58. Studies in the Metabolism of the Strict Anaerobes

8. The Metabolism of Amino-acids by *Cl. welchii**

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(Received 27 April 1942)

It was decided to investigate the amino-acid metabolism of an organism which, unlike those already investigated and described in previous papers in this series, has high saccharolytic and fermentative activities. *Cl. welchii* was chosen as a first example of this type.

Wolf & Harris [1917] and Wolf [1918] showed that *Cl. welchii* fermented sugars with the production of H_2 and CO_2 . When grown on a medium containing protein digest and only traces of carbohydrate, the organism produced relatively little NH_3 and large quantities of H_2 and CO_2 , which suggests that gas may be produced from amino-acid sources.

In the present investigation suspensions of *Cl. welchii* were prepared from cultures grown on the medium considered most likely to give rise to optimal production of amino-acid-dissimilating enzymes. This medium (tryptic caseinogen digest) consists largely of free amino-acids and contains only small quantities of fermentable carbohydrate. Growth was good; more vigorous growth is obtained if glucose is added to the medium, but this was avoided in view of the known inhibitory effect of sugars on the production of amino-acid enzymes in micro-organisms [e.g. Stephenson & Gale, 1937; Gale & Stephenson, 1938; Gale, 1938].

Recently Gale [1941] has shown that *Cl. welchii* grown on the same medium with the addition of 2% glucose develops decarboxylase activity; seven strains of the organism decarboxylated histidine and glutamic acid at *pH* ca. 4-5 with the production of the corresponding amines.

The present work showed that *Cl. welchii* is similar to *Cl. tetanomorphum* [Woods & Clifton, 1937; 1938] in that some amino-acids are decomposed with the formation of gas, but differs from it in that the number of amino-acids attacked is very small. The breakdown of serine requires a special coenzyme-like substance not apparently identical with known coenzymes.

METHODS

Hydrogen and carbon dioxide. These gases were estimated manometrically in an atmosphere of N_2 as described in a previous paper [Woods & Clifton, 1937]. In experiments with cystine and cysteine, $CdSO_4$ absorbers were used to take up the H_2S produced. In certain experiments the total gas production was analysed in a portable Haldane apparatus.

Ammonia. Distillation in a Conway unit followed by titration with a micro-burette [Conway & Byrne, 1933] was used for quantitative work.

Carbohydrate. Total carbohydrate was determined by Pirie's [1936] modification of the orcin method of Tillmans & Philippi [1929], and total reducing substances in the growth medium by the ceric sulphate method of Giragossintz, Davidson & Kirk [1936]. In both cases glucose was used as standard.

* The authors regret the delay in publication of this work, which was completed in 1939.

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Amino-nitrogen. The manometric (constant volume) method of Van Slyke was used; the shaking period was 30 min.

Miscellaneous. Dry weights of bacteria were determined with a photoelectric turbidimeter [Clifton, Mueller & Rogers, 1935] calibrated for *Cl. welchii*. The *dl*-threonine and *dl*-norleucine used were the gift of Dr P. Fildes and Mr R. L. M. Synge respectively. The other amino-acids were Hoffman-la Roche products.

Unless otherwise stated all experiments were carried out at 37° in phosphate buffer pH 7.2 of final concentration 0.05–0.1 *M*. Production of NH₃ is expressed in μ l. (1.6 μ l. NH₃ = 1 μ g. NH₃-N) to facilitate comparison with the H₂ and CO₂ figures.

Growth of organisms. The strain of *Cl. welchii* used for most of the work was *Bacillus welchii* S.R. 9 (National Collection of Type Cultures No. 273). Stock cultures were maintained and washed suspensions prepared as described by Woods [1938].

A few experiments were carried out with another strain (S.R. 12) obtained from Prof. T. Dalling, Department of Animal Pathology, Cambridge.

In some experiments with both strains the cultures were cropped after 5–6 hr. growth, but most experiments were conducted with the 10–11 hr. cultures.

