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Acceleration of Bacterial Glutamic Decarboxylase and Glutaminase by Cetyltrimethylammonium Bromide

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It has been shown in a previous paper from this laboratory (Krebs, 1948) that cetyltrimethylammonium bromide ('cetavlon') can accelerate the decarboxylation of glutamate and the deamidation of glutamine by washed suspensions of *Clostridium welchii*. The present paper is concerned with the mechanism of this effect. As it is known that detergents may alter the permeability of cells (Gale & Taylor, 1947; Hotchkiss, 1946), it was thought that cetavlon might facilitate the entry of the substrates into the cells. To test this hypothesis, the effect of cetavlon on the rate of decarboxylation of glutamate and glutamine by cell-free extracts has been examined, but contrary to expectation the detergent gave the same effect as with intact cells. Thus the effect cannot be due to changes in the permeability of the cell wall.

METHODS

Organisms. *Clostridium welchii* S.R. 12 (National Collection of Type Cultures (N.C.T.C.) no. 6784) was grown for 16 hr. at 40° and a strain of *Escherichia coli* isolated locally and *Proteus morgani* (N.C.T.C. no. 2818) for 24 hr. at 30°, on the medium described by Krebs (1948). *Bacterium cadaveris* (N.C.T.C. no. 6788), *Cl. welchii*, (N.C.T.C. no. 6785), and *Streptococcus faecalis* (N.C.T.C. no. 6783), were grown as described by Gale (1947), but 'Pronutrin' (a commercial casein hydrolysate marketed by Herts Pharmaceuticals Ltd., Welwyn Garden City) was used instead of tryptic casein.

Clostridium septicum (N.C.T.C. no. 547) served as a source of ornithine decarboxylase. The activity of the ornithine decarboxylase was increased 25-fold by reinforcing Gale's (1947) medium with extra ornithine (1 mg./ml. medium).

After growth, the cultures were centrifuged, the cells washed twice with 0.9% NaCl, and suspended in the appropriate buffer (Table 6). Phosphate buffer was used for the pH range from 7.0 to 5.5, acetate buffer for the range 5.5 to 4.0 and lactate buffer for the pH range from 4.0 to 2.5. The final concentration of the buffer was 0.10 M.

Chemicals. The preparations of L-glutamic acid, L-glutamine and cetavlon used in this work were those described by Krebs (1948). Ornithine was prepared from male herring roe, by the following method. The roe (250 g.) was heated on the water bath in a mixture of 150 ml. of conc. HCl and 50 ml. of TiCl₃ (20%). During the heating the roe was broken up with a glass rod and when fairly uniform it was autoclaved at 120° for 45 min. Water and HCl were removed by distillation *in vacuo* on a steam bath. The residual gum was dissolved in 100 ml. of hot water and 40% NaOH added until the pH was 6. The solution was filtered and, after adjustment of the pH to 7.6, an arginase preparation (15 ml. of 10% homogenized fresh rat liver) was added, and the mixture was incubated overnight. Urea was estimated manometrically and the ornithine content calculated from the urea formed.

Chemical determinations. The decarboxylation of glutamate and of glutamine was followed by measuring the evolution of CO₂ in a Warburg manometric apparatus. Buffer and substrate solutions were usually placed in the main compartment of the cup and the cells or extracts (see p. 326) containing the bacterial decarboxylase in the side arm. The gas space

was filled with N_2 , and a stick of yellow phosphorus was put into the centre well to eliminate O_2 . After 10 min. equilibration, the contents of the side arm and the main compartment were mixed. Readings were taken at 2 or 4 min. intervals. The rate of reaction is expressed as Q_{CO_2} , i.e. $\mu l.$ CO_2 /mg. dry wt. of cells/hr. In the case of extracts the Q_{CO_2} was calculated not from the dry wt. of extract used, but from the dry wt. of the cells from which the extract was made. The total volume of the fluid in each Warburg cup was 2.2 ml. and the amount of enzyme material added was generally equivalent to 2.0–5.0 mg. intact cells, varying with the activity of each batch of material, which was determined by a preliminary experiment. The concentration of enzyme material was such that not more than 50% of the added substrate was decomposed in the first 10 min., i.e. not more than 112 $\mu l.$ CO_2 was produced. Within a suitable range the rate of decarboxylation of glutamine in extracts was proportional to the enzyme concentration. Under the conditions used the range was between 1 and 20 mg./cup in the absence of cetavlon and 1 and 10 mg. in its presence. At higher concentrations the rate was too rapid for precise measurement. At lower concentrations the rate fell off after about 5 min., possibly owing to the destruction of the enzyme in dilute solution. Under these conditions the rate was approximately linear for at least 10 min. and the Q_{CO_2} was calculated from the linear rate of gas evolution. Any deviation from this procedure is mentioned later. Ammonia was determined by the method of Parnas & Heller (see Parnas, 1934).

