CXCV. THE DEHYDROGENASES OF *BACTERIUM COLI*.

III. THE COENZYME OF GLUCOSE DEHYDROGENASE.

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THE presence of a coenzyme of glucose dehydrogenase has been demonstrated in *Bact. coli* [Yudkin, 1933]. It was shown that the reduction of methylene blue by a dilute suspension of bacteria in the presence of glucose is much more rapid if a heated preparation of the bacteria is added. The properties and possible identity of the coenzyme are discussed in the present paper. The bacteria were prepared as described in the previous paper [Yudkin, 1933]. The preparation was usually diluted so as to give a reduction time of more than 30 minutes when no coenzyme was added. As standard coenzyme preparation, a suspension of bacteria about twice as strong was heated to 100° for 10–15 minutes. The coenzyme activity of a preparation was tested by comparing the acceleration produced in methylene blue reduction with that produced by a standard coenzyme preparation.

1. *Some properties of the coenzyme.*

The coenzyme is very stable to heat, acid and alkali. Bacteria heated for 6½ hours at 100° showed no loss in coenzyme activity. After heating for another half hour to 120° in an autoclave, it was still active.

The following treatments with acid or alkali did not completely destroy coenzyme activity:

1. The preparation after being heated at 100° for 6½ hours and then autoclaved for half an hour was made *N*/500 with H₂SO₄ or NaOH, heated to 100° for 10 minutes, cooled, neutralised and tested for activity.

2. A fresh preparation was made *N*/50 with H₂SO₄ or NaOH, heated in the autoclave to 120° for 20 minutes, left in the acid or alkali for 3 days, neutralised and tested.

3. A fresh preparation was made *N*/20 with H₂SO₄ or NaOH, heated to 100° for 5 minutes, cooled, neutralised and tested.

4. A fresh preparation was made *N*/2 with H₂SO₄ or NaOH, heated to 100° for 10 minutes, cooled, neutralised and tested.

In the experiments just described, fairly strong suspensions of bacteria were used, more than was necessary to give maximum activation. A partial destruction of the coenzyme would therefore not necessarily be shown. In order to demonstrate partial destruction, a standard preparation of coenzyme was made by heating bacteria to 100° for half an hour in water and diluting so as to give sub-maximum activation. Portions of the same preparation, after treatment with acid or alkali, were diluted to a known extent, and the activity was compared with that of the standard preparation. By this means it was shown that

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treatment with $N H_2SO_4$ or NaOH at 100° for 40 minutes or at 120° for 20 minutes results in more than half the coenzyme being destroyed (Table XIII, p. 1469).

2. *Extraction of the coenzyme from Bact. coli.*

Before attempting to extract the coenzyme, some bacteria were ashed in order to see if the factor was purely inorganic, such as pyrophosphate or magnesium ions. It was found that bacterial ash caused an inhibition of the activity of the glucose dehydrogenase.

The coenzyme cannot be filtered through a bacterial filter. Two types of filter were used—a Seitz filter, made of nickel, and a metal-free porcelain filter, but the filtrates through both always caused an inhibition.

On the other hand, part of the coenzyme remains in the supernatant fluid after centrifuging down the bacteria. Both the clear supernatant fluid and the bacteria activate the glucose dehydrogenase. Repeated centrifuging with fresh quantities of distilled water gradually washes the coenzyme out of the bacteria (Table I).

Table I. *Washing out of coenzyme by water.*

Bacteria heated to 100° for 10 mins.		Reduction time, mins.
Bacteria alone		67
Bacteria <i>plus</i> 1st centrifugate		34
„ „ 4th centrifugate		70
„ „ suspension after 5th centrifuging		30
„ „ suspension after 8th centrifuging		38

Heating the bacteria for a longer time gives an increased amount of coenzyme in the centrifugate (Table II).

