NEUROLOGICAL SIGNS IN MICE FOLLOWING INTRACEREBRAL INOCULATION OF INFLUENZA VIRUSES[1]

NEUROLOGICAL signs have been produced in mice by intracerebral inoculation of allantoic fluids containing active virus of human and porcine influenza. Injection by this route of high-titered infected allantoic fluids or concentrates of virus prepared therefrom leads in 12 to 48 hours to marked hyperirritability in up to 100 per cent. of the animals. When suspended by their tails the mice exhibit marked tremor and clonic convulsions which may change suddenly into tonic convulsions. Death occurs in a high percentage of the infected animals. Mice surviving these attacks are markedly spastic for a few minutes and refractory to repetition of these convulsions for short periods of time. In a number of animals many such attacks have been induced in the course of several days before death occurred and only a few mice survived repeated convulsions for the period of observation (10 days). Depending on the concentration of virus in the inoculum the majority of the experimental animals die within 24 to 72 hours with the described signs either spontaneously or while they are suspended. However, death in convulsions has been noted on occasion as early as 12 hours or as late as 8 days after intracerebral inoculation. Preliminary histological examinations of the brain tissue showed definite changes of meningo-encephalitic nature.

The phenomenon described was considered to be due to influenza virus for the following reasons. Four different strains of mice from unrelated and widely separated sources were used and the reaction could be elicited in each. Control injections of normal allantoic fluid, sterile broth or particulate components of normal chorio-allantoic membranes did not produce any cerebral signs. These experiments serve to exclude the activation in a latent neurotropic virus in the experimental mice.

The cerebral signs were produced under certain conditions with all strains of influenza virus tested, *i.e.*, the PR-8, WS, Weiss, F-12 and F-99 strains of influenza A, the Lee strain of influenza B obtained from two different laboratories, and a strain of swine influenza virus. The uniformity of the results tends to eliminate the possibility of contamination of influenza preparations with an extraneous virus.

The agent responsible for the cerebral signs behaves like influenza virus in every respect studied. It appears to be subject to the same optimal growth conditions in the allantoic sac of chick embryos as described for the influenza viruses[2]: Dilute inocula, which yield allantoic fluids with higher concentrations of influenza virus, produce cerebral signs more readily and in a greater percentage than allantoic fluids derived from more concentrated inocula, which usually show lower titers of influenza virus. The yield of the agent, like that of influenza virus, depends on the time of incubation in that the highest concentrations of both will be found in allantoic fluids collected 48 to 72 hours after injection of dilute inocula whereas a subsequent decrease in titer occurs on further incubation. Consequently, the mortality of mice following intracerebral inoculation may vary from 0 to 100 per cent. according to the history of the infected allantoic fluid used for injection.

The agent may be concentrated by procedures used for influenza virus, *i.e.*, by precipitation with protamine,[3] by sedimentation in the high-speed centrifuge at 20,000 r.p.m. for 20 minutes or by the method of adsorption on and elution from chick red cells.[4]

Cerebral signs may be prevented by neutralization of virus preparations with high-titered immune sera derived from various species. The agent as present in influenza A cultures is neutralized only by anti-influenza A and not by anti-influenza B or anti-swine influenza serum, while the reaction due to influenza B preparations is inhibited only by anti-B and not by the other sera, and so forth. These relationships are shown in Table 1.

TABLE 1

RESULT OF INTRACEREBRAL NEUTRALIZATION TEST

Serum	Virus preparation																		Control saline			
	Influenza A (PR-8)						Influenza B (Lee)						Swine influenza									
Anti-PR-8	O	O	O	O	O	O	D_1	C_1	D_2	D_2	C_2	C_2	D_1	C_1	C_1	C_1	C_1		O	O	O	O
Anti-WS	O	O	O	O	O	O	C_2	C_2	C_2	C_2	C_2	C_2	C_1	C_1	C_1	D_2	D_2	D_3	O	O	O	O
Anti-LEE	C_1	C_1	C_2	C_2	C_2		O	O	O	O	O	O	D_1	C_1	C_1	C_1	D_2	O	O	O	O	O
Anti-Swine influenza	C_2	C_2	C_2	C_2*	O	O	C_1	D_2	D_2	D_2	C_2	C_2	D_1	D_5	O	O	O	O	O	O	O	O
Saline	D_1	C_1	C_2	C_2	C_2	C_4*	C_2	C_2	C_2	C_3			C_1	C_2	C_2	D_5	O	O				

O = no cerebral signs; C_1 = tonic convulsion on first day after inoculation; D_2 = died spontaneously on second day; * = clonic convulsions only.

[1] The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the Children's Hospital of Philadelphia.

[2] W. Henle and G. Henle, *Am. Jour. Med. Sci.*, 207: 705–715, 1944.

[3] L. A. Chambers and W. Henle, *Proc. Soc. Exp. Biol. and Med.*, 48: 481–483, 1941.

[4] G. K. Hirst, *Jour. Exp. Med.*, 76: 195–209, 1942; T. Francis, Jr., and T. E. Salk, SCIENCE, 96: 499–500, 1942.

