related with amount of endotoxin administered, and there was no significant rise of plasma serotonin with a non-lethal dose.

1. Gilbert, R. P., PROC. SOC. EXP. BIOL. & MED., 1959, v100, 346.
2. Gordon, P., Lipton, M. A., *ibid.*, 1960, v105, 162.
3. Armin, J., Grant, R. T., *J. Physiol.*, 1957, v138, 417.
4. Shimamoto, T., Yamazaki, H., Sagawa, N., Iwahara, S., Konishi, T., Madzawa, H., *Proc. Japan Acad.*, 1958, v34, 450.
5. Humphrey, J. H., Jaques, R., *J. Physiol.*, 1955, v128, 9.
6. Waalkes, T. P., Weissbach, H., Bozicevich, J., Undenfriend, S., *J. Clin. Invest.*, 1957, v36, 1115.
7. Sanyal, R. K., West, G. B., *J. Physiol.*, 1958, v144, 525.
8. Davis, R. B., Meeker, W. R., McQuarrie, D. G., *Circ. Res.*, 1960, v8, 234.
9. Braude, A. I., Carey, F. J., Sutherland, D., Zalesky, M., *J. Clin. Invest.*, 1955, v34, 850.
10. Davis, R. B., *J. Lab. Clin. Med.*, 1959, v54, 344.
11. Udenfriend, S., Weissbach, H., Brodie, B. B., *Methods of Biochemical Analysis*, New York, Interscience Publishers, 1958, v6, 95.
12. Davis, R. B., Meeker, W. R., McQuarrie, D. G., *Surg. Forum*, 1959, v10, 401.
13. Barbaro, J. F., *J. Immunol.*, 1961, v86, 377.

Received September 18, 1961 P.S.E.B.M., 1961, v108

Hypothermia in Mice Due to Influenza Virus Infection.* (27064)

YONG T. YANG† AND CHARLES A. EVANS
Department of Microbiology, University of Washington, Seattle

Many of the naturally occurring influenzal infections of man are symptomless, and most of the overt infections are characterized by the occurrence of fever without pneumonia. In the mouse, which has been used extensively in studies of the pathogenesis of influenza, the main criteria of infection have been death and pulmonary consolidation. To determine whether the mouse could serve as an experimental model for the less severe infections that usually occur in man, we studied the temperature response of mice to infection with influenza virus.

Hypothermia, rather than fever, proved to be a striking feature of these infections. It was associated with extensive lung lesions. We were unable to produce in mice a satisfactory model for studying the difference between symptomless infection and illness without pulmonary consolidation.

Materials and methods. Mice. Female mice of an inbred strain, BALB/c, 4 weeks old, were used. Three weeks after birth, they were weaned and separated from the males. Twenty serial passages of lungs failed to show evidence of latent pulmonary viruses. Throughout this study, the room temperature in which mice were kept was maintained at 75° to 80°F.

Viruses. Two sublines of the PR8 strain of type A influenza virus were used. They were derived from stock virus which had undergone 8 passages in ferrets, 593 in mice, and 168 in eggs.

An "egg-line" virus was obtained by inoculating 0.1 ml of a 10^{-3} dilution of the stock virus into the allantoic cavity of 10-day embryonated hen's eggs. After 45 hours incubation at 37°C, infected allantoic fluids were pooled and frozen at −48°C. One ml of the pool contained $10^{7.5}$ infectious doses (ID_{50}) by the allantois-on-shell technique(1) and 512 HA units of virus.

A "mouse-passaged line" was obtained by 7 successive mouse-to-mouse passages of the stock virus at 3-day intervals. Infected lungs from mice of the 7th passage were removed aseptically and kept frozen intact at −48°C until used. Immediately before use, a 10% homogenate was made with Standard Medium(1) by grinding the infected lungs in a mortar with alundum. The homogenate was centrifuged at 2500 rpm for 10 min and the

* This study was supported in part by a grant from Nat. Inst. Health, U.S.P.H.S.

† On leave from Dept. of Microbiology, Chonnam Univ. School of Med., Kwangju, Chonnam, Korea.

supernatant was used as an inoculum.