Preparation of cell-free extracts. The washed cells were broken by grinding with powdered pyrex glass (Wiggert, Silverman, Utter & Werkman, 1940; McIlwain, Roper & Hughes, 1948). The degree of cell disintegration was checked by microscopic examination of a Gram-stained smear of the ground mixture diluted with water. Usually no intact cells were found, and the cell debris of *Cl. welchii* was Gram-negative. The clay-like mass of broken cells and glass was mixed with 0.05-M acetate buffer, pH 4.6, in the proportion of 2 ml. of buffer for 1 g. of wet cells, and centrifuged for 10 min. at about 4000 rev./min. The supernatant was poured off and the residue extracted twice more with the same amount of buffer. The three extracts were combined, centrifuged again, and the slightly opalescent solution stored at about 2° in a stoppered cylinder.

Extracts of *Cl. welchii* contained about 80% of the original glutamic decarboxylase activity (see Table 1). Extracts of equal potency were obtained when the above buffer was replaced by other solutions (0.9% NaCl, 0.05-M acetate buffer pH 5.0 or 6.0, or 0.05-M phosphate buffer pH 7.0). The yield of glutaminase was difficult to measure with any certainty owing to the change in behaviour of the enzyme after extraction. For instance, when the rate of decarboxylation of glutamine was measured at pH 4.9 the yield of glutaminase appeared to be 50–60% without cetavlon and 80–90% when it was added. When the activity was measured at pH 4.1 the yield of glutaminase appeared to be negligible in the absence of cetavlon, but when the detergent was added the activity was often greater than that of the intact cells (see Table 1).

Extracts of *Proteus morganii* decarboxylated both substrates more slowly than the intact cells both in the absence and presence of cetavlon.

Esch. coli gave extracts which under the conditions used appeared to contain 25–40% of the original decarboxylase and 50–100% of the original glutaminase activity.

Stability of the enzyme preparations. As already reported (Krebs, 1948), the decarboxylase and glutaminase in intact

cells of *Cl. welchii* suspended in 0.05-M acetate buffer, pH 4.5, and stored at about 2° keep for several weeks. The glutamic decarboxylase activity of an extract from *Cl. welchii* showed no decrease after 9 days at 2°, but a decrease of 35% after 30 days at 2°. In the same sample, the ability to decarboxylate glutamine fell off more rapidly; 30% of the original activity was lost in 9 days and 55% after 30 days at 2°. In another experiment, in which an extract was incubated without substrate for 20 min. at 40° in 0.1-M acetate buffer pH 4.6, 32% of the glutamic decarboxylase and 80% of the glutaminase activity disappeared. Thus the glutaminase in the extracts is less stable than the glutamic decarboxylase. Most of the work described in this paper was carried out with glutamic acid as the substrate in order to avoid complications arising from instability of the glutaminase.

RESULTS

The effects of cetavlon on the rate of decarboxylation of glutamate and glutamine in intact cells and extracts. Table 1 shows that cetavlon accelerates the decarboxylation of glutamate and glutamine in extracts and in washed cells of *Cl. welchii*, *Proteus morganii* and *Esch. coli*.

Table 1. *The effect of cetavlon on the rate of decarboxylation of glutamate and glutamine by intact cells and extracts*

(Temp. 40°; substrate, 0.0045 M; cetavlon, 0.0025 M; 0.1-M acetate buffer pH 4.6.)

