ON THE "ACTIVATION" OF THE LACTASE OF ESCHERICHIA COLI-MUTABILE[1]

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The studies reported in this paper were directed to an explanation of the puzzling phenomenon that the non-lactose-fermenting *Escherichia coli-mutabile* contains lactase (Deere, Dulaney and Michelson, 1939). Earlier experiments led us to believe that the antiseptics employed "activated" the lactase which was present, but inactive, in living growing cultures of the non-lactose-fermenting (white) form. Use of an antiseptic was at first necessary in determinations of lactase activity since it protected the digest against contaminants and rendered the preparations incapable of attacking glucose and galactose. It was later found that lactase activity of preparations of the white form did not depend on the presence of, or preliminary treatment with, an antiseptic. Drying of cell suspensions by vacuum distillation suffices to "activate" the lactase.

We have investigated more extensively the effect of drying on the various enzyme activities of these organisms. The drying process, unlike the various lactase "activating" antiseptics which we have employed, only partially inhibits the enzyme system or systems concerned in the metabolism of glucose and galactose. Hence, reduction methods for sugars present great difficulties in evaluating results obtained on digests in which the reducing value is being increased by one process (lactose hydrolysis) and

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decreased by another (monose metabolism). Since a portion of the sugar metabolism of *Escherichia coli-mutabile* involves the consumption of oxygen, this oxidative phase apparently being limited to monose metabolism, we have attempted herein to analyze enzyme activities from measurements of oxygen consumption. For brevity we shall designate the enzyme system responsible for oxygen consumption as the "oxidase system." The over-all increase in oxygen consumption by dried organisms in lactose, as contrasted with non-dried organisms in lactose, is to be attributed to a primary hydrolysis of the lactose in the first instance and an only partially destructive effect of the drying process upon the subsequent monose metabolism.

METHODS

Oxygen consumption

Oxygen consumption of cell suspensions was measured in a Warburg apparatus at 37.5°C. Carbon dioxide was absorbed by a roll of filter paper moistened with 0.1 cc. of 20 per cent potassium hydroxide.

Cell preparations

The cells were grown on plain or sugar agar and cell suspensions were prepared as described previously (Deere, Dulaney and Michelson, 1939). One-cubic centimeter quantities of cell suspensions were dried in weighed Warburg flasks by vacuum distillation. The weight of dried cells was determined and 1 cc. of distilled water added to replace that lost in drying. One-cubic centimeter portions of cell suspensions which had not been dried served as controls.

Procedure

To the aqueous suspensions of dried or non-dried cells in the Warburg flasks were added 1 cc. of 1.5 per cent sodium chloride in 0.3 M phosphate buffer (pH 7.0) and 1 cc. of 1.5 per cent sugar solution or (for control) 1 cc. of distilled water. The flasks were attached to manometers and placed in the bath. Fifteen minutes later the vessels were shut off from the air and the pressure

changes recorded at 15-minute intervals. With samples whose oxygen consumption was high, the vessels had to be opened at each 15-minute interval. When this was necessary, 1 minute elapsed between successive intervals.

RESULTS

In experiment 1 (table 1) is reported the oxygen consumption of suspensions of 24-hour plain-agar cultures of Garrett white. In lactose, the dried sample (sample 4) consumed 2.5 times as much oxygen as the non-dried sample (sample 10). The oxygen consumption of dried cells (sample 8) in glucose was about two-thirds that of non-dried cells in glucose (sample 18). These results demonstrate a partial inhibition of the oxidase system by drying but an "activating" effect on lactase. The oxygen consumption of dried cells suspended in glucose is about 3 times that of similarly treated cells suspended in lactose. This finding is not surprising in view of our previous demonstration (Deere, Dulaney and Michelson, 1939) that cells grown in the absence of lactose have only slight lactase activity.

A similar experiment (experiment 2, table 1) was performed using suspensions of Garrett white which had been grown for 24 hours on 1 per cent lactose agar. The results are qualitatively no different from the results reported in experiment 1. Dried cells (sample 8) consumed slightly less oxygen in glucose than non-dried cells (sample 18) but, in lactose, dried cells (sample 4) consumed 3.7 times as much oxygen as non-dried cells (sample 10). Non-dried cells (sample 3) consumed about one-half as much oxygen in the absence of sugar as similarly treated cells suspended in lactose (sample 10). These results demonstrate again the "activating" effect of drying on the lactase of this organism. Comparison of these results with experiment 1 demonstrates the large increase in lactase content which occurs when this organism is grown on lactose.