Table II. *Extraction of coenzyme by heating.*

		Reduction time, mins.
Bacteria alone		95
Bacteria <i>plus</i> standard coenzyme preparation		32
„ „ centrifugate after heating 5 mins.		45
„ „ centrifugate after heating 3 hours		30

The centrifugate from fresh bacteria, which had been standing a short while at room temperature, had a small coenzymic activity (Table III).

Table III. *Washing out of coenzyme from fresh bacteria.*

		Reduction time, mins.
Bacteria alone		31
Bacteria <i>plus</i> standard coenzyme preparation		16
„ „ centrifugate from fresh bacteria		25

The coenzyme is removed from the centrifugate by filtration through a bacterial filter. Drying the centrifugate at 100° does not destroy its activity. Ashing it, however, results in its complete destruction—this ash, as that of the whole bacteria, gives an inhibition.

The coenzyme may be dialysed out of the preparations through a collodion membrane. The dialysis proceeds fairly slowly. After 3 days of dialysis against a large volume of distilled water changed every 12 hours, there is still a slight activity left in the bacterial preparation.

3. *Some properties of glucose dehydrogenase in relation to the coenzyme.*

It was thought that the well-known inhibitory effect of toluene on the enzyme might be due to a destruction of the coenzyme. That this is not so is shown by the fact that bacteria treated with toluene can act as a coenzyme preparation (Table IV).

Table IV. *Fresh bacteria treated with toluene 3 minutes.
Excess toluene removed by passing air.*

	Reduction time, mins.
Bacteria alone	31
Bacteria <i>plus</i> toluene-treated bacteria	16
Toluene-treated bacteria alone	∞

It has often been observed that the glucose dehydrogenase of *Bact. coli* loses its activity more rapidly when left in dilute suspension than in more concentrated suspension. A possible explanation of this is that the coenzyme is removed more completely in the dilute suspension. This was tested as follows. Part of a concentrated suspension was diluted 1 in 50 and left together with some of the undiluted suspension for 2 days in the refrigerator. After this time, the concentrated suspension was also diluted 1 in 50, and both were tested for glucose dehydrogenase activity, with and without coenzyme. The coenzyme preparation was sufficient to give maximum acceleration. It is seen (Table V) that the loss of activity is not completely restored by addition of coenzyme and must therefore be due to an inactivation of the enzyme itself.

	Reduction time, mins.
Bacteria freshly diluted	95
" <i>plus</i> coenzyme	21
Bacteria diluted 48 hours previously	150
" " <i>plus</i> coenzyme	34

4. *Replacement of the coenzyme by other factors.*

The adenylic acids, alone or in combination with pyrophosphate, have been shown to be the coenzymes of several reactions in which glucose is involved, such as the fermentation of yeast [Euler and co-workers: see Myrbäck, 1933] muscle glycolysis (Meyerhof and co-workers) and the dehydrogenation of glucose by the enzyme from liver (Mann, private communication; also Andersson [1934]). The dehydrogenation of lactic acid by an enzyme from heart-muscle involves a coenzyme, which is supposed to be identical with yeast cozymase [Banga and Szent-Györgyi, 1933; Andersson, 1934]. Yeast cozymase is also able to activate several dehydrogenations by enzymes from yeast and cucumber seed [Andersson, 1933]. The fermentations induced by lactic acid and propionic acid bacteria involve a coenzyme, which is probably also cozymase [Virtanen, 1925, 1, 2].

A number of coenzyme preparations from different sources have been tried in an attempt to replace the coenzyme of *Bact. coli*.

Preparations of the lactic coenzyme prepared from heart-muscle were unsuitable owing to the fact that they contained hydrogen donators which caused a rapid reduction of the methylene blue by *Bact. coli* even in the absence of glucose. A preparation of lactic acid coenzyme, made from yeast, caused an inhibition of the bacterial glucose dehydrogenase.

Muscle "Kochsaft" could not be used, since this too contained hydrogen donators. The coenzyme of lactic acid formation in muscle, adenylypyrophos-

phate, was prepared, and although active with muscle, was found also to cause an inhibition with bacteria.

