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John S. Davis IV, Alfred Jay Bollet

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Research Article

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EFFECTS OF ANTIBODIES ON MITOCHONDRIA *

By JOHN S. DAVIS IV and ALFRED JAY BOLLET

(From the Departments of Preventive Medicine and Internal Medicine, University of Virginia School of Medicine, Charlottesville, Va.)

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Since the demonstration of the lupus erythematosus phenomenon by Hargraves, Richmond, and Morton (1), a great deal of attention has been paid to the numerous antibody-like serum factors in patients with active systemic lupus erythematosus (SLE). Until recently, this attention has been concentrated on those factors that react with whole nuclei or nuclear subunits, such as DNA and histone. Asherson (2) and Deicher, Holman, and Kunkel (3) now have demonstrated other common serum factors in SLE that fix complement with cytoplasmic components, including mitochondria and microsomes from a variety of animal and human sources. The observation of low serum complement levels in active SLE (4–10) suggests the possibility that complement-fixing antigen-antibody interactions might occur *in vivo* and play a role in the pathogenesis of the disease.

Antibodies which react with these mitochondria *in vivo* might interfere with important metabolic functions, since mitochondria contain enzymes involved in the regulation of oxidation and high-energy bond formation. A study of the effect of induced antimitochondrial antibodies in rabbits on mitochondrial enzyme activity was therefore undertaken to define the possible metabolic effects of such antibodies preliminary to study of the effects of the antimitochondrial antibodies found in SLE. This report describes several effects of antimitochondrial serum in various immunologic systems; such serum caused inhibition of mitochondrial enzymes, particularly those in the electron transport chain. A heat-labile serum component, presumably complement, potentiated inhibition of DPNH oxidase by mitochondrial antibody.

METHODS

Preparation of mitochondria. Albino Wistar rats weighing 200 to 300 g (Charles River Breeding Laboratories) were killed by a blow on the head; liver and kidney mitochondria were prepared in 0.44 M sucrose by the method of Deicher, Holman, and Kunkel (3), washed three times, and resuspended in a volume of sucrose equal to the original weight of liver or kidney. Microsomes were spun down from the mitochondrial supernatant fraction at 68,000 × G for 60 minutes and washed twice with 0.44 M sucrose. Nuclei for the complement fixation tests were prepared according to Hogeboom (11). Connective tissue, harvested from polyvinyl sponge implants after 10 to 21 days *in situ* (12), was homogenized by use of ground glass, and the mitochondria were similarly prepared.

Preparation of antisera. Five- to 10-pound New Zealand white rabbits, maintained on Purina rabbit chow at will, were given injections of rat liver mitochondria prepared in Freund's adjuvant, made in 25-ml lots that contained 2 g of mitochondrial protein, 10 ml of Bayol F, 5 ml Falba, and 62.5 mg of heat-killed human tubercle bacilli ($H_{37}Rv$). Controls included unimmunized rabbits and rabbits given adjuvant prepared as above except that sucrose was substituted for the mitochondrial suspension. Subcutaneous injections of 2 ml were given at 1, 2, and 4 weeks. At 6 weeks, and at varying intervals thereafter, subcutaneous booster injections of suspensions of mitochondria in sucrose were given to maintain a high antibody titer. Some rabbits received only one injection of adjuvant suspension followed by boosters of graded doses of antigen in sucrose intravenously twice a week. Blood obtained from marginal ear veins was allowed to clot at room temperature; the serum was inactivated at 56° C for 30 minutes and stored at −20° C. Antiserum to a purified bovine enzyme, L-glutamic dehydrogenase, was prepared in a similar fashion by use of an ammonium sulfate suspension (Sigma) as the antigen (13).

Globulin fractions of many sera were prepared by ammonium sulfate precipitation. Ammonium sulfate was added slowly to the serum to a final concentration of 1.6 M. After overnight refrigeration, the precipitate was washed twice in 1.6 M ammonium sulfate solution, dissolved in saline, and dialyzed against at least six changes of 100 volumes of isotonic NaCl.

Immunologic methods. Complement fixation was carried out according to the method of Lennette (14). Stock mitochondria were usually diluted to a concentration of 0.05 to 0.20 mg protein per ml, since this concentration range gave maximum separation of titers of control and antisera.