Substrate added	Q_{CO_2}	
	Intact cells	Extract
<i>Cl. welchii</i>		
Glutamate	270	224
Glutamate and cetavlon	535	405
Glutamine	114	5
Glutamine and cetavlon	270	290
<i>Proteus morganii</i>		
Glutamate	40	44
Glutamate and cetavlon	310	82
Glutamine	45	23
Glutamine and cetavlon	250	56
<i>Esch. coli</i>		
Glutamate	102	11
Glutamate and cetavlon	425	160
Glutamine	89	40
Glutamine and cetavlon	270	270

With intact cells of *Cl. welchii* at pH 4.1 the degree of acceleration of the decarboxylation of glutamate on the addition of cetavlon was from two- to three-fold. In extracts the effect of cetavlon was slightly less than with the intact cells, but of the same order. On the other hand, the decarboxylation of glutamine showed striking differences between intact cells and extracts of *Cl. welchii* (see Table 1). In the absence of cetavlon the extracts were 20% less active than the intact cells even at pH 4.9, which has been shown to be the optimum pH for the decarboxylation of glutamine (Krebs, 1948). The low activity of the

extracts towards glutamine is not due to an irreversible destruction of the enzymes because the addition of cetavlon greatly increases the activity, in some cases to a Q_{CO_2} level higher than that of the intact cells.

In the case of *Proteus morganii* the acceleration by cetavlon was more marked in intact cells than in extracts. It will be shown later for *Cl. welchii* that the pH and the substrate concentration can greatly influence the effect of cetavlon. The role of these factors has not been investigated for *Proteus morganii*.

In *Esch. coli* the effects of cetavlon were very pronounced both on intact cells and on extracts, irrespective of whether glutamate or glutamine was the substrate.

The effect of cetavlon on the pH curve of the glutamic decarboxylase in extracts of *Clostridium welchii*. As shown in Fig. 1 addition of the detergent to extracts

Fig. 1. The effect of cetavlon on the pH curve of the extracted decarboxylase of *Cl. welchii*. 0.1 M-lactate, pH 2.5-3.0; 0.1 M-acetate, pH 4.0-5.0; 0.0025 M-cetavlon if added; substrate 0.0045 M-glutamate; extract corresponding to 2.0 mg. dry wt. of cells; total volume 2.2 ml.; temp. 40°.

of glutamic decarboxylase from *Cl. welchii* changed the shape of the pH curve of the enzyme by broadening the optimum towards the lower pH range. This effect is similar to that found with intact cells (Krebs, 1948). Above pH 4.8 inhibitions varying from 5 to 20 % were found on the addition of cetavlon to both extracts and cells. As the degree of acceleration by cetavlon was greatest between pH 4.0 and 4.2 its effect has been measured at pH 4.1.

It is noteworthy that the pH optimum in the absence of cetavlon is 4.6 with extracts and 4.1 with cells. Similar changes in the optimum pH of the tyrosine decarboxylase on extraction from *Strep. faecalis* have been described by Epps (1944). Another point of interest is that the extracted enzyme has a sharper pH optimum than has the enzyme of the intact cells.

The effect of cetavlon on the stability of glutamic decarboxylase in extracts of *Clostridium welchii*. There was loss of activity of the decarboxylase without the addition of cetavlon when the extract was incubated at 40° at pH 4.9, 4.1, 3.0 and 2.5 (Table 2). Cetavlon had little effect on this loss of

Table 2. The effects of cetavlon on the stability of the glutamic decarboxylase in extracts of *Clostridium welchii*

(Main compartment: 1.0 ml. 0.2 M-buffer, 0.5 ml. extract, equivalent to 2.0 mg. dry wt. cells; if added, 0.1 ml. 2% cetavlon. Side arm: 0.5 ml. 0.01 M-glutamate. Total volume 2.2 ml. Contents mixed after 10 min. or 20 min. at 40°.)

Buffer	Q_{CO_2} at 10 min.		Q_{CO_2} at 20 min.	
	Without cetavlon	With cetavlon	Without cetavlon	With cetavlon
Acetate pH 4.9	264	264	180	180
Acetate pH 4.1	142	250	82	160
Lactate pH 3.0	52	190	26	124
Lactate pH 2.5	33	99	10	43

activity. The accelerating effect of cetavlon after partial destruction of the decarboxylase was about the same as before incubation. The detergent therefore neither destroys the activity of the enzyme nor protects it from destruction.

