our "light" (R_2L) and "heavy" (R_2H) fraction as mitochondrial fragments (including cristae), and vesicles plus membranes, respectively.

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Biochemical Correlates of Respiratory Deficiency. II. Antigenic Properties of Respiratory Particles*

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Received December 30, 1963

Antibodies against respiratory subparticles of yeast produce a differential inhibition of various particulate enzymic activities in the order: succinic dehydrogenase < DPNH dehydrogenase ≃ cytochrome c oxidase < DPNH oxidase < DPNH-cytochrome c reductase. Studies with this antibody-antigen complex have shown that the combining sites for reduced and oxidized cytochrome c cannot be identical, and that the role of the cytochrome in stimulating DPNH-oxidase activity is not that of a mobile carrier between the reductase and the oxidase portion of the chain. "Respiratory" subparticles from *petite colonie* mutants contain a cross-reacting material capable of binding (or reacting with) the anti-DPNH-cytochrome reductase of sera prepared against particles from wild-type cells. This cross-reacting material is not identical with the primary dehydrogenase and is not found in the bulk of the "mitochondrial" fraction of the mutant.

As part of a continuing research program in our three laboratories designed to elucidate the genetic and biochemical basis of respiratory deficiency (Ephrussi, 1953; Slonimski, 1953) in *Saccharomyces cerevisiae*, we have in the present investigation (Mahler *et al.*, 1964a,b) established that wild-type and cytoplasmic mutant strains are similar with regard to: (a) the presence of primary dehydrogenases for D-lactate, L-lactate, succinate, and DPNH; (b) the localization of characteristic enzymatic activities in mitochondria and respiratory particles therefrom derived; (c) the distribution of protein among soluble and particulate fractions; and (d) the qualitative and quantitative pattern of unsaponifiable lipids in the intact cell. They differ most notably with regard to (a) the amount of cytochromes (*including* cytochrome c + c_1) tightly bound to respiratory particles and (b) the ability of these cytochromes to participate in all the enzymatic activities characteristic of a functional respiratory chain (i.e., DPNH- and succinoxidase, DPNH- and succinate-cytochrome c reductase, and cytochrome c oxidase). In the present communication we wish to report on some immunochemical properties of the respiratory particles which have permitted us to unravel certain additional details of electron transport in the wild type, and to demonstrate a cross-reacting material (CRM)[1] in the mutant capable of neutralizing the anti-DPNH-cytochrome reductase of sera prepared against particles isolated from the wild type.

* The investigations here reported have been supported by the following sources: grants H-5457 and GM-K3-1128-Cl-B from the National Institutes of Health to B. M.; grants GM 05942 from the National Institutes of Health and G 8959 from the National Science Foundation to H. R. M.; and grants to P. P. S. The bulk of the investigations here reported were performed during 1962–63 while both B. M. and H. R. M. were on leave, as visiting investigators at the Laboratoire de Génétique Physiologique du C.N.R.S. Indiana University Publication No. 1194.

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Experimental

The various particle preparations used, the enzymological assay procedures, and the source of the

[1] Abbreviation used in this work: CRM, cross-reacting material.

