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Immunologic Relations of the Psittacosis-Lymphogranuloma Group of Viral Agents.*

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Investigations in recent years have revealed a group relationship of viral agents,[1-6] isolated from various sources (man, birds, animals), and morphologically similar to the viruses of psittacosis and lymphogranuloma venereum.[7] Interest in this group has been stimulated through recognition of the frequency of infections in man with several of these agents.[8,9] The need for laboratory procedures which might aid in the recognition and study of these viruses has become increasingly evident.

Serological reactions have been of little value in differentiation and identification of the members of this group. Complement fixation tests, although of value in detecting infection by a member of the group,[9, 10] have indicated a broad antigenic similarity and have failed in general to differentiate sharply between the various agents.[11] The serum neutralization test, which has been perhaps of greatest value in identifying other viruses, was not applicable here because in general it has been the experience of investigators that infection in man and animals,[12] or artificial immunization with these agents,[3,13] does not produce a serum satisfactory for studying the antigenic relationships within the group by the neutralization test.

We have previously reported[13] that a neutralizing and protective antiserum of relatively high titer can be obtained against the Chicago strain of mouse pneumonitis,[14] a member of this group, by repeated intraperitoneal injections of chickens with infected mouse lungs. By similar methods we have more recently produced antiserums against the viruses of meningopneumonitis[15] and lymphogranuloma venereum. In the case of the latter virus we were unable to produce a neutralizing antiserum by injection of infected mouse brain but were successful when infected yolk sac was used. This report records our investigations to date on the antigenic relations of these viruses as studied by means of the serum neutralization test.

The tests were run by mixing 0.3-cc quantities of serial 10-fold dilutions of viral suspensions with equal volumes of serum. A series of virus dilutions mixed with normal chicken serum was always included for purposes of comparison. Following incubation at 22°C for one hour, groups of 6 mice (10 g)

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1 Beck, M. D., Eaton, M. D., and O'Donnell, R., *J. Exp. Med.*, 1942, **79**, 65.

2 Pinkerton, N., and Moragues, V., *J. Exp. Med.*, 1942, **75**, 575.

3 Francis, T., Jr., and Magill, T. P., *J. Exp. Med.*, 1938, **68**, 147.

4 Rake, G., Shaffer, M. F., and Thygeson, P., PROC. SOC. EXP. BIOL. AND MED., 1942, **49**, 545.

5 Thomas, L., and Kolb, E. M., PROC. SOC. EXP. BIOL. AND MED., 1943, **54**, 172.

6 Nigg, C., and Eaton, M. D., *J. Exp. Med.*, 1944, **79**, 497.

7 Rake, G., and Jones, H. P., *J. Exp. Med.*, 1942, **75**, 323.

8 Meyer, K. F., *Medicine*, 1942, **21**, 175.

9 Smadel, J. E., *J. Clin. Inv.*, 1943, **22**, 57.

10 Eaton, M. D., and Corey, M., PROC. SOC. EXP. BIOL. AND MED., 1942, **51**, 165.

11 Rake, G., Eaton, M. D., and Shaffer, M. F., PROC. SOC. EXP. BIOL. AND MED., 1941, **48**, 528.

12 Eaton, M. D., Beck, M. D., and Pearson, H. E., *J. Exp. Med.*, 1941, **73**, 641.

13 Hilleman, M. R., and Gordon, F. B., *Science*, 1943, **98**, 347.

14 Karr, H. V., *J. Infect. Dis.*, 1943, **72**, 108.

15 Hilleman, M. R., and Gordon, F. B., *J. Bact.*, 1944, **47**, 58 (Abstract).

TABLE I.
Representative Serum Neutralization Test with Meningopneumonitis (MP-F97) Virus.

Serums	Final dilutions of virus									Mean Infectivity Scores
	10-1	10-2	10-3	10-4	10-5	10-6	10-7	10-8	10-9	
Normal	5.0*	5.0	5.0	4.5	3.6	1.2	1.0	0.8	0.0	2.9
Antimeningopneumonitis (MP-F97)	5.0	5.0	2.8	1.3	1.0	0.8	0.0	0.0	0.0	1.8
Difference of mean infectivity scores (Numerical value for neutralizing ability of meningopneumonitis antiserum)										1.1

* Numbers represent average infectivity scores of 6 mice.

TABLE II.
Serum Neutralization Tests in Mice with the Psittacosis-lymphogranuloma Group of Viruses Using Chicken Antiserums.