Virus titration. The allantois-on-shell technique described by Fazekas de St Groth and White(1) was applied. Titers were expressed in terms of 50% infectious dose (ID_{50}) per ml.

Several points should be mentioned in applying this technique: 1) For preparation of the allantois-on-shell pieces, 13-day embryonated eggs were used exclusively. 2) Mycostatin (E. R. Squibb and Sons, N. Y.) added to the Standard Medium in a concentration of 20 units per ml effectively controlled contamination by fungi. 3) Titers obtained by this technique were approximately 10 times lower than those of egg infectivity titration (EID_{50}). 4) In determining the amount of virus in lungs of mice, it was found that the virus titers dropped rapidly when a 10% homogenate of the infected lungs was made immediately after removal from mice and kept frozen at −48°C until titration; however, when the infected lungs were kept frozen intact at −48°C and the homogenate was made just before titration, consistently higher titers were obtained.

Inoculation of mice and scoring of lung lesions. A mouse was anesthetized lightly with ether and 0.05 ml of the inoculum was given intranasally. Standard Medium was used as a diluent for the virus inoculum.

Gross lung lesions were scored by the extent of the pulmonary consolidation seen on the lung surface in relation to total lung surface area, i.e., 4+ lung lesion = complete consolidation, 3+ = consolidation of ¾, 2+ = ½, and 1+ = ¼ of total lung field. A numerical score was recorded for each mouse, and the total lung-lesion score of each group of mice was obtained by calculating a percentage of the total possible score(2).

Measurement of rectal temperature. Rectal temperature was measured with a Thermistemp Telethermometer Model TK (Yellow Springs Instrument Co., Yellow Springs, Ohio). For the measurement, a "small animal probe" was inserted into the rectum to a constant depth of 1 cm. About 20 seconds were necessary to obtain a maximum reading. To avoid possible mechanical damage to the rectum, a glycerine and water mixture (1:1) was used as a lubricant for the tip of the probe. Daily measurement was made between 2 and 3 PM.

Results. Temperature patterns in control groups. Four groups of 3 randomly chosen mice were put into 4 cages, and rectal temperatures were taken daily. The mice in Group I had no treatment. On the 4th day after grouping, mice in Group II received light ether anesthesia only, those in Group III were inoculated intranasally with Standard Medium under light ether anesthesia, and those in Group IV were similarly inoculated with normal allantoic fluid from 12-day eggs. Fig. 1 shows the results.

There appeared to be a slight fall in rectal temperature of some mice during the first 3 days after initiation of temperature measurement, but thereafter the temperature was fairly stable. None of the 3 treatments (in Groups II, III, and IV) caused a noticeable effect on rectal temperature of mice during the observation period of 8 days after each treatment.

In view of the results of this experiment, rectal temperatures were taken on 4 successive days before mice were used for experiments. The temperature on the 4th day was referred to as the baseline temperature, and was expressed as 0 in the following experiments. A rise or fall in rectal temperature after virus inoculation was indicated as + or − according to the deviation from baseline temperature.

Temperature changes of mice inoculated with egg-line virus. Six groups of 4 mice were used. After measuring baseline temperatures, mice of each group were inoculated with various amounts of egg-line virus. Rectal temperatures were taken daily. On the 7th day after infection surviving mice were killed, and lung lesions were scored. The results are presented in Fig. 2.

It is clear that more rapid and profound hypothermia was observed in the groups inoculated with larger amounts of virus. A fall in rectal temperature was not found in mice of the 10^{-6} group which received a small amount of virus, approximately 2 ID_{50}; and none of the 4 mice in this group had more than 2+ lung lesions. The total lung-lesion score of this group was 35% at end of experiment. One of the mice inoculated with a large amount of virus (see the 3rd mouse in 10^{-2} group) showed no demonstrable hypothermia in contrast to the findings observed in the