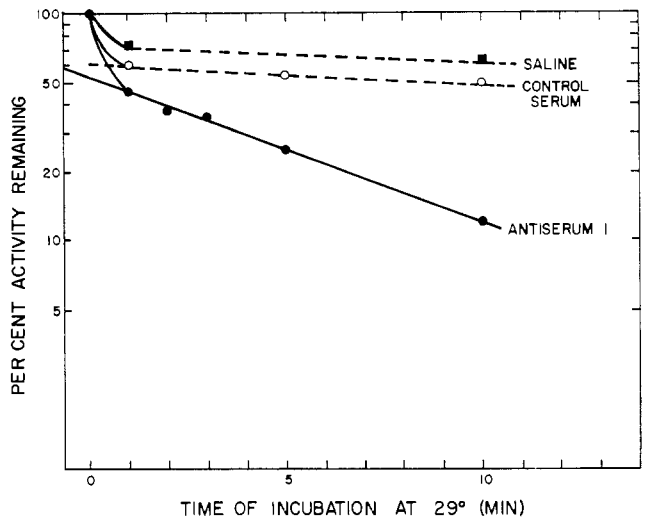


FIG. 1.—Time course of inactivation of DPNH–cytochrome c reductase by anti-R_2L_w serum. All the components of the DPNH–cytochrome c assay, i.e., 0.1 ml of 0.2 M phosphate buffer of *p*H 7.5, 0.1 ml of 0.1 M sodium azide made up in this buffer, 0.05 ml of 2% cytochrome c, and 0.75 ml of H_2O plus 0.02 ml of a 1:25 dilution of R_2L_w No. 12 (5.44 μg total) were incubated with (a) 0.05 ml of 0.9% NaCl, (b) 0.05 ml of control serum in 0.9% NaCl, or (c) 0.05 ml of antiserum No. 1 in 0.9% NaCl at 29° for the periods of time indicated on the graph. At those times 0.01 ml of a 1% solution of DPNH was added to start the reaction and readings were taken every 15 seconds at 550 mμ. Since the rise in optical density under these conditions was linear for the first 2 minutes, the difference in OD between 15 and 75 seconds after initiation of the reaction is taken as the initial velocity. The reaction mixtures were kept at 29° throughout, and the initial velocity of the unincubated uninhibited control was 0.150 min^{-1}.

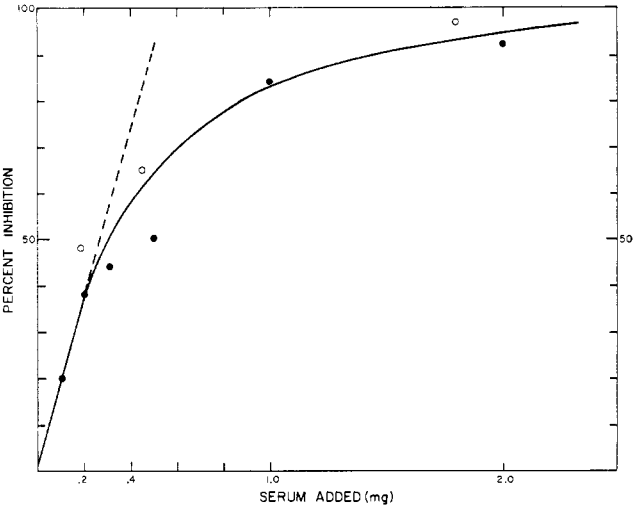


FIG. 2.—Inhibition of DPNH–cytochrome c reductase as a function of antiserum concentration. The assay system was similar to that described in the legend to Fig. 1 except that the different amounts of sera and enzyme but a uniform period of 7.5 minutes' preincubation of R_2L_w with sera at 29° were employed. All rates were compared to the corresponding rates with control sera by using a total volume of 0.1 ml of serum, e.g., 0.1 ml of control, 0.095 ml control plus 0.005 ml of antiserum, 0.09 ml control plus 0.01 ml of antiserum, ... 0.05 ml of control plus 0.05 ml of antiserum, 0.1 ml of antiserum. ●, R_2L_w No. 12 (5.44 μg protein) and serum No. 1; ○, R_2L_w No. 13 (6.0 μg protein) and serum No. 3.

chemicals employed are described in the previous paper (Mahler *et al.*, 1964a).

For preparation of the antisera four rabbits were injected with respiratory subparticles (preparation No. 6 of Mahler *et al.*, 1964a) and a fifth was used as a control. The sera were purified in the manner described by Somlo (1962) and were stored frozen in 0.9% NaCl.

