THE DEMONSTRATION OF LESIONS AND VIRUS IN THE LUNGS OF MICE RECEIVING LARGE INTRAPERITONEAL INOCULATIONS OF EPIDEMIC INFLUENZA VIRUS

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In attempting to induce the adaptation of a strain of human influenza virus, PR8 (1), to the central nervous system of mice, large doses of virus suspension were given intraperitoneally to the animals while simultaneous injections of meat infusion broth were made intracerebrally. No external evidence of infection was noted, but when the mice were sacrificed on the 7th day after inoculation pulmonary lesions of moderate extent were observed. The lesions in the gross were quite characteristic of those produced in the lungs of mice by the intranasal inoculation of influenza virus. They consisted of plum colored areas of edematous pneumonia involving one-fourth to one-half the lung tissue. Since no evidence had previously been obtained to suggest that pulmonary infection occurred with influenza virus administered by other than the intranasal route, further studies were undertaken to determine the distribution of virus in the animal body after inoculation by various routes.

While the present work was in progress, Smorodintsev and his coworkers (2) reported certain results showing that influenza virus could be recovered from the blood of mice for a period after its introduction into the peritoneal cavity but observed no pulmonary lesions. The present report is primarily concerned with the localization and survival of influenza virus in various organs of mice, especially the lungs, after its inoculation by the intraperitoneal route.

Materials and Methods

The PR8 strain of epidemic influenza virus maintained in mice through 150 to 240 serial intranasal passages was used throughout. Intranasal inoculation of

normal mice with 0.05 cc. of a 1:1 million dilution of infected mouse lung suspension almost invariably caused a fatal disease with typical, extensive pulmonary lesions, while with a 1:10 million dilution lesions were invariably produced with but occasional fatalities. The lethal end point of the virus is, therefore, considered to be 1:1 million, and 0.05 cc. of the 10 per cent suspension represents on the average 100,000 intranasal minimal lethal doses (M.L.D.).

Tests for virus were made by emulsifying the lungs of mice in meat infusion broth and subsequently inoculating this material into the nostrils of normal mice lightly anesthetized with ether (1, 3). Mice which received intraperitoneal or subcutaneous inoculations of virus were lightly anesthetized with ether and the material was administered from syringes through needles of 25 or 27 gauge. To avoid leakage and the consequent danger of accidental nasal contamination the site of inoculation was immediately painted with flexible collodion. The efficacy of this procedure in eliminating intranasal infection was shown by experiment in which virus was applied to the skin and the area painted with collodion. The lungs of mice so treated were tested for virus from 2 to 8 days after the application of virus to the skin. In no instance, even after serial passage, was virus demonstrable in the lungs. Other mice treated in a similar manner were tested for immunity by intranasal test 14 days later and were found to be completely susceptible.

Neutralization tests for the identification of virus were carried out with normal serum and with known immune serum according to the technique described by Francis and Magill (4).

The term, minimal lethal doses (M.L.D.), refers throughout to fatal doses as measured by intranasal infection of mice.

The Specificity of Pulmonary Lesions in Mice Following Intraperitoneal Inoculation of Virus

In a preliminary effort to determine whether the pulmonary lesions observed in mice after intraperitoneal injection of influenza virus were associated with the presence of influenza virus in the lungs, the following experiment was conducted.

To each of 4 anesthetized mice was given intraperitoneally 0.5 cc. of a 10 per cent suspension of the lungs of mice infected with the 149th mouse passage of the PR8 strain of epidemic influenza virus. On the 5th day after inoculation the mice were sacrificed and the lungs examined. Pulmonary lesions of +++, ++, ++, + severity, respectively, were noted. Passage made with suspensions of these lungs to normal mice resulted in fatal infections with complete pulmonary involvement. Neutralization tests with known immune serum demonstrated the presence of epidemic influenza virus in the involved lungs.

Attempts to induce similar changes in the lungs of etherized mice by the intraperitoneal inoculation of normal mouse lung suspensions

were unsuccessful. On the other hand, lesions were produced in the lungs of mice after intraperitoneal injection of virus even in the absence of ether anesthesia, demonstrating that etherization played no essential rôle in the development of the lesions. Using similar procedures it was established by repeated experiments that the lesions are associated with the presence of influenza virus in the lungs of the mice although virus may be present in the absence of gross pulmonary damage.