GENERAL SURVEY OF AMINO-ACID BREAKDOWN

The first step in the main experimental work was a survey of all available amino-acids for possible breakdown by suspensions of *Cl. welchii* with production of H₂, CO₂ and NH₃. The results are summarized in Table 1.

Table 1. *Amino-acid breakdown by Cl. welchii*

Amino-acids			Amino-acids		
Attacked with formation of	Mean Q_{NH_3}	Not attacked	Attacked with formation of	Mean Q_{NH_3}	Not attacked
(i) H ₂ , CO ₂ , NH ₃			(ii) CO ₂ and NH ₃		
<i>dl</i> -Serine	390	Glycine	<i>l</i> (+)-Arginine	299	<i>l</i> (+)-Ornithine
<i>dl</i> -Threonine	10	<i>l</i> (+)-Aspartic acid	<i>l</i> (-)-Cystine*	—	<i>l</i> (+)-Lysine
<i>l</i> (-)-Cysteine	8	<i>l</i> (+)-Glutamic acid			<i>l</i> (-)-Tryptophan
		<i>l</i> (-)-Alanine			<i>l</i> (-)-Leucine
		<i>l</i> (-)-Proline			<i>l</i> (-)-Phenylalanine
		<i>l</i> (-)-Tyrosine			<i>l</i> (-)-Hydroxyproline
		<i>l</i> (+)-Valine			<i>dl</i> -Methionine
		<i>l</i> (-)-Histidine			<i>dl</i> -norLeucine

* H₂S is also formed (qualitative tests).

All three determinations were made on the same reaction mixture, the total NH₃ produced being estimated at the end of the experiment after inactivating the organism by tipping H₂SO₄ from the side-bulb. In experiments designed to measure Q_{NH_3} manometers were removed at intervals and the NH₃ estimated. In general the quantities of reactants used were: substrate (0.1 *M*) 0.2–0.3 ml.; bacterial suspension (8–10 mg. dry wt./ml.) 1 ml.; phosphate buffer (0.2 *M*, pH 7.2) 1 ml.; water to 2.5 ml. The substrate was tipped in after equilibration and the reaction followed for at least 2 hr. In each case controls without substrate were put up and all estimations carried out in duplicate. Each amino-acid was investigated with suspensions from at least two different crops of bacteria. Later results with serine showed that it might be important that a relatively large amount of organism should be used, and therefore a few experiments in which less than 8–12 mg. of organism had been used were repeated with this amount. Production of quantities of H₂, CO₂ and NH₃ of less than 0.02 mol./mol. substrate were not considered significant in view of the possibility that the samples of amino-acids used may have contained traces of other amino-acids.

Table 1 shows that, judging by the production of H_2 , CO_2 and NH_3 , only five of the twenty-one amino-acids investigated were attacked by washed suspensions of the organism. It is difficult to conceive of an anaerobic degradation of an amino-acid which would not liberate one or other of these substances. Since all five of the amino-acids attacked give rise to NH_3 the Q_{NH_3} may be used as a comparative measure of their rates of breakdown, although this method of comparison does not take into account the possibility that deamination is not necessarily the primary attack. Table 1 shows that, on the basis of this criterion, only serine and arginine are decomposed at all rapidly.

In view of the surprisingly few amino-acids apparently attacked a number of other possibilities were examined.

(1) Stickland reactions [Stickland, 1934] between pairs of amino-acids might take place. This was investigated by subjecting a mixture of all the amino-acids not attacked singly to the action of *Cl. welchii* suspensions. The experiment was then repeated with the addition in turn of each of the five amino-acids known to be attacked. No production of NH_3 beyond the controls was found in any case. Decomposition of the 'unattacked' amino-acids was not promoted by the addition of glucose (which is rapidly fermented) to the amino-acid mixture. Consequently the possibility of linked reactions between amino-acids and glucose, or its intermediary products of breakdown, appears to be excluded.