RESULTS

Inhibition of Enzymatic Activities by Isologous Antisera

Comparison of Various Activities.—A R_2L_w preparation (No. 6, Table II, previous paper) was used to immunize four rabbits and purified antisera were prepared by the procedure described under Experimental. These antisera were then tested for their ability to inhibit various enzymatic activities of other R_2L_w-particle preparations, isolated in the standard manner and completely comparable to the antigen in their characteristic properties (Nos. 12 and 13, Table II, previous paper). As shown in Table I, under comparable conditions, the extent of inhibition was greatest for DPNH → cytochrome c reductase, which was inhibited essentially completely. The DPNH oxidase, either in the presence or absence of external cytochrome c was inhibited to a maximal extent of 50%, the cytochrome c oxidase approximately 40% and the DPNH dehydrogenase about 33%. Under the conditions used succinic dehydrogenase appeared to be unaffected by the presence of antiserum. Thus the antibodies against R_2L_w appear to be primarily directed against the DPNH → cytochrome c portion of the respiratory chain and, more specifically, against the site(s) responsible for the binding of and/or interaction with cytochrome c when the latter is used as an *external* electron acceptor.

Some Details of the Inhibition of DPNH-Cytochrome c Reductase

Time Course.—Since this appeared to be the reaction most completely blocked by anti-R_2L_w sera we have studied some additional details of this interaction. In Figure 1 is presented its time course at 29°. Except for a more rapid drop during the first minute, largely accounted for by an inhibition of the enzymatic activity by the high salt concentration alone, the inactivation by excess serum appears to be strictly first order with respect to time with an apparent half-life, $t_{1/2}$ = 3.8 minutes. In most of our experiments we have used twice this value, i.e., 7.5 minutes, of preincubation with serum.

Stoichiometry.—In attempts at establishing the stoichiometry of the reaction we have studied the variation of the extent of inhibition produced by different amounts of serum acting upon a constant amount of enzyme for a constant length of time. The curve obtained (Fig. 2) appeared to be a simple hyperbola with half-maximal saturation at a level of about 300 μg of serum for each 5.5 μg of R_2L_w added. Freezing and thawing the enzyme appeared to decrease the maximal inhibition attainable (75% instead of 95%) without affecting either the linear part of the curve or the point of half-maximal saturation.

The above results suggest a 1:1 stoichiometry in the formation of the antibody-enzyme particle complex, i.e., a bimolecular reaction between the two components. If this were so, then in the linear part of the curve (i.e., below 50% inhibition, where the reaction is first order with respect to either component) the extent of inhibition should depend only on the *ratio* of amount of serum added per μg of enzyme, regardless of the absolute *amounts* of either component. That this is indeed so for three different levels of enzyme is shown in Figure 3. The best straight line

TABLE I
INHIBITION OF VARIOUS ACTIVITIES OF R_2L_w PARTICLE[a]

Activity	Enzyme Concn. (μg/ml)	Serum (ml)	S/E (μg/μg)	Inhibition (vs. Control) (%) Serum No. 1	2	3	4
DPNH → cyt c	5.2	0.1	320		86	92	86
	6.0[b]	0.1	280	91	71		
	13.0	0.1	130			58	
DPNH → O_2	13.0	0.1	130	47		38	
	13.0[c]	0.1	130	44			
	13.0	0.2	260		30	47	38
Cyt c → O_2	5.2	0.1	320		11	22	22
	13.0	0.2	320	26			
DPNH → indoph	13.0	0.1	130			29	
	13.0	0.2	260				
Succin → indoph	33.0	0.2	110	0	5	0	0

[a] All activities were measured in the standard assay system (see Experimental) after preincubation of the enzyme (No. 12 R_2L_w) in the absence of substrate for 7.5 minutes with the appropriate serum and compared to a sample preincubated with the same volume of control serum. Except for the DPNH → cytochrome c reaction, preincubation with serum did not affect the various activities when compared to a control incubated with an equal amount of isotonic NaCl; the latter showed an activity ≥70% of the unincubated control. All reactions were started by the addition of substrate and measured at 29°. In the case of the two reactions with indophenol as acceptor, preincubation with substrate and initiation of the reaction with dye had no effect on either the rate or the extent of inhibition by serum. The protein content (mg/ml) of the various sera was as follows: No. 1, 18.1; No. 2, 16.5; No. 3, 20.8; No. 4, 16.9; and Control, 16.0. [b] Enzyme = 13 R_2L_w. [c] Activity measured with cytochrone c omitted from assay.

yields a point for 50% inhibition at 46 μg serum per μg R_2L_w protein.

