STUDIES ON OXIDATION AND REDUCTION BY PNEUMOCOCCUS.

VIII. Nature of the Oxidation-Reduction Systems in Sterile Pneumococcus Extracts.

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(Received for publication, December 4, 1924.)

INTRODUCTION.

Preceding papers (1–7) have described a number of different oxidation-reduction processes which are exhibited by sterile extracts of pneumococci: the production of peroxide, the consumption of molecular oxygen, the reduction of methylene blue, the oxidation of hemotoxin, and the oxidation of hemoglobin with formation of methemoglobin, as well as the oxidation of various endocellular hydrolyzing enzymes.

From the nature of these reactions it is evident that the sterile extracts contain active oxidation-reduction systems. These active systems consist of two components: (1) a thermolabile constituent of the pneumococcus cell which is not removed by washing; (2) thermostable substances which are lacking in washed cells and which are not necessarily of pneumococcus origin, since they may be supplied by muscle infusion and yeast extract.

Two types of sterile pneumococcus extracts have been prepared: (1) broth extracts of unwashed cells, (2) phosphate solution extracts of washed cells. The first type of extract contains both "complete" and active systems for peroxide production, oxygen consumption, hemoglobin oxidation, and reduction of methylene blue and certain other substances. The second type of extract contains only "incomplete" systems which initiate the oxidation-reduction processes only upon the "completion" of the systems by the addition of meat infusion, yeast extract, or certain other substances. The essential difference, then, between the "complete" systems in broth extracts of unwashed cells and the "incomplete" systems in saline extracts of washed cells is the presence or absence of the thermostable constituent; *i.e.*, of autoxidizable or easily oxidized substances. The

distinctions between the two types of pneumococcus extract are graphically presented in Table I.

The activities of the extract which have been reported previously may be grouped under two headings, oxidations and reductions. All of the oxidations reported are examples of "oxygen activation" and represent oxidations of substances themselves not reactive with molecular oxygen. In the absence of oxygen these same sterile extracts manifest strong reducing powers—a characteristic common to all living cells. Thus, the sterile cell extracts exhibit two gen-

TABLE I.

Oxidation-Reduction Systems of Sterile Extracts of Pneumococcus.

Sterile extract of.	Oxidation-reduction systems.	Components of oxidation-reduction systems.		Activity.						
				Oxidation. "Oxygen activation."				*Reduction.*		
		Thermolabile substances (*C**).	Thermostable substances (*RH*†).	Peroxide formation.	Hemoglobin oxidation.	Oxidation of hemotoxin.	Oxidation of enzymes.	Consumption of molecular oxygen.	Reduction of methylene blue.	Reduction of methemoglobin.
Unwashed pneumococcus cells.	"Complete."	+	+	+				+		
Washed pneumococcus cells.	"Incomplete."	+	−	−				−		

* *C* = thermolabile substance of bacterial origin; not removed by washing cells.

† *RH* = thermostable substance removed by washing bacteria; present in heated extracts of yeast, of animal and plant tissues.

eral functions: in the presence of molecular oxygen, the formation of oxidizing agents, or "oxygen activation;" in the absence of molecular oxygen, the establishment of reducing conditions which are manifested in the reduction of such substances as methylene blue and methemoglobin. Moreover, not only are the two types of reactions induced by the same extract, but the active systems themselves which in the presence of molecular oxygen form the oxidizing agents are apparently identical or at least closely related to the systems which establish the reducing conditions in the absence of oxygen (2, 4).[1] These relations may be

[1] In an earlier paper (2) it was suggested that the essential difference between peroxide production and methylene blue reduction consisted in the nature of the hydrogen acceptor or oxygen donator. However, it now seems preferable of interpret the reduction activities of the extract upon the basis of the conception outlined by Clark (8), rather than upon the basis of oxygen or hydrogen transport.

presented schematically as follows, using the symbols *C* to represent the thermolabile cellular constituent and *RH*, the thermostable constituent of the active systems.

1. Pneumococcus extracts in the presence of molecular oxygen.

   $C + RH + O_2 \rightarrow$ oxidizing agents.
   Oxidizing agents + Hb → MetHb.
   " " + hemotoxin or sucrase → inactive hemotoxin or inactive sucrase.

2. Pneumococcus extracts in the absence of molecular oxygen.

   $C + RH \rightarrow$ reducing conditions under which methylene blue and methemoglobin are reduced.