A preparation of muscle-adenylic acid, however, is able to replace the glucose dehydrogenase coenzyme of *Bact. coli*, but it is far less efficient than heated bacteria. As much as 5 mg. are needed in each Thunberg tube in order to give an activation approaching that of a coenzyme preparation containing 0.3 mg. dry weight of bacteria (Table VI).

Table VI. *Muscle-adenylic acid as coenzyme.*

	Reduction time, mins.
Bacteria alone	42
Bacteria <i>plus</i> 0.5 mg. adenylic acid	40
" " 2.5 mg. adenylic acid	35
" " 5 mg. adenylic acid	22
" " coenzyme preparation (0.3 mg. dry weight)	18

Adenylic acid itself acts as a feeble hydrogen donator in the presence of methylene blue.

Since magnesium as well as the adenylic acid cozymase is necessary in yeast fermentation [Lohmann, 1931, 1] the effect of magnesium was tried with bacteria, too. It was found that 1 mg. magnesium gives an inhibition both with and without adenylic acid.

This partial replacement of the coenzyme by muscle-adenylic acid recalls the partial replacement of yeast cozymase by muscle-adenylic acid and *vice versa* [see Lohmann, 1931, 2, 3]. It suggests the possibility that the coenzyme is also related in constitution to adenylic acid. This observation is supported by the observation that the reduction time when both adenylic acid and coenzyme are present is intermediate between the reduction times with either alone (Table VII).

Table VII. *Effect of adenylic acid and coenzyme together.*

	Reduction time, mins.
Bacteria alone	200
Bacteria <i>plus</i> coenzyme	36
" " adenylic acid (5 mg.)	120
" " adenylic acid and coenzyme	48

This suggests the adsorption of both types of adenylic acid molecules at the enzyme, the adsorption of the muscle-adenylic acid hindering the adsorption of the more efficient coenzyme molecules.

A preparation of yeast cozymase (ACo between 10,000 and 20,000) was next tried and was found to replace the coenzyme completely in a concentration of less than 0.1 mg. in each tube. It seems therefore probable that the coenzyme of *Bact. coli* is identical with that of alcoholic fermentation by yeast. The experiments to be described are consistent with this assumption.

It was suggested (Mann, private communication) that the coenzyme of liver glucose dehydrogenase is identical with cozymase. This is supported by the experiments of Andersson [1934]. A preparation of the liver coenzyme [Harrison, 1933] was found to replace the coenzyme of *Bact. coli* (Table VIII).

Table VIII. *Coenzyme of liver glucose dehydrogenase as bacterial coenzyme.*

40 mg. of the preparation of liver coenzyme were used in each tube.

	Reduction time, mins.
Bacteria alone	46
Bacteria <i>plus</i> coenzyme (heated bacteria)	18
" " liver coenzyme	18

Not only can the liver coenzyme replace the bacterial coenzyme in the glucose dehydrogenation by bacteria, but the bacterial coenzyme can replace the former in liver enzyme preparations. That is, heated bacteria accelerate the reduction by liver glucose dehydrogenase to the same extent as the liver coenzyme itself (Table IX).

Table IX. *Bacterial coenzyme as liver coenzyme.*

47 mg. liver enzyme preparation in each tube.

	Reduction time, mins.
Liver enzyme alone	22½
Liver enzyme <i>plus</i> liver coenzyme	8½
„ „ coenzyme (heated bacteria)	8

This lends strong support to the view that the glucose dehydrogenase of *Bact. coli* and of liver are the same and are both identical with yeast cozymase.

The system which oxidises hexosemonophosphate [Warburg and Christian, 1931] has been shown to involve a coenzyme, which more recently was found to consist of more than one factor [Warburg and Christian, 1933]. The crude coenzyme preparation from horse-blood is able to replace the bacterial coenzyme (Table X). It will be seen that the preparation of Warburg's coenzyme itself contains substances capable of acting slightly as hydrogen donators to methylene blue in the presence of *Bact. coli*. This accounts for the time of reduction being less with the Warburg coenzyme (16 minutes) than with the standard coenzyme preparation.