(2) Enzymes catalysing the decomposition of other amino-acids may have been present in the growing culture and their activity lost during the preparation of the washed suspensions. Such a loss might occur in three ways: (a) by the washing out of some diffusible coenzyme-like factor, (b) by actual destruction of enzyme and (c) by loss of extracellular enzymes during washing. The first of these possibilities was investigated by adding a small quantity of the original growth medium (as a source of coenzyme) to the mixture of 'unattacked' amino-acids, but no formation of H_2 and NH_3 beyond the controls was observed.

The question of loss or destruction of deaminating enzymes during the preparation of suspensions could only be investigated indirectly. Total H_2 and NH_3 production during growth on the casein digest medium was compared with the production by washed suspensions on the same medium. If enzymes were destroyed during the preparation of the suspensions it was felt that H_2 production in growth would be greatly in excess of that given by washed suspensions. H_2 produced in growth was measured manometrically by transferring aliquots of the sown medium to sterile manometer vessels. Both growth and suspension experiments were continued until further increase in H_2 and NH_3 was insignificant. A typical experiment gave the following results:

	μ l. product/ml. growth medium	
	H_2	NH_3
During growth	141	363
With suspension	190	454

The activity of the washed suspension is thus apparently greater than that of the growing organism. The growing organism is likely to have greater synthetic powers and may well utilize the products of amino-acid decomposition to build up its cell substance. From these experiments it is considered that no enzymic activity is entirely lost during the preparation of the organism and that no enzymes present at earlier stages in growth had disappeared by the time of cropping. These experiments do not give any indication of possible relative loss of activity of any particular enzyme. It was also shown that younger organisms (cropped from a 6 hr. culture) produced no more H_2 , CO_2 and NH_3 than the usual 11 hr. suspension.

Knowing the general activities of the organism, it was then relevant to consider whether the total production of H_2 , CO_2 and NH_3 from the growth medium corresponded to that expected from the amounts of substrates computed to be present. NH_3 pro-

duction from the growth medium was, in fact, rather less than would be expected from the serine, arginine and cystine contents of the casein from which the broth was prepared. This can only be taken as a general indication since, for example, the serine content of casein is not known with precision and the usually recorded value is probably low.

It was concluded that sufficient carbohydrate and amino-acid substrates are present in the growth medium to account for at least as much gas and NH_3 as was found to be produced over the normal period of growth or by the washed suspensions.

Analysis in the Haldane apparatus showed that the only gases formed from broth in appreciable quantities were H_2 and CO_2 . In Thunberg tube experiments with washed suspensions and serine as substrate, CO_2 and H_2 were the only gases detected. The gas mixture contained 13.9% CO_2 and 85% H_2 .

DETAILED STUDIES OF THE KINETICS OF THE BREAKDOWN OF AMINO-ACIDS

BY CL. WELCHII

Serine

Products. Table 2 shows the quantitative results of a large number of experiments on the rate and total production of H_2 , CO_2 and NH_3 from serine. The results are worked out on the assumption that only the natural isomeride of the amino-acid is attacked.

Table 2. *Summary of quantitative results with serine*

Q_{H_2}	No. of exps. 13	Range 197-535 mol. product/mol. serine	Mean 332
H_2	17	0.78-0.93	0.84
CO_2	6	0.82-0.91	0.88
NH_3	7	0.85-1.11	0.99

The relative activity (Q_{H_2}) of different batches of the organism varied over a wide range, but the total extent of decomposition was fairly constant. Deamination is practically complete; H_2 and CO_2 production is equimolecular, and the amount of each is slightly less than that required for 1 mol. per mol. of substrate.

Fig. 1. Effect of pH on the rate of H_2 -formation from serine, threonine and cysteine.

●—●, serine; ■—■, threonine; ▲—▲, cysteine.

Effect of pH. The pH-activity curve is given in Fig. 1, together with those for cysteine and threonine. The results justify working at pH 7.1 in comparisons of the H_2 production from the three substrates.

