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Susceptibility of Convalescent Ferrets to Reinfection with Influenza Virus in Absence of Specific Antibodies.*

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It has been suggested[1] that during the early stages of convalescence from influenza virus infection, resistance to reinfection might be related more closely to a non-specific cellular refractoriness than to immunological activity of the blood. That suggestion is supported by the demonstration[2,3] that, following the intranasal inoculation of ferrets with the PR8 strain of influenza virus, the nasal epithelium was destroyed and was replaced by one of abnormal type, and that 7 to 8 days after the infection the abnormal epithelium was resistant to injury by the reinoculation of the same strain of influenza virus, and, also, was resistant to injury by a chemical stimulus which completely destroyed the nasal epithelium of the normal ferret. However, under the conditions of those experiments, animals which possess resistant nasal epithelium in all probability also would possess antibodies specific for the inoculated virus. It has not been shown that those animals would be more resistant to infection than would a normal animal of the same species, if inoculated with a strain of virus against which they possessed no antibodies.

In the experiment to be reported ferrets were infected with either influenza A or influenza B virus. Then, they were tested for immunity by intranasal inoculation of those viruses during that period of convalescence when resistant nasal epithelium would be expected to be present.

Pools of allantoic fluid infected either with the Czech strain of influenza B virus or with the PR8 strain of influenza A virus were collected and stored in the CO_2-ice chest until immediately before use. Twelve ferrets were anesthetized by intraperitoneal injections of nembutal. While anesthetized, they were bled from the heart and then were given 1.5 cc of inoculum intranasally. Six of the animals were infected with the Czech (B) strain of virus and 6 were infected with the PR8 (A) strain. One week later, 3 of the Czech infected animals and 3 of the PR8 infected animals were tested for immunity by intranasal inoculations with the Czech virus; the other 6 animals were tested by intranasal inoculations with PR8 virus.

In addition to the bleedings made at the start of the experiment, blood was obtained immediately preceding and one week following the second inoculation. The virus neutralizing antibody content of each serum was determined by means of the mouse protection test; the titers are expressed in terms of the initial dilution of serum which protected 50% of the mice from death[4]. The data are shown in Chart 1 and in Table I.

Chart 1 shows that the ferrets were susceptible to reinfection with the heterologous virus when inoculated 7 days after the initial infection. For example, all of the animals convalescing from infection with Czech, showed a definite febrile response when challenged with PR8. Also, the animals originally infected with PR8 responded, when subsequently inoculated with Czech, with a temperature course that was little different from that shown by normal animals inoculated with that virus. On the other hand, none of the animals gave a febrile reaction when reinoculated with the homologous virus.

Table I shows that by the time of the

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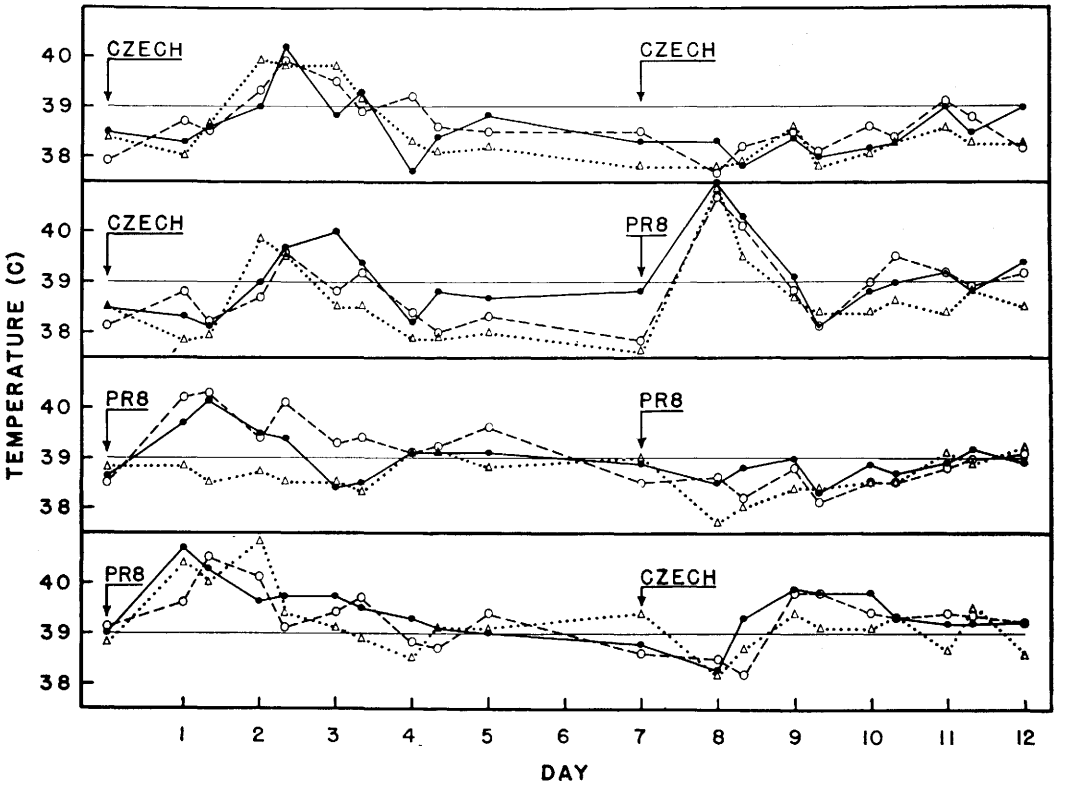