Table X. *Warburg's coenzyme as bacterial coenzyme.*

50 mg. crude coenzyme in each tube.

	Reduction time, mins.
Bacteria alone	46
Bacteria <i>plus</i> coenzyme (heated bacteria)	18
„ „ Warburg's coenzyme	16
„ „ Warburg's coenzyme (without glucose)	100
Bacteria alone (without glucose)	>120

The interchanging of the coenzymes was further studied, and it was shown that although Warburg's coenzyme can replace the liver coenzyme, the latter cannot replace the former. Liver enzyme with Warburg's coenzyme gave a reduction time of 7 minutes (compare Table IX). These results suggest that the complex which we have described as Warburg's coenzyme contains cozymase and, in addition, other essential factors which are not present in the liver coenzyme preparations.

5. Presence of cozymase in preparations of the coenzyme.

It has been shown that cozymase is able to replace the coenzyme in *Bact. coli*. The converse is also true. Heated suspensions of the bacteria are able to reactivate washed yeast (apozymase).

The fermentations were measured in the Barcroft manometer at 30°. A *M*/10 solution of sodium hexosediphosphate was made in *M*/20 phosphate buffer, *p*_H 6.5, containing 10 % glucose. To 0.2 g. of apozymase in the left-hand cup were added 1 ml. of this hexosediphosphate solution and 1 ml. of water or the solution to be tested for cozymase activity. The right-hand cup contained 2 ml. of water. In these conditions, the apozymase preparation with no cozymase added showed no measurable output of CO₂ in the course of the experiments (about 1 hour).

In order to obtain thick suspensions of bacteria, they were grown on tryptic caseinogen broth containing 0.5 % glucose and aerated during growth. The washed suspensions were heated for 15 minutes before determining the cozymase activity. Three other species of bacteria which had been found to contain the coenzyme of the glucose dehydrogenase of *Bact. coli* (see below) were also tested, as well as a preparation of dried baker's yeast and the cozymase preparation used in the experiments described previously. The results are expressed in ml. of CO₂ per hour per g. dry weight of preparation (Table XI). The dry weight of the bacteria was calculated after determination of the nitrogen content of the suspensions [Yudkin, 1932].

The cozymase activities of the preparation are thus expressed in the same form as by Euler and Myrback [see Myrback, 1933]. The conditions used in these experiments were, except for the use of the Barcroft manometers, the same as those used by these authors. The values, however, are about one-tenth those expected. For example, dried yeast should have ACo about 80-90. But in spite of this, the relative concentrations of cozymase in the different preparation can be seen from the values given.

Table XI.

Preparation	Dry weight g.	CO ₂ ml. per hour	CO ₂ ml. per hour per g.
<i>Bact. coli</i>	0.033	0.875	26.5
<i>Bact. lactis aerogenes</i> (strain 124)	0.071	2.34	33
<i>Bact. alkaligenes</i>	0.051	1.70	33
<i>Bact. dispar</i>	0.035	1.90	54
Dried yeast	0.200	1.62	8
Cozymase	0.001	1.16	1160

Cozymase is known to occur in other bacteria, *e.g.* in *Streptococcus lactis* [Myrback and Euler, 1924, 1] and in *B. casei* and propionic acid bacteria [Virtanen, 1925, 1, 2]. Myrback and Euler failed to find any cozymase in *Bact. lactis aerogenes* and they deduce from this that the initial splitting of the glucose molecule is different from that produced by *Streptococcus lactis*, or by baker's or Torula yeasts. Such deductions are somewhat hazardous. For example, *Bact. alkaligenes*, although it possesses no glucose dehydrogenase [Quastel and Wooldridge, 1925] and does not ferment glucose at all, is active both as glucose dehydrogenase coenzyme (see below) and cozymase. The negative results of Myrback and Euler may be due to their having used a different strain of the organism, but may also be due to the fact that these authors used a preparation of bacteria dried in air and not a heated suspension. The cells of *Bact. lactis aerogenes* are surrounded by a thick capsule which is probably not destroyed by drying but is by heating, thus liberating the cozymase.

