dehydrogenase. The activity has been determined by the methylene blue technique as before but in this case the Q_{MB} values do not correspond to the activities of a single enzyme. As in the case of hydrogenase the potential activity shows a restricted degree of *p*H compensation over the central part of the growth range only.

Glucozymase. Glucozymase is a constitutive enzyme of *E. coli* whose formation is roughly doubled by the presence of glucose during growth [Stephenson & Gale, 1937*b*]. To avoid difficulties arising over the value of the *p*H during growth in glucose, the constitutive part of the enzyme only has been studied, growth taking place in the absence of glucose. Activities were studied by estimating the disappearance of glucose incubated anaerobically in the presence of bacterial suspensions and buffer, using the ceric sulphate method of glucose estimation [Giragossintz, Davidson & Kirk, 1936] adapted for the present work along lines similar to those devised by Trim [1938]: activities are expressed as $Q_{glucose} = \mu$l. glucose removed/hr./mg. dry weight organism. Figs. 12*a* and 12*b* show

that the optimum activity *p*H is approximately 6 and the potential activity is greatest when the growth *p*H lies between 7·5 and 8 so that compensation occurs over the middle of the growth range only.

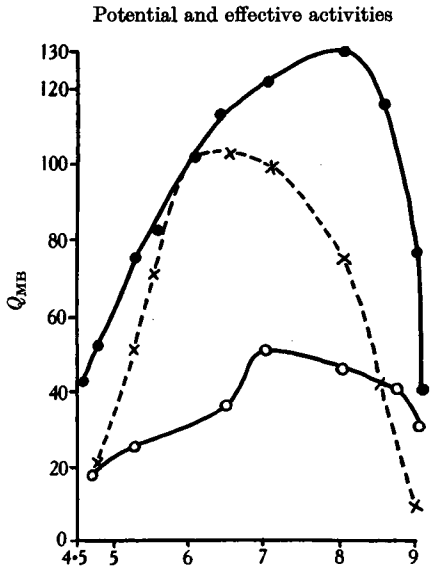

Fig. 11 *a*.

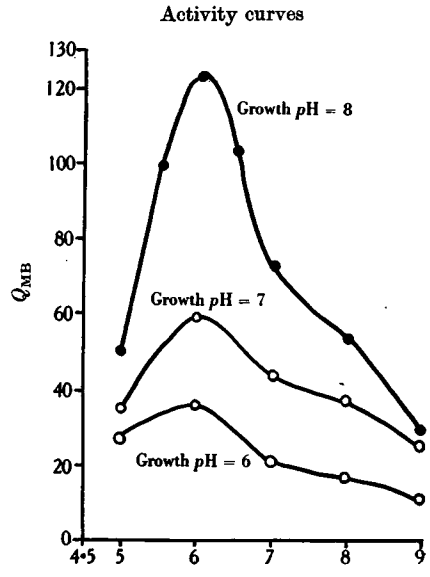

Fig. 11 *b*.

Fig. 11. Hydrogenase and succinic dehydrogenase. (*a*) Variation of activity with growth *p*H. •—• Potential activity: hydrogenase. o—o Potential activity: succinic dehydrogenase. ×- - - × Effective activity: hydrogenase. (*b*) Activity-*p*H curves for hydrogenase •—• and succinic dehydrogenase o—o.

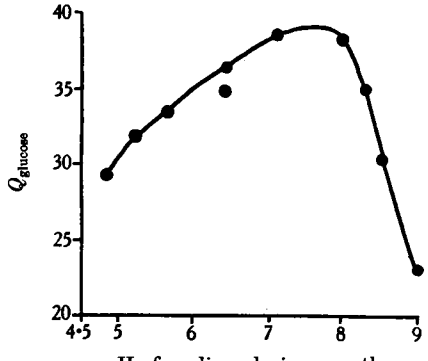

Fig. 12 *a*.

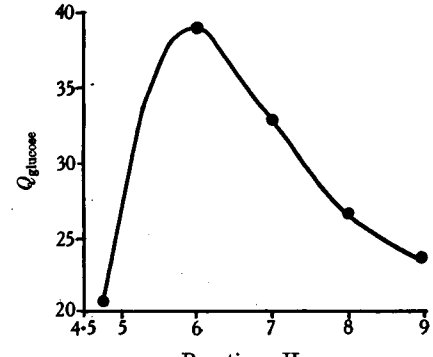

Fig. 12 *b*.

Fig. 12. Glucozymase. (*a*) Variation of activity with growth *p*H. $Q_{glucose}$ = μl. glucose disappearing/hr./mg. dry weight organism. (*b*) Activity-*p*H curve for culture grown at *p*H 7.