Agglutination of sucrose-suspended whole mitochondria was performed on glass slides and read by gross in-

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spection. The mitochondrial antigen was concentrated to about 80 mg protein per ml. The sera or globulin fractions were serially diluted in an 0.1 M NaOH-glycine buffer at pH 8.6; isotonic saline was not a satisfactory diluent because of nonspecific clumping of mitochondria. A drop of diluted serum was placed on a slide and a drop of antigen added. These were mixed with an applicator stick, the slide was repeatedly tilted, and the agglutination read on a scale of 0 to 4+ after 2 minutes. Mitochondria, with a diameter of about 0.8 μ by electron microscopic measurements, do not change greatly in size in 0.44 M sucrose; the particles, therefore, do not differ significantly in size from latex particles. Agglutination was also performed with mitochondrial fragments prepared by treatment for one minute with an Ultra-Turrax model TP 18/2.[1]

Precipitation reactions were carried out on mitochondria fragmented by ultrasonic oscillation in a Raytheon 10-kc sonic oscillator for 30 minutes. The largest remaining particles were discarded after centrifugation at 37,000 × G for 10 minutes; 0.4 ml samples of the remaining suspension of fragments—similar to the electron transport particles of Green (15)—were incubated for 30 minutes at room temperature with an equal volume of undiluted whole or fractionated serum. The mixture was then refrigerated overnight after the addition of 0.4 ml of 5 per cent bovine serum albumin in isotonic saline, or 0.4 ml of saline alone. After the tubes were inspected for gross precipitation, they were spun at 37,000 × G for 5 minutes and the degree of precipitation determined by assay of the supernatant fluid for disappearance of DPNH oxidase activity; 0.1 ml was incubated for 30 minutes at 37° C in a total volume of 1.5 ml containing 1.3 ml of 0.05 M phosphate buffer at pH 7.6 and 0.3 or 0.6 μmole of DPNH. The reaction was terminated by boiling and 2.0 ml of 0.1 M NaOH-glycine buffer at pH 10.6 was added. After centrifugation at 37,000 × G for 30 minutes, the remaining DPNH was determined fluorimetrically by the method of Lowry, Roberts, and Kapphahn for autofluorescence of DPNH (16). These assays were frequently confirmed by a spectrophotometric assay of DPNH disappearance at 340 mμ; for these determinations 0.1 ml of supernatant fluid, 2.8 ml of 0.05 M phosphate buffer at pH 7.6 and 0.1 ml of 3×10^{-3} DPNH were used. The spectrophotometric method lacked sufficient sensitivity in some instances.

Other enzyme methods. L-Glutamic dehydrogenase was assayed by the method of Strecker (17), as well as by a more sensitive modification employing a 37° C incubation for 30 minutes and a fluorometric assay for DPNH (16). Potassium cyanide in a final concentration of 0.001 M was added if there was significant DPNH oxidase activity in the enzyme preparation. Since no glutamic dehydrogenase activity was demonstrable in whole mitochondria, the supernatant fractions of sonicated preparations were routinely assayed after centrifugation at 37,000 × G for 30 minutes.

[1] Janke and Kunkle, Staufen, Breisgau, West Germany.

DPNH-cytochrome C reductase and succinate-cytochrome C reductase were measured at 37° C with a 5-cm light path in a Zeiss model PMQ II spectrophotometer. A constant slit width of 0.1 mm was employed. Samples of 0.1 ml of diluted mitochondria suspensions, antiserum, and a complement source or saline were mixed and allowed to stand in a cuvette for 5 minutes at room temperature. The increase in optical density at 550 mμ was recorded at 30 seconds and then at 1-minute intervals at 37° C after addition of 2.7 ml of a solution that gave a final concentration of the following reagents: 0.045 M phosphate buffer at pH 7.6, 1×10^{-3} M KCN, 2.6×10^{-5} M cytochrome C, and either 1.0×10^{-4} M DPNH or 1.7×10^{-2} M succinate.