Relation between cozymase and coenzyme activities. It has been shown that the coenzyme is fairly stable to heating in acid or alkali. Yeast cozymase appears to be less stable, particularly when purified [Myrback and Euler, 1924, 2], and it might have been expected that it would be completely destroyed in some of the experiments described above. However, in all cases where coenzyme activity persists after treatment, cozymase activity can also be demonstrated, although often greatly diminished (Table XII).

It will be seen that the treatments in Exp. 3 decrease the activity to 1/4 or less. Estimation of the glucose dehydrogenase coenzyme in the same suspensions shows that at least more than half the activity is lost. A was diluted so as to give sub-maximum activation (Table XIII).

Table XII. *Cozymase activity of Bact. coli after treatment with acid or alkali.*

		ml. CO ₂ per hour
Exp. 1.	Heated bacteria	650
	After 10 mins. in <i>N</i> /30 H ₂ SO ₄ at 100°	425
	" 10 " <i>N</i> /30 NaOH ,,	135
	" 20 " <i>N</i> /30 H ₂ SO ₄ at 120°	170
	" 20 " <i>N</i> /30 NaOH ,,	150
Exp. 2.	Heated bacteria	580
	After 20 mins. in <i>N</i> /2 HCl at 120°	84
	" 20 " <i>N</i> /2 NaOH ,,	125
Exp. 3.	A. Heated bacteria	310
	B. After 40 mins. in <i>N</i> H ₂ SO ₄ at 100°	72
	C. " 40 " <i>N</i> NaOH ,,	70
	D. " 20 " <i>N</i> H ₂ SO ₄ ,,	75
	E. " 20 " <i>N</i> NaOH ,,	56

Table XIII. *Activities of coenzyme after treatment with acid or alkali (see Table XII).*

	Reduction time, mins.
Bacteria alone	>180
Bacteria <i>plus</i> A (1 in 300)	60
" " B (1 in 150)	100
" " C (1 in 150)	120
" " D (1 in 150)	120
" " E (1 in 150)	140

The coenzyme activity of B is rather greater than that of the others although its cozymase activity is the same as those of C and D. But these small differences, which have been observed in several other cases, may be due to the formation during the treatment of substances which inhibit either the dehydrogenase or the yeast fermentation. Inhibition of the dehydrogenase for example was observed in certain cases after alkali treatment, where even in fairly high concentrations, the preparation never gave more than a certain sub-maximum activation.

From these experiments, we are justified in concluding that the destruction of the cozymase activity of bacterial suspensions is more or less parallel with the destruction of the coenzyme.

6. *Presence of the glucose dehydrogenase coenzyme of Bact. coli in other bacteria.*

Three other species of bacteria have been tested for the presence of the coenzyme: *Bact. dispar*, *Bact. lactis aerogenes* and *Bact. alkaligenes*. They were grown in tryptic caseinogen broth, and preparations for the testing of coenzyme activity were made by heating washed suspensions, as with *Bact. coli*. All three species contain the coenzyme (Table XIV).

Table XIV. *Coenzyme of Bact. coli in other bacteria.*

		Reduction time, mins.
Exp. 1.	<i>Bact. coli</i> alone	100
	<i>Bact. coli</i> + heated <i>Bact. coli</i>	26
	„ + heated <i>Bact. alkaligenes</i>	26.5
Exp. 2.	<i>Bact. coli</i> alone	>180
	<i>Bact. coli</i> + heated <i>Bact. coli</i>	36
	„ + heated <i>Bact. alkaligenes</i>	34
Exp. 3.	<i>Bact. coli</i> alone	50
	<i>Bact. coli</i> + heated <i>Bact. coli</i>	27
	„ + heated <i>Bact. dispar</i>	28

7. *Coenzyme of glucose dehydrogenase of other bacteria.*

The abnormal falling off in activity of the glucose dehydrogenase on dilution, which suggested the presence of the coenzyme in *Bact. coli* [Yudkin, 1933], is also observed with *Bact. lactis aerogenes* and *Bact. dispar*.