Extent of Inhibition.—In Table I we have indicated that the extent of inhibition for DPNH-cytochrome c reductase, DPNH-oxidase, cytochrome oxidase, and DPNH-dehydrogenase at saturation, with serum No. 3 in excess, are in the order of 90:45:25:25, i.e., 4:2:1.1:1.1. For the same four activities but with one-fourth as much serum, i.e., in the linear range, the ratios are 50:25:17:20, or 4:2:1.4:1.6. To a first approximation the extent of relative inhibition for the four indicator activities remains constant in going from the saturation to the first-order region. This would indicate either that all activities are blocked as a result of the formation of a single species of antigen-antibody complex, or that up to four different species of antibody (one for each reaction) can react with the particulate antigen but exhibit approximately equal affinity for the latter. The first alternative would appear somewhat more plausible.

Presence of CRM in Respiratory Particles of "Cytoplasmic Petites"

It has been possible to demonstrate serum-blocking power; i.e., the presence of CRM related antigenically to the antigen(s) in the R_2L_w of wild-type yeasts in similar particles obtained from respiratory deficient mutants. Experiments leading to this conclusion are summarized in Table II. Two types of experiments have been performed: *direct competition experiments* (experiments 1 and 2) in which the decrease in inhibition of DPNH-cyt c reductase of R_2L_w (wild type) by serum after preincubation of the latter with particles from the mutants is measured with all components present in the assay mixture, and *centrifugation experiments* (experiments 3 and 4) in which the CRM-serum complex is first removed by ultracentrifugation prior to the addition of the other components.

The results of both types of experiments are qualitatively similar and may be summarized as follows: (a) R_2L_w particles from the mutant contain CRM which can combine with serum and as a result decrease the inhibitory effect of the latter when tested against the DPNH-cytochrome reductase of R_2L_w particles from the wild type. When tested in the region where

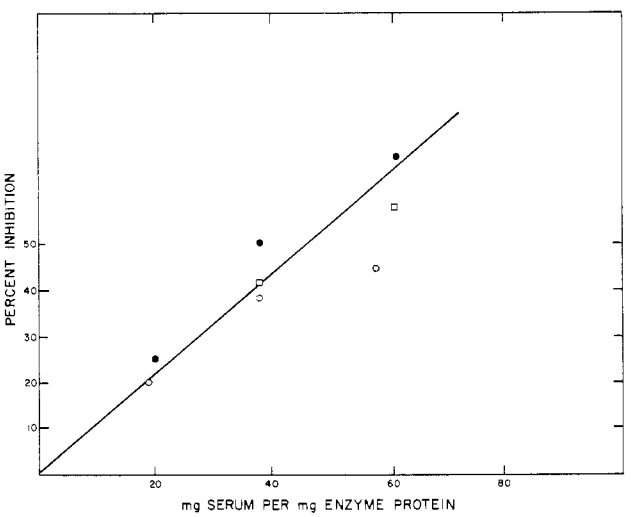


FIG. 3.—Inhibition of DPNH–cytochrome c reductase at different ratios of serum to enzyme. The assay system was analogous to that described in the legends to Figs. 1 and 2. The ratios of enzyme (R_2L_w No. 12) and serum No. 3 were varied in the manner indicated and preincubation was for 7.5 minutes at 29°. □, 2.7 μg enzyme protein; ○, 5.4 μg enzyme protein; ●, 13 μg enzyme protein.