TABLE I

Variation in Pulmonary Lesions Produced by Different, Large Intraperitoneal Doses of Virus

Group No.	Virus suspension	Quantity given	Approximate number of intranasal M.L.D.	Day of autopsy	Number of mice	Pulmonary lesions noted							
	per cent	*cc.*											
1	5	0.5	500,000	6th	5	+	0	0	0	0			
2	2	0.5	200,000	6th	5	+++	++	+	0	0			
3	1	0.5	100,000	6th	5	±	0	0	0	0			
4	2	0.5	200,000	6th	9	++	++	++	+	±	0	0	0 0
5	10	0.5	1,000,000	4th	7	±	0	0	0	0	0	0	
6	10	0.1	100,000	4th	8	±	0	0	0	0	0	0	0
7	5	0.5	500,000	4th	7	++	++	+	+	+	+	+	
8	5	0.2	200,000	4th	8	++	++	++	++	+	+	+	0
9	2	2.0	800,000	4th	5	++	++	++	+	0			
10	2	1.0	400,000	4th	8	++	++	++	++	++	++	+	+
11	2	0.5	200,000	4th	8	+	+	±	±	0	0	0	0
12	2	0.1	40,000	4th	8	+	0	0	0	0	0	0	0
13	1	1.0	200,000	4th	8	+	+	+	+	0	0	0	0
14	1	0.5	100,000	4th	8	++	+	+	0	0	0	0	0
15	0.5	2.0	200,000	4th	8	+	+	0	0	0	0	0	0
16	0.5	0.5	50,000	4th	8	+	+	0	0	0	0	0	0

The Frequency of Occurrence of Virus and Lesions in the Lungs of Mice after Intraperitoneal Inoculation of Virus

In Table I is presented the incidence of pulmonary lesions in 16 groups of mice receiving relatively large doses of virus intraperitoneally. Considerable variation was observed in the frequency with which pulmonary lesions occurred in different groups of mice receiving the same dose of virus. Within the range of dosage employed in this series (50,000 to 1 million intranasal M.L.D.), no consistent relationship between the size of the intraperitoneal dose and the extent of pul-

monary involvement was detectable. Furthermore, the variation exhibited by animals of the same group suggests that within these limits the individual reaction is perhaps of greater importance in determining pulmonary involvement than the dose of virus employed. This fact is clearly illustrated by the following experiment.

TABLE II

Titrations of Amount of Virus Present in the Lungs of Individual Mice 4 Days after Intraperitoneal Inoculation

Mouse No.	Lung lesions after intraperitoneal inoculation	Results of intranasal inoculation of mice with different dilutions of test suspensions					
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
1	0	0	0	0	0	0	0
		0	0	0	0	0	0
2	0	0	0	0	0	0	0
		0	0	0	0	0	0
3	0	5	7	7	++	++	+
		7	7	8	++	++	0
4	0	5	6	8	6	+	+
		7	7	9	9	+	0
5	±	4	4	6	7	7	+++
		4	6	7	7	++++	+

In Tables II, III, IV, VII.

Numerals indicate day of death of test mice with typical ++++ lesions.

+ to ++++ = progressive degrees of gross pulmonary involvement in surviving mice autopsied on 10th day.

0 = no gross pulmonary lesions.

Five mice were given 0.5 cc. of 1 per cent mouse lung virus suspension (100,000 intranasal M.L.D.) intraperitoneally. The mice were sacrificed and autopsied on the 4th day after inoculation. The lung lesions were noted and titrations of the virus content of the individual lungs were made.

The results are shown in Table II. It is seen that neither virus nor lesions were present in the lungs of 2 of the mice, while virus was present in large amounts (1,000 to 100,000 M.L.D.) in the lungs of the other 3 mice although in only 1 was evidence of gross pulmonary involvement observed.

Owing to variation in individual mice the minimal intraperitoneal dose of virus which results in the appearance of lesions or virus in the lungs has not been subject to determination with the same accuracy as in the case of virus administered intranasally. There has been, nevertheless, a relatively sharp boundary between effective and non-effective doses. The injection of 100,000 M.L.D. intraperitoneally is invariably followed by the appearance of virus in the lungs of a large proportion of the inoculated mice. With 10,000 M.L.D. virus was recovered in only one of several attempts. When intraperitoneal doses of 1,000 M.L.D. were given, virus was not demonstrable in the lungs over a period of 1 to 4 days, even with the secondary passage technique employed by the Russian investigators (2). Paralleling to a certain extent the presence of virus in the lungs, lesions have been observed in the lungs, but for one exceptional instance, only when doses greater than 10,000 M.L.D. have been given intraperitoneally.

It is readily apparent, therefore, that the intraperitoneal injection of influenza virus is followed by the appearance of virus and the associated lesions in the lungs of mice only after large amounts of virus are used. When less than 10,000 intranasal M.L.D. were given intraperitoneally, no evidence of the presence of virus in the lungs was obtained.