If the active system be considered from this view-point, the inactivation of the extract by exposure to air might be assigned to any one of several different causes. If the thermostable constituents represented by *RH* were irreversibly oxidized, the inactivation of the system would be a natural sequence of the oxidation of the extract. However, the oxidation of the extract may not result in the exhaustion of *RH* substances, either because of the presence of an excess of these substances or because they represent, at least in part, molecules which (in the presence of *C*) may be alternately oxidized and reduced. If this is the case, the inactivation of the extract would seem to be due to the destruction or inactivation of the thermolabile cellular substance which has been designated *C*. It has been shown in preceding papers that other labile substances such as the intracellular enzymes and the endohemotoxin which are contained in these same extracts, although themselves not directly involved in the oxidation processes, are in turn destroyed by the oxidizing agents which are formed when the "complete system" type of pneumococcus extract is exposed to air. Hence the thermolabile cellular component of the oxidation system, although not actually oxidized in the initial reaction, may be inactivated by the oxidizing agents which accumulate in the extract by reactions analogous to those which under similar conditions inactivate sucrase and hemotoxin.

As indicated above, the inactivation of the described oxidation-reduction systems of the sterile pneumococcus extracts may be due to the destrcution or exhaustion of either of the two proposed constituents of these systems. The following experiments are designed to determine which of these constituents is inactivated during the oxidation of pneumococcus extracts. The experiments consist in large part of attempts to "reactivate" extracts which have been inactivated through oxidation, by adding to them fresh meat infusion (*RH*) or unoxidized washed cell extract (*C*). In addition, the relative ease

of oxidation of the cellular component of the oxidation systems is compared with the susceptibility to oxidation of other labile substances of pneumococcus origin, such as the hemotoxin and the hydrolyzing enzymes contained in these extracts. The investigation has been confined to the methylene blue-reducing, and hemoglobin-oxidizing systems, as type examples of the reducing and oxidizing activities of the sterile extract.

EXPERIMENTAL.

Methods.

The sterile pneumococcus extracts used in this investigation have been prepared in exactly the same manner described in a previous paper (7). Both types of extract have been used. To emphasize the fact that only the broth extract of unwashed cells contains the "complete" oxidation-reduction systems, this type of extract is frequently referred to in the text as the "complete system" type of extract. For a similar reason, the phosphate solution extract of washed cells is termed "incomplete system" type of extract. As in previous papers, the term "reduced" extract applies to extracts which have been protected from oxidation; extracts which have been exposed to air are termed "oxidized" extracts. The sterility of all preparations has been carefully controlled.

The procedure employed in tests of methylene blue reduction, hemoglobin destruction, hemolysis, as well as the tests of activity of the hydrolyzing enzymes, was the same as that previously described (3–6). The meat infusion used in this study is also described in a former paper (2).

Inactivation of Pneumococcus Oxidation-Reduction Systems in Unwashed Cell Extracts Exposed to the Air.

Sterile broth extracts of unwashed pneumococci contain the "complete" oxidation-reduction systems. When this type of extract is exposed to the air, marked oxidative reactions occur, such as peroxide production, hemoglobin oxidation, and oxygen consumption. One of the results of these oxidation processes is the complete inactivation of the systems responsible for the oxidation-reduction reactions. If stored in the reduced state protected from molecular oxygen, these extracts retain their activity for many months even at room temperature. These facts may be recalled from previous reports, which it is unnecessary to review in detail.

Attempts to "Activate" Extracts Which Have Been Rendered Inactive by Exposure to Air.

1. Failure of Activation of Oxidized Extracts by the Addition of the Thermostable Component (RH).

If the inactivation of extracts which have been exposed to air is due to the irreversible oxidation of the thermostable component (*RH*) (*i.e.* to the exhaustion of easily oxidized molecules), the addition of meat infusion to the oxidized extract should result in its activation in

TABLE II.

Effect of the Addition of the Thermostable Component to Pneumococcus Extracts Which Have Been Inactivated by Exposure to Air.