DPNH dehydrogenase in sonicated mitochondrial particles was measured at room temperature with potassium ferricyanide at a final concentration of 3.3×10^{-4} M as the electron acceptor. The rate of decrease in optical density at 340 mμ was determined in a volume of 3.0 ml that contained 1×10^{-4} M DPNH, 1×10^{-3} M KCN, and 0.048 M phosphate buffer at pH 7.6. In some instances 3.3×10^{-5} M $K_3Fe(CN)_6$ was substituted for cytochrome C in the method described for DPNH-cytochrome C reductase. Succinic dehydrogenase activity in diluted whole mitochondria was determined at room temperature at 400 mμ. Final concentrations of reagents were: 1.3×10^{-2} M succinate, 1.0×10^{-3} M $K_3Fe(CN)_6$, 1.0×10^{-2} M KCN, and 0.045 M phosphate buffer at pH 7.6 in a volume of 3.0 ml. The method of Racker (18) was used to determine fumarase activity. Acid phosphatase was determined by the micromethod of Lowry and colleagues (19), with 0.2 M acetate buffer at pH 5.0.

RESULTS

1. *Tests for antimitochondrial antibodies*

A. Complement fixation. Sera from rabbits immunized with rat liver mitochondria fixed complement with whole mitochondria isolated by differential centrifugation, with mitochondria subjected to sonic disruption, and with the clear supernatant fluid remaining after centrifugation of the sonically fragmented particles (Table I). Titers of less than 1 : 16 were not considered significant because of the nonspecific reaction of control sera at these dilutions. In order to determine the specificity of the antibodies to mitochondria, nuclei and microsomes were diluted to the same final protein concentration, .05 mg per ml, and complement fixation titers determined (Table II). Relative specificity for mitochondria was apparent, although immunization caused a significant rise in titer with the other subcellular particles. Control sera (not shown) gave a constant nonspecific reaction at low dilutions. Complement fixation was also demon-

TABLE I

Complement fixation titers with rat liver mitochondria and subunits

Antigen*	Globulin†	Globulin dilution (reciprocal) 16	32	64	128	256	512
A. Whole rat mitochondria	Immune (B8)	4+	4+	4+	4+	3+	0
	Control (BC11)	±	0	0	0	0	0
B. Sonicated mitochondria	Immune (B8)	4+	4+	4+	4+	3+	0
	Control (BC11)	0	0	0	0	0	0
C. Supernatant fraction ‡ of sonicated mitochondria	Immune (B8)	4+	4+	4+	4+	3+	0
	Control (BC11)	0	0	0	0	0	0

* Dilutions of 1:256 of various rat liver mitochondrial fractions.
† Reconstituted ammonium sulfate precipitates brought to original volume of serum.
‡ Obtained from sonicated mitochondria after centrifugation at 100,000 × G for 2 hours.

strated with mitochondria from rat kidney and connective tissue (Table III), indicating a lack of organ specificity. Titers up to 1:8,192 were obtained in systems containing rat liver mitochondria as antigen; titers up to 1:64 were obtained with 1:32 dilutions of rat serum, a possible contaminating antigen. The data in Tables I and III were obtained with sera from two rabbits injected with mitochondria and from two controls. A total of 16 rabbits given mitochondria gave comparable results, fixing complement in titers 4 to 512 times higher than sera from rabbits given no injections, adjuvant alone, or sucrose alone. Reconstituted globulin fractions obtained by ammonium sulfate precipitation of antisera gave results comparable to whole serum in the complement fixation tests. With pooled human serum as a nonspecific control antigen, the complement fixation titers of both control and antimitochondrial sera were similar, indicating that there was no specific increase in "normal" cross-reactivity to this unrelated antigen as a result of immunization.

B. Agglutination. A slide agglutination test was devised as a screening procedure for the presence of antibodies to mitochondria. The specific antisera produced agglutination in dilutions of 1:5 to 1:10; control sera sometimes caused agglutination in titers up to 1:4. Globulin fractions obtained by ammonium sulfate precipitation reduced nonspecific agglutinating effects of all sera, apparently by eliminating albumin. Results of some of these assays are listed in Table IV. In addition, some globulin fractions of control and immune sera were reconstituted to a concentration of 1 g per cent; agglutination of mitochondria occurred only in the presence of antimitochondrial globulin, thus ruling out a nonspecific effect of increased globulin titers.

