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Reduction of Group Reactivity of Complement Fixing Antigen of Meningopneumonitis Virus by Potassium Periodate.* (20697)

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Past attempts at demonstrating the specific antigen in viruses of the psittacosis-lymphogranuloma group have yielded negative or inconclusive results. In this laboratory several approaches have been tried including extraction of the antigen with solvents, differential centrifugation, and search for the specific component during the early phase of virus multiplication (before the appearance of the new mature virus). The preliminary reports by Barwell(1,2) suggested that potassium periodate could uncover the specific complement fixation antigen, presumably by virtue of destroying the group reactive "component". It was therefore decided to employ this substance in our investigations. The results of these studies agree in principle with Barwell's recently published observations(3). However, in our experiments it was found that the unmasking of the specific antigen was limited to certain conditions. Some of the results are presented in this paper.

Materials and methods. Virus and antisera. Meningopneumonitis (MP) virus and antisera were prepared as described in a previous communication(4). Human convalescent lymphogranuloma venereum (LGV) sera were obtained from patients. All sera were inactivated by heating at 56°C for 30 minutes. They were stored at −20°C. *Antigens.* Crude and purified antigens were prepared from infected allantoic fluid as described in the previous report(4). *Treatment with potassium periodate.* Virus was deposited in lusteroid tubes by centrifugation at 13000 RPM for 30 minutes. The

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TABLE I. Comparison of the Effect of KIO_4 on Various Preparations of MP Antigen.

Serum *vs.*	Antigen dil.	Antigen: Crude heated (Prep. 1) No KIO_4 treatment	Crude heated (Prep. 1) Treated with KIO_4	Purified heated (Prep. 2) No KIO_4 treatment	Purified heated (Prep. 2) Treated with KIO_4	Purified unheated (Prep. 3) No KIO_4 treatment	Purified unheated (Prep. 3) Treated with KIO_4
MP	1:2	120*	20	40	<10	120	40
	1:4	120	20			40	P20
LGV	1:2	960	960	960	<30	1920	60
	1:4	960	960			960	<30

* Reciprocal of highest dilution of serum showing complete fixation.
P = Partial (2+) reaction.

sediments were resuspended in one-half the original volume of veronal buffer (pH 7.3) containing periodate in a final concentration of 0.005 M. On a few occasions, higher and lower concentrations of periodate were used but 0.005 M appeared to be most satisfactory. Action of periodate was allowed to proceed at 37°C for 30 minutes. Shorter time intervals were also employed but they gave less clear-cut results. The activity of periodate was neutralized by the addition of an amount of 5% glucose equal to the volume of periodate. A control preparation consisted of deposited virus suspended in a volume of veronal buffer (pH 7.3) equivalent to half the volume of starting material and also placed at 37°C for 30 minutes. An equal amount of 5% glucose was added to this virus suspension. Treatment with potassium periodate was carried out on four types of preparations: 1) Crude heated antigen. 2) Purified heated antigen obtained from 2 cycles of differential centrifugation. 3) Purified unheated antigen obtained from 2 cycles of differential centrifugation. 4) "Reconstituted crude" antigen. Representative findings obtained with preparations 1, 2, and 3 are shown in Table I.

Experimental. Effect of periodate on crude heated virus: (Preparation 1). Infected allantoic fluid was centrifuged at 1000 RPM for 10 minutes and the supernatant was heated at 100°C for 20 minutes. The heated preparation was then centrifuged at 13000 RPM for 30 minutes. The sediment was exposed to periodate in a manner described above. Periodate seemed to have *little or no effect when the antigen was tested with the heterologous system.* In the homologous system periodate brought about a 4- or 8-fold decrease in CF activity. Other experiments have consistently yielded similar results as regards the heterologous system. However, with the MP virus-MP antiserum combination, the results were irregular. Sometimes periodate reduced the activity of the crude antigen, other times it appeared to have no effect. The irregular decrease of CF activity observed in the homologous system cannot yet be explained.

Effect of periodate on purified heated antigen: (Preparation 2). Preparation was purified in the following manner: 20 ml of virus were centrifuged at 1000 RPM for 10 minutes and the resultant supernatant was centrifuged at 13000 RPM for 30 minutes in a Sorvall Angle centrifuge. The sediment was resuspended to the original volume in cold veronal buffer (pH 7.3) and the procedure was repeated. The sediment obtained from the second high speed centrifugation was resuspended in veronal buffer to the original volume and was heated at 100°C for 20 minutes. Treatment with periodate was as described under *Materials and methods.*

The results show a striking difference from those obtained with Preparation 1. Both preparations were strictly group reactive—the specific antigen having been destroyed by heating; yet the group reactivity of the first preparation persisted while that of the second preparation disappeared following treatment with KIO_4.

Effect of periodate on purified unheated antigen: (Preparation 3). Virus was washed by means of 2 cycles of differential centrifugation (same as preparation 2) and the final sediment treated with periodate in the manner described. The material was not heated and therefore contained, prior to treatment, both the specific and group reactive components. Following treatment, this preparation showed

TABLE II. Effect of KIO_4 on "Reconstituted MP Crude" Antigen.

Serum *vs.*	Antigen dil.	Purified heated antigen, control	Purified heated antigen + .005M KIO_4	Antigen reconstituted— in 1st & 2nd washing, heated, control	Antigen reconstituted— in 1st & 2nd washing, heated + .005M KIO_4
MP-hamster	Und.	80	20	80	80
	1:2	40	<20	40	20
	1:4	P40	—	<20	<20
LGV-human*	Und.	160	20	160	160
	1:2	80	<20	40	20 P40 P80
	1:4	P40	—	<20	<20

* Pool of sera from 3 patients.

no group reactivity or only a slight amount of such reactivity. It retained, however, a considerable amount of specific activity. Thus, with the unheated treated antigen preparation shown in Table I the heterologous serum reacted to only 3% or less of the original titre, while the homologous serum still exhibited a titre of 33% of the original.