The effect of cetavlon on the solubility of the extracted decarboxylase of *Clostridium welchii*. On adjusting the pH of extracts to below pH 4.3 with acetate buffer a precipitate was formed. About 60 % of the decarboxylase activity was precipitated at pH 4.1, and both the precipitate and the mother liquor gave the cetavlon effect (Table 3, Exp. 1). In the presence of cetavlon almost all the decarboxylase was in the precipitate (Table 3, Exp. 2), and the activity of the washed precipitate was not increased by the addition of cetavlon. At pH 4.6, the addition of cetavlon produced a precipitate, which, even after washing, contained decarboxylase whose activity was again unaffected by the addition of further cetavlon. About 70 % of the activity was recovered in the precipitates formed when cetavlon was present, but quantitative assay was difficult as the precipitates were very sticky and adhered to pipettes and test tubes. The material was insoluble in acid and neutral buffers and lost activity if the pH was above 7.0. On the other hand, precipitates obtained by adjusting the pH of the extract to pH 4.1 without the addition of cetavlon redissolved at pH 6 and were stable even at pH 9. Above pH 9 the activity was lost rapidly.

If the extract was adjusted to pH 4.1 in the presence of methyl orange the precipitate was stained orange when cetavlon was absent, and this orange colour was removed slowly by washing. When cetavlon was present the precipitate was bright yellow and the colour was not removed after four

Table 3. *The effect of pH and cetavlon on the precipitation of the glutamic decarboxylase*

(Each fraction was transferred in 0.5 ml. water to the side arm; 0.0045 M-glutamate, 0.1 M-acetate buffer pH 4.1 and cetavlon, if added, 0.0025 M, were in the main compartment. Total volume 2.2 ml.)

Condition of precipitate	Fraction tested	Q_{CO_2}	
		Without cetavlon	With cetavlon
Control. Original extract not precipitated		108	344
Exp. 1. Extract taken to pH 4.1 with acetate; left 15 min. at 2°; centrifuged; precipitate washed once with 0.05 M-acetate buffer pH 4.1	Supernatant	59	118
	Precipitate	88	188
Exp. 2. Extract precipitated as above but in the presence of 2.5×10^{-3} M cetavlon; precipitate washed once with 0.005 M-acetate buffer pH 4.1	Supernatant	0	0
	Precipitate	258	258
Exp. 3. Extract adjusted to pH 4.6, diluted to the same volume as the above experiments and cetavlon added (final concentration 2.5×10^{-3} M). Precipitate washed as above	Supernatant	0	0
	Precipitate	262	262

washings with 0.05 M-acetate buffer pH 4.6 or 4.1, or with 0.05 M-phosphate buffer, pH 7.0. Hartley (1923) has shown that some cationic detergents change methyl orange to a bright yellow colour. This colour change is not due to any change in pH and is connected with micelle formation by the detergents. The fact that methyl orange becomes yellow when absorbed on to precipitated enzyme material indicates that detergent-protein complexes have been formed (Putman & Neurath, 1945; Lundgreen, 1945).

Microscopic examination showed that the precipitate obtained by acidifying the extract to pH 4.1 was composed of very fine granules when cetavlon was absent, and coarse granules when the detergent was present. When these precipitates were shaken with glutamate at 40° the one without cetavlon did not change in appearance, whilst the one with cetavlon became coarser. This effect is similar to that found by Klarman & Wright (1946) on the clumping of bacteria by detergents. Thus cetavlon neither solubilizes nor peptizes the enzyme-containing material, but precipitates it and forms complexes.

The effect of cetavlon on the rate of decarboxylation at varying concentrations of glutamate. At 30° the rate of decarboxylation of glutamate (2 mg. dry cells in 2.2 ml.; substrate concentrations 0.0023–0.046 M) was found to be steadier than at 40°, and the following experiments were therefore carried out at 30°. Table 4 shows the effect of cetavlon on the rate of decarboxylation at different glutamate concentrations by washed cells and extracts. It will be seen that the effect of the detergent was greatest at the lowest substrate concentrations, whilst at high substrate concentrations when the enzyme is almost saturated with substrate it has little or no effect. The increase being, in the case of the cells, 147% at 0.0023 M-glutamate and only 13% at 0.046 M-glutamate. These results can be explained on the assumption that cetavlon increases the apparent affinity of the substrate for the enzyme. The concentrations of substrate giving half the maximum velocity, determined graphically from the results

given in Table 4, are as follows: intact washed cells, without cetavlon, 0.009 M; with cetavlon, 0.004 M. Extract of cells, without cetavlon, 0.0095 M; with cetavlon, 0.0045 M.

