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Activation of Latent Mouse Pneumonitis Virus by Human Serum.*

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During an investigation of specimens from a patient with primary atypical pneumonia, pleural fluid was inoculated intranasally in albino Swiss mice. Many of these animals showed extensive pulmonary consolidation, during the second week after inoculation. When impression films of the consolidated areas were stained by the Macchiavello method, numerous red-staining intracellular granules were seen which bore a close morphological resemblance to the elementary bodies of psittacosis.

Serum freshly obtained from this patient was also inoculated intranasally in mice, and the majority developed a similar type of pneumonitis. Subsequently, several samples of serum freshly obtained from normal persons were inoculated in mice. Almost all of the mice which received normal human serum intranasally were found to have developed pulmonary lesions which were indistinguishable from those produced by the pleural fluid. Moreover, structures resembling elementary bodies were seen in impression films prepared from many of their lungs. Similar lesions

* The Bureau of Medicine and Surgery of the United States Navy does not necessarily undertake to endorse views or opinions which are expressed in this paper.

were not encountered in the lungs of normal uninoculated mice. When either the pleural fluid or the serum was heated at 56°C for 30 minutes and then inoculated, no pulmonary lesions were produced in mice.

Because of the possible relation of these observations to the general problem of latent viruses, and also because of their obvious importance in relation to the interpretation of results of attempts to recover viruses from human material by the intranasal inoculation of mice, this phenomenon was studied further.

Samples of fresh human serum from 6 normal adults were each inoculated intranasally into groups of mice under light ether anesthesia. After 8 days some of the mice were killed, and in about 80% of the animals the lungs were found to contain irregular circumscribed areas of greyish or pearly, and often lobulated, consolidation, varying in extent from pinhead-sized foci to lesions involving the major portions of 4 lobes. The lesions were found usually at the hilar region, and extended outwards therefrom, but sometimes occurred also at the periphery. Similar areas of consolidation were seen in the lungs of the remaining mice which were killed after 16 days, although in most instances the pneumonitis was less extensive at this time. Impression films prepared from the cut surface of the pulmonary lesions, stained by the Macchiavello technic, showed structures resembling elementary bodies in about one-half of the preparations studied. These bodies were sometimes present in abundance, but more often a prolonged search was necessary before they could be found. The microscopic pathology of the pulmonary lesions was characterized by areas of dense parenchymal infiltration with polymorphonuclear cells, and lesser numbers of cells of the mononuclear series. The smaller bronchi and alveoli were often filled with cellular exudate which consisted chiefly of polymorphonuclear cells.

Transmission of the disease in mice was attempted by the inoculation of 10% suspensions of consolidated lung tissue intranasally, intracerebrally or intraperitoneally in other groups of mice. No evidence of disease resulted from either intracerebral or intraperitoneal inoculations. In many instances, intranasal inoculation also gave negative results, or produced areas of consolidation in only a small percentage of animals. On 3 occasions, however, intranasal passage resulted in pulmonary lesions in about 50% of inoculated mice, and in 2 instances it was possible to continue transmission of the disease for 3 serial passages. In both series the 4th passage yielded negative results. Attempts to transmit the disease directly from mice to cotton rats, guinea pigs and Syrian hamsters were unsuccessful.

Suspensions of consolidated lung tissue from mice which had received serum were inoculated on the chorioallantoic membrane of 8-day-old chick embryos, and serial passages were made from embryo to embryo by this route at 3-day intervals. A total of 12 different pools of consolidated lung tissue from mice which had received normal human serum were passed in this manner. From 2 of these pools (No. 6 and No. 7) an elementary body virus was obtained after the third membrane passage. This virus was morphologically indistinguishable from the virus of psittacosis.