inhibition is a linear function of the amount of serum added the release of inhibition by CRM can be made virtually quantitative. (b) There is essentially no effect when R_2H particles from the same preparation of mutant yeast are substituted for the R_2L_w particles. This not only constitutes a significant internal control[2] but is the more surprising since, as calculated from Tables II and VII of the preceding paper, the R_2H particles exhibit greater total and specific enzymic activity than do R_2L_w particles with respect to all the reactions examined *including the DPNH dehydrogenase* and the residual DPNH-cytochrome reductase; i.e., they are to be considered as containing the bulk of the "mitochondrial" mass of the cell. The amount of the residual cytochrome reductase present in the

[2] The amount of R_2H and R_2L actually used in these experiments was so chosen as to supply an amount of dehydrogenase activity equal to that of the wild-type enzyme.

TABLE II

PROTECTION OF R_2L_w (GRANDE) BY PRETREATMENT WITH R_2L_w (PETITE)[a]

Expt. No.	Additions: R_2L_w (No. 13) Wild Type	Additions: R_2L_w (1P) Mutant	Additions: R_2H (1P) Mutant	Additions: Antiserum	Activity (1000 × OD/105 sec)	Inhibition (%)	Release (%)
1	+	−		−	250		
	−	+		−	15		
	+	+		−	300		
	+	−		+	80	68	
	+	+		+	147	51	25
2	+		−	−	173		
	−		+	−	10		
	+		+	−	187		
	+		−	+	72	59	
	+		+	+	78	59	0
3	+	+		[b]	397		
	+	+		−	312		
	+	−		++	90	71	
	+	+		++	139	56	21
	+	++		++	225	28	60
	+	−		+	163	48	
	+	+		+	248	20	58
	+	++		+	295	6	88
4	+		++	−	175		
	+	−	−	++	56	68	
	+		+	++	66	62	9
	+		++	++	72	59	11

[a] In experiments 1 and 2 the procedure was as follows: cuvets containing in 1.0 ml all the components of the conventional DPNH → cytochrome c assay but with DPNH omitted were incubated for 5 minutes at 29° in the presence of 0.05 ml of control serum (−) or antiserum 3 (+) (1 mg total); where shown they also contained either 16.5 μg of R_2L_w or 15 μg of R_2H of preparation 1P (from "petite" yeast, Mahler *et al.*, 1964a). These quantities were chosen to give indophenol activities approximately equal to that given by the amount of R_2L_w of preparation 13 used in the experiment. At the end of the preincubation period 5.4 μg of R_2L_w of preparation 13 (from wild-type yeast, Mahler *et al.* 1964a) was added and the incubation was continued for 5 minutes longer whereupon the reaction was started by the addition of DPNH. In experiments 3 and 4 the following procedure was used: To a final volume of 1.6 ml were added 0.4 ml of 0.2 M phosphate buffer, *p*H 7.2; 0.1 ml of control serum (−); 0.1 ml (++) or 0.05 ml (+) of antiserum 3 plus particles from preparation 1P; either 32 μg (+) or 64 μg (++) of R_2L_w in experiment 3; 60 μg (+) or 120 μg (++) of R_2H in experiment 4. The mixtures were incubated for 10 minutes at 29° and then centrifuged in the No. 40 rotor of the Spinco Model L for 30 minutes at 40,000 rpm. Supernatant (0.8 ml) from each tube was then placed in a cuvet, 0.5 ml of 2% cytochrome c, 0.10 ml of 0.1 M azide, 0.10 ml of H_2O, and 5.4 μg of R_2L_w of preparation 13 were added, and the mixtures were incubated for 5 minutes at 29°. The reaction was again started by the addition of DPNH. [b] No serum added.

R_2L_w of the *petite* is completely insufficient by several orders of magnitude to account for the results observed in the centrifugation experiments.