Search for Virus and Lesions in the Lungs of Mice at Daily Intervals after Intraperitoneal Inoculation

The foregoing results were based on observations made to a great extent on the 4th day after intraperitoneal inoculation of virus. To determine the length of time through which virus and lesions could be detected in the lungs after the introduction of virus into the peritoneal cavity, the following experiments were conducted.

Each of 15 mice in series A was given 0.5 cc. of 2 per cent virus suspension (200,000 M.L.D.) intraperitoneally with the aid of ether anesthesia. To 29 mice of series B, 0.5 cc. amounts of 10 per cent virus suspension (1 million M.L.D.) were given intraperitoneally without the use of ether. Groups of 3 to 5 mice were killed at daily intervals, the presence of pulmonary lesions noted, and the pooled lungs of each group made into 20 per cent suspensions which were then tested for the presence of virus by intranasal inoculation into 3 normal mice. These test mice were observed for 10 days and the day of death and the lung lesions were recorded. All survivors were sacrificed and examined on the 10th day. The results are tabulated in Table III.

In both series of mice virus was recovered from the lungs as early as 24 hours after its intraperitoneal administration and, as judged by the time of death of the test mice, reached its height on the 4th day, at which time pulmonary lesions were first observed. In series B a gradual decrease in the amount of virus occurred after the 4th day so that by the 7th day virus was not detectable, and the lesions observed

TABLE III

Search for Virus and Pulmonary Lesions in the Lungs of Mice at Daily Intervals after Intraperitoneal Inoculation of Virus

Day mice killed after intraperitoneal inoculation	Series A					Series B						
	Number of mice in group	Lesions noted			Day of death of test mice with ++++ lesions	Number of mice in group	Lesions noted					Day of death of test mice with ++++ lesions
1st	3	0	0	0	6, 7, 8	3	0	0	0			8, 8, 8
2nd	3	0	0	0	4, 4, 5	3	0	0	0			6, 6, 7
3rd	3	0	0	0	3, 6, 6	4	0	0	0	0		6, 6, 7
4th	3	+	+	0	3, 3, 4	5	+	+	+	+	0	5, 5, 6
5th	3	++	++	0	4, 4, 5	5	++	0	0	0	0	7, 8, 8
6th	—		—		—	5	+	+	0	0	0	8, 9, 10
7th	—		—		—	4	++	+	+	0*		0, 0, 0

* Lesions of a greyish appearance commonly seen in mice surviving sublethal intranasal doses.

in the lungs at that time were of a pale greyish appearance characteristic of the healing process. It was also of interest that the use of ether exerted no apparent influence upon the outcome of the experiment.

Subsequent studies were then conducted to ascertain by titration the relative amounts of virus present in the lungs of mice at daily intervals after intraperitoneal injection.

Intraperitoneal injections of 0.5 cc. amounts of 1 per cent virus suspension were made in 21 mice. The animals were sacrificed in groups of 3 at daily intervals from 1 to 7 days after inoculation. No pulmonary lesions were observed. The lungs of each group were pooled and broth suspensions in dilutions of from 10^{-1} to 10^{-6} were inoculated intranasally into test mice. The mice were observed for 10 days at the end of which time the survivors were sacrificed and the presence or

absence of pulmonary lesions noted. The results of the titrations are presented in Table IV.

It is seen that while virus was present in the lungs 24 hours after its intraperitoneal administration the amount was comparatively small.

TABLE IV

Titration of Amounts of Virus Present at Daily Intervals in the Lungs of Mice after Intraperitoneal Inoculation

Day after intraperitoneal inoculation	Day of death of test mice with ++++ lesions in different dilutions					
	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
1st	9	0	0	0	0	0
	++	0	0	0	0	0
	+	0	0	0	0	0
2nd	4	4	4	5	8	++
	4	4	4	8	++++	++
	4	8	6	8	++	+
3rd	4	4	5	7	7	++
	4	4	5	8	7	0
	5	6	5	9	9	0
4th	7	7	10	++	0	0
	7	8	++++	+	0	0
	8	8	++++	0	0	0
5th	5	5	9	+++	+	+
	7	6	++++	++	+	0
	8	7	++++	++	0	0
6th	4	5	5	7	+++	+
	4	5	6	10	++	0
	5	6	10	++++	++	0
7th	7	8	+++	+	0	0
	8	10	++	+	0	0
	8	10	+	0	0	0

At 48 and 72 hours, however, as much as 100,000 M.L.D. of virus was detected in the lungs. Thereafter, virus was found in the lungs throughout the course of the experiment although in somewhat smaller amounts.

