Table II summarizes results obtained when mice were exposed to oxygen at 4 atm. press. for 5 minutes. Of a total of 144 animals, 2 convulsed during the first pressurization (1.3%). None of the mice pressurized once a day or on alternate days died. In the group receiving 2 exposures a day, a mouse died on the second day and another on the fourth day (4.2%). Two of 24 mice (8.3%) exposed 4 times a day died after the first day. On autopsy all of these mice exhibited the classic "liverlike" lungs observed in oxygen toxicity. Convulsions occurred at any time from the beginning of full pressurization to the start of decompression. The average time of onset of convulsions ranged from 2.0 to 4.5 minutes.

Microscopic examinations of the testes showed no testicular degeneration in any of the mice exposed to either 3 or 4 atm. press. In all the experiments on the second exposure to hyperbaric oxygen, convulsions occurred in a significant number of mice although they had no apparent reaction to an earlier identical first exposure. This effect still persisted 6 days after the initial pressurization. Aside from an increased mortality rate in the groups of mice exposed to 4 atm. press., there was no apparent pattern of cumulative toxic symptoms or tolerance to hyperbaric oxygen. Some of the animals did not convulse after a second exposure, indicating a wide range of individual tolerance to hyperbaric O_2, a finding which is well documented(3). Administration of chlorpromazine (20 mg/kg intraperitoneally) to the mice before the first exposure did not alter the incidence of convulsions following a second exposure.

The results of the present experiments provide additional evidence that there is a sensitization of the central nervous system of rodents to the toxic effects of hyperbaric oxygen. The mechanism of this phenomenon and the extent of its presence in man remain to be established.

Summary. Mice that did not convulse after an initial exposure to oxygen at 3 or 4 atmospheres of pressure (absolute), convulsed in significant numbers after a second exposure. This residual effect of "hyperbaric oxygen" lasted as long as 6 days.

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Received April 26, 1965. P.S.E.B.M., 1965, v119.

Role of Interferon and Partial Immunity in Influenza Virus Infection of Mouse Lung. (30302)

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Isaacs and Hitchcock(1) reported that a virus inhibitor similar or identical with interferon (ITF), produced in the lungs of nonimmune mice infected with a sublethal dose of influenza virus (PR8), promoted recovery. The presence of ITF in the lung prior to the appearance of neutralizing (NT) antibody in the serum supported this view. Loosli *et al*(2) showed that mice immunized with influenza virus (PR8) and exposed to a lethal aerosol of homologous virus became moderately ill but survived, whereas nonimmune controls died. In the present study, these findings were reexamined and extended by studying simultaneously the effect of ITF and NT antibody on influenza virus (PR8) infection of partially immunized mice.

Materials and methods. Viruses. The PR8 strain of influenza A virus was obtained through the courtesy of Merck, Sharp and Dohme Laboratories, West Point, Pa. The virus had received 128 allantoic passages in chick embryos and 4 mouse lung passages to produce a stock pool having a lethal mouse

lung (LD_{50}) titer of $10^{4.7}$ to $10^{4.9}$ and an infectious egg (EID_{50}) titer of $10^{7.7}$ to $10^{8.2}$ per 0.05 ml, respectively. The strain of vesicular stomatitis virus (VSV) was supplied by Dr. S. Baron of the National Institute of Infectious Diseases and Allergy, Bethesda, Md. The virus received 2 passages in chick embryo fibroblast tissue cultures to produce a stock pool having a titer of $10^{7.0}$ plaque forming units (PFU) per ml in mouse L 929 cells.

Mice. Male, albino Swiss mice, supplied by Dierolf Farms, Doylestown, Pa., weighed 10 to 12 g when vaccinated and 24 to 26 g at time of challenge.

Vaccine. Monovalent PR8 virus vaccine was produced and inactivated with formalin (CP) as described elsewhere(3). Infectious virus was not detected in the vaccine following 3 blind passages in chick embryos. The vaccine had a potency of 500 CCA (chicken cell agglutination) units per ml(3) and was stored at 4°C. Mice received a single intraperitoneal injection of 30 to 40 CCA units or formol saline.

Interferon studies. Fifteen days after vaccination, the mice were infected intranasally under light ether anesthesia with 10^3 to 10^6 EID_{50} per 0.05 ml of PR8 virus. Groups of 10 immunized and control mice were sacrificed at various intervals after infection and the lungs removed. Supernates from 20% lung suspensions were acid-treated and ITF titers determined by the 50% plaque reduction method with VSV(4,5).