Broth extract of unwashed cells which had been inactivated by exposure to air.		Reduced broth extracts of unwashed cells (thermostable component).		Meat infusion (thermostable component).	PO_4 solution.	Methylene blue (1:2,000) or HbO_2 (2.0 mM).	Methylene blue reduction.	Hemoglobin oxidation (formation of methemoglobin).	
Unheated.	Heated.	Unheated.	Heated.					5 min.	8 hrs.
cc.	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*			
		0.4			0.4	0.2	Complete, 2 hrs., 30 min.	+	+
0.8						0.2	Partial, 3 days.	—	—
0.4			0.4			0.2	" 3 "	—	—
0.4				0.4		0.2	" 3 "	—	—
	0.4		0.4			0.2	" 3 "	—	—
	0.4		0.4	0.4		0.2	" 3 "	—	—

the same manner as does the addition of meat infusion to the washed cell extracts (2, 3, 5).

Similarly, the addition of an extract of unwashed cells in which the thermolabile component has been destroyed by heat should have the same effect as the addition of meat infusion, since both contain only the thermostable substances. If it is the thermostable substances which are destroyed as a result of the oxidation, the addition of fresh substances of a similar nature should restore the activity of the oxidized extract. A number of experiments have been made to test these relations; a typical example is given below.

Broth extract of unwashed cells ("complete system" type of extract) was exposed to air for 18 hours at 37°C. The extract upon aeration formed peroxide, but after 18 hours the peroxide had practically disappeared. As shown in previous experiments, the extract after oxidation no longer contains "complete" or active systems for methylene blue reduction and hemoglobin destruction. The thermostable component of the oxidation systems (as represented by fresh meat infusion and by heated "reduced" broth extract) was added to the oxidized extract and tests made of the methylene blue-reducing and hemoglobin-oxidizing powers of the mixtures.

Results of experiments of this nature (Table II) prove that the activity of cell extracts which has been destroyed through oxidation cannot be restored by the addition of the thermostable components present in meat infusion or heated extracts. Therefore the exhaustion of the thermostable component cannot explain the inactivation of these extracts by oxidation.

2. Activation of Oxidized Extracts by the Addition of the Thermolabile, Cellular Component.

The failure to "reactivate" oxidized extracts by the addition of the thermostable component of the oxidation systems indicates that the thermolabile, cellular constituent of the system is injured by the oxidation processes. Provided the thermolabile component alone is destroyed, the oxidized extract will still contain the thermostable component. To investigate this question, experiments have been conducted in which washed cell extracts, which have been shown to contain only the thermolabile component, have been added to extracts previously inactivated by oxidation. The experiments, therefore, are tests of the possibility of "reactivating" the oxidized, inactive systems by the addition of the thermolabile, cellular component.

Thermolabile Component.—The thermolabile, cellular component was obtained by the use of an extract of washed cells which had previously been found to contain only the thermolabile component and to be by itself inactive.

Thermostable Component.—The thermostable component was obtained by destroying the thermolabile component by heating and by oxidation. As controls, both the oxidized and unoxidized extracts were used in the heated state.

Test Mixtures.—Mixtures were made of known amounts of the thermolabile and thermostable component and the activity of the resultant mixture was determined by observations of the methylene blue-reducing and hemoglobin-oxidizing powers. The results are given in Table III.

In the previous experiment it was shown that the addition of the thermostable component to an oxidized extract does not restore its activity. In the present experiment, however, it is evident that the addition of the thermolabile component to the oxidized extract results in the complete restoration of activity. Hence, the loss in activity of an oxidized extract represents the inactivation of the thermolabile component. On the other hand, the thermostable component

TABLE III.

Effect of the Addition of the Thermolabile Component to Pneumococcus Extracts Which Have Been Inactivated by Exposure to Air.

PO_4 solution extract of washed cells (thermolabile component).		Broth extract of unwashed cells previously inactivated by exposure to air.		"Reduced" broth extract of unwashed cells (thermostable component).	Meat infusion (thermostable component.)	Methylene blue reduction.	MetHb formation.	
Unheated.	Heated.	Unheated.	Heated.	Heated.			30 min.	8 hrs.
cc.	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*			
	0.4	0.4				Incomplete, 3 days.	−	−
0.4		0.4				Complete, 3 hrs., 15 min.	+	+
0.4			0.4			" 3 " 15 "	+	+
0.4				0.4		" 3 "	+	+
0.4					0.4	" 3 " 15 min.	+	+
	0.4				0.4	Incomplete, 3 days.	−	−

remains relatively unaffected not only by oxidation but by heat, since in the present experiment, the heated and unheated oxidized extracts exhibit comparable activity when suitably complemented by the thermolabile component. It is interesting to note that the thermostable component is but little if at all affected by the oxidation of the extract. In the presence of the thermolabile constituents, the thermostable substances in oxidized extracts function practically as well as do those present in extracts which have been heated but never exposed to oxi-

dation. Although not quantitative, these data suggest that the thermostable constituents are either present in great excess, or that they consist, in part, of substances which may be alternately oxidized and reduced.