Since in the preceding experiment no end point was reached as to the number of days virus persisted in the lungs, the procedure was repeated and extended to 11 days. The results were quite parallel to those given above, especially in regard to the low titer of virus in the lungs at the end of 24 hours and the rapid increase to a maximum in 48 to 72 hours. In addition, sufficient virus was present in the lungs of mice 8, 9, and 10 days after intraperitoneal injection for a dilution of 1:1,000 to cause fatal infection in test mice, while on the 11th day no virus was demonstrable. Obviously, however, the duration of virus in the lungs of mice in different experiments is variable.

The presence of small amounts of virus in the lungs on the 1st day after intraperitoneal inoculation, the rapid increase in amount on the 2nd and 3rd days, and the persistence of virus over a prolonged period suggest that multiplication of the virus has taken place in the lungs. Owing to the large amounts of virus injected, the possibility remains, however, that the virus has selectively localized in the lungs. This hypothesis would demand acceptance of the idea that either the virus is capable of surviving in the body in a relatively inert state for several days or that it is multiplying in other parts of the body and being transported to the lung. In any case, it is apparent that following intraperitoneal inoculation of influenza virus the lung of a mouse may, without showing any evidence of pulmonary injury due to virus, contain virus in sufficient amount to kill 100,000 mice if inoculated intranasally.

Observations upon the Distribution of Virus after Intraperitoneal Injection

In order to evaluate the significance of the recovery of relatively large amounts of virus from the lungs of mice receiving influenza virus intraperitoneally, it was necessary to study further the distribution of virus in other organs and in the circulating blood. If virus is consistently present in the blood, its presence in other organs and the mode of its transport to the lungs is readily explained.

Presence of Virus in the Blood.—

Three groups of 5 mice each were given approximately 100,000 M.L.D. of virus intraperitoneally. At 1, 3, and 4½ hour intervals, respectively, 10 drops of blood

were obtained from the tail vein of each mouse of one group. Blood from each group was pooled, ground with alundum in 1.0 cc. of broth, and the supernatant fluid was inoculated into the nostrils of 3 normal mice. When the mice which received the blood were killed 3 days later no lung lesions were seen, but a second serial passage made with lungs of these mice resulted in death of all second passage mice with influenza virus infection as confirmed by neutralization tests.

Thus the presence of virus in the blood was demonstrated from 1 to 4½ hours after its introduction into the peritoneal cavity. Using the same procedures, however, repeated attempts to demonstrate virus in the blood from 24 hours to 6 days after inoculation have been uniformly unsuccessful.

TABLE V

Virus Demonstrated in Blood and Lungs of Individual Mice after Intraperitoneal Inoculation

Virus in blood 3 hrs. after inoculation..	+N	0	0	0	0	0	+	+	0	+
Virus in lungs 3 days after inoculation.	+	0	0	+	0	+N	0	+	+N	0

In Table V.
+ = virus present.
0 = no virus present.
N = positive neutralization test with influenza immune serum.

A further experiment was done in which 9 mice were injected intraperitoneally with approximately 10,000 intranasal M.L.D. by the same technique as above. The blood of each mouse was tested for the presence of virus 3 hours after inoculation and the lungs of each mouse were tested on the 3rd day after injection. Virus was detected in the blood of 4 of the 9 mice and the nature of the virus confirmed by neutralization tests in mice. No virus was found in the lungs. This dose of virus is, of course, below the threshold which ordinarily results in demonstrable virus in the lungs.

The results of a similar experiment in which 100,000 M.L.D. of virus were given intraperitoneally to 10 mice are briefly summarized in Table V. Virus was demonstrated in the blood of 4 of the 10 mice 3 hours after intraperitoneal injection, and in the lungs of 5 of the 10 mice 3 days later. In 2 of the mice virus was found in the blood and again subsequently in the lungs. In 2 of the mice virus was found

in the blood but was not in the lungs 3 days later. In 3 instances virus was present in the lungs although not demonstrable in the earlier tests of the blood. In 3 instances virus was not demonstrated in either the blood or lungs. These experiments demonstrate that shortly after the intraperitoneal inoculation of virus, the agent can be recovered from the circulating blood. Little information is elicited, however, as to the relation between circulating virus and the presence of virus in the lungs. That virus can be obtained from the lung following intravenous injection will be subsequently shown (Table VIII). The significance of the results will be discussed more thoroughly in that connection.