When 10^{-1} or 10^{-2} dilutions of suspensions of infected embryo membranes were inoculated intranasally in mice, a fulminating pneumonitis resulted which caused death of all animals within 36 hours. Higher dilutions of the membrane suspensions, *i.e.*, 10^{-3} through 10^{-6}, produced only chronic infections in mice, and extensive pulmonary lesions were still present 6 weeks after inoculation. The pathology of the pulmonary lesions produced appeared to be identical with that seen after infection by either the virus of mouse pneumonitis (Nigg[1]), or cat pneumonitis (Baker[2]). Both of these latter agents have been shown to be members of the psittacosis-lymphogranuloma group.[3,4]

The embryo passage virus, like the Nigg and Baker viruses, was pathogenic for mice only when inoculated intranasally. Intracerebral or intraperitoneal inoculations with high con-

1 Nigg, C., *Science*, 1942, **95**, 49.

2 Baker, J. A., *Science*, 1942, **96**, 425.

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

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

TABLE I.
Results of Cross Complement Fixation Tests with Strains No. 6 and No. 7, and Members of the Psittacosis-Lymphogranuloma Group of Viruses.

Immune serum against	Complement fixation titer against indicated antigen					
	Strain No. 6	Strain No. 7	Psittacosis virus	Baker virus	Nigg virus	Normal yolk sac
Strain No. 6	1:128	1:128	1:64	1:64	—	0
" " 7	1:16	1:16	1:16	1:8	1:8	0
Psittacosis virus	1:256	1:256	1:256	1:256	1:256	0
" "	1:64	1:64	1:64	1:16	1:32	0
Baker "	1:64	1:64	1:128	1:128	1:128	0
Nigg "	1:16	—	1:8	1:16	1:64	0
Normal serum	0	0	0	0	0	0

centrations of the virus produced no evidence of disease, and the virus could not be detected in either the brains or spleens of mice following such inoculations. The embryo passage virus was pathogenic for both cotton rats and hamsters when inoculated intranasally, but not by other routes.

Hamster lung tissue containing the virus was employed for the immunization of hamsters by both the intranasal and intraperitoneal routes. Following immunization antisera were obtained which in high dilution were capable of fixing complement with yolk sac antigens prepared from either of the 2 embryo passage strains.

Cross complement fixation tests with other members of the psittacosis-lymphogranuloma group of viruses were carried out. Yolk sac antigens as well as hamster antisera against the Nigg virus, the Baker virus, and the two embryo passage strains (strains No. 6 and No. 7) were prepared by methods which have been described previously.[4] Psittacosis virus antigen was prepared from chick embryo tissue culture by the method used as a routine in this laboratory.[5] Antiserum against psittacosis virus was obtained from immunized mice, as well as from human patients convalescent from psittacosis.

The results of these cross complement fixation tests are shown in Table I. Sera from animals immunized with either strain of virus, obtained from mice inoculated with human serum, fixed complement with the homologous as well as with each of the heterologous virus antigens tested. Moreover, antigens prepared from each of these strains caused fixation of complement in the presence of either the homologous or the heterologous antisera. These results indicate that both strains of virus are related antigenically to the Nigg, Baker, and psittacosis viruses.

The factor in certain human sera which leads, on intranasal inoculation, to the development of pneumonitis in mice has not been identified, nor is the mechanism by which this pneumonitis is induced understood. This serum factor was found to be thermolabile, and was destroyed completely by heating at 56°C. Attempts to restore the property to heated sera by the addition of unheated serum from either mice or guinea pigs were unsuccessful. That the factor may be present in the serum of species other than human beings is suggested by results obtained in preliminary experiments with ferret serum. Pneumonitis was produced in mice by intranasal inoculation of ferret serum in dilutions as high as 1:16.

Summary and Conclusions. The intranasal inoculation of fresh human serum in mice resulted in the development of pulmonary consolidation in a high percentage of animals. Structures resembling elementary bodies were seen in impression films prepared from these lesions. Serial passage in mice yielded irregular results, although in 2 series consolidation recurred in each of 3 passages. Serial passage on the chorioallantoic membrane of chick embryos resulted in the isolation of 2 strains of a pneumotropic elementary body virus. Cross complement fixation tests demonstrated that this virus was related antigenically to other members of the psittacosis-lymphogranuloma group of viruses. These observations suggest the possibility that this virus

[5] Smadel, J. E., *J. Clin. Invest.*, 1943, **22**, 57.

may be present in latent form in a considerable number of laboratory mice. The finding that the virus can be activated by the intranasal inoculation of fresh human serum seems of considerable interest.