Effect of Exposure to Air upon the Subsequent Activity of Washed Cell Extracts.

The preceding experiments have demonstrated that it is the thermolabile component (an actual constituent of the pneumococcus cell) which is destroyed during the oxidation of the "complete system" type of extract. In previous studies it has been found that during the oxidation of these extracts other thermolabile substances of cellular origin, such as pneumococcus hemotoxin, sucrase, amylase, raffinase, and inulase, are also destroyed. In spite of the destruction of the hemotoxin and enzymes in oxidized unwashed cell extracts, in the "incomplete" or washed cell extracts these substances proved relatively stable when exposed to air. The following experiment was conducted to determine the effect of exposure to air upon the subsequent activity of the thermolabile cellular component of the oxidation-reduction systems in washed cell extracts.

Phosphate solution extract of washed pneumococci (the "incomplete system" type of extract) was exposed to air in a shallow layer in an Erlenmeyer flask, held in a water bath at 37°C. After 1 hour, 3 hours, and 24 hours exposure, 0.6 cc. of the aerated extract was placed in duplicate reduction tubes together with 0.6 cc. of meat infusion or 0.6 cc. of yeast extract, and 0.2 cc. of 1:2,000 methylene blue was added to each tube. The reduction tubes were sealed and incubated at 37°C. The time required for dye reduction by the extract previously exposed to air was compared with that required by the extract which had not been exposed to air.

The effect of exposure to air upon the subsequent hemoglobin-oxidizing power was tested by the usual method. The results are given in Table IV.

The results of experiments of this type (Table IV) indicate that when exposed to air in the washed cell extract, the thermolabile constituent of the methylene blue-reducing and hemoglobin-oxidizing systems suffers a gradual loss in activity. This loss is extremely slow, and demonstrable only after prolonged periods of exposure. This is in striking contrast to the rapid inactivation which occurs

whenever the "complete system" type of extract is exposed to air. However, the fact that the potential activity of this thermolabile cellular substance is injured at all under these conditions is evidence of greater susceptibility to oxidation than that exhibited by the enzymes and hemotoxin in the same extracts. Although both are extremely labile substances, neither pneumococcus hemotoxin nor sucrase is inactivated to a detectable degree when washed cell extracts are exposed to molecular oxygen for 24 to 48 hours. Since it is gradually destroyed by prolonged exposure to air in the washed cell extracts, the thermolabile component of pneumococcus oxidation-

TABLE IV.

The Effect of Exposure to Air upon the Subsequent Activity of Washed Cell Extracts.

Washed cell extract exposed to air at 37°C. for.	Meat infusion added to extract after exposure to air.	Activity of washed cell extract after exposure to air.			
		Methylene blue reduction (time for reduction).	Hemoglobin oxidation (methemoglobin formation) after following periods at 37°C.		
			30 min.	1 hr.	4 hrs.
hrs.					
0		2 hrs., 30 min.	+	++	++
1		2 " 30 "	+	++	++
3		2 " 30 "	+	++	++
24		3 " 15 "	−	+	++

reduction systems appears to be the most easily oxidized among all of the labile constituents of the pneumococcus cell which have yet been described.

Comparison of the Influence of Heat and of Oxidation upon the Subsequent Activity of the Various Labile Cellular Substances in Sterile Extracts of Washed Pneumococci.

The results of a previous investigation showed that the known endocellular enzymes of Pneumococcus vary in their relative resistance to oxidation, their resistance decreasing in the following order: peptonase, lipase, bacteriolytic enzyme, sucrase, and other carbohydrate-hydrolyzing enzymes. This order of relative resistance is maintained whether the oxidizing agents employed be the reagent hydrogen peroxide or the oxidizing agents formed during the oxidation of pneumo-

coccus extracts. It was further found that the order of resistance of these enzymes to oxidation agrees with the relative order of resistance of the same enzymes to heat. Data previously presented which are collected together in the following pages, demonstrate that this relation does not apply to the thermolabile cellular constituent which is concerned in methylene blue reduction and hemoglobin oxidation.