Presence of Virus in Organs Other than the Lung.—In an experiment to determine the distribution of influenza virus in the body after the intraperitoneal injection of large doses of the agent, mice so treated were killed after 3 hours, and others on the 3 following days. In addition to the blood and peritoneal washings, the lungs, nasal turbinates, liver, spleen, and kidneys were examined for the presence of virus, utilizing 2 serial passages in normal mice for this purpose.

Blood was examined by the procedure previously described. Peritoneal washings were obtained by flushing the abdominal contents with approximately 1.0 cc. of broth which was then inoculated in 0.05 cc. amounts intranasally to test mice. The abdominal organs were vigorously washed 5 times in 20 cc. amounts of Locke's solution, an amount thought to be sufficient to eliminate any virus that might be present on the surface of the organs. A 10 per cent suspension of each organ in broth was then given intranasally to mice.

Three hours after intraperitoneal inoculation, virus was present in small but demonstrable amounts in the blood; it was present in larger amounts in the peritoneal washings and lungs, but not demonstrable in the nasal turbinates. On all 3 days thereafter virus was recovered from the peritoneal washings and lungs but not from the turbinates.

Although positive results were obtained in examination of liver, spleen, and kidneys the difficulty in eliminating direct contamination from the peritoneal cavity makes their evaluation uncertain. This is emphasized by a control experiment in which 2 mice were killed and immediately given 1.0 cc. of 1 per cent virus suspension intraperitoneally. After abdominal massage, liver, spleen, and kidney were removed, subjected to the same amount of washing, and then tested for the presence of virus. Virus was recovered from all the organs.

The tendency of the virus to remain in the peritoneal washings and in the abdominal viscera suggested that the virus is taken up by the serosal cells of the peritoneum and the capsules of the abdominal organs. This suggestion was tested in the following experiment.

Six mice were injected intraperitoneally with 100,000 M.L.D. of virus. The mice were bled and killed in groups of 2 on the 1st, 3rd, and 6th days following inoculation. Peritoneal washings were obtained in the manner already described. The intestines were removed and abdominal cavities vigorously flushed 14 times

TABLE VI

The Distribution of Virus after Intraperitoneal Injection in Mice

Organs examined	Deaths and lesions in test mice								
	1st day			3rd day			6th day		
	A	B	C	A	B	C	A	B	C
Blood	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0
Peritoneal washings	6, 6	8, 8	− −	+ +	10, 10	4, 4	0 0	0 0	0 0
Mesentery and parietal peritoneum	+ 0	++ 0	4, 6	4, 4	4, 4	− −	+ +	7, 7	5, 5
Lungs	5, 5	6, 7	− −	4, 4	4, 5	− −	0 0	0 0	0 0
Turbinates	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0

In Tables VI and VIII.

A = day of death with ++++ lesions or lesions seen when mice were killed on 4th day after inoculation; primary passage mice.

B = day of death with ++++ lesions or lesions seen when mice were killed on 10th day after inoculation; primary passage mice.

C = day of death with ++++ lesions or lesions seen when mice were killed on 10th day after inoculation; secondary passage mice.

with 12 cc. of saline per flushing, after which pieces of mesentery and parietal peritoneum were made into 10 per cent suspension for inoculation into test mice. Peritoneal washings and suspensions of blood, lungs, and turbinates were likewise inoculated. Except in cases where death had already occurred or the test mice were obviously ill, on the 4th day following inoculation 2 of the group were killed (A) and secondary passage made to 2 additional mice (C); the remaining 2 of the 4 original test mice (B) were kept until the 10th day when survivors were killed and lesions observed.

As observed in Table VI virus was found in the peritoneal washings as long as 3 days after its injection, while in the cells of the mesentery and parietal peritoneum virus was demonstrable as late as 6 days after

injection. The latter observation shows clearly the capacity of the virus to survive and perhaps to multiply in the peritoneal cells. In contrast to these results is the absence of virus from the blood and nasal turbinates. Virus was not recovered from the lungs after the 3rd day, a result perhaps due to the factor of individual variation in the mice.

The Development of Resistance in Mice Receiving Large Doses of Influenza Virus Intraperitoneally

It has been clearly shown that as a result of the intraperitoneal inoculation of mice with the larger doses of influenza virus a high concentration of the virus and gross pathological changes are found in the lungs of the animals. In view of these facts it is noteworthy that in a large series of observations upon mice so inoculated no deaths from influenza virus infection have occurred. This result is even more striking when it is recalled that as much as 100,000 intranasal lethal doses of virus may be recovered from the lung of a mouse which appears perfectly healthy after the intraperitoneal injection of virus. It must be assumed either that the virus reaches the lungs by a route which prevents its attacking the pulmonary tissues in the same manner as after intranasal inoculation (2), or that an alteration in the reactivity of the animal host occurs as a result of the pararespiratory injection so as to inhibit the virus from exerting its full pathogenic influence upon the pulmonary tissues to which it has gained access. The fact that the pulmonary lesions observed after intraperitoneal inoculation of virus are rarely of more than moderate extent seems to support the latter point of view. Moreover, the animals become solidly immune to subsequent intranasal tests with as much as 100,000 M.L.D. of virus.

