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Detection of Antibody by Complement-Fixation in Sera of Man and Monkey Convalescent from Mumps.*

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The importance of mumps as a disease among military personnel is considerable. In the last war it stood third among the diseases causing time lost from duty, being surpassed only by venereal diseases and influenza.[1, 2]

Although the work of Johnson and Goodpasture,[3] using *Macaccus mulatta* as the experimental animal, established the etiological agent of mumps as a filterable virus, the practical implications of their findings have not been realized. Thus, no laboratory tests for the diagnosis of the infection or tests for susceptibility are available. Moreover, no means are at hand by which the potency of convalescent sera which have been employed as prophylaxis may be assayed. Accordingly, we undertook the repetition of the basic experiments of Johnson and Goodpasture with the object of extending them in these directions. In this paper we report briefly on a complement-fixation test for the detection and estimation of antibody in the sera of man and monkey convalescent from mumps.

About 3 cc of saliva collected on the 1st and 2nd days of the disease from a typical case of epidemic parotitis was introduced into Stensen's duct of a monkey (*Macaccus mulatta*). On the 5th day the gland, which was somewhat enlarged, was removed under ether anesthesia, weighed and emulsified by grinding in infusion broth to make a 20% suspension. This was then inoculated into both ducts of another monkey. The animal, on the 7th day following inoculation, developed marked swelling of both parotid glands with considerable subcutaneous edema of the cheeks. In this way the virus has been passed in series through 5 monkeys—the last of

* This work in part has been carried out as one of the projects of the "Chemical, Clinical and Immunological Investigations of Human and Other Serum Globulins," under contract with the Committee on Medical Research of the Office of Scientific Research and Development.

1 Michie, H. C., *The Medical Department of the United States Army in the World War*, Vol. IX, pp. 451-462, Government Printing Office, Washington, D.C., 1928.

2 Wesselhoeft, C., *New England J. Med.*, 1942, **226**, 530.

3 Johnson, C. D., and Goodpasture, E. W., *J. Exp. Med.*, 1934, **59**, 1.

which received material obtained by filtration of the parotid suspension through an Elford collodion membrane of 515 mμ pore diameter. The filtrate, which was shown to be bacteriologically sterile by aerobic and anaerobic incubation in suitable media, produced a disease in all respects similar to the unfiltered suspensions. Examination of sections of the parotid glands from all the infected monkeys revealed the pathological changes described by Johnson and Goodpasture.[4] Such changes were not observed in sections of glands removed from monkeys dying either of poliomyelitis or dysentery.

Antigens for use in complement-fixation tests were prepared by grinding with sand or alundum weighed portions of infected glands removed at the height of the disease. Sufficient physiological salt solution was added to make a 20% suspension. The suspension was then centrifuged at about 750 rpm for 10 minutes and the supernatant fluid was stored in sealed tubes in a CO_2 box (infected parotid suspension). Parotid glands removed from monkeys infected with dysentery bacilli or the virus of poliomyelitis were treated in the same manner to obtain suspensions of parotid to be used as controls (non-infected parotid suspension). At the time a test was set up, parotid suspensions were diluted with 5 times their volume of salt solution and centrifuged at 3500 rpm for 30 minutes in an angle centrifuge. Of the supernatant fluids, amounts of 0.2 cc were used in the tests.

The sera which have been tested for the presence of complement-fixing antibody have been obtained from monkeys before and at various times after infection with the virus of mumps and from human beings during acute stage of the disease and convalescence. To remove the anticomplementary activity which was occasionally marked, particularly in the monkey sera, the latter were heated at 62.5°C for 20 minutes. Satisfactory results were usually obtained with human sera which were heated at 60°C for 20 minutes. This procedure, suggested by Casals and Palacios,[5] was effective not only in removing the anticomplementary effect of sera, *per se*, but also in eliminating a certain amount of non-specific fixation which sometimes occurred with parotid suspension from monkeys not suffering from mumps. Sera were diluted by two-fold decrements and 0.25 cc of each dilution was employed in the test.

Pooled guinea pig serum was used as complement. This was preserved in the CO_2 box where its titer appeared to remain essentially unchanged for at least 2 weeks. Two units of complement contained in 0.25 cc were employed.

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[4] Johnson, C. D., and Goodpasture, E. W., *Am. J. Path.*, 1936, **12**, 495.

[5] Casals, J., and Palacios, R., *J. Exp. Med.*, 1941, **74**, 409.

The hemolytic system consisted of 0.5 cc of a 1% suspension of sheep red blood cells sensitized with 2 units of antisheep cell rabbit serum.

The test was carried out in the following manner: Diluted, inactivated serum, complement, antigen (parotid suspension from infected monkey) and sufficient saline to bring the volume to 1 cc were added in that order. The controls included in each test were as follows:

1. Complement alone
2. Serum alone
3. Serum and complement
4. Serum + complement + non-infected parotid
5. Infected parotid alone
6. Infected parotid + complement
7. Non-infected parotid alone
8. Non-infected parotid + complement
9. Salt solution

The mixtures were kept at 4 C for about 18 hours, when the sensitized cells were added. After incubation for ½ hour at 37°C in the water bath, the mixtures were again placed in the icebox and final readings made after the unhemolyzed cells had settled. In the tables dilutions of serum denote those which existed in the mixtures before the addition of sensitized cells.