TABLE V.

Comparison of the Influence of Heat and of Oxidation upon the Subsequent Activity of the Various Cellular Substances in Sterile Pneumococcus Extracts.

Labile constituent of pneumococcus cell.	Resistance to heat.	Resistance to oxidation.	
		Exposed to the oxidation products which are formed when unwashed cell extracts are exposed to air.	Exposed 24 hrs. to 0.003 per cent H_2O_2.
Labile cellular constituent of methylene blue-reducing and hemoglobin-oxidizing systems.	Completely destroyed. 10 min at 65°C.	Completely destroyed.	Completely destroyed.
Lipase.	Completely destroyed. 10 min. at 65°C.	Unaffected.	Unaffected.
Sucrase.	Completely destroyed. 10 min. at 55°C.	Partially destroyed.	Partially destroyed.
Hemotoxin.	Completely destroyed. 1 min. at 55°C.*	Completely destroyed.	Partially destroyed.

* Unpublished observation.

The oxidized portion of extract was prepared as follows: A titrated dilute solution of "Dioxogen" was added to washed cell extract in amount sufficient to give a concentration of 0.003 per cent of hydrogen peroxide. The peroxide-treated extract was sealed with vaseline and gave a faint peroxide test with benzidine. The heated extract was exposed to 55°C. for 10 minutes in a sealed tube.

The activity of the various cellular constituents in portions of the original extract, heated extract, and peroxide-treated extract was tested and described in a preceding paper (6).

The results of the above experiments, together with data from preceding papers, are summarized in Table V. It is evident (Table V)

that the thermolabile cellular constituent which is involved in the described oxidation reactions is extremely sensitive to hydrogen peroxide, an oxidizing agent similar in nature to those which accumulate in aerated pneumococcus extracts. In a preceding paper (6) a study was made of the resistance of different enzymes of Pneumococcus to various concentrations of hydrogen peroxide. The resistance of the different enzymes to this oxidizing agent was found to be comparable to the relative resistance of the same enzymes to heat; *i.e.*, enzymes which proved more resistant to heat were also more resistant to hydrogen peroxide. Quite different relations are evident in the above results as concerned the labile cellular component of the oxidation-reduction system. This labile constituent of Pneumococcus is far more sensitive to oxidation than is the pneumococcus lipase although both exhibit approximately equal heat resistance. Further, hemotoxin and sucrase, although much more heat-labile than the cellular constituent of the oxidation systems, are less susceptible to inactivation by oxidation.

Destruction of the Thermolabile Constituent of the Oxidizing-Reducing Systems in Aerobic Cultures of Pneumococcus.

The destruction of hydrolyzing enzymes which occurs during the oxidation of sterile pneumococcus cell extracts is reflected in differences in the enzyme activity of the filtrates of anaerobic and aerobic cultures (7). The endocellular substances freed by autolysis are destroyed by oxidation in aerobic cultures in a manner apparently analogous to the destruction of the same substances when exposed to the oxidation products formed in the sterile cell extracts. In another paper from this laboratory (9) it has been shown that the methemoglobin-forming system is inactivated in aerobic cultures just as in "oxidized" cell extracts. The rapid destruction of both the methylene blue-reducing and hemoglobin-oxidizing systems when the cell extracts are oxidized has been shown in the present paper to be due to the inactivation of the thermolabile cellular constituent. Experiments were made to determine if the destruction of this constituent is also the cause of the inactivation of the systems in the case of aerobic culture filtrates. Since the cellular component of the oxidation-

reduction system is endocellular, the tests were made on autolyzed cultures.

It was found that filtrates of autolyzed anaerobic cultures possess an active methylene blue-reducing system, as well as the hemoglobin-oxidizing system reported previously, while filtrates of aerobic cultures do not possess these active systems. The inactivity of the filtrates of the aerobic cultures is due to the destruction of the thermolabile cellular substances, probably by the same reactions as those which inactivate the sterile cell extracts.

DISCUSSION.