Virus	Antiserums against:		
	Meningopneumonitis (MP-F97)	Mouse pneumonitis (Chicago)	Lymphogranuloma venereum
Meningopneumonitis (MP-F97)	+	0	
Ornithosis (207-Meyer)	+	0	
Mouse pneumonitis (Chicago)	0	+	0
Mouse pneumonitis (Atherton)	0	+	
Feline pneumonitis (Baker)	0	0	
Lymphogranuloma venereum	0	0	+

\+ = neutralization.
0 = absence of neutralization.

were inoculated intranasally under light ether anesthesia with 0.03 cc amounts of each mixture. On the 10th day all mice yet alive were autopsied and the extent of lung consolidation noted. Table I shows the results obtained in a representative titration, that of meningopneumonitis virus with its homologous antiserum. A numerical value for the infectivity of each mixture is obtained by averaging the infectivity scores of the individual mice, determined by the method of Horsfall.[16] By averaging the scores of a single serum a mean infectivity score is derived. By subtracting this mean infectivity score for immune serum from that of the normal, a figure is derived which gives a numerical value (1.1, Table I) to the neutralizing capacity of the serum for any given virus. This is admittedly a crude method but the single figure has the advantage of allowing a simple comparison between two serums or between the neutralizing capacity of a single serum for different viruses.

Using the 3 antiserums prepared in chickens cross neutralization tests were run and other members of this group of agents were tested in the combinations indicated in Table II. It will be seen that meningopneumonitis antiserum neutralized ornithosis virus as well as the homologous strain, but none of the other viruses against which it was tested. Antiserum produced against the Chicago strain of mouse pneumonitis neutralized not only the homologous strain but also the Atherton II strain.[6] Lymphogranuloma venereum antiserum failed to neutralize mouse pneumonitis (Chicago) the only heterologous virus tested to date.

It is noteworthy that no partial neutralizations occurred with these chicken antiserums. In the 2 cases of heterologous neutralization the serums neutralized to full titer, *e.g.*, the difference of mean infectivity scores for meningopneumonitis antiserum against meningopneumonitis virus was 1.1 (Table I); against ornithosis virus it was 1.3. The 2 corresponding figures for the mouse pneumonitis antiserum were 2.0 for the homologous strain (Chicago) and 1.8 for the heterologous (Atherton). Likewise where neutralization did not occur the figures were 0.0 or nearly so, indicating no difference between immune and normal serum. Our tests therefore indicate complete antigenic similarity or dissimilarity insofar as the neutralizing antibody is con-

[16] Horsfall, F. L., *J. Exp. Med.*, 1939, **70**, 209.

cerned. Serums of pigeons infected with psittacosis have likewise been reported as being highly specific.[17,18] Chickens have been found advantageous for the preparation of antiserums against a variety of substances (See reference 13).

The relations within this group as revealed by serum neutralization tests are in agreement with the relations established on other bases by other authors.[1,2,5,6,11,19] Our experiments suggest that neutralization tests with this type of serums is a more satisfactory method for studying the antigenic relations of this group of agents than those methods heretofore described.

17 Eddie, B., and Francis, T., Jr., PROC. SOC. EXP. BIOL. AND MED., 1942, **50**, 291.

18 Smadel, J. E., Wertman, K., and Reagan, R. L., PROC. SOC. EXP. BIOL. AND MED., 1943, **54**, 70.

19 Hamre, D. M., and Rake, G., *J. Bact.*, 1944, **47**, 312.

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Comparison of the Acid Humoral Intermediation of Stimulation in Respiratory and Non-Respiratory Muscles.*

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The strength of contraction of the rectus abdominus muscle of the frog immersed for varying periods (15-60 seconds) in weak acetylcholine solution (about 1 part in 500,000 parts of Ringer's solution, depending on the sensitivity of the preparation) was found to increase with the hydrogen ion concentration of the environment. Such effects occurred within a range of pH 7.5 to pH 5.0. Lactic, phosphoric, and carbonic acids produced comparable effects. These results agree with those previously reported in the rectus abdominus.[1,2] However, when the sartorius muscle was used it was found to respond with a weaker contraction under increasing hydrogen ion concentration of the environment. This unexpected finding led us to make comparative observations on a number of muscles of the frog, turtle and alligator. The rectus abdominus, mylohyoid and geniohyoid of the frog, mylohyoid of the turtle, and diaphragm of the alligator, muscles which contribute to the respiratory act, were found to show a progressively increasing strength of contraction with an increasing cH of the acetylcholine-containing environment, while the sartorius, peroneus longus and gracilis minor of the frog, muscles possessing primarily a locomotor function, showed either a consistent depression or an initial augmentation followed by an early depression.

This differential effect of acid on these two groups of muscles is of interest in suggesting, for the lower forms at least, that the control of breathing is not strictly a central reflex phenomenon but is supported by a peripheral motor adjustment as well. The chemical (acid) control of respiration may thus be extended to the respiratory muscles as well as to the carotid body and the intra-cranial portions of chemically sensitive control centers. How the differential humoral response of respiratory and non-respiratory muscle to cH has been attained is a problem requiring further study. The observation of Carey[3] that expansion and extension of the processes of the motor end plate of intercostal muscles of the rat occurs during hypercapnia suggests

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1 Mason, A., and Gesell, R., *Fed. Proc.*, 1942, **1**, 58.

2 Gesell, R., Mason, A., and Brassfield, C., *Am. J. Physiol.*, 1944, **in press.**

3 Carey, E. J., PROC. SOC. EXP. BIOL. AND MED., 1941, **47**, 67.