DISCUSSION

Number and Type of Antigens Present in Particles from Wild Type

Brief mention has already been made of experiments which can be interpreted in terms of the presence of a single species of antibody in the serum capable of interacting with the R_2L_w antigen. If this interpretation is accepted, then the combination of the antigen with this antibody presumably occurs at a site identical with, or closely adjacent to, the one responsible for the binding of and interaction with external cytochrome c when the particle functions as a DPNH-cytochrome c reductase.

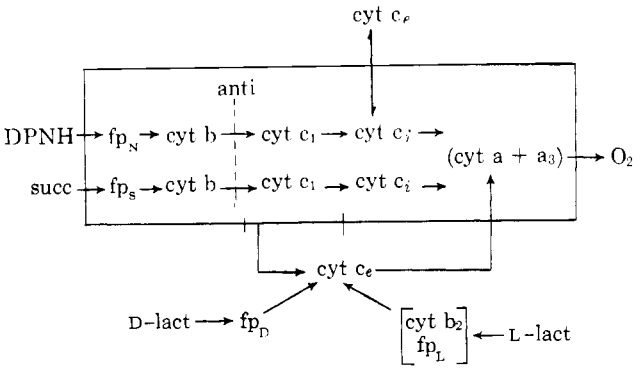
As a consequence of this interaction there takes place a structural alteration in or on the surface of the particle which impedes its ability to combine with, and/or transport electrons to and from, other substrates and acceptors. In other words, the particulate antibody-antigen complex is altered structurally and functionally as compared to the native particle and exhibits enzymatic properties which may vary from complete inhibition for one substrate, utilizing sites identical with or proximal to the antibody-combining site, to very little or no inhibition for substrates utilizing sites and reaction sequences distal to the combining site.

Conclusions Regarding Electron Transport

These can best be discussed in terms of Scheme I, which incorporates the results obtained by previous investigators as well as those obtained by us and described in this and the preceding publication. The most characteristic features follow.

Nonidentity of Combining Sites for Oxidized and Reduced Cytochrome c.—When oxidized cytochrome c is added to the preparation it can be reduced by the dehydrogenases contained therein. These involve either the respective primary dehydrogenases only, in the case of D- or L-lactate, or the dehydrogenase plus other members of the electron-transport chain (including at least those linked by the antimycin A–sensitive site) in the case of DPNH or succinate. Cytochrome c reduced *in situ* or reduced cytochrome c added externally can then be used as a substrate for the particulate cytochrome c oxidase. The question naturally arises whether the combining sites for oxidized cytochrome c (of the succinate- and DPNH-cytochrome c reductase activities) and for reduced cytochrome c (of the cytochrome oxidase) are identical. The answer appears to be clear cut: *they are not.* With excess serum DPNH-cytochrome c reductase is inhibited essentially quantitatively while cytochrome

Scheme I
Electron Transport in *S. cerevisiae*



⇀↽, reversible binding, direction of equilibrium indicated. →, direction of electron transfer. fp_N, DPNH (NADH) – dehydrogenase; fp_S, succinate dehydrogenase; fp_D, D-lactate dehydrogenase (D-lactate–cytochrome c reductase); fp_L cyt b_2, L-lactate dehydrogenase–cytochrome b_2; cyt, cytochrome; cyt c_i, "internal" cytochrome c; cyt c_e, "external" or added cytochrome c; succ, succinate; lact, lactate; anti, antimycin A.

oxidase is only partially inhibited; in the linear range the former is inhibited 52% while the latter is inhibited only 17%. We therefore indicate spatially separate sites for cytochrome c in the "reductase" and the "oxidase" portions in the scheme but can make no statement as to their precise localization.