Effect of concentration of organism. It may be seen (Table 3) that there is a very sharp fall in activity with dilution of the suspension. Rate of H_2 production fell to zero at a concentration of organisms only 1/10 of that giving optimal results, although 242 μ l. H_2 /hr. would have been expected if the rate had fallen linearly with decreasing concentration of organism.

Table 3. *Effect of concentration of organism on the rate of H_2 production from serine*

Dry wt. organism used mg.	H_2 formed μ l./hr.	H_2 expected from rate with 5.6 mg. μ l./hr.	% found of expected
5.60	2418	—	—
2.24	686	969	71
1.12	36	484	7
0.56	0	242	0

This finding immediately suggested that there may be a diffusible coenzyme factor necessary for the deamination of serine by *Cl. welchii*. Attempts were made to reactivate the diluted organism by adding small quantities of substances which are already known to act as coenzyme factors in other reactions. H_2 production was taken as the criterion of activity. The results of these experiments are summarized in Table 4: It was found that even the most active preparation was further activated by the addition of boiled organism. The highest activity of any particular batch of organism obtained by the addition of the optimum quantity of boiled organism was taken as a standard, and the activity obtained with the addition of other factors was expressed as a percentage of that figure.

Table 4. *Activation of serine decomposition by various agents*

	Experiment				
	1	2	3	4	5
Max. Q_{H_2} with boiled <i>Cl. welchii</i>	573	435	536	884	710
	Q_{H_2} as % of maximum Q_{H_2} with boiled <i>Cl. welchii</i> added				
Boiled <i>Cl. welchii</i> (6 mg./ml.), 0.9 ml.	100	100	100	100	100
" " " 0.5 ml.	—	—	—	—	90
" " " 0.2 ml.	—	—	—	—	78
" " " 0.1 ml.	—	—	—	—	48
" " " 0.05 ml.	—	—	—	—	22
Supernatant from boiled <i>Cl. welchii</i> (6 mg./ml.), 0.9 ml.	—	—	92	—	—
Boiled <i>Bact. coli</i> (6 mg./ml.), 0.9 ml.	—	—	—	98	—
Coenzyme I, 25% pure (mg.), 0.9	—	—	—	—	52
" " " 0.5	—	60	96	78	59
" " " 0.1	—	—	—	—	23
" " " 0.05	—	—	—	—	21
Coenzyme II (mg.), 1.0	—	27	—	—	—
Flavin-adenine dinucleotide (mg.), 0.8	67	56	—	—	—
" " " 0.1	—	15	—	—	—
Coccarboxylase (mg.), 0.5	—	—	—	18	—
Muscle adenylic acid (<i>M</i> /100), 0.5 ml.	—	—	—	44	—
Yeast adenylic acid (<i>M</i> /100), 0.5 ml.	45	—	25	39	—
Adenosine (<i>M</i> /100), 0.5 ml.	—	—	—	31	—
Lactoflavin (sat. soln.), 0.5 ml.	13	—	—	—	—
" + adenylic acid	33	—	—	—	—
Nicotinamide (<i>M</i> /100), 0.5 ml.	—	—	—	18	—
Uracil	—	—	—	7	—
No activator	1	26	3	16	8

The supernatant solution from the boiled organism was almost as potent an activator as the boiled whole suspension, as was also a boiled suspension of *Bact. coli* of the same concentration. This would suggest that either the same or an equally potent coenzyme for anaerobic serine deamination exists in *Bact. coli*.

Stephenson & Gale [1938] showed the presence of a diffusible heat-stable coenzyme factor for the deamination of serine by *Bact. coli* both aerobically and anaerobically. Inactivation produced by washing with distilled water at 0° could be prevented if boiled *Bact. coli* or washings from *Bact. coli* were added to the suspension. It would appear that the factor is similar to that now recorded for the strict anaerobe *Cl. welchii*.