Variation of potential activities of washed suspensions in buffer

The variation in potential activity of certain enzymes so far described has occurred during growth at various medium *p*H values of the organisms containing them. The question arises whether similar variations can occur without growth by keeping the washed suspensions in buffers at various values within the growth range. To test this, we estimated the activities of two group I enzymes, formic and alcohol dehydrogenases, and

one group II enzyme, hydrogenase. A washed suspension of *E. coli* was prepared from a culture grown at *p*H 6 and the initial activities determined as described above. Four tubes were then set up, each containing 3 ml. washed suspension of 4 mg. dry weight organism/ml., and 1 ml. of buffer at *p*H 4·5, 6·0, 8·0 and 9·0 respectively added one to each tube. The tubes were then left at 27° for 2 hr., the organisms spun down in each and made up again in water to 4 ml. suspension. The formic, alcohol and hydrogenase activities were then determined, at the optimum activity *p*H in each case, for each suspension. Table 3 shows that there has been a general decrease in all activities but that the loss of activity has been greatest for all three enzymes at *p*H 4·5 and 9 so that there is no evidence of a compensatory formation of enzyme, as discussed above, in the absence of growth.

Table 3. *Variation of activities on standing in buffer*

Enzyme	*Q* unit	*p*H of buffer during 2 hr. at 27°				Initial activity
		4·5	6	8	9	
Formic dehydrogenase	O_2	50	54	44	29	110
Alcohol dehydrogenase	M.B.	13	41	36	24	63
Hydrogenase	M.B.	8	47	37	32	100

Absolute nature of the variation of potential activity with growth p*H*

Before attempting to place any interpretation upon these results it is necessary to determine whether the variations in potential activity described ('formation curves') represent alterations in enzyme content of the cells with growth *p*H, or varying degrees of enzyme-substrate accessibility in cells of constant enzyme content. In the absence, at present, of any satisfactory method of disintegrating *E. coli* cells and of making quantitative cell-free preparations of many of the enzymes mentioned, we have carried out analogous experiments with *M. lysodeikticus* to investigate this point. Fleming [1922] found that *M. lysodeikticus* is rapidly lysed when incubated with lysozyme, which is readily obtained by the addition to the suspension of a few drops of egg-white solution in saline. Penrose & Quastel [1930] found that this lysis involves the destruction of many enzymic activities such as formic and alcohol dehydrogenases, while other enzymes such as catalase, urease and fumarase remain unimpaired or even enhanced in activity after lysis. The organism grows thickly on the surface of nutrient agar but to obtain cultures in liquid media, in which the *p*H can be controlled, we had to adopt the measures described above. The organism will not grow in liquid media which have been adjusted to *p*H values $< 6{\cdot}5$ but is able to grow as far as *p*H 9·5 on the alkaline side. Quastel [1937] used a final concentration of 1/500 egg-white for lysis, incubating at 40° until the process was complete as judged by the disappearance of any turbidity from the suspension. Except in the case of urease we have found that such a procedure is satisfactory but allowance has to be made for wide variations in the lysozyme content of eggs.

Catalase. Estimations were carried out as described above but as *M. lysodeikticus* suspensions have about 10 times the catalase activity of equivalent *E. coli* suspensions, the amount of organism used was adjusted to 0·01 mg. dry weight; in the case of the lysed suspensions 0·001 mg. of material was sufficient. Fig. 13 shows that the potential activity exhibits a variation with growth *p*H similar to that obtained for this enzyme in *E. coli* and that the curve obtained with lysed suspensions is parallel with that obtained with the corresponding intact organisms. The catalase activity increases about 10 times on lysis of the organisms, an effect noted by Penrose & Quastel [1930], so the activities of the lysed suspensions have been reduced 10 times in Fig. 13. The constant ratio of the activity of the intact organism to that of the lysed suspension proves that the variation of potential activity with growth *p*H is due to an alteration of actual catalase content of the cells.

Urease. Penrose & Quastel [1930] state that the urease activity of *M. lysodeikticus* increases on lysis; we have been unable to confirm this but find, on the other hand, that unless lysis takes place in the presence of a high concentration ($M/24$) of urea, the urease

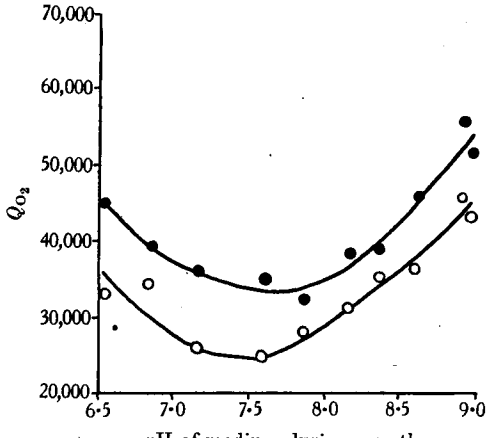

Fig. 13.