Antibody studies. At the time of lung harvests for ITF studies, groups of 10 immunized and control mice were also sacrificed and sera and lungs from infected mice were harvested for determination of NT antibody content. Sera and supernates from 20% lung suspensions were stored at —70°C. On the day of test, they were thawed rapidly and inactivated at 56°C for 30 minutes. Lung supernates or sera were titrated for NT antibody content against 10 or 100 EID_{50} of PR8 virus, respectively(6). The tests were carried out utilizing 2-fold dilutions of inactivated serum mixed with an equal volume of a constant amount of virus. After incubation for one hour at 4°C, 0.2 ml volume of each mixture was injected into the allantoic cavity of 10- to 11-day-old embryonated white Leghorn chick embryos (5 eggs/group). The eggs were incubated at 36°C for 44 to 48 hours, then chilled at 4°C for 3 hours. Allantoic fluid from each egg was tested for the presence of virus hemagglutinin (HA) by addition of an equal volume of 0.5% suspension of chicken erythrocytes. NT antibody titers were expressed as the reciprocal of the initial dilution of serum showing complete inhibition of HA.

Virus titrations. Supernates from 10% infected lung suspensions from immunized and control mice were stored at —70°C prior to EID_{50}, LD_{50} and HA titrations(7,8). EID_{50} and LD_{50} titers were computed by the method of Reed and Muench(9).

Results. Interferon, immunity and virus growth. The effect of partial immunity on production of ITF and parameters of growth of virus in mice infected with a sublethal challenge of PR8 virus is summarized in Table I. The results indicated that ITF was not detected in extracts of lungs from immunized mice. The data also indicated that serum antibody did not prevent virus growth, as detected by the presence of infectious virus in the mouse lung on day 2 and day 3, nor did it inhibit subsequent production of lung consolidation. However, under these conditions, virus growth was not demonstrated when determined by the less sensitive mouse lethal and HA tests. In control mice, virus growth preceded the detection of ITF. Thereafter, virus titers remained level as ITF reached a peak on day 5. Although significant but declining infectious and lethal virus titers were demonstrated on day 7, ITF was not detected. Lung consolidation reached a maximum subsequent to these events. Finally, serum NT antibody was present in trace amounts on day 5, then increased in titer prior to its detection in lung extracts.

Virus growth and interferon. The effect of dose of virus challenge on production of ITF and growth of PR8 virus in partially immunized and control mice is presented in Table II. The data indicated that a linear response in virus growth to size of challenge was noted in all groups. Partially immunized

mice survived a challenge of 10^6 EID_{50} and responded with production of high levels of infectious and lethal virus prior to detection of ITF. When the virus challenge was reduced to 10^4 EID_{50}, resistance increased and virus growth was revealed by the detection of infectious virus. However, virus growth was not demonstrated by tests for lethal and HA virus. Finally, mice were resistant to a challenge of 10^3 EID_{50} and virus growth and ITF was not demonstrated. In control mice, production of ITF, virus titers and survival were related to size of challenge.

Discussion. In general, the present study confirms previous reports(10,11) and indicates that in the infection of mouse lung with the PR8 strain of influenza virus, the presence of NT antibody in the serum before virus infection plays an important role in resistance. The results also confirm findings of Isaacs and Hitchcock(1), and Baron *et al* (12) and indicate that ITF fails to persist during the later stages of viral replication. Yet, as reported by Isaacs(13, a causal relationship may exist between the time of decline in virus titers and demonstration of maximum levels of ITF prior to detection of NT antibody in the serum. However, in the absence of data which demonstrate virus growth in the lung without concomitant pro-

TABLE I. Growth of Influenza Virus Strain PR8 in Lungs of Partially Immunized and Control Mice.

Group*	Virus challenge EID_{50}	Lung harvest post-infection day	Pulmonary titers ITF[a]	EID_{50}[b]	LD_{50}[b]	HA[c]	Avg lung score[d]	NT antibody† Lung[a]	Serum
Immunized	10^3	1	$<10^0$	<1.0	<1.0	<1.0	.0	5	320
		2	ND‡	3.5	<1.0	<1.0	.0	ND	ND
		3	$<10^0$	4.0	<1.0	<1.0	.0	5	320
		5	$<10^0$	<1.0	<1.0	<1.0	.3	5	320
		7	$<10^0$	<1.0	<1.0	<1.0	.4	5	320
		9	ND	<1.0	<1.0	<1.0	.2	ND	ND
		11	ND	<1.0	<1.0	<1.0	.4	5	640
Control	10^3	1	$<10^0$	7.0	3.5	<1.0	.0	<5	<5
		2	ND	7.5	5.2	5.0	.0	<5	<5
		3	8	8.0	5.2	5.0	.1	<5	<5
		5	32	7.5	5.2	5.0	.5	<5	<5§
		7	$<10^0$	6.7	3.0	<1.0	1.5	<5	5
		9	$<10^0$	5.5	<1.0	<1.0	2.3	5	80
		11	ND	5.7	<1.0	<1.0	2.2	5	80