The next most potent activators were the preparations of coenzymes I and II and of flavin-adenine dinucleotide, and of these the coenzyme I preparation was the most effective, while the coenzyme of the amino-acid oxidase of animal tissues (flavin-adenine dinucleotide) was the least. The interpretation of these results is difficult as none of the preparations was pure.

Muscle and yeast adenylic acid and adenosine preparations of approaching 100% purity gave considerable activation, while lactoflavin gave some slightly enhanced activity. The effects noticed with the purine nucleosides and nucleotides were partially obscured by the fact that these substances are themselves decomposed by the organism.

So far the evidence suggests that the coenzyme, while not identical with those already known, may be similar to them and is probably widespread among bacterial organisms.

Fig. 2 shows the effect of the concentrations of boiled *Cl. welchii* and of cozymase on the degree of activation. With the boiled organism there is a linear relation between activity and concentration until saturation is reached, as would be expected in a case of

Fig. 2.

Fig. 3.

Fig. 2. Effect of boiled *Cl. welchii* and cozymase on the rate of decomposition of serine. ●—●, ml. boiled *Cl. welchii* (6 mg./ml.); or ■—■, mg. cozymase I (25% pure).

Fig. 3. Partial restoration by boiled organism of activity towards serine lost on storage. ●—●, fresh suspension (1 mg. dry wt./ml.) + boiled organism; ■—■, 2 days old suspension (1 mg. dry wt./ml.) + boiled organism; ▲—▲, 2 days old suspension (1 mg. dry wt./ml.).

simple catalytic activation. With cozymase this effect is not seen because either (1) the effective concentration of the catalyst is continuously removed by other metabolic processes (as with adenylic acid and adenosine), or (2) some kind of partial inhibition by impurities is taking place.

The nature of the other chemical groupings which make the natural coenzyme resistant to decomposition (as would seem to be the case) and highly active was not discovered. The

experiments with the addition of nicotinic acid amide together with purine derivatives suggest either that the factor does not contain the amide or that the organism is unable to synthesize the factor from its components. Similar experiments with lactoflavin suggest that it may be one of the components of the natural factor.

The presence of this type of essential factor in *Cl. welchii* partly explained the fact that its activity towards serine falls off on storage of the washed suspension. The activity of a freshly prepared suspension was compared with that after storage for 2 days at 0°, H₂ production being measured both in the presence and absence of the optimal quantities of boiled organism from the same crop. Fig. 3 shows that the lost activity can be partially restored by the addition of the heat-stable factor (which does not lose its activity appreciably on storage).

Experiments on NH₃ production showed that the coenzyme was also concerned with deamination, giving further evidence of the interdependence of the deamination and oxidation of amino-acids by the organism.

Growth experiments gave valuable evidence on the importance of the factor at various stages in the life history of the organism. The following figures illustrate this point:

	Q_{H_2} with serine
Suspension prepared from 10 hr. culture	332
Suspension prepared from 6 hr. culture, crop 1	31
Suspension prepared from 6 hr. culture, crop 2	20

Previous observations suggested three possible explanations of this phenomenon: (a) the enzyme itself is not developed in the younger organism; (b) the coenzyme is not developed in the younger organism; (c) the coenzyme is much more easily lost by diffusion in the younger organism. Combinations of these effects might also be the cause.

To investigate this problem the history of the development of the serine enzyme system was followed in a large scale growth experiment. A 5 l. flask containing several litres of growth medium was set up in a tank at 37°. An O₂-free atmosphere was maintained by passing in a stream of H₂ previously passed over heated Cu turnings. The flask was heavily inoculated, and after about 1 hr. growth became visible. A sample of the suspension was siphoned off sterily and the growth measured. Thereafter, at convenient intervals, large samples of the fluid were removed and immediately chilled in ice to stop further growth. The concentration of the organism was measured and the organism centrifuged and washed in the usual way. The suspension was stored in the ice chest, and as soon as possible the H₂ and NH₃ production from serine by each sample of organisms was measured.