TABLE II

Complement fixation titers with three subfractions of rat liver

Rat liver antigen	Immune serum	Serum dilution (reciprocal) 16	32	64	128	256	512	1,024	2,048	4,096
Nuclei	B62	4+	4+	4+	3+	2+	1+			
	B64	4+	4+	4+	3+	1+				
	B66	4+	4+	4+	3+	±				
	B68	4+	4+	4+	3+	2+				
Microsomes	B62	4+	4+	3+	2+					
	B64	4+	4+	3+	2+					
	B66	4+	4+	3+	2+					
	B68	3+	3+	2+	1+					
Mitochondria	B62	4+	4+	4+	4+	4+	4+	4+	3+	2+
	B64	4+	4+	4+	4+	4+	4+	4+	2+	2+
	B66	4+	4+	4+	4+	4+	4+	4+	3+	1+
	B68	4+	4+	4+	4+	4+	4+	4+	1+	0

TABLE III

Complement fixation titers with kidney and connective tissue mitochondria

Antigen	Test material	Dilution (reciprocal) 16	32	64	128	256	512
Rat kidney mitochondria*	Globulin†						
	Immune (B7)	4+	4+	4+	3+	1+	0
	Immune (B8)	4+	4+	4+	4+	3+	0
	Control (BC10)	2+	0	0	0	0	0
	Control (BC11)	0	0	0	0	0	0
Rat connective tissue mitochondria‡	Serum						
	Immune (B7)	3+	2+	0	0	0	0
	Immune (B8)	4+	4+	4+	2+	0	0
	Control (BC10)	2+	±	0	0	0	0
	Contol (BC11)	±	0	0	0	0	0

* Diluted 1:128.
† Reconstituted ammonium sulfate precipitates brought to original volume of serum.
‡ Diluted 1:64.

C. Precipitation. It was noted that whole mitochondria or sonicated mitochondrial particles settled out of solution after prolonged incubation at 4° C with immune serum. Disappearance of DPNH oxidase activity from the supernatant fluid was a convenient method for quantifying the precipitation of mitochondria. Whole serum or reconstituted globulin fractions from antigen-injected rabbits caused a marked diminution in DPNH oxidase activity of the supernatant fluid; compared to tubes incubated with control sera, a decrease of about 90 per cent of the enzyme was consistently found (Figure 1). When the precipitate was resuspended in 0.44 M sucrose, about three-fourths of the activity lost from the supernatant fluid could be recovered. No significant loss of DPNH oxidase activity occurred in the control tubes during the procedure.

TABLE IV

Agglutination of mitochondrial particles by rabbit antisera

Rabbit*	Dilution 1:4	1:8	Rabbit†	Dilution undil.	2	4
B62‡	4+	1+	B7‡	3+	1+	0
B64‡	4+	1+	B14‡	4+	1+	0
B66‡	4+	1+	BC15‖	0	0	0
B68‡	4+	1+	BC50§	1+	0	0
BC61§	0	0				
BC63§	±	0				
BC65§	±	0				
BC67§	0	0				

* Whole serum.
† Globulin fractions reconstituted to original volume of serum.
‡ Injected with rat liver mitochondria in adjuvant.
§ Injected with adjuvant alone.
‖ Uninjected control.

2. Effect of antimitochondrial sera on enzyme activity

L-Glutamic dehydrogenase prepared from rat liver mitochondria was assayed in the presence of control and immune sera. No inhibition could be demonstrated when the enzyme activity was in the range of 10 to 30×10^{-9} moles of DPN reduced per minute at 25° C (about 1.0 mg of mitochondrial protein). Serum obtained from rabbits injected with crystalline bovine L-glutamic dehydrogenase in adjuvant did inhibit this amount of enzyme activity, and it was subsequently found that assay for inhibition was more sensitive when smaller amounts of enzyme were used. Accordingly, the antimitochondrial sera were assayed with lower substrate concentrations and as little as 13×10^{-9} g of purified bovine enzyme incubated for 12 hours at 37° C; the enzyme used in