* Mice challenged intranasally 15 days post-vaccination with PR8 virus.
PR8 EID_{50} titer = $10^{8.0}$/0.05 ml.
† Reciprocal of initial dilution. ‡ ND = Not done. § Trace of NT antibody in undiluted serum.
[a] Reciprocal of dilution of 20% lung supernate. [b] Log_{10} [c] Log_2 [d] Lung consolidation score: 5+ = death and 100%; 4+ = 100%; 3+ = 75%; 2+ = 50%; 1+ = 25%; ± = <25%; 0 = 0%.

TABLE II. Growth Patterns of PR8 Virus in Lungs of Partially Immunized and Control Mice.

Group*	PR8 challenge EID_{50}	Pulmonary virus titers 24 hr post-infection EID_{50}†	LD_{50}†	HA‡	No. survivors /No. infected	Pulmonary ITF titers§ post-infection on day: 1	3	5	7
Immune	10^3	<1.0	<1.0	<1.0	10/10	$<10^0$	$<10^0$	$<10^0$	$<10^0$
	10^4	6.5	<1.0	<1.0	10/10	ND	ND	ND	ND
	10^6	7.8	4.8	2.0	10/10	$<10^0$	4	16	$<10^0$
Control	10^3	7.0	3.5	<1.0	9/10	$<10^0$	8	32	$<10^0$
	10^4	8.5	4.6	3.0	7/10	ND	ND	ND	ND
	10^6	8.8	5.6	8.0	0/10	$<10^0$	16	64	4

* Mice challenged intranasally 15 days post-vaccination with PR8 virus.
EID_{50} titer = $10^{8.0}$/0.05 ml.
† Log_{10} ‡ Log_2 § Reciprocal of dilution of 20% lung supernate.

duction of ITF, it cannot be stated with certainty that the presence of high levels of ITF prior to recovery did indeed accelerate the decline of virus growth. Thus, recovery may not result primarily from production of ITF, but may also be a consequence of other nonimmune host reactions, *e.g.*, replacement of infected and necrotic lung cells with newly formed cells resistant to infection(14), increase in local acidity(15) and reduction in oxygen tension(16).

In the immunized group, resistance to infection is mediated chiefly by serum NT antibody, and the mice survive a challenge dose of virus which is lethal for nonimmune mice. However, the presence of NT antibody does not inhibit virus growth nor prevent subsequent production of ITF. Although ITF is not detected in the lungs of immunized mice receiving a sublethal infection, it may have been produced in small local areas of the lung as a consequence of the limited virus growth. Therefore, under circumstances of immunized mice receiving high or low challenge, production of ITF in the lung possibly augments resistance.

Finally, the data demonstrate that specific serum NT antibody produced late in the pathogenesis of a primary influenza virus infection does not play a role in recovery. These results support the observations of other investigators(16). Moreover, the data indicate that in a primary infection of mouse lung with PR8 virus, low levels of NT antibody are detected in the lung after its appearance in the circulation. These findings suggest serum origin of the NT antibody rather than local production in the lung and agree with the observations of Fox(17) and Fazekas(18).

Summary. The mechanism of recovery from infection with the PR8 strain of influenza virus in nonimmune mice and in mice partially immunized with formalin-inactivated PR8 vaccine, was investigated. The results demonstrated that in the lungs of immunized mice limitation of virus growth is mediated primarily by specific serum NT antibody. ITF was not detected in the lungs of the immunized group challenged with a sublethal dose of virus but was demonstrated in the lungs of control mice from day 3 through day 5. It was not detected on day 7 when NT antibody appeared in the serum. Pulmonary virus growth, as indicated by tests for infectious, lethal and HA virus, was maximal from day 2 to day 5 at which time ITF content reached a peak. Thereafter, virus titers remained at a high level though declining when ITF was no longer demonstrated. The time of maximum lung consolidation was subsequent to these events. Partially immunized mice survived a lethal challenge of 10^6 EID_{50} and ITF was detected in the lung on day 5, whereas in the lungs of infected controls, higher ITF titers were present over a longer period but the mice did not survive. The implication of these findings is discussed.

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Received March 5, 1965. P.S.E.B.M., 1965, v119.