Table 5. *Development of the serine enzyme system during the growth of the organism*

Growth time hr.	Amount of growth mg. dry wt. organism/ml.	Dry wt. suspension mg./ml.	Q_{H_2} of suspension with serine	H ₂ formed μ l./30 min.	NH ₃ formed μ l./30 min.	μ l. H ₂ /μl. NH ₃
1	2	—	—	—	—	—
2	24	2.5	4	5	5	1.0
2.25	42	2.45	12	15	20	0.8
2.75	84	2.45	25	31	30	1.0
3.25	140	2.45	212	259	289	0.9
3.75	165	2.45	220	268	289	0.9
4.75	192	2.30	290	309	358	0.9
5.75	196	2.30	277	291	341	0.9
7.75	196	2.45	188	206	252	0.8
9.25	208	2.45	154	170	216	0.8

Table 5 summarizes the results of this experiment. Care was taken to use bacterial suspensions of similar concentration to avoid dilution effects. A marked increase of deaminase activity and closely linked increase of the rate of H₂ production were observed.

The rate of increase of activity was linear and greatest during the logarithmic phase of growth, and it would appear that deamination is slightly ahead of H_2 production.

The curves for the relation between growth and activity (Fig. 4) show that in the early stages either the enzyme is not fully developed or the coenzyme is not being produced in sufficient quantities or is very easily lost by diffusion. Further experiments showed that *both* enzyme and coenzyme are relatively less available to the younger organism.

Fig. 4. Development of serine enzyme system during growth. ■—■, bacterial growth; ●—●, hydrogen production from serine by suspensions.

Table 6 summarizes the results of an experiment (similar to that of Table 5) in which the main samples were taken after 2 and 6 hr. growth respectively, and activities were estimated with and without the addition of boiled organism. The 2 hr. culture alone has a very low activity which is increased fifteenfold by the addition of the coenzyme. The 6 hr. culture alone has a much higher activity which is still somewhat increased by

Table 6. *Effect of boiled organism on the activity of suspensions prepared from cultures of different ages*

Age of culture (hr.) ...	2	6
Amount of growth (mg. dry wt./ml. culture)	54	210
Dry wt. of washed cells used for Q_{H_2} (mg.)	3	3
Q_{H_2} with serine: (a) suspension alone	12	288
(b) suspension + 0.8 ml. boiled organism	172	381

boiled organism of the concentration used for activating the younger suspension. The fully activated 2 hr. sample does not, however, attain the activity of the older organism without additional coenzyme. It is certain, therefore, that the amount of enzyme present per unit of organism increases with growth. In addition, the coenzyme is either produced in increasingly greater quantities with ageing or is much more easily lost by diffusion from the younger organism.

Arginine

Table 7 summarizes the quantitative results obtained for the decomposition of arginine by *Cl. welchii*.

Of the amino-acids attacked arginine is peculiar in three respects: (1) no H_2 is produced, (2) the amounts of CO_2 and NH_3 produced in the complete reaction are not equimolecular, (3) the quantities of CO_2 and NH_3 finally produced vary with the age of the culture used in preparing the suspension. The smaller yield of products obtained with the older organism is not due to the exhaustion of the enzyme, since another similar quantity of products was obtained by tipping in more substrate at the end of the reaction.

Table 7. *Summary of quantitative results with arginine*

	No. of exps.	mol. product/mol. arginine		Q_{CO_2} or Q_{NH_3} Mean
		Range	Mean	
(a) 10-11 hr. organism				
H_2	5	—	0	—
CO_2	5	0.29-0.36	0.33	90
NH_3	5	1.28-1.34	1.31	299
Ratio NH_3/CO_2	5	3.72-4.4	3.98	—
(b) 6 hr. organism				
H_2	3	—	0	—
CO_2	3	0.58-0.60	0.59	—
NH_3	3	1.49-1.79	1.64	—
Ratio NH_3/CO_2	3	2.52-3.04	2.78	—

It seems significant that with organisms of both ages the excess of NH_3 over 1 mol./mol. arginine is equal to the total molar quantity of CO_2 produced per mol. of arginine.