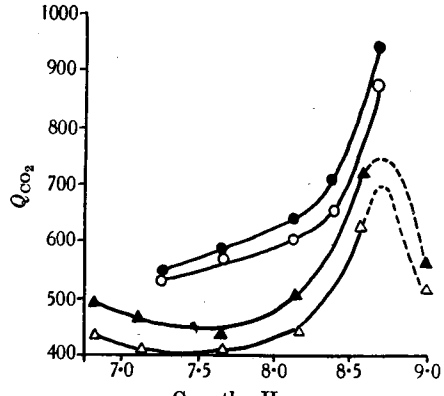

Fig. 14.

Fig. 13. Variation of potential catalase activity, estimated at *p*H 6, with growth *p*H (*M. lysodeikticus*). •—• Intact organism. o—o Lysed suspension (reduced 10 ×).

Fig. 14. Variation of potential urease activity, estimated at *p*H 6·0, with growth *p*H (*M. lysodeikticus*). 2 series. •—•, ▲—▲ Intact organism. o—o, △—△ Lysed suspension.

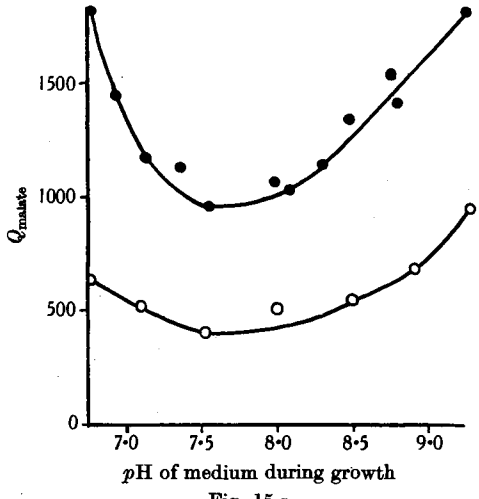

Fig. 15 *a*.

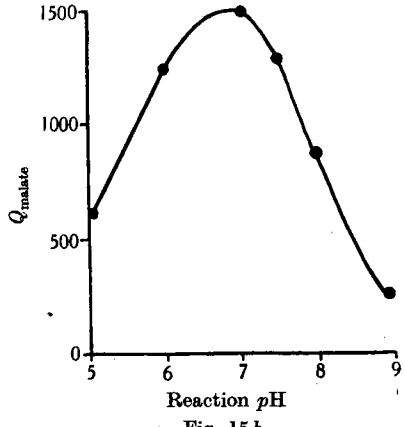

Fig. 15 *b*.

Fig. 15. Fumarase (*M. lysodeikticus*). (*a*) Variation of potential activity with growth *p*H. Q_{malate} = μl. malate formed/hr./mg. organism. •—• Lysed suspension. o—o Intact organism (increased 10 ×). (*b*) Activity-*p*H for lysed suspension of organism grown at *p*H 7.

activity is mostly destroyed during lysis. We eventually determined the activity manometrically, using $M/15$ phosphate buffer *p*H 6 (1·2 ml.), 1 ml. bacterial suspension of dry weight 1 mg., 0·5 ml. $M/4$ urea, in the main cup and 0·3 ml. egg-white solution in buffer in the side bulb. The manometers were shaken in a bath at 37° and readings taken every 5 min. until the rate of CO_2 evolution was steady in each case; then the egg-white was

tipped in from the side bulb and readings resumed after a 5 min. interval during which lysis took place. After lysis the rate of CO_2 evolution remains steady for 20–30 min. or until the buffer is exhausted. When cultures were grown as usual in urea-free media, the results were very irregular from day to day and in the response to growth *p*H but these irregularities ceased when 0·5% urea was incorporated in the growth medium. Fig. 14 shows that the potential activity exhibits a fully compensated type of variation (group I type) with growth *p*H although the compensation tends to break down when the growth *p*H is higher than 9. The activity of the lysed suspensions is slightly lower than that of the intact organism suspensions but there is a constant ratio between these activities for each culture, indicating that we are dealing with variations in actual enzyme content.