CHART 1.
Temperature courses following intranasal inoculation of influenza virus.

TABLE I.
Antibody Titers and Febrile reactions Following Intranasal Inoculation of Influenza Virus.

Ferret No.	1st day Antibody titers Czech	1st day Antibody titers PR8	1st day Virus strain used for inoc.	2nd to 7th day. Fever	8th day Antibody titers Czech	8th day Antibody titers PR8	8th day Virus strain used for inoc.	9th to 14th day. Fever	15th day Antibody titers Czech	15th day Antibody titers PR8
1	0	0	Czech	+	90	0	Czech	—	1000+	0
2	0	0	"	+	47	0	"	—	1000+	0
3	0	0	"	+	47	0	"	—	1000+	0
4	0	0	"	+	74	0	PR8	+	1000+	305
5	0	0	"	+	54	0	"	+	1000+	360
6	0	0	"	+	22	0	"	+	1000+	360
7	0	0	PR8	+	0	98	"	—	0	1000+
8	0	0	"	+	0	178	"	—	0	1000+
9	0	0	"	—	0	360	"	—	0	1000+
10	0	0	"	+	0	305	Czech	±	23	1000+
11	0	0	"	+	0	360	"	+	74	1000+
12	0	0	"	+	0	218	"	+	19	1000+

second inoculation, all of the ferrets had developed antibodies specific for the virus used for the original infection, while none of the animals possessed demonstrable antibodies reactive with the heterologous virus. Thus, during the period of convalescence, when a resistant nasal epithelium would be expected to be present, the animals were susceptible to reinfection with a strain of influenza virus against which they did not possess antibodies, although they were immune to clinical reinfection with a strain of

influenza virus against which they did possess antibodies. If the mechanisms of infection with those 2 viruses are the same, non-specific cellular resistance resulting from infection with one virus should have conferred increased resistance to infection with the antigenically distinct virus. The results, therefore indicate that following an original influenza virus infection either (1) non-specific cellular refractoriness plays little, if any part in the resistance of ferrets to reinfection with that virus or (2) the mechanisms of infection with the A and B viruses are not the same.

It is of interest that when PR8 was used for inoculation (Chart 1), the animals previously infected with Czech showed a higher fever, an earlier peak and a less prolonged febrile reaction than did the normal animals. If that difference in response was not due to chance these results might be interpreted as an indication that the convalescing animals were even more susceptible to infection than were the normal ferrets.

Summary. Ferrets which had been infected with either influenza A or influenza B virus were tested for immunity to those 2 viruses on the eighth day of convalescence. They were susceptible to infection when reinoculated with the heterologous virus against which they possessed no antibodies, but were immune to infection when reinoculated with the homologous virus against which they possessed antibodies. If the mechanisms of infection with the A and B viruses are the same the results indicate that non-specific cellular refractoriness plays little part in the resistance of ferrets to reinfection with influenza virus.

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Observations on the Action of Streptomycin *in vitro* (I).*

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Since the discovery of streptomycin by Waksman,[1] a number of reports on the sensitivity of microorganisms to this antibacterial agent have appeared.[2-8] The sensitivities have been expressed in terms of units per cc or micrograms of streptomycin base per cc necessary for inhibition of growth. The sensitivities reported have varied widely depending upon the individual strain tested. Strains which have been considered sensitive to streptomycin and which have been inhibited in some laboratories by a few tenths of a microgram per cc have in other laboratories required from 1 to 10 μg per cc or more for inhibition. These differences have depended upon the experimental conditions, and upon the criteria of inhibition used.

It was early demonstrated by Waksman,[2,3] Donovick,[9] and others[10,11] that medium influ-

* A part of this work was presented before the Society of American Bacteriologists, May, 1946.

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