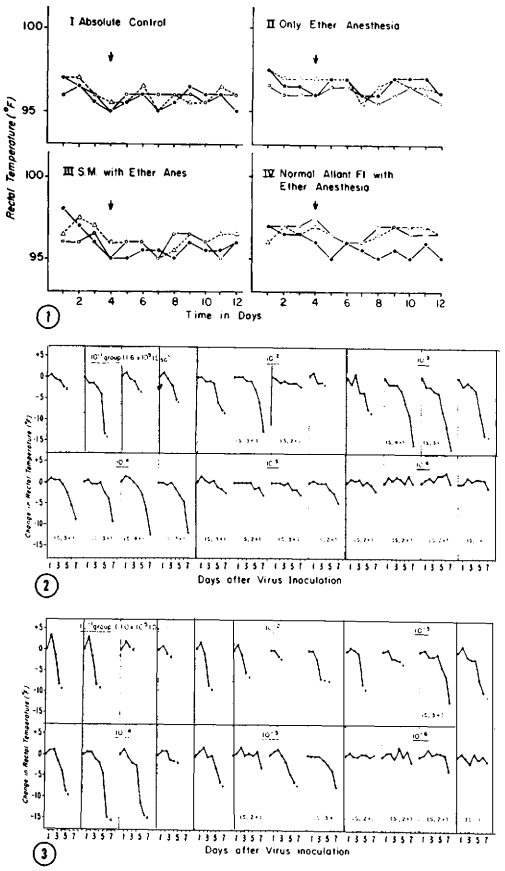

FIG. 1. Temperature pattern in the control groups of mice. Arrow indicates the time (day) of each treatment. S. M. = Standard Medium. Allant. Fl. = Allantoic fluid.

FIG. 2. Temperature changes of mice inoculated with egg-line of PR8 strain of influenza virus. 10^{-1} group (1.6×10^{5} ID_{50}) indicates that mice in this group were inoculated with a 10^{-1} dilution of egg-line virus, and calculated amount of virus inoculum per mouse was 1.6×10^{5} ID_{50}. 0 = Baseline temperatures of mice measured before virus inoculation. × = Death. (S,3+) = Survival with 3+ lung lesion at end of experiment.

FIG. 3. Temperature changes of mice inoculated with mouse-passaged line of PR8 strain of influenza virus. See footnotes, Fig. 2.

rest of the mice in this group. The mouse had 2+ lung lesion when killed at end of experiment.

Temperature changes of mice inoculated with mouse-passaged line of virus. In this experiment, mice, grouped as in the preceding experiment, were inoculated with various amounts of mouse-passaged virus. The results are shown in Fig. 3.

As in the preceding experiment with egg-line virus, mice responded to increased amounts of virus inoculum with more rapid development of hypothermia. There was a significant increase in mortality as compared with the preceding experiment. Early death obscured the occurrence of hypothermia in 3 of the mice that received the 10^{-1} and 10^{-2} inocula. The steepness of the temperature curve showing a sharp fall in rectal temperature by 3 days after infection was characteristic of these groups.

In general, it appeared that the mouse-passaged virus caused more rapid development of hypothermia than occurred with egg-line virus. For example, in mice given a 10^{-4} dilution of mouse-passaged virus, hypothermia developed about 24 hours earlier than in those given the same dilution of egg-line virus.

In a subsequent experiment, 3 mice were inoculated with a 10^{-7} dilution of mouse-passaged virus. For a period of 6 weeks their temperatures remained at the level of the baseline temperature even though autopsies made at the end of a 6-week period revealed that they had pulmonary lesions involving ¼ or less of the total lung field.

The course of hypothermia was observed for 6 weeks in a mouse infected with a 10^{-5} dilution of mouse-passaged virus. Its temperature remained at or below the −5 (°F) level for 2 weeks, then gradually returned to baseline temperature. At the end of the 6-week period, the rectal temperature of this mouse was close to the baseline temperature and the mouse appeared healthy. However, it was found at autopsy to have well-defined and markedly constricted lesions involving nearly half of the total lung area.

Relation of hypothermia to amount of virus in the lungs and to extent of pulmonary lesions. To study the relationship of hypothermia as shown in Fig. 2 to virus multiplication in the lungs and to the extent of lung lesions, the following experiments were carried out.