The related compounds, ornithine, citrulline, guanidine and urea were investigated in an attempt to throw light on the mechanism of the arginine decomposition, but no attack on these compounds could be detected.

Experiments on the effect of concentration of organism and of the addition of boiled organism on the rate of the reaction indicated that no diffusible coenzyme of the type required for serine is necessary for the attack on arginine. The results of these experiments are summarized in Tables 8 and 9.

Table 8. *Effect of concentration of organism on CO_2 and NH_3 production from arginine*

Product	Amount of bacterial suspension (8 mg./ml.) used ml.	Found μ l./80 min.	Expected from rate with 0.2 ml. organism μ l./80 min.	% found of expected
CO_2	0.2	174	—	—
	0.1	80	87	92
NH_3	0.2	594	—	—
	0.1	277	287	96

Table 9. *Effect of boiled organism on rate of CO_2 and NH_3 production from arginine*

Product	Amount of bacterial suspension (8 mg./ml.) used ml.	μ l. product in 80 min.		
		Arginine alone	Arginine + 0.8 ml. boiled organism 8 mg./ml.	Accelerating effect of boiled organism
CO_2	0.2	174	164	-10
	0.1	80	62	-18
NH_3	0.2	594	578	-16
	0.1	277	310	33

Changes in pH between 6.1 and 8.0 had a very small effect upon the rate of CO_2 and NH_3 production from arginine; within this range the activities measured never fell below 90% of the optimum.

Cystine and cysteine

Both cystine and cysteine were attacked by *Cl. welchii* with the production of CO_2 and NH_3 , and H_2 was formed from cysteine. Of the two amino-acids cystine was the more slowly decomposed and in no experiment was its complete decomposition obtained. In both cases the activity of the organism was low compared with that for serine and arginine (see Table 1). The activity towards both substrates was increased about twofold by the addition of boiled organism (cf. serine) and also by the addition of small quantities

Table 10. *Summary of quantitative results with cysteine, cystine and threonine*

	No. of exps.	mol. product/mol. substrate (mean)			Q_{H_2} mean
		H_2	CO_2	NH_3	
Cysteine	5	0.52	0.76	0.86	14
Threonine	3	0.72	0.80	0.95	12
Cystine	3*	—	0.37	0.25	—

* These experiments did not reach completion.

of the culture medium. Some slight activation was obtained by the addition of purine derivatives. The quantitative results given in Table 10 were obtained in experiments in which the organism was activated in this way. After some 3 hr. the NH_3 production without added amino-acid began to increase rapidly, and results obtained with the organism in this state were not considered significant. Further, in spite of the fact that substrate remained the activity of the enzymes fell off sharply. Fig. 5 shows the effect of boiled organism and of growth medium on the H_2 production; the catalytic effect is definite although small. As in the case of serine, the NH_3 and H_2 productions are parallel and both processes are activated to a comparable extent. The effect of pH is not marked, and, as with serine and arginine, there is no clear optimum (cf. Fig. 1). H_2S production was indicated by the orange staining of the CdSO_4 absorbers in all cases in which they were used.

With the exception of the production of H_2S and the lower Q_{H_2} , the decomposition of this amino-acid is qualitatively and quantitatively similar to that of serine. The non-production of H_2 from cystine suggests that it is necessary to reduce cystine to cysteine before it may be attacked by the organism. The H_2 produced from cysteine might then be utilized to reduce more cystine. It was found that molecular H_2 will not reduce cystine in this way.

Fig. 5. Effects of broth and boiled organism on rate of H_2 formation from cysteine. 1, broth alone; 2, cysteine alone; 3, cysteine + broth; 4, cysteine + optimum amount of boiled organism (boiled organism control deducted).