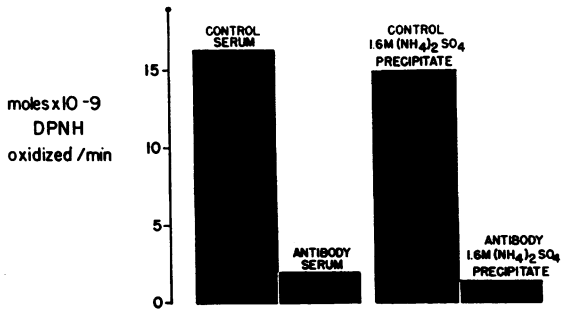

FIG. 1. DPNH OXIDASE ACTIVITY IN SUPERNATANT FRACTION AFTER INCUBATION OF MITOCHONDRIAL PARTICLES WITH WHOLE SERA AND RECONSTITUTED GLOBULIN FRACTIONS.

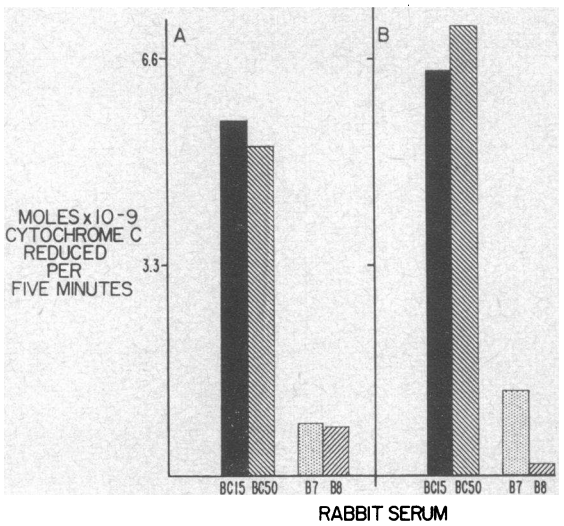

FIG. 2. A) DPNH-CYTOCHROME C REDUCTASE ASSAYS AFTER INCUBATION OF FRAGMENTED MITOCHONDRIA PARTICLES WITH TWO CONTROLS (BC15, BC50) AND TWO ANTIMITOCHONDRIAL RABBIT SERA (B7, B8).

B) SUCCINATE-CYTOCHROME C REDUCTASE ASSAYS AFTER INCUBATION OF WHOLE MITOCHONDRIA SUSPENSION WITH THE SAME FOUR SERA USED IN A.

these assays reduced between 1 and 3 $\times$ 10^{-6} moles of DPN during this prolonged incubation period. Inhibition of the activity of the bovine and rat liver enzymes was then demonstrable when immune sera were compared with sera from uninjected or adjuvant-injected rabbits. No inhibition of fumarase or acid phosphatase by antimitochondrial sera was demonstrable.

DPNH oxidase activity of whole and sonicated mitochondrial particles was inhibited by antimitochondrial sera. Sonication increased the DPNH oxidase activity of the mitochondria 5- to 10-fold; with either sonicated or whole mitochondria, inhibition was equivocal with preparations having enzyme activity in the range of 15 to 50 $\times$ 10^{-9} moles of DPNH oxidized per minute at 25° C. Moderate degrees of inhibition of DPNH oxidase activtiy occured when antimitochondrial sera were incubated for 10 minutes at room temperature with sonicated preparations diluted to one-fifth to one-tenth as much enzyme activity.

A sensitive method for assaying the DPNH-cytochrome C reductase portion of the DPNH oxidase system was devised that permitted use of smaller amounts of enzyme in the assay; as little as 0.01 mg of sonicated mitochondrial protein in a final volume of 3.0 ml gave satisfactory enzyme activity. Up to 88 per cent inhibition of enzyme activity by antimitochondrial serum was demonstrated (Figure 2,A). In a system using whole mitochondria, inhibition of succinate-cytochrome C reductase activity was also clearly evident (Figure 2,B). Previously it had been found that sonically or physically disrupted mitochondria most readily reduced cytochrome C with DPNH as substrate. On the other hand, succinate more readily reduced cytochrome C in intact mitochondria, although the rate of this reaction was only about one-third to one-quarter the former. In both systems, marked inhibition of these reactions could be shown after incubation of the respective particles with antimitochondrial serum. The DPNH-cytochrome C reductase activity was inhibited by an average of 85 per cent in these assays; the succinate-cytochrome C reductase activity was inhibited by an