The phenomenon has been observed, thus far, only with active influenza virus preparations. Inactivation by ultraviolet irradiation, heat or formalin rendered the preparations innocuous. On the other hand, irradiated preparations have given evidence of interference in accord with experience gained with the influenza viruses.[5] When high concentrations of irradiated virus were injected simultaneously with active homologous or heterologous virus by the intracerebral route reduction in the incidence of convulsions was noted as compared to the controls inoculated with active virus alone.

Although only active virus has been found to elicit the phenomenon, no propagation of the influenza virus in the brain tissue could be demonstrated. When brains were harvested from mice immediately after intracerebral inoculation of PR-8 or Lee virus, or 24, 48 and 72 hours later when cerebral signs were apparent, titration of emulsions of these brains in eggs revealed that the amount of influenza virus did not increase in the brain but rather decreased to less than 0.1 per cent. of the amount of virus found in the brain immediately after inoculation. Furthermore, attempts to pass the agents from mouse brain to mouse brain at three day intervals failed in several trials. No neurological signs were noted in the second brain passage and subcultures in eggs from the third cerebral passage failed to demonstrate the presence of influenza virus.

The phenomenon described can be elicited not only by influenza viruses grown in the allantoic cavity of the developing chick, but also by strains which have been maintained continuously by mouse lung passage. Potent suspensions of infected mouse lungs and concentrates therefrom behave essentially similar to infected allantoic fluids.

Neurological signs of similar nature caused by influenza virus have been reported previously by Stuart-Harris[6] and Francis and Moore,[7] who were able to adapt certain strains to mouse brain passage. The results of these authors differ from ours in various respects: Infected chick brain tissue cultures or mouse lungs served as starting material; the virus multiplied in the brain tissue; neurological signs developed only after from 3 to 12 cerebral passages; the incubation period varied from 3 to 11 days; and only two strains (WS and Melbourne) could be established in this way, while the PR-8 strain gave negative results. In contrast to these observations we used mainly allantoic fluid preparations of influenza virus which caused the indicated signs on first passage to the mouse brain, usually within 24 to 72 hours and in exceptional cases only as late as 6 to 8 days after inoculation; the agent could not be passed from brain to brain in series, and finally, all strains of influenza virus tested gave the result, provided enough virus was present.

These apparent differences may possibly be explained by the assumption that influenza virus in sufficient concentration is toxic for the brain tissue without showing propagation in the CNS. As shown by the other workers,[6,7] only a few strains are able to multiply in the brain tissue, in which case enough virus accumulates gradually to elicit the toxic reaction. This would imply a separation of the propagating property from the toxic activity. In this regard it is of interest to note that toxic properties of another group of viruses have been observed recently by Rake and Jones.[8]

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[5] W. Henle and G. Henle, SCIENCE, 98: 87–89, 1943; *Am. Jour. Med. Sci.*, 207: 717–733, 1944. J. E. Ziegler, G. I. Lavin and F. L. Horsfall, Jr., *Jour. Exp. Med.*, 79: 379–399, 1944.

[6] C. H. Stuart-Harris, *Lancet*, 1: 497–499, 1939.

[7] T. Francis, Jr. and A. E. Moore, *Jour. Exp. Med.*, 77: 717–728, 1940.

[8] G. Rake and H. P. Jones, *Jour. Exp. Med.*, 79: 463–486, 1944.

THE INHIBITING EFFECT OF SODIUM AZIDE ON MOLD GROWTH[1]

THE growth of molds on a variety of materials is of great economic and military significance. In addition to this well-known activity, infections of man and animals, particularly of the skin, are matters of considerable importance. In view of these facts, the chance observation that high dilutions of sodium azide (NaN_3) prevented the growth of *Penicillium notatum* seemed worthy of further investigation. The inhibiting action of sodium azide on biological processes was first described by Keilin and Hartree[2] in respect to catalase. Snyder and Lichstein[3] suggested the addition of this chemical to bacteriological media employed for the isolation of gram-positive organisms from specimens of urine and feces containing a preponderance of gram-negative bacteria since its selective inhibition for the gram-negative coliform bacteria had been demonstrated by Bryan, Devereux, Hirschey and Corbett.[4]

In determining the concentration of NaN_3 which would inhibit the mold growth, modified Chapek-Dox media[5] containing varying concentrations of NaN_3

[1] From the Hygienic Laboratory, University of Michigan, Ann Arbor.

[2] D. Keilin and E. F. Hartree, *Nature*, 134–933, 1934.

[3] M. L. Snyder and H. C. Lichstein, *Jour. Inf. Dis.*, 67: 113, 1940.

[4] C. S. Bryan, E. D. Devereux, W. C. Hirschey and A. C. Corbett, *North. Am. Vet.*, 20: 41, 1939.

[5] W. Kochalaty, *Jour. Bact.*, 44: 469, 1942. Constituents: $NaNO_3$ 2.0 g, KH_2PO_4 1.0 g, KCl 1.0 g, $MgSO_4$ 0.5 g,