The Function of Internal vs. External Cytochrome c During the Oxidation of DPNH by O_2.—The particulate preparations studied by us can function as aerobic oxidases for the four substrates D-lactate, L-lactate, succinate, and DPNH. As discussed in detail in the first paper of this series, the first two substrates differ qualitatively from the third. Their oxidation is mediated exclusively by external cytochrome c functioning in conjunction with the particulate cytochrome oxidase, while the oxidation of succinate procedes through the particulate components of the chain exclusively and its rate is virtually unaffected by the presence of added cytochrome c in the reaction system. DPNH occupies an intermediate position: its oxidation is stimulated (to the extent of about 2-fold) by the addition of external cytochrome c. The question then arises whether the function of this "external" cytochrome c is that of a shuttle between the "reductase" and the "oxidase" portions of the chain. We have previously presented arguments based on the level of this cytochrome required which appeared to rule out this possibility and to suggest instead that its function is to replenish the pool of "internal" cytochrome c depleted during the course of isolation and purification of the particle. The experiments reported here provide additional support for this contention: If the reduction of external cytochrome c were a constituent and rate-limiting step in cytochrome c–stimulated DPNH oxidation then the extent of inhibition of the overall should reflect that of the partial reaction. Under identical conditions (i.e., with cytochrome c at saturating concentrations[3]) the reductase step does appear to be rate limiting (Tables IV and VII of the previous paper); yet while the former is inhibited essentially completely the latter reaches a maximum of only about 50% at saturation with antibody. One might argue that this discrepancy is explicable in terms of inhibition of *only* the reaction involving external cytochrome c, i.e., exclusively that portion of the overall oxidase which is stimulated by cytochrome c. Yet the data of Table I show that the maximal inhibition attainable is *identical* for both the intrinsic and the cytochrome c–stimulated DPNH-oxidase. It may be added that both the extent of stimulation by added cytochrome and of the inhibition produced by antibody are unaffected by the substitution of reduced for oxidized cytochrome c. Thus in the antibody experiments added cytochrome c behaves in a manner indistinguishable from that of "internal" cytochrome, and probably provides a source of the latter.

Identity of Succinate and DPNH Electron-Transport Chain.—We have already briefly discussed the lines of evidence which suggest that the chain for these two substrates, at least prior to the cytochrome-oxidase portion, may not be identical. In the experiments with antibody there did not appear to be any inhibition of the succinic dehydrogenase of the particle. Since, however, the maximal inhibition even of DPNH dehydrogenase was relatively small, and since the experiments involving succinate required of necessity a 2-fold greater amount of enzyme, and hence a smaller antibody-particle ratio an inhibition $\leq 10\%$ might have remained undetected.

Nature and Significance of the CRM in Respiratory Particle Obtained from Mutants

Fractionation of cell-free extracts from mutants under conditions identical to those devised for the separation of active respiratory subparticles from their wild-type counterparts leads to the isolation of two fractions (R_2H and R_2L) similar to their wild-type counterpart in their sedimentation behavior, protein content, and distribution of certain primary dehydrogenases.[4] Yet only R_2L contains the CRM to DPNH-cytochrome c reductase in detectable amounts; it is of course R_2L which in the wild type is to be regarded as the respiratory subparticle fraction of high activity. It will be interesting to study the relationship, if any, between the R_2L_w particle of mutants and the mitochondrial-precursor particles obtainable for the wild type under conditions of incomplete adaptation to O_2, described by others

[3] These concentrations are an order of magnitude greater than those used in the assay of cytochrome c–stimulated DPNH-oxidase. Thus if there were any competition between the cytochrome and the antibody for combining sites the inhibition of the DPNH-cytochrome reductase should be, if anything, diminished when compared to the DPNH-oxidase.

[4] If anything, in the mutant the R_2H is the more "active" particle fraction in terms of both specific and total activities, and is most representative of the bulk of the mitochondrial protein.

(Linnane *et al.*, 1962; Schatz, 1963), and to determine whether the latter also contains a similar CRM.