In the preceding papers of this series (1–7), the oxidizing and reducing activities of sterile pneumococcus extracts have been described. The nature of these reactions is determined by the presence or absence of molecular oxygen. In the presence of molecular oxygen the active extracts have been shown to exhibit the following activities: consumption of molecular oxygen, formation of peroxide, oxidation of hemoglobin, oxidation or destruction of the endohemotoxin and intracellular enzymes contained in pneumococcus extracts. All of these oxidations are examples of "oxygen activation" whereby substances themselves not reactive with molecular oxygen are easily oxidized by agents formed during the oxidation of other substances. If molecular oxygen is absent or the supply restricted, these same extracts establish conditions which result in the reduction of methylene blue and methemoglobin. Analysis of these processes shows that the active systems consist of two components, one of which is thermolabile, the other thermostable. The thermolabile component is an actual constituent of the Pneumococcus and is not removed by washing the cells. The thermostable component is not necessarily of pneumococcus origin and may be supplied by adding yeast extract or meat infusion to extracts prepared from washed bacteria. The marked oxidation reactions which occur when an active extract is exposed to air, result in the inactivation of the oxidizing and reducing systems. The results of the present study show that the thermostable substances are still active in oxidized extracts and that the inactivation of the oxidation-reduction systems is due to the destruction of the thermolabile cellular constituent. This cellular substance is not only rapidly de-

stroyed by products formed during oxidation but is gradually inactivated by molecular oxygen itself. Moreover, the labile component of the oxidation system is more susceptible to the action of the reagent hydrogen peroxide than is any other known intracellular substance of pneumococcus origin.

In the interpretation of the interaction of these two components with molecular oxygen, the formula $C + RH$ has been used to represent the system in these extracts by which peroxide is formed and hemoglobin oxidized. It is assumed that the thermostable substances (RH) are the actual source of the oxidizing agents and that the thermolabile cellular substance (C) serves as a catalyst in accelerating the reactions involved. The fact that the cellular component is inactivated by oxidation is not a serious objection to its catalytic nature. Evidence presented in another paper makes it clear that thermolabile cellular substances of ferment nature, such as the hydrolyzing enzymes, which are not concerned in oxidation and reduction, are inactivated by agents formed during the oxidation of pneumococcus extracts. In view of the high degree of susceptibility to peroxides of the thermolabile component, it is reasonable to assume that the oxidation-reduction systems may be destroyed by the same reactions which inactivate the hemotoxin and enzymes contained in the extracts. In fact, the inactivation of catalytic agents by products of their own activity is a common phenomenon. The order of reactions would seem to be analogous to that previously proposed for the oxidation of hemoglobin, hemotoxin, and sucrase.

(1) $C + RH + O_2 \rightarrow$ oxidizing agent.
(2) Oxidizing agent $+ C \rightarrow$ inactive C.

Here it is inferred that C functions in equation (1) as a thermolabile, cellular catalyst. Its inactivation in equation (2) is assumed to represent simply the destruction of a labile substance which is very easily inactivated by the oxidizing agents present in the aerated extract.

While our knowledge of the oxidation-reduction systems of Pneumococcus is still incomplete, the work thus far accomplished has resolved the system into two components, the nature of which may be characterized as follows: The component RH represents substances which are not present in the Pneumococcus after thorough washing. These

substances are relatively heat-stable, resisting boiling for prolonged periods of time. They are present in water or alcohol extracts of muscle, yeast, and vegetable tissue. By themselves, these substances react slowly with molecular oxygen to form oxidizing agents, and, in the absence of molecular oxygen, they establish conditions under which methylene blue and methemoglobin are slowly reduced. Although separately these substances are but slowly reactive, in the presence of the second component the reactions of oxidation and reduction are markedly accelerated. This labile cellular component, referred to as *C*, represents substances which are resident in the Pneumococcus and are not removed by washing the cells. This component is thermolabile, being inactivated by exposure to 65°C. for 10 minutes. By itself it is wholly non-reactive with molecular oxygen and possesses no reducing power. This cellular component seems to be catalytic in nature, since it greatly accelerates reactions of oxidation and reduction in the presence of the other component of the system. When present together, these substances constitute systems responsible for many of the biological oxidations and reductions of the living cell.

SUMMARY.

1. The systems responsible for oxidation and reduction in the pneumococcus cell have been shown to consist of two components, the nature of which has been discussed.

2. The oxidizing and reducing power of active extracts of pneumococci is inactivated by exposure to air. This loss in activity is due to an inactivation of the thermolabile cellular component consequent on secondary reactions analogous to those which under similar conditions destroy the hemotoxin and enzymes in pneumococcus extracts.

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