Fumarase. Penrose & Quastel [1930] state that the fumarase activity of *M. lysodeikticus* increases greatly on lysis. Since fumarase activity is estimated by the formation of malic acid from fumaric acid, the smaller activity of the intact organism is probably explained by the removal of malic acid in this case by enzymes which are destroyed during lysis. Fumarase activity was estimated as follows: sufficient organism can be obtained from one Roux bottle to provide 7 ml. of suspension of dry weight 6–8 mg./ml.; this was mixed with 7 ml. buffer and 7 ml. *M*/2 fumarate and the whole shaken at 37°. At 30 and 60 min. after mixing, 10 ml. were withdrawn from each container and added to 10 ml. 14·2% ammonium molybdate solution followed by the addition of 1 ml. glacial acetic acid. The samples were left in the dark for 2–3 hr., the precipitate filtered off and malic acid estimated polarimetrically by the method of Auerbach & Kruger [1923]; activities expressed as $Q_{\text{malate}} = \mu$l. malate formed/hr./mg. dry weight organism. Figs. 15*a* and 15*b* show that fumarase has a fully compensated group I type of variation with growth *p*H with minimal formation occurring when the growth *p*H coincides approximately with the optimum activity *p*H. The variation is shown equally well with the lysed suspension or the apparently less active intact organism.

M. lysodeikticus does not appear to possess aspartase, alanine deaminase or glucozymase when tested under the usual conditions for these enzymes.

The absolute nature of the variation of enzyme formation with growth *p*H is shown also in the case of certain extracellular enzymes of *Cl. welchii* studied by Gale & van Heyningen [1942]: the production of hyaluronidase shows a typical group I variation with growth *p*H while that of α and θ toxins shows a group II variation.

Discussion

The enzymes investigated in the course of this work can be divided into two main groups according to the type of variation they exhibit with alteration of the *p*H of the medium during growth.

Group I: those enzymes for which a compensatory formation takes place as the growth *p*H deviates from the optimum activity *p*H so that the effective activity remains approximately constant whatever the growth *p*H.

Group II: those enzymes which are formed best when the growth *p*H approximates to the optimum activity *p*H. In some cases where the optimum activity *p*H is near 7, a compensatory formation takes place over the middle of the growth range so that the effective activity is constant in this region but falls off rapidly towards the extremes of the range.

The enzymes of *E. coli* and *M. lysodeikticus* so far studied are distributed between the two groups as follows:

Group I	Group II	
Formic dehydrogenase	Hydrogenase	Serine deaminase
Alcohol dehydrogenase	Succinic dehydrogenase	(Aspartase)
Catalase	Glucozymase	Arginine decarboxylase
Urease	Tryptophanase	Ornithine decarboxylase
Fumarase	Alanine deaminase	Lysine decarboxylase
(Formic hydrogenlyase)	Glutamic acid deaminase	Histidine decarboxylase

The effective activities have not been determined in the case of the deaminases owing to the change in their activities with the nature of the buffers used. Kocholaty & Hoogerheide [1938] give an activity-*p*H curve for alanine dehydrogenase of *Cl. sporogenes* which shows two optima, at *p*H 7 and 9·5, when the whole range is covered with phosphate buffers. We find that phosphate systems are such unsatisfactory buffering agents for *p*H values higher than 8 that such figures must be accepted with reserve. With alanine deaminase of *E. coli* the activity-*p*H curve determined in veronal buffers appears to have only one optimum (Fig. 2*b*) and that at approximately 9·5. If these deaminases can be regarded as mechanisms complementary to the decarboxylases, which have optimum activity *p*H values ranging from 2·5 to 5·5, then the alkaline values obtained in veronal and borate could be regarded as the true optimum activity *p*H figures, whilst phosphate has a 'salt effect' resulting in a shift of the optimum. We cannot solve this problem satisfactorily until the enzymes concerned have been obtained in a cell-free state. Whatever may be the true interpretation, the formation of the serine, alanine and glutamic acid deaminases is greatest when the *p*H of the external growth environment approaches this more alkaline optimum activity *p*H, whilst the alanine and serine curves show signs of an increased formation also at the optimum activity *p*H of 7·0–7·5 found in phosphate. These considerations justify the inclusion of these deaminases in group II. Aspartase is unusual in that its potential activity displays some considerable degree of compensatory formation for growth *p*H values 5–7 but the shape of the curve (Fig. 5*a*) outside these values places the enzyme in group II. Aspartic acid would seem to be the only amino-acid of those studied which can be utilized by the cell as a source of N at acid growth *p*H values.