The data of Table II suggest that the R_2L_w particles from mutants possess approximately one-third the combining ability for antibody (on a protein basis) as do the corresponding antigenic particles from the wild type. If we make the highly questionable assumption that the association constant for the formation of the complex is the same for particles regardless of genetic constitution, it follows that the level of CRM is about one-third that of the wild-type antigen in commercial yeast. It is perhaps not inappropriate at this point to recall the observation that the actual level of the DPNH-cytochrome c reductase in the parent wild type (59 R) is also only one-third of that of commercial yeast (Table VI of the previous paper). Thus the amount of CRM in the mutant may be quite comparable to that of the antigen in the corresponding wild-type strain.

Finally we may make some suggestions concerning the probable nature of the cross-reacting protein. We know the mutant contains mitochondrialike structures (Yatsuayanagi, 1963) and a particulate fraction not too dissimilar from that of the wild type; the latter can be used as a source of a respiratory subparticle having a content of proteins in general and of primary dehydrogenase in particular, again quantitatively similar to that observed in the analogous structure from the wild type (Mahler *et al.*, 1964a). The mutant cell shows a lipid pattern qualitatively and quantitatively similar to that of the wild-type (Mahler *et al.*, 1964b), although we do not yet know whether these lipids are integrated in the normal manner into the matrix of the respiratory particles. The data on the distribution of protein and primary dehydrogenase between R_2H and R_2L particles from wild-type and mutant cells, taken in conjunction with the small extent of inhibition of the dehydrogenase as compared to the reductase and the virtual absence of CRM in the R_2H particles from the mutant, appear to exclude either the primary dehydrogenase or the structural protein(s) of the particles as a likely candidate for the CRM. Thus antigen and CRM appear to be localized in the cytochrome b–cytochrome c_1 region of the respiratory chain. Their exact nature is currently under investigation.

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The Structure of a Myoinositol Mannoside from *Mycobacterium tuberculosis* Glycolipid*

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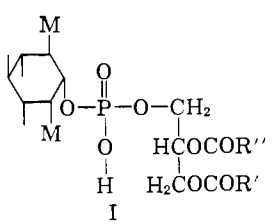
Received January 21, 1964

The retention times on gas chromatography of the four possible isomeric pentamethylmyoinositol ethers have been determined. Using these reference values, it has been possible to identify the pentamethylmyoinositol obtained by methanolysis of permethylated myoinositol monomannoside which had been prepared from the glycolipid of *Mycobacterium tuberculosis*. It is established that the mannose is attached to the 2-hydroxyl group of the myoinositol ring.

It has been established (Lee and Ballou, 1964) that "manninositose," the myoinositol dimannoside obtained from a *Mycobacterium* glycophospholipid by alkaline degradation (Anderson *et al.*, 1938), has the mannoses attached to the 2- and 6- positions of the myoinositol ring. In combination with other work (Ballou *et al.*, 1963), this makes it possible to write structure I for the intact lipid:



I

* This work was supported in part by a grant (AM 00884) from the National Institute of Arthritis and Metabolic Diseases, United States Public Health Service.

to which may be assigned the definitive name 1-phosphatidyl-L-myoinositol-2,6-bis-α-D-mannopyranoside. There is a discrepancy between this structure and one suggested by others (Vilkas and Lederer, 1960; Angyal and Shelton, 1963), but the reasons for accepting structure I are well documented.

Among the products of alkaline degradation of the crude *Mycobacterium* phospholipid, one also finds a myoinositol monomannoside (Vilkas, 1960). This substance forms a crystalline acetate, mp 178–180°, with a specific rotation in chloroform of $[\alpha]_D$ + 20.5°. The existence of galactinol (Brown and Serro, 1953), a galactoside of myoinositol which has the galactose on the D-1- position of the myoinositol ring (II) (Kabat *et al.*, 1953), suggests that the myoinositol mannoside might have the analogous structure. In fact Angyal and Shelton (1963) report the synthesis of such a compound by coupling tetra-*O*-acetyl-D-mannosyl bromide: