CXXXIV. VARIABILITY IN THE ACTIVITY OF BACTERIAL ENZYMES.

I. THE EFFECT OF THE AGE OF THE CULTURE.

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Many papers have appeared during the last few years dealing with the dehydrogenase reactions induced by suspensions of washed bacterial cells. Up to the present no attempt has been made to examine systematically the activities of these enzymes in relation to any variations that might result from growing the organism under different conditions. In the past it has been more or less assumed that when grown on one of the usual bacteriological nutrient media an organism will exhibit a characteristic range of enzymic behaviour, although instances have been cited in which differences in this behaviour appeared to develop. Thus Klotz [1906] showed that a non-lactose-fermenting organism, *B. perturbans*, if grown upon a solid medium containing lactose, produced colonies which developed lactose-fermenting papillae. When the organism was sub-cultured from the central non-lactose-fermenting colony the resulting cells did not ferment this sugar, whilst subcultures from the lactose-fermenting papillae again formed colonies with a non-lactose-fermenting centre but with lactose-fermenting papillae. It would seem that under the conditions of these experiments an organism which was apparently unable to ferment lactose had, during growth upon a medium containing this sugar, developed the necessary enzymes to effect this change. Karström [1930] extended this work and showed that of the enzymes of bacteria that attack sugars some, the so-called "constitutive" enzymes, form an invariable part of the cellular biochemical structure, whilst others, the "adaptive" enzymes, are absent from the cells which have not been grown in the presence of their substrate. Stephenson and Stickland [1932] demonstrated that a suspension of washed cells of *Bact. coli* grown in broth containing added formate possess a hydrogenlyase which will decompose formate with the evolution of hydrogen, whilst the cells obtained from the same organism grown on plain broth do not possess this enzyme. Yudkin [1932] developed this work further. These results indicate the possible importance to its enzymic make-up of the constitution of a medium upon which an organism grows. In the present series of papers it is hoped to extend the enquiry into an analysis of various factors that might influence enzyme formation. The work described in this particular paper serves to show that the activities of dehydrogenases, when tested by the usual procedure, are not constant throughout the life of a culture, but appear to vary considerably with the time the organism is allowed to grow before it is reaped for enzyme examination.

Experimental.

The organisms examined in this paper were grown upon the usual nutrient media, *viz.* heart broth, caseinogen digest broth or nutrient agar. With the liquid medium good aerobic conditions were secured by placing 120 ml. in Roux bottles

and incubating them on their sides. For a particular series of experiments all flasks were inoculated, as far as possible, at the same time; if this was not possible the stock inoculum was stored at 0° for the short time (a few hours at most) during which it was being used. Every flask of medium received equal amounts of the stock inoculum, which was sometimes a broth culture and sometimes a suspension of the organism in quarter strength Ringer solution. The inoculated flasks were incubated at 37°, and a sufficient number were removed at the required intervals to ensure an adequate supply of washed organisms for the enzymic examinations. The cell suspensions were obtained in the usual way by centrifuging, or washing the cellular deposit twice with saline, suspending finally in quarter strength Ringer solution, aerating for 20 min. and examining within a short time for dehydrogenase activity. The number of inoculated flasks required to give sufficient organisms after a short period of growth was naturally much larger than the number required for longer periods of growth.

The Thunberg tubes contained 1 ml. of bacterial suspension, 1 ml. 1/10,000 methylene blue solution, 0·5 ml. substrate solution at p_H 7·4 and 0·5 ml. of phosphate buffer, p_H 7·4. The tubes were evacuated at the water-pump, immediately incubated at 45° and the time required for decoloration of 90 % of the methylene blue noted. The end-point was obtained by matching a similar mixture of organism and nitrate containing only 1/10 as much methylene blue. All the bacterial suspensions of a given series were standardised by turbidity comparisons with standard suspensions of *Bact. coli* cells and were usually adjusted to an opacity of 5×10^9 cells per ml. of suspension. With *Bact. pullorum* and *Bact. suipestifer* thicker suspensions were required. All bacterial suspensions were examined within an hour or two of their preparation.