The following experiment was undertaken to determine whether after the intraperitoneal inoculation of virus any evidence could be obtained of a rapidly developing resistance which might serve to protect mice against the characteristic virus effect.

Suspensions of 2 per cent mouse lung virus were prepared and stored in a freezing mixture (5) at −79° C. until used. Every day for 5 days a different group of 10 mice each was given 0.5 cc. of the virus suspension intraperitoneally. On the 3rd day all mice, regardless of whether or not they had yet received the virus

intraperitoneally, were given 0.05 cc. amounts of a 1:100,000 dilution of virus intranasally. In addition one group of 10 mice received only the intranasal inoculation, while another group received only the intraperitoneal injection of virus. The mice were observed for 10 days at which time all survivors were sacrificed and the lungs examined. The results are summarized in Table VII.

As evidenced by the fact that all survived and that only low grade pulmonary lesions were found at autopsy, the mice which received the intraperitoneal inoculation of virus 2 days before intranasal infection exhibited a well marked resistance. This is especially im-

TABLE VII

The Effect of Intraperitoneal Injection of a Large Dose of Virus before and after the Intranasal Inoculation of a Small Infective Dose of the Same Virus

Time relation of intraperitoneal to intranasal inoculation	Day of death with ++++ lesions or lesions seen in mice surviving 10 days after intranasal inoculation									
2 days before	++	++	+	+	+	+	+	±	0	0
1 day before	7,	7	++	++	+	+	+	+	+	+
Same day	6,	6,	7	+++	+++	++	++	++	++	++
1 day after	7,	7,	7,	7,	7,	7,	7	++	++	0
2 days after	6,	7,	7,	7,	7,	7,	9,	9,	10	++++
Intranasal only	7,	7,	7,	8,	8,	8	+++	++	++	++
Intraperitoneal only	+	+	+	+	0	0	0	0	0	0

pressive when one considers that the intranasal infection was given at about the time when the amount of virus recoverable from the lung following intraperitoneal injection reaches its maximum. Although to a lesser extent, a similar effect was observed in the mice receiving virus intraperitoneally 24 hours before intranasal infection. Thereafter, the severity of the pulmonary lesions and the frequency of death increased so that animals receiving intraperitoneal virus after the intranasal infection showed no evidence of resistance. Similar results were obtained when these observations were repeated using 10 per cent virus concentration for the intraperitoneal injection.

Since the resistance developed so rapidly it was of further interest to ascertain what correlation, if any, obtained between the active resistance of the animals and the development of circulating antiviral substances. Accordingly, each of 45 mice was given the equivalent

of 50,000 to 100,000 intranasal M.L.D. intraperitoneally. At intervals of approximately every 3 days from 1 to 27 days thereafter, groups of 5 mice were bled from the tail veins. The serum of each group was pooled and tested for the presence of antibodies to influenza virus by means of the protection test in mice. No antibodies were detected in the serum taken 1 or 3 days after intraperitoneal inoculation. On the 5th, 7th, and 10th days sufficient antibody was present in undiluted serum to protect test mice against fatal infection with 1,000 lethal doses of virus even though some pulmonary involvement was observed. On the 13th day the serum had a protective titer between 1:100 and 1:200, and this level was maintained until the termination of the experiment on the 27th day.

It appears, therefore, that the resistance exhibited to intranasal virus infection by mice which receive large doses of influenza virus intraperitoneally is effective at the very time maximal amounts of virus tend to collect in the lungs and, on the basis of the preceding experiment, before neutralizing antibodies are detectable in the blood. This apparently non-specific resistance may possibly represent an example of the interference phenomenon similar to that described for yellow fever virus (6), when the neurotropic strain of virus given to monkeys intraperitoneally protects against an otherwise fatal infection with the viscerotropic strain of virus. Further investigation of the nature of the resistance observed in the present experiments will be required.