Table 4. *Effect of cetavlon on the rate of decarboxylation at varying concentrations of glutamate*

(In each Warburg cup, 0.1 M-acetate buffer (pH 4.1), glutamate solution, 0.0025 M cetavlon, if added; enzyme in the side arm; total volume 2.2 ml.; temp. 30°.)

Glutamate (M)	Q_{CO_2}	
	Without cetavlon	With cetavlon
Intact washed cells		
0.046	890	1003
0.023	790	990
0.011	535	745
0.0046	268	455
0.0023	109	268
Extract of the above cells		
0.046	535	675
0.023	492	582
0.011	330	465
0.0046	211	362
0.0023	125	231

Assuming that the half-maximum velocity is a measure of the apparent affinity of the enzyme for the substrate, the addition of cetavlon increased the affinity of the decarboxylase about twofold, both with intact cells and with extracts.

Changes in the apparent affinity of enzyme for substrate can be brought about by competitive inhibitors, addition of a competitive inhibitor decreasing, and its removal increasing, the apparent affinity. The effect of cetavlon could thus be explained by the assumption that it removes a competitive inhibitor which accompanies the bacterial decarboxylase and glutaminase. This conception is supported by the graph shown in Fig. 2, in which a plot is made of the reciprocal of the velocity $1/v$ against the reciprocal of the substrate concentration $1/S$ (Lineweaver & Burk, 1934). The two lines referring to extracts show the same ordinate inter-

cept, i.e. the same maximum velocity, but the addition of cetavlon has decreased the slope. According to Lineweaver & Burk (1934) this is expected if a competitive inhibitor is removed.

Fig. 2. Data in Table 4 plotted as suggested by Lineweaver & Burk (1934); v =reaction velocity, expressed as Q_{CO_2} ; S =substrate concentration (M). $\times-\times$, intact cells without cetavlon; $o-o$, intact cells with cetavlon; $\triangle-\triangle$, extract without cetavlon; $\square-\square$, extract with cetavlon.

The plot of the data obtained with intact cells, when cetavlon is absent and the substrate concentration is low, does not give a straight line. This suggests that under these conditions the rate of entry of glutamate into the cell limits the rate of decarboxylation. This conclusion is further supported by the fact that a straight line is obtained from the data with extracts.

The plot obtained from the results with intact cells in the presence of cetavlon shows two points of interest. First, the line is straight, suggesting that cetavlon eliminates diffusion as a controlling factor. Secondly, the line is almost parallel to that showing the effect of cetavlon in the extract. This means that although cetavlon shows an effect on the rate of entry of glutamate into the cell, the apparent affinity of the enzyme in intact cells is also increased, in the same way as in extracts. The fact that the acceleration by cetavlon in intact cells of *Cl. welchii* is slightly higher than that found in extracts (see Table 1) can probably be explained by an additional effect of the detergent on the permeability of the cell.

The effect of cetavlon on the rate of decarboxylation of glutamine at varying substrate concentrations. The rates of decarboxylation at varying concentrations of glutamine by extracts of *Cl. welchii* are shown in Table 5. A concentration of substrate sufficient to saturate the enzyme was not reached, as the solu-

bility of glutamine at 30° is approximately 0.23 M. The decarboxylation is extremely slow at low concentrations of substrate without cetavlon and is increased by the addition of cetavlon, the cetavlon effect decreasing as the substrate concentration is raised. This suggests that the apparent affinity of the glutaminase is increased in a manner similar to that of the decarboxylase.

Table 5. *The effect of cetavlon on the rate of decarboxylation of varying concentrations of glutamine by extracts of Clostridium welchii*

(0.1 M-acetate buffer pH 4.1; glutamine and 0.0025 M-cetavlon in the centre; enzyme in the side arm; total volume 2.2 ml.; temp. 40° .)