Threonine

Threonine was also broken down slowly at a rate comparable with that for cysteine. The quantitative yields of H_2 , CO_2 and NH_3 are given in Table 10. The rates of H_2 and NH_3 production were found to be enhanced to the same extent as with cysteine by boiled organism and broth. As with the other amino-acids the pH (see Fig. 1) has no marked effect on the activity over the range 6.1–8.0.

Results with strain S.R. 12

All substrates were tested with the S.R. 12 strain of the organism, and it was found that activities were in all cases qualitatively similar to those of the S.R. 9 strain; the actual rates of the reactions, however, varied widely.

DISCUSSION

The few amino-acids which are attacked by *Cl. welchii*, under the conditions used in the present work, fall into two main groups.

(a) *Serine, threonine, cysteine and cystine*. These amino-acids are broken down with formation of NH_3 , CO_2 and, with the exception of cystine, H_2 . Coenzyme-like factors are involved in the breakdown of these amino-acids. These amino-acids, except threonine which was not then available, were among those found to be broken down (again with formation of H_2 , CO_2 and NH_3) by *Cl. tetanomorphum* [Woods & Clifton, 1937]. With both organisms no H_2 was found with cystine. *Cl. tetanomorphum* attacks a wider range of amino-acids than *Cl. welchii*, for, in addition to the above, glutamic acid, aspartic acid, histidine, tyrosine and methionine are also attacked. With *Cl. tetanomorphum* there was no reason to suspect that a coenzyme-like factor might be involved; in the light of the results obtained in the present work a further investigation might be profitable. The coenzyme-like factor involved in the decomposition of this group of amino-acids is present in boiled organism and also in boiled *Bact. coli*. There is evidence that it has some relation to the purine nucleotides and nucleosides, but it does not appear to be identical with any of the known coenzymes.

(b) *Arginine*. This amino-acid appears to be decomposed by *Cl. welchii* suspensions in a rather different way from those considered under (a) above. Arginine, like serine, is rapidly attacked by the organism but no H_2 is formed. The amounts of CO_2 and NH_3 produced are not equimolecular (as with serine), and furthermore, both the absolute amounts and relative proportions of these products vary with the age of the culture from which the bacterial suspension is made. No evidence was found of the necessity of a coenzyme-like factor in the decomposition of arginine. This amino-acid is not attacked by *Cl. tetanomorphum*.

Cl. welchii, like *Cl. tetanomorphum* [Woods & Clifton, 1937] has no formic hydrogenlyase enzyme and no H_2 is produced from formate. Formate is usually considered to be the immediate precursor of H_2 formed by bacteria, but, as pointed out by Woods & Clifton, this cannot be so in the case of the H_2 produced from amino-acids by *Cl. tetanomorphum*. The same seems also to be true in the case of H_2 production from amino-acids by *Cl. welchii*.

Recently Gale [1941] has found that if suspensions are prepared from cultures grown in the presence of glucose and the tests carried out at pH 4-5, *Cl. welchii* (7 strains including S.R. 9) decarboxylates histidine and glutamic acid with formation of the corresponding amines.

SUMMARY

Cl. welchii, grown under the best known conditions for the production of amino-acid deaminases, attacks only five of the twenty-one commoner amino-acids investigated.

Serine was actively attacked, giving rise to H_2 , CO_2 and NH_3 in roughly equimolecular proportions. A coenzyme, apparently of the purine-nucleotide type, is necessary for this reaction. This coenzyme does not appear to be identical with coenzymes I and II or with flavin-adenine dinucleotide.

Cystine, cysteine and threonine are attacked in a similar manner and there is some evidence of the necessity of a coenzyme factor.

Arginine is also attacked with production of NH_3 and CO_2 but no H_2 is formed. The final yield of NH_3 and CO_2 in the complete reaction varies with the age of the culture from which the bacterial suspension is prepared. No evidence for the necessity of a coenzyme was found with this amino-acid.

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