An activation occurs with *Bact. dispar* on adding, to a dilute suspension of the bacteria, heated preparations of any of the four bacteria mentioned, or by cozymase (Table XV).

Table XV. *Glucose dehydrogenase coenzyme of Bact. dispar.*

	Reduction time, mins.
<i>Bact. dispar</i> undiluted	22
" diluted 1 in 2	80
" + heated <i>Bact. coli</i>	33
" + heated <i>Bact. lactis aerogenes</i>	33
" + heated <i>Bact. dispar</i>	31.5
" + 1 mg. cozymase	31
" + 0.5 mg. cozymase	45

Bact. dispar requires more cozymase to reactivate its glucose dehydrogenase than either *Bact. coli* or *Bact. lactis aerogenes* (Table XVI). It is interesting to compare this with the relatively larger amount of cozymase present in the former (Table XI).

In the case of *Bact. lactis aerogenes*, activation does not occur on the addition of heated bacteria but only on the addition of cozymase. This is caused by inhibiting substances present in the heated preparations. That this effect is due to the glucose dehydrogenase of *Bact. lactis aerogenes* which is sensitive to these substances and not to differences in the nature of the heated bacteria is shown by the fact that heated *Bact. coli* also inhibit the enzyme of *Bact. lactis aerogenes* (Table XVI). On the other hand, heated *Bact. lactis aerogenes* do not cause an inhibition of the enzyme of *Bact. coli* (Table XIV) or of *Bact. dispar* (Table XV). Moreover, the activation of the enzyme of *Bact. lactis aerogenes* by cozymase is nullified by the presence of heated bacteria. A decrease in the concentration of the heated bacteria, while decreasing the amount of coenzyme, also decreases the concentration of the inhibitory substances, and a greater activity is observed.

Table XVI. *Glucose dehydrogenase coenzyme of Bact. lactis aerogenes.*

	Reduction time, mins.
Exp. 1. <i>Bact. lactis aerogenes</i> undiluted	14
" diluted 1 in 2	70
Exp. 2. <i>Bact. lactis aerogenes</i> alone	16
" + boiled <i>Bact. lactis aerogenes</i>	70
" + boiled <i>Bact. lactis aerogenes</i> (1 in 2)	55
" + boiled <i>Bact. lactis aerogenes</i> (1 in 3)	40
" + boiled <i>Bact. coli</i>	71
" + boiled <i>Bact. coli</i> (1 in 2)	49
" + cozymase (0.15 mg.)	12
Exp. 3. <i>Bact. lactis aerogenes</i> alone	32
" + boiled <i>Bact. lactis aerogenes</i>	50
" + cozymase (0.2 mg.)	21
" + boiled <i>Bact. lactis aerogenes</i> + cozymase (0.2 mg.)	43

8. *Participation of the coenzyme in other reactions with glucose.*

(a) *The oxidation of glucose by oxygen.* The oxygen uptakes of the bacteria with and without coenzyme were measured in the Warburg manometer at 37°.

Five manometers were used; each contained 1 ml. of bacterial suspension, 1 ml. of *M*/20 phosphate buffer, p_H 7.0, and in addition the following:

Manometer	Glucose	Coenzyme	Cozymase (1 mg.)	Water
1	1	0	0	2
2	0	0	0	3
3	1	1	0	1
4	0	1	0	2
5	1	0	1	1

The results are shown in Table XVII together with the reduction times of methylene blue by the same mixtures.

Table XVII.