Glutamine (M)	Q_{CO_2}	
	With cetavlon	Without cetavlon
0.23	1160	440
0.12	850	250
0.046	490	143
0.023	330	78
0.0124	260	24
0.0046	215	1.0-5.0
0.0023	144	1.0-5.0

Treatment of the results, according to Lineweaver & Burk (1934), does not give as clear a result as in the case of the decarboxylation of glutamate. Whilst the plot of $1/S$ against $1/v$ is a straight line for extract with cetavlon, that for the extract alone gives an S-shaped curve. If the results for extract alone are plotted in the form $1/v$ against $1/S^2$ the line is straight. With a single enzyme this result suggests that either a ternary collision was involved or that an inhibitor was present.

The effect of cetavlon on other enzyme systems. No acceleration of any other enzyme system has been found. The systems tested and the methods of testing are summarized in Table 6.

DISCUSSION

The acceleration by cetavlon of the decarboxylation of glutamate and glutamine, which had previously been reported with intact cells (Krebs, 1948), has now been shown to take place with extracts of *Cl. welchii*, *Proteus morganii* and *Esch. coli*. The effects are generally of the same order as with intact cells; thus the main action cannot be due to an effect on permeability of the cell walls. At low substrate concentrations the degree of acceleration with intact cells was slightly higher than with extracts, and this may be due under these conditions to an additional effect of cetavlon on cell permeability.

Examination of the cetavlon effect at varying substrate concentrations showed that the detergent increased the apparent affinity of the decarboxylase for glutamate. Changes in the affinity of an enzyme for its substrate can be brought about by the

Table 6. *The effect of cetavlon on the reaction rates of enzymes other than glutamic decarboxylase and glutaminase of Clostridium welchii*

Name of enzyme	Source of enzyme	Method of preparation and experimental conditions	pH at which the system was tested	Effect of cetavlon (0.0025 M and 0.01 M final concentration)
Ornithine decarboxylase	Washed intact cells of <i>Cl. septicum</i>	Gale (1947)	5.5, 5.0, 4.0	None
Tyrosine decarboxylase	Washed intact cells of <i>Strep. faecalis</i>	Gale (1947)	5.5, 5.0, 4.0	None
Tyrosine decarboxylase	Extract of acetone dried cells of <i>Strep. faecalis</i>	Epps (1944)	5.5, 5.0, 4.0	None
Arginine decarboxylase	Washed intact cells of <i>Esch. coli</i>	Gale (1947)	5.0, 4.0	None
Arginine decarboxylase	Extract of <i>Esch. coli</i> disintegrated by grinding glass	Tested as with intact cells	5.0, 4.0	None
Histidine decarboxylase	Washed intact cells of <i>Cl. welchii</i> strain (6785)	Gale (1947)	5.0, 4.0, 3.0	None
Lysine decarboxylase	Washed intact cells of <i>Bact. cadaveris</i>	Gale (1947)	5.0, 4.0	None
Glutamic decarboxylase	Extracts of carrots and squash	Schales, Mims & Schales (1946)	5.0, 4.0, 3.0	None
Urease	Extract of Jack-bean meal		5.0, 4.0	None

following mechanisms: (a) increased concentration of substrate in the vicinity of the enzyme (Van Slyke, 1942); (b) an alteration in the properties of the enzyme by reaction with an added substance; (c) removal of an inhibitor.

It seems unlikely that mechanism (a) is involved, as transport of material by surface-active agents has been demonstrated only in the case of lipophilic substances (Dean & Vinograd, 1942), and neither glutamate nor glutamine is lipophilic.

The reaction of surface-active agents with enzymes usually leads to inactivation of the enzyme (Putman, 1948; Valko, 1946). On the other hand, Bodin & Hill (1945) found that the addition of anionic detergents to prototyrosinase increases the activity; they suggested that the increase was due to formation of enzyme material from a precursor, but did not investigate the effect of detergents on the affinity of the enzyme.

The data on the effect of cetavlon at varying substrate concentrations strongly suggest that a competitive inhibitor of the glutamic decarboxylase is removed by cetavlon, either by solubilization of the inhibitor on micelles (for this effect see Alexander & Trim, 1946), or by combination with the inhibitor to form a complex.