Variable dehydrogenase activity of Bact. coli *grown on heart broth.*

When suspensions of cells of *Bact. coli* grown on heart broth are prepared and examined in the way previously described it is found that their dehydrogenase activities are not constant but may vary considerably with the age of the culture from which the organism was obtained. This variability is not the same for all the enzymes as an examination of the ratio of the reduction times for any two donators throughout the series will show. Nevertheless, there seems to be a general tendency for the dehydrogenase activities to increase at first, reach a maximum and subsequently to decrease. These observations are illustrated by the results recorded in Table I. It will be seen that whilst the activity of the formic dehydrogenase remains practically constant throughout the course of the experiment, the activities of all the other enzymes examined vary to a greater or less degree. The reduction times obtained with the 4, 24, 48 and 72-hour organisms are respectively 8·9, 2·9, 2·5 and 3·5 min. for lactate, and 5·2, 3, 23 min. and $>$ 2 hours for glucose; results which clearly illustrate both the variability of the activity of individual enzymes and the fact that this variability is not the same with each enzyme. This difference in variability is further emphasised by the ratios given in Table I, comparing one series of reduction times with another. With the amino-acid enzymes the peak in the activity is very marked. Of those examined the most active suspension, considering the enzymes collectively, appears to be that obtained from the organism grown for 24 hours. A comparison of the various reduction times obtained with this and with other suspensions (*vide* last column, Table I), emphasises the differences that may be obtained. In fact the differences may be so great that it would be a

Table I. *Dehydrogenase activities of suspensions of* Bact. coli *cells obtained from cultures of variable ages grown on heart broth.*

Experimental details described in the text. The reduction times are given in minutes. In this and subsequent tables the shortest time for each donator is given in heavy type. Temperature 45°. All the bacterial suspensions were standardised to a turbidity $\equiv 5 \times 10^9$ *Bact. coli* cells per ml.

Substrate	Period of growth in hours						Ratio 4 hr./ 24 hr.
	4	6	12	24	48	72	
Control	>120	113	68	>120	>120	>120	—
Formate	2·7	2·9	3·0	2	2	2	1·35
Lactate	8·9	7	3·7	2·9	**2·5**	3·5	3·07
Glucose	5·2	4·1	3·5	**3**	23	>120	1·73
Succinate	18·5	14·3	6	**3**	6	8·3	6·17
Glutamate	37	29	7	**5**	100	>120	7·4
Alanine	29	20·5	16·5	**9·5**	>120	>120	3·05
Trytophan	>120	43	32·5	**20**	>120	>120	>6
Ratios:							
Lactate/formate	2·93	2·42	1·20	1·45	1·25	1·75	—
Glucose/formate	1·93	1·41	1·17	1·5	11·5	> 60	—
Glucose/lactate	0·58	0·59	0·95	1·03	9·2	> 34	—
Glutamate/formate	13·7	10·0	2·33	2·5	50	> 60	—

pardonable error to conclude that the suspensions were in reality obtained from quite different organisms. Thus the times for the 72-hour suspension suggest that it was derived from an organism possessing active formic, lactic and succinic dehydrogenases but lacking the enzymes for sugars and amino-acids, whilst the results with the 24-hour suspension suggest an organism of a different type, showing marked activity with all the substrates.

Bact. coli *grown on caseinogen digest broth.*

Another strain of *Bact. coli* was grown similarly on a caseinogen digest medium and the activities of its dehydrogenases were examined. The results obtained are similar in character to those described with heart broth (Table II).

Table II. *Dehydrogenase activities of* Bact. coli *grown on caseinogen digest for variable periods of time.*

Details as for Table I. ∞ = >180 min.

Substrate	Period of growth in hours							
	5	10	17	24	48	72	96	160
Control	∞	∞	63	∞	∞	∞	∞	∞
Formate	2·4	**2·3**	3·5	2·7	2·5	3·5	6	19·3
Lactate	15	10	10·5	7·3	**6·3**	7·5	17	52
Glucose	6	5·7	**4**	7·7	7·7	11	∞	∞
Succinate	11	13	**4·5**	6·5	9·5	14	58	∞
Glutamate	5·5	6·3	**5·6**	7	9	11	16	210
Alanine	112	106	**18**	84	∞	∞	∞	∞
Leucine	150	146	**21**	90	95	∞	∞	∞

The formate enzyme again shows the least variation but there are signs of a falling off in its activity after growth for 96 hours, a diminution which is quite definite after 160 hours' growth. With many of the enzymes there are indications of an increasing activity of the dehydrogenases during the early hours of growth.