The amino-acid decarboxylases appear to be adaptive enzymes which are formed only when the organism grows under acid conditions, when the growth *p*H approaches the optimum activity *p*H. In such an acid medium, the carboxyl groups of the amino-acids are presumably undissociated, while in an alkaline medium the —COOH groups will be ionized and the —NH_2 groups undissociated. Thus we find that the organism grown under alkaline conditions can no longer produce decarboxylases but attacks certain amino-acids by deamination. Both decarboxylases and deaminases can therefore be looked upon as adaptive enzymes where the specific substrate stimulating enzyme formation is the undissociated form of the molecule involved. Likewise they may be neutralization mechanisms, the decarboxylases being formed in response to an acid medium and by their action tending to neutralize that acidity, and certain deaminases being formed under opposite conditions and having a neutralizing effect on alkaline growth conditions. That such neutralizing mechanisms do exist is shown by the drift of the *p*H of unbuffered media towards neutrality during growth of the organisms. The action of these mechanisms will so modify the *p*H of the internal environment of the cell that this will not be subject to such wide variations as shown by the growth range of *p*H measured in the external environment. A probable consequence of this is that, for enzymes which are most active under strongly acid or alkaline conditions, the optimum activity *p*H determined with intact cells and measured in the external environment will differ from the true figure for the cell-free enzyme by an amount determined by the capacity of the cell to control the *p*H of its internal environment. We intend to publish data demonstrating this point later.

Formic hydrogenlyase is placed in group I on account of the general shape of its potential activity curve (Fig. 10*a*) although the minimal enzyme formation does not take place when the growth *p*H coincides with the optimum activity *p*H. Formic acid is obviously toxic to *E. coli* under acid conditions of growth and formic hydrogenlyase acts by removing the inhibitory substance under these conditions. It is obvious that the enzymes of group I play a different part in the economy of the bacterial cell from those of group II, since their formation is so controlled that they should be equally effective under all conditions of growth. Their activity would seem to be essential to the existence

of the cell. Formic hydrogenlyase is formed by the cell only under anaerobic growth conditions, in response to the presence of formic acid, when it may be assumed that the formic dehydrogenase will be inefficient. Likewise it has been recorded that formic dehydrogenase has an unusually high affinity for its substrate [Stickland, 1929] and undergoes little variation with the age of the culture [Wooldridge, Knox & Glass, 1936]. Thus the cell has evolved mechanisms for dealing with this substance under almost all conceivable growth conditions. The substrates of other enzymes in group I such as urea, alcohol and H_2O_2 are known to be toxic to bacteria and so it would seem that some at any rate of these group I enzymes are protective or detoxication mechanisms. It is too early to generalize as yet: there is little evidence that fumaric acid is toxic to bacteria. Further, the strain differences in the distribution of these enzymes are difficult to explain unless it may be that the organisms produce these enzymes to protect themselves from the toxic products of their normal metabolism.

We see from these results in general that bacteria react to an alteration in their external environment by an alteration in their enzymic constitution. The changes appear to involve two principles: (1) an attempt to counter the adverse external change and (2) an attempt to maintain essential activities at a constant value. In other words, bacteria react to a change in their external environment in such a way that the resultant change in their internal environment is at a minimum.

Summary

1. *E. coli* can grow in casein digest adjusted to any *p*H between 4·5 and 9.

2. Change in the external *p*H during growth is followed by an alteration in the enzyme content of the cells. The enzymes can be divided into two groups according to the type of their variation with growth *p*H.

Group I: those enzymes whose formation undergoes a variation so that their activity per cell is constant whatever the medium *p*H. This means that the potential activity of the cells in respect of these enzymes increases as the growth *p*H deviates from their optimum activity *p*H.

Group II: those enzymes whose formation is greatest when the growth *p*H approaches their optimum activity *p*H. In cases where the optimum activity *p*H lies between 6–8, a small degree of compensatory formation takes place over the centre of the growth range so that the effective activity is constant over that part of the range but falls off rapidly towards the extremes.

3. The group I enzymes appear to have a protective function in the cell in that they remove inhibitory substances, e.g. urease, catalase, formic dehydrogenase and hydrogenlyase, etc.

4. Group II enzymes include the amino-acid decarboxylases and deaminases so that the former are produced in response to an acid growth environment and the latter in response to an alkaline growth *p*H. In this manner they act as neutralization mechanisms.

5. These variations in enzyme content do not occur in the absence of growth.

6. Similar variations in the enzymes of *M. lysodeikticus* are not affected in nature by lysis of the cell suspensions with lysozyme; therefore the variations observed are due to alterations in actual enzyme content of the cells concerned.

7. In general, a change in the external *p*H is followed by an alteration in the enzymic constitution of the cells such that (1) an attempt is made to counter the external change and (2) certain essential activities are maintained at a constant level.

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