CXXXIX. CELL STRUCTURE AND ENZYMIC ACTIVITY

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With the majority of enzymes, the activity is proportional to the concentration of the enzyme. In a few instances, dilution brings about a disproportionately rapid decrease in the activity of the enzyme solution or suspension and very soon the activity is completely lost. The respiration of the laked erythrocytes of the goose decreases rapidly on dilution [Warburg, 1911]. Krebs [1935] found that the *l*-amino-acid deaminase of kidney brei, unlike the *d*-amino-acid enzyme, is sensitive to dilution; a twofold dilution decreased the ammonia formation from *l*-aspartic acid in some experiments by as much as 95%. Similar results have been recorded with bacterial enzymes; both the glucose dehydrogenase [Yudkin, 1933] and the lactic dehydrogenase [Yudkin, 1937] of *Bact. coli* show a great diminution in activity when the bacterial suspension is diluted. These results were shown to be due to the "washing out" of a coenzyme of the dehydrogenases from the bacterial cells on dilution. The activity of the enzymes was restored by the addition of suspensions of heated bacteria, and the coenzymes of both reactions were shown to be probably identical with the cozymase of yeast fermentation [Yudkin, 1934; 1937].

Several enzymes have been described the activity of which depends on the integrity of the cell. Cytolysis results in a considerable and often complete loss in the activity of these enzymes. Now lysis of a cell is invariably accompanied by a dilution of its contents and it seems therefore likely that, in some instances at any rate, the results of lysis are due to dilution. Thus, the glyoxalase activity of human red blood cells was almost invariably lost in the cytolysis experiments of Jowett & Quastel [1933], whilst Warburg & Christian [1931], in whose experiments the resultant dilution was not so high, found quite an appreciable residual glyoxalase activity. Moreover, just as the results of dilution with the dehydrogenases of *Bact. coli* have been explained by the presence of a coenzyme, so Jowett & Quastel were able to restore the glyoxalase activity of the lysed erythrocytes by the addition of glutathione, the coenzyme of glyoxalase.

The behaviour of bacterial enzymes after cytolysis has been studied by Young [1929] and Penrose & Quastel [1930]. Working with several strains of *Bact. coli*, lysed by repeated freezing and thawing, Young showed that although the first two or three freezings had little effect on the enzymes, some dehydrogenases, including those of glucose and lactic acid, were destroyed, whilst others such as the formic and succinic dehydrogenases were unaffected. Penrose & Quastel used *Micrococcus lysodeikticus* which is readily lysed by saliva or egg-white. Amongst the effects of cytolysis were a complete destruction of glucose dehydrogenase, a 70% decrease in lactic dehydrogenase activity, no effect on glutamic dehydrogenase and peroxidase, and an increased activity of fumarase and urease.

The loss of enzymic activity in these bacterial cytolysis experiments has been attributed by the workers concerned to the enzyme being bound up with the structure of the cell wall, destruction of the latter involving destruction of the former. We have seen, however, that the loss of erythrocyte glyoxalase activity

on lysis is to be explained by the "washing out" of a coenzyme. It seemed likely, therefore, that the loss of bacterial dehydrogenase activity with cytolysis might similarly be explained, particularly in view of the fact that the enzymes most affected, glucose and lactic dehydrogenases, have been shown, in *Bact. coli* at any rate, to be associated with a coenzyme. The experiments here described were undertaken in an attempt to show whether the decreased activity of the bacterial dehydrogenases on lysis was due to the washing out of a coenzyme or to their being bound up with the structure of the cell in some unknown way.

EXPERIMENTAL

The organisms used were *Micrococcus lysodeikticus* (National Collection of Type Cultures) and a laboratory strain of *Bact. coli*. They were grown for 18–20 hr. in Roux bottles on an agar medium containing a tryptic digest of casein, washed twice by centrifuging and suspended in distilled water. The activity of the dehydrogenases was determined by the methylene blue technique as described in previous papers.

Experiments with Bact. coli

A washed suspension of *Bact. coli* was frozen in liquid air and thawed in cold running water, the freezing and thawing operations each occupying 2–3 min. The process was repeated a number of times and the activity of the suspension in respect of glucose and lactic dehydrogenases was determined and compared with that of an untreated portion of the same suspension. The results (Table I) show that the lactic enzyme is unaffected by this process, whereas the glucose dehydrogenase is inactivated by more than 50%. The difference between these results and those recorded by Young is probably due to differences in the organisms used. Table I further shows that the addition of coenzyme in the form of heated bacteria or a solution of cozymase did not restore the activity of the glucose enzyme.

Table I. *Effect of freezing and thawing on the dehydrogenases of Bact. coli*

	Reduction time, min.	
	Exp. 1 (frozen 6 times)	Exp. 2 (frozen 25 times)
Glucose dehydrogenase:		
Before freezing and thawing	6	5.5
After freezing and thawing	15.25	35
After freezing and thawing + heated <i>Bact. coli</i>	14	35.5
After freezing and thawing + cozymase	16	—
Lactic dehydrogenase:		
Before freezing and thawing	6	7
After freezing and thawing	6	7.5

An attempt was also made to destroy the action of the enzymes by grinding the bacteria in a bacterial mill. However, after grinding for 35 min., the activity of several dehydrogenases, including those of glucose and lactic acid, were unaffected.