This variability in enzyme activity was also shown aerobically in the Barcroft differential manometer. Table III illustrates the rate at which oxygen was absorbed by similar *Bact. coli* suspensions in the presence of certain substrates.

Table III. *The rate of oxygen absorption by* Bact. coli *grown for variable periods on caseinogen digest.*

Experimental details for the preparation of the bacterial suspensions (1 ml.≡5 × 10^9 cells) as given in the text. The figures represent the oxygen absorption in 1 hour, expressed in μl. at N.T.P., by the various bacterial suspensions in the presence of the named substrates. Both cups of the manometers contained bacterial suspensions and buffers but only the right-hand cup contained the substrate. The usual technique was followed [Dixon, 1934].

	Period of growth in hours							
Substrate	2	5	10	17	24	48	96	160
Formate	80	71	70	72	72	69	—	23
Glucose	86	99	182	169	178	87	42	32
Glutamate	176	178	240	300	238	200	36	45
Alanine	12	19	59	41	142	—	—	20

The activity of the formic enzyme is again remarkably constant but falls off after prolonged growth, whilst the other enzymes examined exhibit more variable activities which at first increase but later diminish in a way similar to that demonstrated anaerobically.

Variation in the dehydrogenase activities of other organisms with the period of growth.

The reduction times obtained with various substrates in the presence of suspensions of *Bact. aerogenes*, *Bact. pullorum* and of *Bact. suipestifer* that have been grown for variable periods of time on nutrient media are displayed in

Table IV. *The dehydrogenase activities of* Bact. aerogenes *grown on heart broth.*

Details as for Table I. Each Thunberg tube contained 1 ml. of bacterial suspension corresponding in turbidity to a *Bact. coli* suspension containing 7 × 10^9 cells per ml.

	Period of growth in hours											
Substrate	0	3	6	12	18	25	36	48	72	96	120	144
Control	40	40	45	>120	>120	>120	>120	>120	>120	>120	>120	>120
Formate	4	3·7	7	11·7	11·7	11·3	11·5	14	14	14·3	44	63
Lactate	15	4	5	6·5	6·5	3·3	3	3·5	4	4	9	10·5
Glucose	6	3·7	3·7	6·5	6	5·3	5·5	5·3	9·3	11	120	>120
Succinate	11·5	11	11·3	25	29	9	7·7	10	21	23	>120	>120
Glutamate	6·5	4·7	4	5	5·7	6·5	9	9·5	14	17·5	>120	>120
Tryptophan	>80	24	13	30	30	32	34	—	>120	>120	>120	>120

Table V. *Dehydrogenase activities of* Bact. pullorum *grown on nutrient agar for variable periods of time.*

Experimental details as for Table I, except that the original suspension was obtained by washing off the bacteria from the solid medium with a few ml. of sterile saline. The suspensions used were equivalent to *Bact. coli* suspensions containing 16 × 10^9 cells per ml. ∞ = >120 min.

	Period of growth in hours							
Substrate	5	9	12	18	24	48	72	120
Control	∞	∞	∞	∞	∞	∞	66	∞
Formate	—	∞	90	26	18·5	30	40	—
Lactate	27	21	20	9·7	11·7	4	7	27
Glucose	—	27	18	20	6	4	8·5	13
Succinate	—	—	—	35	8·7	16	9	—
Glutamate	—	29	22	22	13·3	8·5	6·5	26
Glycine	—	—	52	22·3	24·3	100	—	∞
Alanine	—	—	—	60	40	15	29	∞
Tryptophan	—	∞	∞	85	48	∞	—	∞

Table VI. *Dehydrogenase activities of* Bact. suipestifer *grown on nutrient agar for variable periods of time.*

Experimental details as for Table V. The bacterial suspensions contained 20×10^9 cells per ml.

	Period of growth in hours					
Substrate	5	8	12	24	54	72
Control	∞	∞	∞	30	46	∞
Formate	4·6	6·8	**5·3**	7·2	7·7	29
Lactate	36·5	5	**3·5**	4·5	5·8	15
Glucose	∞	5·8	**4**	5·3	8·6	27
Succinate	53	8·5	**6·7**	9·8	9·5	34
Glutamate	∞	7·5	**7·3**	7·5	10·1	31

Tables IV, V and VI. The results exhibit a similar variability to those previously obtained with suspensions of *Bact. coli*. With these three organisms the formate enzyme is comparatively weak but it is especially so with *Bact. pullorum*.

Discussion.

It has been shown that the dehydrogenase activity of many, if not all, bacterial cells may vary considerably with the period between inoculation and reaping of the organism. There appears to be a general tendency for the enzyme activities to increase at first and subsequently to decrease. Whether the maximum activity has any relationship to the logarithmic phase of growth cannot be stated at present, although it usually occurs within the first 24 hours of growth. That the variability of dehydrogenase activity is not identical with each enzyme is clearly shown by an examination of the ratios of reduction times given in Table I. The curves in Fig. 1 show that, although many bacteria exhibit varia-

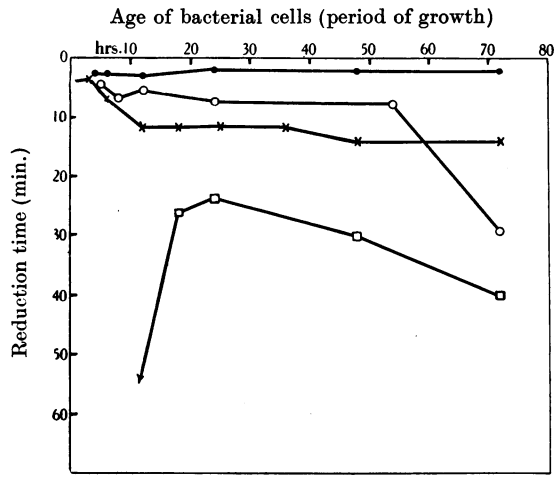

Fig. 1. Variability of the formic dehydrogenase of different organisms. •—• *Bact. coli* (heart broth); ×—× *Bact. aerogenes* (heart broth); □—□ *Bact. pullorum* (nutrient agar); o—o *Bact. suipestifer* (nutrient agar).

tions for particular dehydrogenases (*e.g.* the formic dehydrogenase), the variations obtained are not necessarily the same with the different organisms. It is not our intention in this paper to assign causes for these variations but several possible factors that might influence the enzymic activity of such cellular suspensions as have been examined may be cited. Thus the activity might be influenced

by the proportion of viable to total cells in the culture medium or in the actual cell suspension used for the tests, by differences in permeability and other properties associated with the "condition" of the cell with respect to its growth phase, by variation in the concentration of co-enzymes, by differences induced in the cell by variations in the chemical or physical nature of the medium during the course of growth or by the effect of these earlier conditions upon the ability of the cells to undergo some change in the Thunberg tube, such as a change in shape or cell division, which change might affect the rate of reduction of the methylene blue. It is hoped to examine the effects of many of these factors in further work, meanwhile it is important to emphasise that the apparent enzymic complex of bacterial suspensions prepared and examined in the way originally described by Quastel and Whetham [1924] does not necessarily indicate a range of activities characteristic of that organism throughout its life (as was more or less implied by Quastel and Wooldridge [1925]) for it appears not only that the order of the activities of the various enzymes will differ among themselves but that certain enzymes which seem to be inactive at one time may be very active at some other stage in the life of the organism. The importance of this fact and its bearing upon the question of "constitutive" and "adaptive" enzymes is obvious. Thus an "adaptive" enzyme may simply be a "constitutive" enzyme which is active apparently only during a part of the life of the cell and is not normally recognised by the technique most usually employed in the enzymic examination of the organism. By suitably altering the conditions of growth of the organism, however, the concentration and stability of such an enzyme may be increased so that it becomes readily detectable by the usual technique.

SUMMARY.

1. It is shown that the activity of most bacterial dehydrogenases may vary considerably with the time the organism is allowed to grow before reaping.

2. The activity of most of these enzymes appears to increase at first, to reach a maximum and subsequently to diminish. This variability is generally least with the formic acid enzyme, *vide* the results with *Bact. coli*, but even with this enzyme it may be great, especially with some other organisms such as *Bact. pullorum*.

3. As the series of reduction times obtained with a standard cell suspension and several substrates may differ considerably with the initial period of growth of the cells of the suspension, it is concluded that to secure a true picture of the enzymic make-up of organisms such as bacteria it is necessary to examine the organism at different stages in its growth.

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