Manometer containing	Exp. 1		Exp. 2		Exp. 3
	O ₂ uptakes ml./hour	Reduction time mins.	O ₂ uptakes ml./hour	Reduction time mins.	O ₂ uptakes ml./hour
1. Bacteria + glucose	6	45	11.5	33	7
2. Bacteria alone	3	∞	6.5	∞	—
3. Bacteria, glucose and coenzyme	39	22	28.5	23	18
4. Bacteria and coenzyme	11.5	∞	11.5	∞	—
5. Bacteria, glucose and cozymase	—	—	—	—	17

The oxygen uptakes are seen to be greatly increased by the presence of coenzyme. The relative acceleration is much greater than for the reduction of methylene blue; *e.g.* in Exp. 1, the oxygen uptake is accelerated more than six times by the coenzyme, whereas the reduction time is only halved. It will be seen that there is a comparatively large uptake with bacteria and coenzyme without glucose (4) although there was no reduction at all of the methylene blue after 5 hours. This difference in behaviour between the oxidations by methylene blue and oxygen is not yet explained.

As with the methylene blue experiments, the coenzyme may be replaced by cozymase.

(b) *The liberation of hydrogen from glucose.* In order to obtain a suspension of bacteria containing the enzyme glucose hydrogenlyase capable of performing this reaction, *Bact. coli* was grown on a glucose broth medium in Roux bottles and the activity of the enzyme measured in the Barcroft manometer [Yudkin, 1932].

The action of glucose hydrogenlyase also involves a coenzyme which is present in heated bacteria and is replaceable by cozymase (Table XVIII).

Table XVIII. Coenzyme of glucose hydrogenlyase.

	H ₂ , μ l. per hour
Bacteria alone	0
„ + heated bacteria	25
„ + 1 mg. cozymase	15

The demonstration of the coenzyme with this enzyme is not so satisfactory as with the dehydrogenase, since when the bacteria have been diluted sufficiently to wash out the coenzyme, the activity even in the presence of coenzyme is very low.

DISCUSSION.

The results described above are all in agreement with the hypothesis that the glucose dehydrogenase coenzyme of *Bact. coli* is identical with cozymase. Moreover, they support the view of Andersson that the coenzyme of liver glucose dehydrogenase is also cozymase.

It has been stated that the liver coenzyme is partially activated by adenylypyrophosphate from muscle [Harrison, 1933], whereas we have seen that the enzyme of *Bact. coli* is inhibited by this. This does not necessarily imply that the coenzymes cannot be the same. If the enzymes are different, one can imagine that both may be activated by the same substance which occurs naturally with them, but that a second substance might produce an activation with one and an inhibition with the other. The glucose dehydrogenases of liver and *Bact. coli* are certainly very different. For example, the affinity of the former for glucose is unusually low, the optimum concentration being 0.25 *M* [Harrison, 1931], whereas the affinity of the bacterial enzyme is so high that it cannot be measured.

SUMMARY.

1. It is suggested that the coenzyme involved in the dehydrogenation of glucose by *Bact. coli* is identical with the cozymase of yeast. The evidence for this is:

(a) Bacterial coenzyme may be replaced by very small concentrations of cozymase.

(b) Liver coenzyme, which is itself replaceable by cozymase, can replace the bacterial coenzyme. This supports the view of Andersson that the liver coenzyme is also identical with cozymase.

(c) The bacterial coenzyme can replace the liver coenzyme.

(d) Warburg's coenzyme, which can replace the liver coenzyme, can also replace the bacterial coenzyme.

(e) Preparations which contain the coenzyme also contain cozymase, and the destruction of one involves the destruction of the other.

2. Warburg's coenzyme appears to contain cozymase as one of its components.

3. The coenzyme of the glucose dehydrogenase is also contained in three other bacteria—*Bact. alkaligenes*, *Bact. dispar* and *Bact. lactis aerogenes*. The latter two species also need a coenzyme for the dehydrogenation of glucose, which is replaceable by cozymase.

4. All four species of bacteria examined contain cozymase.

5. A coenzyme, found in heated bacteria, and replaceable by cozymase, is involved in two other reactions in which glucose is attacked by *Bact. coli*. These are the oxidation of glucose by oxygen, and the production of hydrogen from glucose by the enzyme glucose hydrogenylase.

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