Experiments with Micrococcus lysodeikticus

Preliminary experiments showed that an ordinary fairly active preparation of yeast cozymase and a heated suspension of *Bact. coli* accelerated the reduction of methylene blue by both intact and lysed *Micrococcus lysodeikticus* even in the absence of any substrate and were therefore unsuitable for testing for coenzyme activity. It appears that these preparations contain substances capable of acting as hydrogen donors in the presence of these organisms. It was found that a purer preparation of cozymase or a heated suspension of *M.*

lysodeikticus centrifuged four or five times instead of the usual twice, did not produce this effect, although both preparations were able to reactivate the lactic and glucose dehydrogenases of a diluted *Bact. coli* suspension. These preparations were therefore used in the following experiments.

(a) *Experiments with intact organisms.* If strict proportionality exists between enzymic activity and dilution, then the product of enzymic activity and dilution should be constant for all dilutions. Experiment showed that both the lactic and the glucose dehydrogenase of *Micrococcus lysodeikticus*, as those of *Bact. coli*, are inactivated by dilution; the product of activity (reciprocal of the reduction time) and dilution, which is given an arbitrary value of 100 for the undiluted suspension, falls off considerably (Table II). The activity is restored by the addition of a heated suspension of *Micrococcus lysodeikticus* (Table III).

Table II. *Effect of dilution on the dehydrogenases of intact Micrococcus lysodeikticus*

Concentration of suspension	Glucose dehydrogenase		Lactic dehydrogenase	
	Reduction time min.	Activity $\times$ dilution	Reduction time min.	Activity $\times$ dilution
1	5	100	5	100
1/2	15	67	12	83
1/3	30	53	25	60
1/4	74	27	—	—
1/5	>120	<20	82	31
1/8	—	—	>120	<30

Table III. *"Coenzyme" in dehydrogenations by intact Micrococcus lysodeikticus*

	Glucose dehydrogenase		Lactic dehydrogenase	
	Reduction time min.	Activity $\times$ dilution	Reduction time min.	Activity $\times$ dilution
<i>M. lysodeikticus</i> , undiluted	14	100	11.5	100
" diluted 1/3	120	36	85	41
" diluted 1/3 + heated <i>M. lysodeikticus</i>	38	110	32	107

Table IV. *Dehydrogenases in lysed Micrococcus lysodeikticus*

	Reduction time, min.		
	Exp. 1	Exp. 2	Exp. 3
Control (no substrate):			
Lysed bacteria alone	>120	>120	>120
Lysed bacteria + heated bacteria	>120	>120*	>120
Lysed bacteria + cozymase solution	>120	—	—
Glucose dehydrogenase:			
Unlysed bacteria	16	20	3
Lysed bacteria	>120	>120	110
Lysed bacteria + heated bacteria	>120	>120*	100
Lysed bacteria + cozymase solution	>120	—	—
Lactic dehydrogenase:			
Unlysed bacteria	12.5	17	2
Lysed bacteria	>120	>120	120
Lysed bacteria + heated bacteria	>120	>120*	120
Lysed bacteria + cozymase solution	>120	—	—

* Slightly more reduced after 120 min. than corresponding tubes containing no heated bacteria.

(b) *Experiments with lysed organisms.* Lysis of the bacteria was effected by the addition of an equal volume of 1 in 300 egg white to a washed suspension of the organism and allowing the mixture to stand in an incubator at 37° for 1 hr. [see Penrose & Quastel, 1930]. After this time the activity of the glucose and lactic dehydrogenases was determined in the lysed preparation and in the original suspension diluted with an equal volume of water. It was found that the activity of both enzymes was reduced considerably by lysis and that the activity was not restored by the addition of coenzyme in the form of either purified cozymase or a heated preparation of *M. lysodeikticus* (Table IV).

The slight acceleration in reduction in the tubes marked with an asterisk in Table IV is due to the presence of reducing substances in the preparation of heated bacteria used in Exp. 2, since it occurs also in the control tube.

DISCUSSION

The loss of activity of the enzyme on cytolysis may be due to the dilution which simultaneously occurs and depends in these instances on the fact that there is a coenzyme involved in the reaction. We may quote the more general words of Krebs [1935, p. 1643]: "The effect of dilution is explained by the assumption that a ternary collision, or a collision of a higher order, plays a role in these enzymic systems which become inactive on dilution. The probability of a ternary collision decreases in proportion to the dilution." An example of such a simple physicochemical explanation of the dependence of enzymic activity on the integrity of the cell membrane is the glyoxalase of human erythrocytes, the activity of which is lost on lysis and dilution and is regained on the addition of the coenzyme of the reaction—glutathione [Jowett & Quastel, 1933]. However, the results described in the present work have shown that this explanation does not cover all the facts and that there may also be enzymes which are in some way bound up with the structure of the cell so that rupture of the cell involves destruction of the enzyme.

SUMMARY

Cytolysis often results in the loss of activity of certain enzymes. Since cytolysis involves dilution, and dilution results in inactivation of those enzymes in which a coenzyme plays a part, it was thought possible that the loss of bacterial dehydrogenase activity on lysis might be due to dilution and the consequent "washing out" of the coenzyme. However, the decrease in activity of the glucose dehydrogenase of *Bact. coli* by freezing and thawing, and of the glucose and lactic dehydrogenases of *Micrococcus lysodeikticus* by lysis with egg white, are not restored by the addition of the coenzyme necessary for their action. In these instances the enzymes appear to be linked in some way with the structure of the cell.

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