Effect of periodate on "reconstituted crude" antigen: (Preparation 4). In one experiment, virus was washed as described in the previous sections. The final sediments were suspended either in a volume of veronal buffer or in a 1 to 1 mixture of the supernatants obtained from the first and second high speed centrifugations. Both preparations were heated and portions separated and treated with periodate in the following fashion: 9 ml of antigen were mixed with 1 ml of a 0.05 M periodate solution and the mixture incubated at 37°C for 30 minutes. The effect of periodate was stopped by the addition of 1.1 ml of a 50% glucose solution. The results in Table II compare the purified heated antigen with the "reconstituted crude" heated antigen. They show that while periodate had an effect on the purified preparation, it had little or no effect on this antigen when the purified virus was resuspended in the supernatants derived from the washing procedure. It would appear therefore that some undetermined component or factor associated with the viral antigen was shed in the washings. This is probably the component responsible for the resistance of the original crude heated preparation to the action of periodate.

Discussion. It was found that under certain conditions the group reactive "component" is susceptible to the action of periodate and can thereby be reduced or eliminated from an antigen of the MP virus. The specific component appeared to be fairly resistant. In order for KIO_4 to exert its full effect, the antigens had to be washed. The absorption experiments with crude and purified antigens(4), the presently recorded experiments on the effect of periodate on such preparations as well as studies now in progress on the effect of heat and phenol on CF activity of MP antigen preparations which were subjected to various procedures of washing and sonic vibration point to a heterogenous nature of the group reactive "component". It appears that the "component" is made up of at least one loosely bound constituent which is resistant to periodate and at least another constituent which is intimately bound to the virus particle and which is susceptible to periodate.

It is not clear at the present time whether the loosely bound component which is removed by washing and which is resistant to periodate is a naturally occurring group reactive constituent of the fresh antigen or whether it is artificially effected by the heating of the material with the resultant conversion of the specific component to a group reactive antigen or whether it is a gradually accumulating substance resulting from natural denaturation of the virus.

The effect of KIO_4 on antigens of other viruses of the psittacosis-lymphogranuloma group has not yet been determined in this laboratory.

Summary. It was found possible to reduce the group reactivity of the MP CF antigen by treatment with KIO_4. Three kinds of results were obtained depending on the state of the original antigen. 1) The group reactivity of

the crude antigen was left practically unaltered. 2) Most of this reactivity was removed by KIO_4 from the purified antigen. Such preparations became inactive or only slightly active in the CF test with either homologous or heterologous antisera since the specific activity was previously destroyed by heating. 3) Antigens possessing the heat-labile specific component as well as the KIO_4-susceptible group-reactive "component" could be rendered fairly specific by treatment with KIO_4. It appears that the group reactive "component" is not a single entity, but is made up of at least 2 antigenic constituents.

1. Barwell, C. F., *Nature,* 1948, v162, 460.
2. ———, *ibid.,* 1949, v164, 1013.
3. ———, *British J. Exp. Path.,* 1952, v33, 268.
4. Pollikoff, R., and Sigel, M. M., *J. Immunol,* in press.

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Adsorption of A_2isoagglutinogen-like Substances of Infectious Agents on Human Erythrocytes.* (20698)

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The A_2isoagglutinogen-like substance is the term applied to a polysaccharide complex which is either extracted from or secreted by animal parasites and bacteria. This substance is characterized by the following immunological properties: 1) The polysaccharide, as isolated chemically from the infectious agent, inhibits the a_2 isoagglutinins when added to human blood serums. The agglutinin titer in the serums against erythrocytes of Group A_2 (a_2 agglutinin titer), as observed at 37°C, becomes negative. There is also a slight inhibition of the a_1 isoagglutinins, but this may be due to the fact that the a_1 and a_2 antibodies may be partially bound together(1). 2) The polysaccharide is secreted when the living infectious agent is incubated in human serums. The titer of the a_2 isoagglutinins in the serums becomes negative. There is also a slight reduction in titer of the a_1, but no effect on the β isoagglutinins(2). 3) When the polysaccharide is injected intravenously into rabbits and monkeys, the titer of the a_2 isoagglutinins, as detected at 37°C, is reduced to negative for a period of 24 hours, and remains low for a period of 96 hours. The titer of the agglutinins acting at 6°C (cold agglutinins) against human and sheep erythrocytes, is also reduced after inoculation of the polysaccharide(3).

The term A_2isoagglutinogen-like substance seems therefore, appropriate, since the polysaccharide reacts specifically with the a_2 isoagglutinins in the serums. Studies on the possible relationship of the A_2isoagglutinogen-like substance with certain pathological conditions are being continued. Attempts were made to adsorb the polysaccharide onto human red blood cells to determine whether the A_2isoagglutinogen-like property could be transmitted to the erythrocytes.

Materials and methods. The erythrocytes utilized in this study were obtained from blood donors attending the Blood Bank of the School of Medicine, at San Juan, P. R. The red blood cells were washed at least 3 times with buffered saline (Bacto Hemagglutination Buffer, Difco) before using. Polysaccharides were extracted from dry and pulverized *Ascaris lumbricoides* from pig, larval forms of *Trichinella spiralis* from rat muscle, and adult *Taenia saginata*. The method of preparation has been reported previously(3). Glycogen, prepared in the same way, and rice starch, were used as controls. Ten mg of polysaccharide were suspended in 1 cc of buffered saline, to which 0.5 cc of packed erythrocytes was then added. The mixture was incubated at 37°C, or stored at 6°C, for one hour, with frequent shakings,

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