TABLE V

Summary of antibody effects on enzyme systems

Enzyme system	Rate*	Inhibition
	moles/min	
DPNH oxidase	2.0 $\times$ 10^{-8} DPNH	−
DPNH oxidase	2.0 $\times$ 10^{-9} DPNH	+
DPNH-cytochrome C reductase	1.0 $\times$ 10^{-9} cyt. C †	+
Succinate-cytochrome C reductase	1.0 $\times$ 10^{-9} cyt. C †	+
DPNH dehydrogenase	2.0 $\times$ 10^{-9} DPNH *	−
Succinic dehydrogenase	5.0 $\times$ 10^{-8} $K_3Fe(CN)_6$	−
Glutamic dehydrogenase	3.0 $\times$ 10^{-8} DPN	−
Glutamic dehydrogenase	3.0 $\times$ 10^{-9} DPN †	+
Fumarase	1.0 $\times$ 10^{-8} fumarate	−
Acid phosphatase	5.0 $\times$ 10^{-8} *p*-nitrophenol †	−

* Expressed as moles of substrate or cofactor reacting per minute. Rates given for systems inhibited by antiserum are the maximal rates assayed at which inhibition occurred; rates given for those systems not inhibited are the minimal rates assayed.

† Assayed at 37° C; all other systems assayed at 25° C.

average of 89 per cent. In systems using ferricyanide to accept electrons from DPNH or succinate, inhibition by antiserum could not be demonstrated.

The enzymes assayed for inhibition by the rabbit antimitochondrial serum and the conditions used are summarized in Table V.

3. *Effect of complement on enzyme inhibition by antibody*

It was found that DPNH oxidase inhibition was consistently enhanced by the addition of fresh guinea pig, rabbit, or human sera to the incubation system. Most or all of this enhancing effect was lost on heating the sera at 56° C for 30 minutes. The heat-labile, antibody-activating factor therefore resembles complement. Although the term complement is used for brevity, it is apparent that the effective factor (or factors) has not yet been proved to be part of the complement system.

Fresh human serum enhanced the inhibitory effect of rabbit antimitochondrial serum on DPNH oxidase activity to a greater extent than did reconstituted guinea pig serum (Figure 3). The enhancing effect of fresh rabbit serum was variable, but under the conditions of room temperature incubation used in these studies, rabbit serum was always less effective than human serum, and usually less effective than guinea pig serum. These findings were in sharp contrast to the relative titers of complement in the standard hemolytic assay in which the guinea pig serum was most potent, as has been reported previously (20). Figure 4 shows the influence of various dilutions of pooled fresh human serum on the inhibition of DPNH oxidase activity by rabbit antimitochondrial serum; 1:4 dilutions of human serum with saline gave optimal potentiation of enzyme inhibition. Figure 5 shows the enzyme inhibitory effect of varying dilutions of rabbit antiserum in a system containing a 1:4 dilution of fresh human serum. The degree of inhibition was determined by comparison to mitochondrial particles incubated with heat-inactivated serum from control rabbits; addition of fresh human serum to control rabbit serum did not affect mitochondrial enzyme activity. Addition of fresh human serum to rabbit antisera did not potentiate inhibition of the DPNH

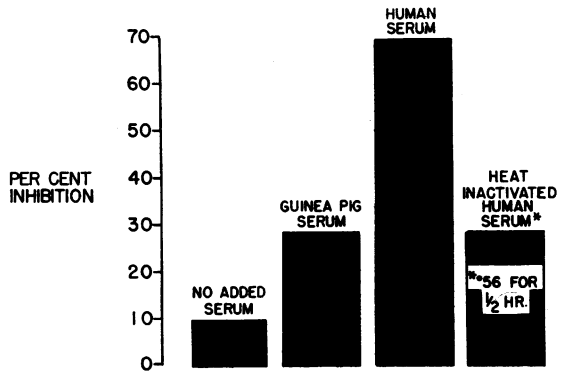

FIG. 3. INHIBITION OF DPNH OXIDASE ACTIVITY OF SONICATED MITOCHONDRIAL PREPARATIONS INCUBATED WITH ANTIMITOCHONDRIAL SERA IN THE ABSENCE OF ADDITIONAL SERUM, AND IN THE PRESENCE OF GUINEA PIG AND HUMAN SERUM. Inhibition by antibody expressed as percentage of activity in the control system, which contained same components except that serum from uninjected rabbits was substituted for antiserum. There was no loss of activity in the control sample during the incubation with normal rabbit serum.