We then turned our attention to the behavior of the lactose-fermenting (red) strain in similar experiments. Experiment 3 (table 1) gives the results obtained with a suspension of a 24-hour growth of Garrett red on 1 per cent lactose agar. It will be noted

TABLE 1

Oxygen consumption of suspensions of Escherichia coli-mutabile

Experiment Number..	1				2					3				4		5			6		
Strain used.....	White				White					Red				Red		White			Red		
Culture medium.....	Plain				Lactose					Lactose				Plain		Glucose			Plain		
Sample number.....	10	4	18	8	3	10	4	18	8	18	4	8	10	16	18	7	16	18	7	16	10
Treatment of cells.....	Non-dried	Dried	Non-dried	Dried	Non-dried	Non-dried	Dried	Non-dried	Dried	Non-dried	Dried	Dried	Non-dried	Dried	Non-dried	Dried	Non-dried	Non-dried	Dried	Dried	Non-dried
Weight of sample, mgm.....	—	9.3	—	9.2	—	—	9.1	—	8.9	—	9.1	9.0	—	10.0	—	7.9	—	—	8.9	8.4	—
Sugar present in respirometer.....	Lactose	Lactose	Glucose	Glucose	None	Lactose	Lactose	Glucose	Glucose	None	Lactose	Glucose	Lactose	Glucose	Glucose	Glucose	Glucose	None	Glucose	Lactose	Lactose
Time	Oxygen consumption in cubic millimeters per 10 mgm. of preparation																				
minutes																					
0–15	16	46	164	126	7	19	89	230	212	7	10	0	120	89	175	−3	199	16	112	46	27
15–30	13	41	183	125	19	23	87	196	189	8	3	1	148	86	170	1	205	19	108	42	7
30–45	17	42	196	117	7	22	83	201	196	12	4	6	166	80	167	5	193	16	116	52	30
45–60	15	33	183	119	7	19	90	175	167	13	0	3	165	88	161	8	199	19	112	50	16
60–75	11	35	167	102	10	26	95	161	157	10	2	1	173	79	154	8	185	14	117	56	29
75–90	16	33	172	110	13	28	91	160	157	9	−3	0	173	69	87	5	167	13	100	55	19
90–105	14	38	168	116	14	25	95	132	134	9	−1	1	168	78	139				121	62	36
105–120	15	39	156	102	12	28	96	103	107	7	4	6	166	76	118				103	60	28
Total oxygen consumption.....	117	307	1,389	917	89	190	726	1,358	1,319	75	19	18	1,279	645	1,171				889	423	192

that only a slight oxygen consumption by dried preparations occurs in lactose or glucose (samples 4 and 8). These results indicated that destruction of the oxidase system had occurred during the drying procedure, earlier work (Deere, Dulaney and Michelson, 1939) having demonstrated that lactase is not damaged by drying. Drying had not destroyed the oxidase system of the white strain (experiments 1 and 2). The most plausible explanation of the difference in behavior of the two strains appeared to be the difference in pH of the medium from which the

TABLE 2

Oxygen consumption of suspensions of Garrett red grown on lactose agar

Samples 10, 16 and 18 washed in $NaHCO_3$ to bring pH to 7.6

Substrate—0.1 M phosphate, pH 7.0, 0.5 per cent sodium chloride, and 0.5 per cent sugar

Sample Number...	4	7	8	10	16	18
Treatment of cells..	Dried	Dried	Non-dried	Dried	Dried	Non-dried
Weight of sample, mgm.....	5.6	5.6	—	4.7	4.4	—
Sugar present.....	Lactose	Glucose	Glucose	Lactose	Glucose	Glucose
Time interval	Oxygen consumption in cubic millimeters					
minutes						
0–15	2	1	147	1	4	138
15–30	2	0	124	0	2	123
30–45	0	2	111	−2	4	117
45–60	1	1	101	1	−1	96
Total oxygen consumption	5	4	483	0	9	474

cells had been harvested. 24-hour growth on lactose agar produced a pH of about 5 for the red and above 7 for the white strain. It appeared unlikely that more than traces of organic acids would remain in the cells of the red strain during the process of washing, once in saline and twice in distilled water. This possibility, however, was now tested.

A 26-hour growth of Garrett red on 1 per cent lactose agar was harvested in saline as usual and the resulting suspension divided into two parts. One part was centrifuged without further

treatment. The second part was treated with an equal volume of M/10 sodium bicarbonate and then centrifuged. The supernatant fluid from the bicarbonate-treated sample had a pH of 7.6; the untreated sample had a pH of 4.9. Both samples were washed twice in distilled water in the usual manner and portions prepared for study of the effect of drying on oxygen consumption. The results (table 2) demonstrate a similar behavior of the non-treated and bicarbonate-treated samples. The non-dried samples consumed oxygen rapidly in glucose but the dried samples consumed negligible quantities of oxygen in lactose or glucose. Thus, it appeared that the oxidase system of the red strain was peculiarly susceptible to destruction by drying or that growth on a fermentable sugar produced a susceptibility to destruction, a susceptibility which was not altered by neutralization of the products of fermentation before drying.

We therefore tested the effect of drying on the oxidase activity of a plain agar culture of the red strain. A 23-hour growth of Garrett red gave a pH of 7.3, a reaction similar to that of a lactose agar culture of the white strain. The results (experiment 4, table 1) demonstrate the failure of the drying technique to paralyze the oxidase system of cells of the red strain grown in this manner. It appears then that growth of the red strain on a fermentable sugar is responsible for the susceptibility of its oxidase system to destruction by drying. It seemed unlikely that this behavior was peculiar or characteristic of only the red strain. This indicated the advisability of studying the behavior of suspensions of the white strain which had been grown on a fermentable sugar.

A 24-hour culture of Garrett white on 1 per cent glucose agar was treated in the usual manner and the effect of drying on the oxygen consumption of cells suspended in glucose was determined. The destructive effect of drying on the oxidase system of these cells is apparent (experiment 5, table 1). Our original plan of comparing the effect of drying on the lactase activity of the two strains could then be carried out. This required a dry preparation which retains oxidase activity which could be obtained with the red strain only by growing it on plain agar.

Study of a 25½-hour plain-agar culture of Garrett red revealed that drying doubled the oxygen consumption of cells suspended in lactose (experiment 6, table 1). Comparison of these results with those of experiment 1 and 2 demonstrates a similarity in the activating effect of drying on the lactase activity of both the white and red strains though the activation is more marked with the white.

It occurred to us that the increase in lactase activity which takes place on drying might be due to release of lactase from cells into the surrounding fluid. An experiment was designed to test this hypothesis. A 25-hour growth of Garrett white was harvested from 1 per cent lactose agar, the pH being 7.2. The cells were washed in the usual manner and resuspended in water. Four 1-cc. portions of the suspension were measured into Warburg flasks and set aside for respiration studies, after one of them, no. 7, had been dried by vacuum distillation. The weight of dry material was 7.1 mgm. Two 3 cc. portions of the suspension were measured into centrifuge tubes and used for preparation of extracts A and B.

The sample used for preparation of extract A was dried in a 50 cc. centrifuge tube by vacuum distillation, the weight of dry material being 21.3 mgm. After the dried sample had stood for 2½ hours, 3 cc. of 1.5 per cent sodium chloride in 0.3 M phosphate buffer were added to it. The cells were resuspended and 15 minutes later the sample was centrifuged. One cubic centimeter of the supernatant fluid represents the extract from 7.1 mgm. of dried cells and is designated as extract A.

A second 3-cc. portion of the original suspension was centrifuged and the supernatant fluid removed as completely as possible. Then 3 cc. of the phosphate-sodium chloride solution were added, the cells resuspended, and 5 minutes later the sample was centrifuged. One cubic centimeter of the supernatant fluid represents the extract from 7.1 mgm. of non-dried cells and is designated extract B.

For determination of the effect of these extracts on the oxygen consumption of cells, samples containing cells alone, extract alone, and cells in the presence of extract were prepared. Lac-

tose was added to all samples. The results of the experiment are presented in table 3.

Extract B (of non-dried cells) consumes no oxygen in the presence of lactose (sample 16). Addition of extract B to non-dried cells suspended in lactose (sample 8) results in no greater oxygen consumption than was observed in the absence of extract (sample 10). These facts are interpreted as indicating the absence of lactase in extract B.

TABLE 3

The lactase activity of an extract of dried cells of Garrett white

Substrate—0.1 M phosphate, pH 7.0, 0.5 per cent sodium chloride, and 0.5 per cent lactose

Extract A is an extract of dried cells. Extract B is an extract of non-dried cells

Sample Number...	8	16	10	4	7	18
Type of cells present.....	Non-dried	None	Non-dried	Non-dried	Dried	None
Extract present....	B	B	None	A	None	A
Time interval	Oxygen consumption in cubic millimeters					
minutes						
0–15	6	−2	4	19	62	−3
15–30	11	2	14	17	62	−7
30–45	8	−1	5	18	65	0
45–60	11	0	12	17	64	−1
60–75	10	0	10	18	56	−2
75–90	11	0	10	18	56	0
Total oxygen consumption	57	−1	55	107	365	−13

Extract A (of dried cells) produces gas when added to lactose (sample 18). Since potassium hydroxide was present to absorb carbon dioxide, it seems probable that some hydrogen was produced. Addition of extract A to non-dried cells suspended in lactose (sample 4) results in an oxygen consumption 1.9 times that observed in the absence of extract (sample 10). We attribute these results to the presence of lactase in extract A.

DISCUSSION

The results reported here confirm previously reported findings that the difference in the two strains of *Escherichia coli-mutabile*

does not lie so much in lactase content as in lactase activity, the lactase of the white strain being inactive in uninjured cells. Several possible explanations of the presence of an inactive enzyme in a cell can be offered. One explanation is the presence of a specific enzyme inhibitor or antienzyme as postulated by Stewart (1926 and 1928). Our results do not contradict this hypothesis. In fact, our results could be explained easily if it could be shown that an antienzyme is, in reality, present. However, if this explanation is correct, the antienzyme is very labile for it is destroyed by antiseptics or by drying. We have considered trying to extract such a substance but, if it is present, its extreme lability has made the possibility of extracting it exceedingly remote.

Stewart believed that the variants were formed through the loss of this hypothetical antienzyme by growth on lactose. He assumed, but did not prove, the presence of lactase in the white form of *Escherichia coli-mutabile*. Lewis (1934) does not accept Stewart's view. Lewis believes he has demonstrated that the mutation of *Escherichia coli-mutabile* occurs continuously, irrespective of the medium.

A possible explanation which seems plausible to us is based on permeability of the bacterial cell. Is it not possible that the failure of the white strain to metabolize lactose is due to impermeability of the cell to lactose rather than to inactivity (antienzyme suppression) of its lactase? Search of the literature has revealed no answer to the question of the permeability of bacterial cell membranes to sugars. Lactase of this group of organisms is generally agreed to be intracellular. The methods which we have used for demonstration of lactase in *Escherichia coli-mutabile* undoubtedly injure the cells. Since uninjured cells of the white strain do not metabolize lactose, and since injury which is known to influence permeability renders them active towards lactose, it seems reasonable to suggest that the inability of the uninjured cells to metabolize lactose is due to their impermeability to lactose. We have demonstrated that significant amounts of lactase can be extracted from injured (dried) cells (table 3) but change in permeability of the cell to lactose may be of more consequence than change in permeability to lactase.

An hypothesis based upon permeability of the cells must explain the following facts:

1. The white strain contains lactase but is unable to ferment lactose. Impermeability of the cells to lactose would satisfactorily explain this fact.

2. Prolonged growth of the white strain in the presence of lactose results in the production of lactose-fermenting variants (reds). This transformation could be explained as a change from a cell impermeable to lactose to one permeable to it.

3. Treatment of cells of the white strain with antiseptics or simply drying them renders their lactase active. These treatments are injurious to the cell and cell injury is known to alter permeability. These facts fit well in the hypothesis.

4. Drying cells of the red strain increases their lactase activity as judged by their oxygen consumption in the presence of lactose. This fact can be reconciled with the permeability hypothesis on the assumption that the uninjured cells are difficultly permeable to lactose and that the drying process increases this permeability.

SUMMARY

Studies on the oxygen consumption of preparations of *Escherichia coli-mutabile* have demonstrated the following facts:

1. Lactase activity of preparations of the white strain does not depend on the presence of or preliminary treatment with an antiseptic. High vacuum drying suffices to increase the oxygen consumption in lactose to 3 to 6 times that observed with non-dried preparations.

2. Our previous demonstration of the necessity of the presence of lactose in the medium on which cells are grown in order to obtain high lactase activity is confirmed by the present study.

3. High-vacuum-dried preparations of the red strain harvested from lactose agar consume no oxygen when suspended in lactose. This behavior was shown to be due to inactivation of the oxidase system by the drying procedure.

4. Drying cells of the white strain which have been grown on glucose results in inactivation of their oxidase system. This phenomenon was observed with both the white and red strains

when they had been grown on fermentable carbohydrate and was not observed when they were grown in the absence of fermentable carbohydrate. The significance of this observation is not apparent.

5. Drying cells of the red strain which have been grown on plain agar yields a preparation which consumes more oxygen when suspended in lactose than does the corresponding non-dried preparation. In this case the increase in oxygen consumption is not as marked as with the white strain.

6. Extracts of dried cells of the white strain increase the oxygen consumption of non-dried cells suspended in lactose. A similar extract of non-dried cells does not have this effect.

An hypothesis of altered membrane permeability which attempts to explain the observed facts is presented.

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