The experiments on the effect of cetavlon on the apparent affinity of the glutaminase do not give such a clear picture as those on the glutamic decarboxylase. However, the results suggest that cetavlon accelerates the glutaminase by the removal of an inhibitor.

SUMMARY

1. Cell-free extracts containing glutamic decarboxylase and glutaminase have been prepared from *Clostridium welchii*, *Proteus morganii* and *Escherichia coli* by grinding the cells with powdered glass, adding buffers to the ground mass and centrifuging.

2. Of the original decarboxylase of the intact cells, about 60% was recovered in extracts from *Cl. welchii*, 30–40% from *Proteus morganii* and 25–40% from *Esch. coli*. The glutaminase activity of extracts was between 50 and 100% of that of the intact cells.

3. The activity both of the decarboxylase and of the glutaminase in the extracts was increased by the addition of cetavlon.

4. The acceleration of the decarboxylase by cetavlon in intact cells and extracts of *Cl. welchii* became smaller as the substrate concentration was raised, i.e. the apparent affinity of the decarboxylase for glutamate seems to be increased by the addition of cetavlon.

5. Bacterial decarboxylases acting on ornithine, tyrosine, arginine, lysine and histidine are not accelerated by the addition of cetavlon, nor is the glutamic decarboxylase of *Daucus carota* (carrot) or *Cucurbita pepo* (squash).

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Factors Influencing the Polysaccharide Content of *Escherichia coli*

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It is known that bacteria build up reserves of polysaccharides within the cell, but little work has been done on the factors which influence their accumulation. Our object was to investigate factors influencing polysaccharide storage and the role which storage plays in the metabolism of the bacterial cell. Dawson & Happold (1943) reported that D-phenylalanine caused increased storage of polysaccharide when *Escherichia coli* was grown on a glucose, but not on a mannose, medium. Gale (1947) has stated that polysaccharide formation occurs within the cell when *Esch. coli* is allowed to metabolize glucose in excess, but this material is itself metabolized as soon as the external glucose is exhausted. To investigate this, storage was examined where onset of the stationary phase was determined by both glucose and nitrogen exhaustion in the medium.

Additional interest in variations of polysaccharide content was occasioned by frequent observations that a fall in bacterial mass, as indicated by turbidity measurements, occurred in the early stationary phase. Monod (1942) also observed decrease in turbidity, attributed to a decrease in cell size, in this phase, and it was of interest to see whether this was related to polysaccharide content.

EXPERIMENTAL

The organism used throughout this work, unless otherwise stated, was no. 5928 of the National Collection of Type Cultures.

Growth cycles were followed turbidimetrically, using a Spekker photoelectric absorptiometer with neutral filters, H 508, and microcells. Since we have shown that values for the polysaccharide content are dependent on the stage reached in the stationary phase we have been careful to ensure that samples were taken at the same phase of growth. In every experiment the inoculum has been taken from a medium containing the same constituents as the medium to be used in the experiment, and from the early stationary phase, under which conditions minimum lag is obtained (Hinshelwood & Lodge, 1943).

Flasks were incubated in a thermostat at 38°. Determinations of pH were made with a Marconi type TF 717A pH meter. Dry-weight determinations were made on 1 ml. bacterial suspension placed on a watch glass and heated overnight in an electric oven (80–90°) to constant weight.

For polysaccharide determinations portions of at least 250 ml. culture were centrifuged and the supernatant reserved for pH and sugar determinations. The cells were twice suspended in distilled water and again centrifuged, taken up in 10 ml. distilled water, filtered through glass wool and used for polysaccharide and dry-weight determinations. No sugar was detected in the supernatant after final centrifugation.

Media

Medium A, a simple salt medium containing: 5.4 g. KH_2PO_4 , 1.2 g. $(\text{NH}_4)_2\text{SO}_4$, 6.6 g. glucose and 0.66 g. $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}/\text{l.}$; pH adjusted to 7.12 with NaOH.

Medium B, a complex amino-acid medium containing: 0.1 g. L-cystine, 0.5 g. glycine, 1 g. DL-valine, 5 g. sodium glutamate, 15 g. Na lactate, 5 g. NaCl, 2.5 g. $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$, 0.35 g. KH_2PO_4 , 0.30 g. $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 3.5 mg. $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}/\text{l.}$; pH adjusted to 7.5 with NaOH.