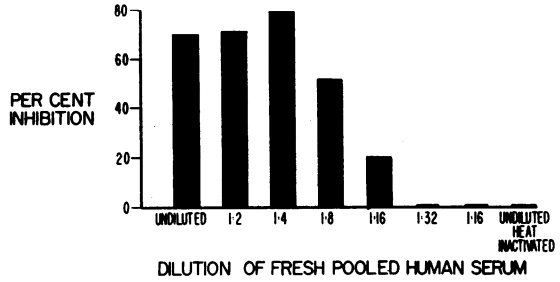

FIG. 4. INHIBITION OF DPNH OXIDASE ACTIVITY BY UNDILUTED RABBIT ANTIMITOCHONDRIAL SERUM INCUBATED WITH VARIOUS DILUTIONS OF FRESH POOLED HUMAN SERUM. Inhibition expressed as in Figure 3.

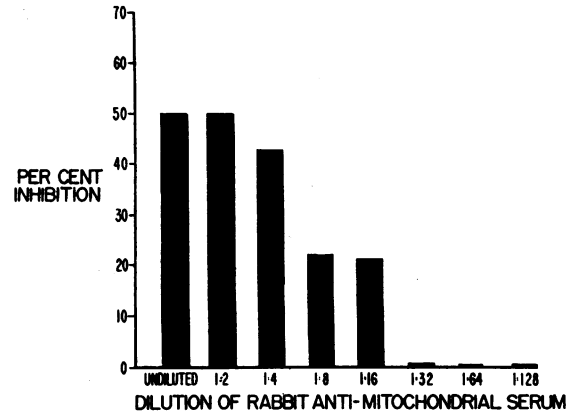

FIG. 5. INHIBITION OF DPNH OXIDASE ACTIVITY BY SERIAL DILUTIONS OF RABBIT ANTIMITOCHONDRIAL SERUM IN THE PRESENCE OF FRESH POOLED HUMAN SERUM DILUTED 1:4. Inhibition expressed as in Figure 3.

and succinate-cytochrome C reductase system, nor the DPNH dehydrogenase.

DISCUSSION

Morgan, Perlmann, and Hultin (21) demonstrated antibody formation in response to the injection of subcellular particles; injection of rat liver microsomes and cell sap into rabbits produced antisera with a fair degree of fraction specificity. To date, all rabbits injected with rat liver mitochondria in complete Freund's adjuvant have responded with a significant rise in antibody titer, as measured by complement fixation or by the less sensitive agglutination and precipitation tests. The moderate amount of reactivity with nuclei and microsomes was not unexpected, since it is likely that they share many common antigens with mitochondria. It is also likely that the mitochondrial antigen was contaminated to some extent by these particles, particularly by nuclei that would not be washed out if they happened to be carried over into the mitochondrial fraction after the first preparative centrifugation. The minor degree of reactivity found when these sera were tested for complement fixation with rat serum might be due in part to contamination of the rat mitochondrial antigen preparations with serum. The definite cross-reactivity with rat kidney mitochondria revealed a close antigenic relationship between the mitochondria of different organs. Since the antisera inhibited mitochondrial enzyme activity, it is notable that a lack of organ and species specificity of antibodies to enzymes can be demonstrated (13).

The activity of the multi-enzyme mitochondrial electron transport system was affected by these antimitochondrial sera; DPNH oxidase, DPNH-cytochrome C reductase and succinate-cytochrome C reductase were consistently inhibited. It is not clear why DPNH oxidation was not inhibited when ferricyanide was used as electron acceptor; presumably the transfer of electrons in some way bypasses the block caused by the antiserum in the other assays. The addition of cytochrome C to the DPNH oxidase preparation increased the over-all reaction rate, and decreased the percentage of inhibition by antisera. Progesterone, which had been shown by Yielding and Tompkins (22) and by them, Munday, and Cowley (23) to inhibit the electron transport system, slowed the DPNH oxidase reaction but did not significantly alter the percentage of inhibition caused by the antisera.