Distribution of Virus after Subcutaneous or Intravenous Administration

The foregoing experiments have revealed that after the intraperitoneal inoculation of mice with large amounts of influenza virus the virus can be recovered from the blood for a short time and from the lungs in relatively high concentrations for a number of days. When the same procedures were applied after the subcutaneous inoculation of similar amounts of virus, virus was not demonstrable in the blood or lungs of the mice. Moreover, one large dose of virus subcutaneously results not only in a less effective active immunity among the treated mice but also in a lower titer of neutralizing antibodies in the blood than when the same amount of virus is given by the intraperitoneal route. Virus was readily recovered, however, from the re-

gional lymph nodes in large quantities after 4 hours, still demonstrable but considerably diminished after 24 hours, but no longer detectable after 48 hours. The results suggest that in mice following subcutaneous inoculation of influenza virus, the virus is absorbed in regional lymphatics and is there destroyed without invasion of the blood stream or of the lungs in contradistinction to the course of events after intraperitoneal inoculation.

Attempts to determine the effects produced by virus administered *intravenously* to mice were impeded at first by a toxic effect of suspensions of mouse lung tissue which resulted in the immediate death of most mice with symptoms of acute "pulmonary" shock. It was subsequently found that prolonged centrifugation of the material reduced its toxicity sufficiently to permit the inoculation of larger groups of mice although toxic symptoms with occasional deaths still occurred. The following experiment illustrates the results obtained.

Five mice anesthetized with ether were given into the tail veins 0.25 cc. of 5 per cent suspension of mouse lung virus in 10 per cent normal mouse serum in Locke's solution. The suspension had been previously centrifuged for 1 hour at 2,500 R.P.M., and when titrated intranasally in mice a dilution of 1:100,000 caused death. Therefore, approximately 25,000 intranasal M.L.D. were administered intravenously. Individual mice were exsanguinated from the heart after 30 minutes, and 1, 2, 4, and 6 days following inoculation. The abdominal viscera were removed at the same time. The blood was ground with 1.0 cc. of broth and 10 per cent suspensions made of liver, spleen, kidney, and lung, care being taken to avoid cross contamination of organs. Groups of 4 test mice were inoculated intranasally with 0.05 cc. amounts of each blood and organ suspension.

The results of the present experiment, summarized in Table VIII, show that following intravenous administration of large doses of virus, virus may be demonstrated consistently in the lungs, and typical influenzal pulmonary lesions may occur. Results obtained from examination of the other organs and the blood must be considered as preliminary observations. They are included, however, because of the interesting tendency of the virus to localize and persist in the liver. While no explanation can be offered at present in regard to this phenomenon, the similarity in embryological origin of the lung and liver as well as certain structural similarities might be borne in mind. It is of further interest as well that virus administered intravenously is

TABLE VIII

The Distribution of Virus after Intravenous Inoculation in Mice

Organs examined	Deaths and lesions in test mice																	
	30 min.				1 day			2 days				4 days				6 days		
	A	B		C	A	B	C	A	B		C	A	B	C	A	B		C
Blood	0 0	0	0	0 0	0 0	0 0	0 0	0 0	0	0	0 0	0 0	0 0	0 0	0 0	0	0	0 0
Liver	+ ±	++	+	4, 4_N	0 0	0 0	0 0	+ +	+++	++	4, 6	+ ±	6, 6	4, 4	0 0	0	0	0 0
Spleen	0 0	+	0	0 0	0 0	0 0	0 0	0 0	0	0	0 0	0 0	0 0	0 0	0 0	0	0	0 0
Kidney	0 0	+++	++	3, 4_N	0 0	0 0	0 0	0 0	0	0	0 0	0 0	0 0	0 0	0 0	0	0	0 0
Lungs	+ ±	7, 8		3, 3_N	5, 6_N	6, 7	—	4, 5	5,	5	—	4, 4	4, 5	—	+ +	++	++	4, 4
Lesions seen in lungs		0				0			0				±			++		

rapidly removed from the blood but persists in relatively high concentration in the lungs. The virus has undoubtedly been transported to the lung by way of the blood stream and concentrated there. These observations may aid in understanding the route by which virus from the peritoneal cavity reaches the lung. It is impossible at present, however, to decide whether the virus in the lung reaches its concentration merely by selective localization or to what extent it represents a result of virus multiplication.

DISCUSSION

It has been shown in the foregoing experiments that, following the intraperitoneal inoculation of mice with large doses of epidemic influenza virus, the virus reaches the lungs and may there produce macroscopic lesions of epidemic influenza virus infection. These results are obtained only when virus is administered intraperitoneally in amounts equivalent to more than 10,000 intranasal lethal doses. It is quite remarkable, however, that although as much as 100,000 M.L.D. of virus may be recovered from the lungs of mice and the virus may persist in the lungs from 4 to 10 days, pulmonary lesions of more than moderate extent have not been observed, nor have any fatalities due to virus infection occurred among the many mice subjected to the procedure. This is in striking contrast to the effects observed after intranasal inoculation of mice in which instance minute amounts of influenza virus cause a fatal disease with complete pulmonary involvement. The capacity of the virus to inflict pulmonary damage is obviously influenced by the route by which it reaches the lung, and the mechanism of its action must differ under the two conditions.