About 40 mice were inoculated with a 10^{-4} dilution of egg-line virus. At various intervals after infection, 3 mice were killed and the lung lesions were scored. The infected lungs were removed aseptically and kept frozen intact at −48°C until all specimens were titrated

simultaneously at the end of this experiment. Fig. 4 shows the results.

Following intranasal inoculation, there was no detectable virus in the lungs at 2 and 4 hours. These results constituted a typical lag phase in virus multiplication in the mouse lung as described by Davenport and Francis (3). The virus titer in the lungs reached a maximum level 48 hours after infection. A marked drop in virus titer was observed after the 6th day, presumably due to the action of antibodies produced in the mouse(4).

Lung lesions were seen first on the 2nd day and increased gradually thereafter. The total lung-lesion score was 60% at end of experiment.

From a comparison of the findings presented in Fig. 4 with those in Fig. 2 (see

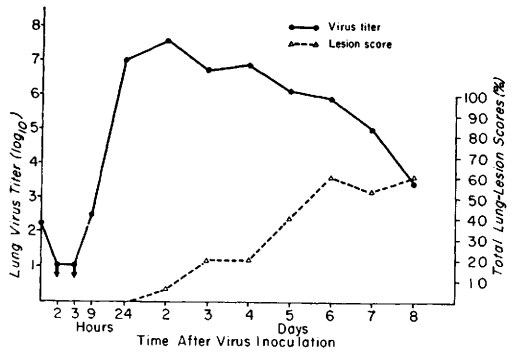

FIG. 4. Virus multiplication and development of lung lesions in mice inoculated with a 10^{-4} dilution of egg-line of PR8 strain of influenza virus. Virus titers were averages of the titers obtained from lungs of 3 mice killed at various intervals after inoculation.

10^{-4} group), it appears that as a rule hypothermia developed 3 to 4 days after a maximum concentration of virus was present in the lung. Furthermore, hypothermia apparently did not occur in the first several days during which lung lesions were present and relatively limited in extent. Onset of definite hypothermia on the 5th to 6th day corresponded to the time when the total lung-lesion score reached a level of about 50% and was continuing to increase.

In a similar experiment, mice were inoculated with a lesser amount of the same virus, i.e., a 10^{-6} dilution, which was approximately 2 ID_{50}. At 48 hours after infection the virus titer in the lungs reached the highest level, but in this case the titer was about one-hundredth of that obtained in mice inoculated with a 10^{-4} dilution of the virus. The virus titer remained at this level until the 7th day and then dropped sharply. Lung lesions were seen first on the 3rd day and increased more slowly than in the mice inoculated with a 10^{-4} dilution of the virus. Eventually the total lung-lesion score was about 40% at end of this experiment. As noted in discussing Fig. 2, mice in the 10^{-6} group did not show hypothermia during the experimental period.

Discussion. Among homeothermal animals, the mouse is known to have a relatively poor heat control mechanism. The control mechanism of body temperature may vary from one strain of mice to another as shown by McLaren(5). It has long been known that moderate changes in environmental temperature cause a significant change of body temperature of the mouse(6,7).

Mice have been shown to respond with hypothermia rather than with febrile responses in several kinds of experimental infection(8, 9,10,11). Therefore, it has been suggested that hypothermia is the outstanding characteristic of the thermal response of mice to endotoxins, as well as to a variety of other infectious and toxic agents(9).

In our study it appeared that hypothermia was not related directly to the level of virus multiplication but was related to the extent of pulmonary lesion. In mice inoculated with a 10^{-4} dilution of egg-line virus, hypothermia did not occur until the 5th to 6th day although the virus titer in the lungs was at its maximum level at 48 hours after infection. The initiation of hypothermia coincided with development of pulmonary lesion equal to 50% or more total lung-lesion score.

A transitory febrile response in mice was observed by other investigators in an early stage of some experimental infections which resulted in definite hypothermia in a later stage(9,10). Halberg and Spink(9) have shown that the rise in rectal temperature of mice after administration of *Brucella* endotoxin did not exceed the limit of the "physiological range." In our study, of 24 mice given mouse-passaged virus, 18 mice exhibited an elevation of rectal temperature of at least 1°F at 24 hours after infection, but in all cases

the temperature was well within the range observed in uninoculated mice.