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Effect of Sulfonamides on Toxic and Antigenic Actions of Endotoxins of Certain Gram-Negative Bacteria.

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Four independent groups of workers[1-4] have reported that sulfonamide compounds protect mice to a limited extent against the lethal effects of various bacterial toxins, especially the endotoxins* of gram-negative bacteria. On the other hand, some workers[5-7] failed to observe protective effects in similar experiments.

Hutner and Zahl[3] found that orally administered sulfanilamide protected mice against between one to ten LD_{50} of the endotoxin of *Salmonella typhimurium*. The object of the present study was: (1) to obtain a quantitative evaluation of the relative degree of protection provided by sulfanilamide, sulfathiazole, sulfapyridine, sulfadiazine, sulfamerazine, and sulfaguanidine,† (2) to ascertain if this protective action of the sulfonamides affects the immunizing properties of the endotoxins of gram-negative bacteria, as determined by the degree of resistance to the endotoxin developed by mice immunized while receiving sulfonamide treatment.

Relative Protection by Various Sulfonamides. The partially purified endotoxins of *Salmonella typhimurium* and *Shigella paradysenteriæ* Flexner were chosen as representative of endotoxins of gram-negative bacteria generally. These organisms were grown in large quantities in synthetic culture media, using methods described elsewhere.[8] The

[1] Levaditi, C., and Vaisman, A., *C. R. Soc. Biol.*, 1938, **128**, 463; Levaditi, C., Vaisman, A., and Reinié, L., *Ann. Inst. Pasteur*, 1938, **61**, 635.

[2] Carpenter, C. M., Hawley, P. L., and Barbour, G. M., *Science*, 1938, **88**, 530; Carpenter, C. M., and Barbour, G. H., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 255; Carpenter, C. M., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 354; Carpenter, C. M., *Rep. Proc. 3rd Internat. Congress Microbiology*, 1940, 595.

[3] Hutner, S. H., and Zahl, Paul A., *Science*, 1942, **96**, 563; Zahl, Paul A., and Hutner, S. H., PROC. SOC. EXP. BIOL. AND MED., 1942, **51**, 285.

[4] Birger, O. G., and Hal'perin, E. P., *Zeit. Mikrobiol., Epidemiol., Immun.* (U.S.S.R.), 1941, **5-6**, 94.

* For most gram-negative bacteria, the toxic factor present in the bodies of the organisms is probably part of the toxic principle somatic O antigen. In *Neisseria*, some species of *Hemophilus* and *Brucella*, and possibly the rough phase of most gram-negative bacteria, the toxin may be part of a nucleoprotein, different in chemical pattern from the O antigens studied by Boivin, Morgan and Partridge, and others. However, the mode of pathogenesis of both toxins appears similar, if not identical. We have therefore for these studies preferred the more inclusive term "endotoxin" or "toxic factor" rather than the more precise immunochemical descriptions.

[5] Long, P. H., and Bliss, E. A., *The Clinical and Experimental Use of Sulfanilamide, Sulfapyridine, and Allied Compounds*, 1939, Macmillan Company, New York City.

[6] Buttle, G. A. H., Parish, H. J., McLeod, M., and Stephenson, D., *Lancet*, 1937, **1**, 681.

[7] Gross, P., Cooper, F. B., and Lewis, M., *J. Inf. Dis.*, 1938, **63**, 245.

† We wish to express our appreciation to Lederle Laboratories, Inc., and to the American Cyanamide Company for generous gifts of sulfanilamide, sulfathiazole, sulfapyridine, and sulfadiazine, and to Sharp and Dohme for sulfamerazine.

[8] Zahl, Paul A., and Hutner, S. H., *Am. J. Hyg.*, in press.