Although the mechanisms of the inhibition of the electron transport system by antibody to mitochondria is not definite from these data, it is evident that sites of access for DPNH were not totally blocked, since the DPNH-ferricyanide system was not inhibited.

The labile serum factor inhibiting the DPNH oxidase system in the presence of antibody was presumably related to the complement system. It was of particular note that human serum had a greater potentiating effect than guinea pig or rabbit serum, and is now being used routinely to increase the sensitivity of assays for DPNH oxidase inhibition by antisera. Dilution of antimitochondrial sera or their globulin fractions results in loss of inhibitory activity; addition of fresh human serum to these dilute systems restores the inhibitory effect of the antisera. This phenomenon suggests that serum complement in the presence of antibody results in an inhibitory effect on a particle-bound enzyme system. Whether complement binding can enhance the inhibition of crystalline enzyme by antibody remains to be demonstrated; we have been unable to show such an effect when assaying crystalline L-glutamic dehydrogenase after incubation with its specific rabbit antibody. It seems unlikely that inhibition of enzyme activity by antibody was due to physical coating or agglutination of the mitochondrial particles, in view of the selective effects on various parts of the electron transport pathway. In addition, there is evidence for reaction with single mitochondrial enzymes, such as glutamic dehydrogenase. Evidence has been presented that antibody to an enzyme can inhibit activity by reaction with substrate binding sites and that such antibody can cross-react with similar sites on analogous enzyme from other organs and species (13).

Although the addition of the heat-labile serum factor greatly increased the inhibition of the DPNH oxidase system, no increase in inhibition of the DPNH-cytochrome C or succinate-cytochrome C reductases was demonstrable. These selective effects suggest that the action of the complement-like factor was not simply due to further coating of the particles by additional proteins, causing physical interference or agglutina-

tion of particles. Gross agglutination of the particles was not evident in these dilute suspensions, an observation analagous to the sheep red cell-rabbit anti-sheep red cell system in which "antisera generally exhibit a high ratio of hemolytic to agglutinating activity" (20). A possible explanation for the effect of the labile serum factors in the presence of antibody is an action that results in increased permeability of the electron transport particles to some essential part of the electron transport chain. The possibility that increased permeability to cytochrome C occurs is being investigated.

Several mitochondrial enzymes and one lysosomal enzyme, acid phosphatase, were not inhibited by these antisera; these enzymes might not have been sufficiently antigenic under the conditions used, or the assay system employed may not have been sufficiently sensitive. These considerations apparently applied in the studies with glutamic dehydrogenase, since antiserum obtained by injection of purified enzyme was a much more potent inhibitor of the enzyme than antibody induced by whole mitochondria, but the enzyme could be inhibited by antimitochondrial serum when very small amounts of enzyme activity were used in the assays.

Studies of antimitochondrial antibodies in sera of patients with SLE have confirmed the existence of factors that will react with rat liver mitochondria in complement fixation assays; the mitochondrial agglutination test useful in following titers of antimitochondrial rabbit sera was not sensitive enough for use with human sera. Complement fixation by SLE sera was demonstrable with intact mitochondria and with the soluble supernatant material obtained after sonic disruption of mitochondria. Precipitation of whole mitochondria by SLE sera was demonstrable by assay for disappearance of DPNH oxidase activity. Mitochondrial enzyme inhibition by SLE sera is currently under study by use of the most sensitive assay conditions found by study of the induced rabbit antimitochondrial antibodies.

SUMMARY

1. Antisera produced in rabbits by injection of rat liver mitochondria fixed complement and caused agglutination and precipitation when incubated with mitochondria *in vitro*. Some reactivity at lower titers was also noted with rat liver microsomes and nuclei.

2. Several parts of the mitochondrial electron transport chain were effectively inhibited by antimitochondrial sera.

3. A factor in fresh human serum was found to increase greatly the inhibition of mitochondrial DPNH oxidase activity by rabbit antimitochondrial antibody. A similar but less potent factor was demonstrated in guinea pig and rabbit serum. This activity could be destroyed by heating the sera at 56° C for 30 minutes, and was presumably related to the complement system.

ACKNOWLEDGMENT

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