Summary and Conclusions. 1. Hypothermia rather than fever was the characteristic thermal response of mice to intranasal inoculation of the PR8 strain of type A influenza virus. 2. Mice showed marked hypothermia with fatal termination in response to inoculation of large amounts, e.g., 1,000 or more infectious doses (ID_{50}), of 2 sublines of the virus, one adapted to embryonated eggs and the other to mice. 3. Inoculation of mice with a small amount of virus, e.g., one to two ID_{50}, did not cause hypothermia. In mice inoculated with about 2 ID_{50} of egg-line virus, the maximum level of virus in the lungs was lower than in mice inoculated with larger amounts of virus, and pulmonary lesions were present but failed to reach the level of 50% total lung-lesion score. 4. In mice inoculated with a moderate amount of egg-line virus, e.g., about 200 ID_{50}, the maximum virus level in the lungs was reached 3 to 4 days before the occurrence of hypothermia. Time of occurrence and degree of hypothermia appeared to be closely related to the extent of pulmonary lesions. 5. The mouse-passaged virus caused more rapid development of hypothermia than the egg-line virus. 6. It is concluded that hypothermia is related to the extent of pulmonary lesion rather than to the extent of virus increase *per se*.

1. Fazekas de St Groth, S., White, D. O., *J. Hyg.*, Camb., 1958, v56, 151.

2. Cohen, A., *Lancet*, 1960, v2, 791.

3. Davenport, F. M., Francis, T., Jr., *J. Exp. Med.*, 1951, v93, 129.

4. Ishida, N., Mizuno, H., Chiba, A., Nakazima, S., *Tohoku J. Exp. Med.*, (Japan), 1959, v71, 137.

5. McLaren, A., *Quart. J. Exp. Physiol.*, 1961, v46, 38.

6. Congdon, E. D., *Arch. Entw. mechan. der Organismen*, 1912, v33, 703.

7. Larson, W. P., Levine, M., Bieter, R. N., McLimans, W. F., *J. Bact.*, 1940, v39, 45.

8. ———, Bieter, R. N., Levine, M., McLimans, W. F., PROC. SOC. EXP. BIOL. & MED., 1939, v42, 649.

9. Halberg, F., Spink, W. W., *Lab. Invest.*, 1956, v5, 283.

10. Weir, J. A., *Proc. Iowa Acad. Sci.*, 1947, v54, 383.

11. Anderson, W. H., Brodersen, R., PROC. SOC. EXP. BIOL. & MED., 1949, v70, 322.

Received September 18, 1961 P.S.E.B.M., 1961, v108

Neutralization of "Long-Acting Thyroid Stimulator" of Graves' Disease by Antisera to Bovine Pituitary Thyrotropin.* (27065)

SIDNEY C. WERNER AND JOAN TIERNEY†

Department of Medicine, Columbia University College of Physicians and Surgeons, and Presbyterian Hospital, New York

Adams and Purves(1) assayed the serum of patients with Graves' disease for thyrotropin, in guinea pigs given I-131 to label the thyroid and then thyroxine to suppress endogenous release of thyrotropin. They observed the test animal's response to patients' serum to be different from that to standard thyrotropin or to serum from patients with myxedema. Injection of standard thyrotropin or of myxedema serum increased the circulating level of I-131 and a maximum value was reached after a few hours. The level then decreased and was significantly lessened by 16 hours. The serum from Graves' disease on the other hand caused little increase in level early after injection but produced a maximal response at 16 hours or later.

These findings have been amply confirmed in the mouse, similarly prepared for assay purposes except that the responses can be measured at 2 hours and 9 hours after injection(2). The term "long-acting thyroid

* Aided by grants from Division of Arthritis and Metabolism, Nat. Inst. Health, U.S.P.H.S.

† The authors are indebted to R. Siegal, C. H. R. Lange, and N. Gross for technical assistance.