The results so far obtained may be summarized as follows: The sera of monkeys taken before inoculation with the virus of mumps or at the height of the disease have not fixed complement in the presence of the suspension of infected parotid. Sera obtained from 6 monkeys during various periods of convalescence have, in every instance, shown various capacities specifically to fix complement. The titers, taken as "two-plus" fixation, have ranged between 1:128 and 1:2048. Complement-fixing antibodies persist in the sera of

TABLE I.

Complement-fixation Tests on Specimens of Serum from a Monkey Inoculated with the Virus of Mumps.

Time serum drawn Days after inoculation	Reciprocal of serum dilution							Control Non-infected parotid + serum 1:16
	16	32	64	128	256	512	1024	
0	0	0	0	0	nd	nd	nd	0
6†	tr	0	0	0	0	0	0	0
19	++++	++++	++++	++++	++++	+++	+++*	0
34	++++	++++	++++	++++	++++	+++	++	0

++++ = complete fixation
tr = trace of fixation
0 = complete hemolysis
nd = not done

*A subsequent titration of this specimen gave ++ fixation when diluted 1:2048.
†This specimen was taken during the height of the disease.

TABLE II.
Complement-fixation Test on Sera from Human Cases of Mumps.

Patient	Time serum drawn Days after onset	Reciprocal of serum dilution 8	16	32	64	128	256	512	1024	Control Non-infected parotid + serum 1:16
A	4	tr	0	0	0	0	0	0	nd	0
	17	tr*	++	+±	0	0	0	0	nd	0
	26	+++±	+++±	+++±	+±	tr	0	0	nd	0
O	6	+++±	+++±	+++	+±	0	0	0	nd	0
	24	++++	++++	++++	++++	+++	+	tr	nd	0
E	4	0*	+	+±	+	tr	0	0	nd	0
	23	++++	++++	+++	++±	++	+±	+	0	0

++++ = complete fixation.
tr = trace of fixation.
0 = complete hemolysis.
nd = not done.
*A suggestion of prozone occurred in these sera which gave very weak reactions.

monkeys for at least 3½ months following infection. The results of complement-fixation tests done on samples of serum obtained from one monkey at various times are presented in Table I.

The results of titrations carried out in the same manner on sera obtained from human beings during the acute and convalescent stages of mumps are presented in Table II.

It will be observed that whereas the specimen obtained from patient "A" during the active disease exhibited practically no fixation, slight evidence for the presence of antibody was obtained in the "acute" serum of patient "E" and definite indication of such a factor in that of the corresponding serum of patient "O". These findings strongly suggest that complement-fixing antibody may appear early in certain individuals in contrast to others in whom its development may be delayed. The alternative hypothesis that antibody was present at the onset of the disease in the blood of patient "E" is manifestly improbable because of the weak reactions and relatively low titer of the acute serum compared with those of the specimen taken during convalescence. In the case of patient "O" this possibility definitely exists, but again is unlikely because of the rise in titer and increase in intensity of fixation shown by the specimen obtained on the 24th day. Certainty in respect to the question of whether or not complement-fixing antibody may sometimes be present at the onset of clinical symptoms must await the accumulation of additional data.

Although complement-fixing antibody is not always correlated with the protective capacity of antisera against viral agents, this is frequently the case. Its assay, then, in convalescent and normal human sera might serve as a tentative index to their value as pro-

phylactic agents until a satisfactory technic for the evaluation of protective antibody becomes available.

The complement-fixation test should also prove useful in determining the presence or absence of virus in attempts to propagate the latter in tissue cultures, the developing chick embryo or in material obtained from animals hitherto regarded as insusceptible.

Finally, it is not impossible that when appropriate surveys are completed on the incidence of positive and negative reactions in various age groups and in groups of persons giving positive and negative histories of the disease, the complement-fixation test may be found to be of value in the detection of the susceptible individual.

It is evident, however, that the extensive application of the test will depend upon the discovery of a more readily available and cheaper source of antigen.

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A Method for Demonstrating an Antidiuretic Action of Minute Amounts of Pitressin: Statistical Analysis of Results.

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Using single rats rendered diuretic by the administration of water and alcohol, it has been possible to detect the antidiuretic action of as little as 0.02 milliunit or 20 microunits of pitressin given intravenously. The method is conservative of time and material. As an assay technic, it is at least as accurate as others heretofore reported.

Healthy male rats, of unselected stock, weighing 180-220 g were used. They were fed a diet of dog chow cubes, with biweekly additions of fresh vegetables. Prior to the test, they were starved for from 12-18 hours, but water was allowed *ad libitum*. At the time collections of urine were begun, the environmental temperature was brought to 29-30°C, and was maintained constant by the use of an electric reflector heater. Solutions to be administered by gavage were warmed to 40°C.

All containers used for handling pitressin had been carefully washed in sodium hypochlorite solution 5% (commercial), rinsed with hot and cold tap water and distilled water, and dried with alcohol and ether. Ampoules from a single lot of pitressin (No. 161) were