It is difficult to decide whether the virus reaches its relatively high concentration in the lung as a result of multiplication in that organ or whether it is concentrated in the lung as a result of selective localization on transport from other parts of the body. In favor of the latter hypothesis are the facts that the virus does not exert its complete, pathogenic action in the lung after intraperitoneal inoculation, and that an available source for feeding virus to the lung is in the peritoneal cells where virus has been shown to persist, if not multiply, for several days. Furthermore, virus is recovered from the lungs only after maximal intraperitoneal doses, indicating that for it to reach the

lung a flooding of the body must occur. On the other hand, a mode of transport is not readily apparent, for virus has not been recovered from the blood later than 24 hours after its introduction into the peritoneal cavity. Moreover, since such large amounts of virus intraperitoneally are required to permit the detection of virus in the lung, it may be that any virus which reaches the lung can subsequently multiply. This is suggested by the fact that the highest concentration of virus in the lung is usually encountered 24 to 48 hours after virus can be recovered from the blood. At present, however, no final conclusion appears warranted.

As previously stated, mice whose lungs contain relatively high contents of influenza virus and present macroscopic lesions subsequent to the intraperitoneal injection of virus show no external evidence of infection. They do not die but become staunchly immune to infection by the intranasal route. The failure of the virus to induce fatal infection in mice under these conditions immediately suggests that the animal develops an increased resistance capable of inhibiting the pathogenicity of the virus which is abundantly present in susceptible tissues. It was shown, in fact, that mice which were given large doses of virus intraperitoneally 48 hours before the intranasal introduction of a fatal dose of virus resisted the fatal infection. The lethal intranasal dose of virus was given at the time when the highest concentration of virus in the lung is reached as a result of the intraperitoneal inoculation. It is extremely interesting that instead of lending an added effect to the intranasal dose, an antagonistic effect was observed. On the basis of the experiments recorded above, the resistance is active before demonstrable antiviral substances are present in the blood. The studies of Hoskins (6) with yellow fever virus are of interest in suggesting an interpretation of the present results. Hoskins observed that when the neurotropic strain of yellow fever virus was given to monkeys by the intraperitoneal route, the animals were rendered resistant to fatal infection by the viscerotropic strain of virus given as much as 24 hours previously. It may be in the present instance that the virus of influenza entering the pulmonary tissues *via* the peritoneal route renders the cells refractory to fatal infection with the same virus administered 48 hours later by the intranasal route.

Following the same reasoning it might be suggested that the solid immunity to intranasal infection which follows intraperitoneal vaccination with active influenza virus is entirely attributable to a localization of the immunizing virus in the lungs, and that unless virus reaches and multiplies in the lungs no immunity is produced. This concept is immediately eliminated by reference to the successful vaccination of mice with inactive formalized virus (7). Furthermore, in the course of the present studies it has been possible to show that fully active immunity can be induced by the intraperitoneal injection of active virus cultivated in tissue culture medium (8, 9) although it has not been possible to detect its presence in the lungs of vaccinated mice, even after repeated passages.

SUMMARY

Following the intraperitoneal inoculation of mice with large doses of epidemic influenza virus (50,000 to 1 million intranasal M.L.D.) it can be recovered from the lungs in high concentration, and pulmonary lesions of moderate extent may be observed. The virus reaches its highest titer in the lungs 48 to 72 hours after intraperitoneal injection and may persist for 10 days. Virus may be recovered from the blood in the first 24 hours, but is readily detected in the omentum and peritoneum for 5 to 6 days. Mice which as a result of the intraperitoneal injection of virus show a high concentration of virus in the lungs do not die but become solidly immune to intranasal infection. Moreover, as early as 24 to 48 hours after intraperitoneal inoculation of large amounts of virus the animals may exhibit resistance to infection with fatal doses of virus given intranasally.

Influenza virus given intravenously to mice is rapidly removed from the blood but persists in the lungs and induces pulmonary lesions. Virus can also be recovered from the liver for several days. With subcutaneous inoculation of influenza virus, however, the virus does not reach the blood or lungs in detectable amounts although the regional lymph nodes may yield considerable quantities of the agent.

A brief consideration is presented of the mechanisms of infection and resistance which may be involved.

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