THE SYNERGISM OF HUMAN INFLUENZA AND CANINE DISTEMPER VIRUSES IN FERRETS

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(Received for publication, June 18, 1940)

The investigations of Smith, Andrewes, and Laidlaw (1) and those of Francis (2) have demonstrated that the vaccination of ferrets with influenza virus does not result in the development of complete immunity even against the same strain of virus although partial immunity may be produced. Evidence was presented by Horsfall and Lennette (3) concerning the effectiveness of a formalinized complex vaccine prepared from lungs and spleens removed from ferrets late in the course of concurrent infection with the viruses of human influenza and canine distemper. It was shown that this vaccine, when given to normal ferrets, stimulated the production of a high titer of neutralizing antibodies against influenza virus and resulted in the development of solid immunity against antigenically different strains of this virus. It was also reported that preliminary tests indicated that the complex vaccine was effective in stimulating the production of neutralizing antibodies against influenza virus in man.

In searching for an explanation of the effectiveness and the broad antigenicity of this vaccine, a study was made of the individual components which had entered into it. It was found that vaccines prepared similarly with tissues infected with either influenza virus or distemper virus alone, irrespective of whether they were obtained early or late in the course of the ferret disease, were ineffective in reproducing the results obtained with the original complex vaccine. It was also found that vaccines prepared from the tissues of ferrets individually infected with either influenza virus or distemper virus and mixed *in vitro* were ineffective as immunizing agents. Only when vaccines were prepared from the lungs and spleens of ferrets infected simultaneously with influenza and distemper viruses could an adequate neutralizing antibody response and active immunity to infection by different strains of influenza virus be demonstrated.

These observations seemed of sufficient theoretical and practical importance to justify a comprehensive investigation in order to find some

logical explanation for the results obtained with the complex vaccine. Three possibilities appeared worthy of consideration; namely, (*a*) that some antigenic relationship existed between influenza and distemper viruses, (*b*) that quantitatively more influenza virus was produced by ferrets suffering from both infections, or (*c*) that the concurrent infections resulted in the production of a qualitatively different influenza antigen. A series of experiments was designed to test these various possibilities. It is the purpose of this paper to present the experimental results obtained and to offer evidence for the occurrence of what appears to be a hitherto undescribed phenomenon.

Methods

Viruses.—The W.S. (4), PR8 (5), 149, and 188 (6) strains of human influenza virus were studied. With the first two strains both serial ferret passage and mouse-adapted varieties were used, while with the latter two strains only ferret passage viruses were utilized. Two strains of canine distemper virus which have been designated as X and Y were studied. The X strain was encountered accidentally in the ferret stock of this laboratory in November, 1939, and has since been passed numerous times in this species. The Y strain was obtained directly from the spleen of an infected dog[1] and has been passed only twice in ferrets. Stock organ suspensions from animals infected with the various viruses, stored in a low temperature gas cabinet at −76°C., served as a source of infectious material throughout this investigation.

Ferrets.—Normal ferrets, between 6 and 12 months of age and not vaccinated against canine distemper, were held in isolation quarters for an observation period of at least 2 weeks prior to use. Blood specimens were obtained by cardiac puncture before any experimental procedure was instituted and at intervals thereafter as required. Normal serum obtained from the initial bleeding was tested for the presence of neutralizing antibodies against influenza virus; in no instance did such a serum contain antibodies against this virus.

Ferrets were inoculated under light ether anesthesia and, unless stated otherwise, 1 cc. of the desired virus dilution was administered intranasally.

Isolation.—Inoculated ferrets were individually isolated in relatively air tight metal cubicles which were ventilated by forced draft through filters and kept in a special isolation room (7). This isolation technique and the equipment which made it possible were essential to the study because of the well known infectiousness of both viruses under investigation and especially because of the extreme infectiousness of distemper virus. Inoculated ferrets were examined twice daily; their temperatures were taken and pertinent symptoms recorded. At the desired interval after inoculation, infected animals were anesthetized with ether, bled by cardiac puncture, and killed with chloroform. The lungs and spleens were then removed aseptically.

Virus Titrations.—The presence of influenza virus and its concentration in infected ferret tissues were determined by the inoculation of mice. When ferrets had been

[1] We are grateful to Dr. C. N. Leach of Montgomery, Alabama, for sending us this infected spleen.

inoculated with mouse-adapted strains of influenza virus, serial decimal dilutions of the ferret tissue suspensions were made and were inoculated intranasally into groups of either four or six Swiss mice anesthetized with ether. Inoculated mice were observed for a period of 10 days. Those which died were autopsied and their lungs examined for the presence and the extent of pulmonary consolidation. Mice which survived were killed at the end of the observation period and their lungs examined. The virus titer was determined by the method previously described (8), and in the tables below the 50 per cent mortality end point will be given. In the case of ferrets which had been inoculated with ferret passage strains of influenza virus, the presence of the virus in their tissues was also determined by the inoculation of mice. However, in such cases it was not possible to determine accurately the concentration of virus present inasmuch as the ferret passage strains of virus, although capable of producing pulmonary lesions in mice at high dilution, seldom caused fatal consolidation in these animals even when very low dilutions were used. The fact that pulmonary lesions produced in the mice were actually the result of influenza virus infection was demonstrated by neutralization with known anti-influenza virus ferret serum. The presence of distemper virus in the lungs and spleens of infected ferrets was determined by the inoculation of normal ferrets. Determinations of the concentration of distemper virus in these tissues were not made because of the impracticability of such a procedure and the very large number of ferrets which would have been required.

Antibody Titrations.—The neutralizing antibody titers of sera against influenza virus were determined by means of the quantitative technique which has been described previously (8). Four mice were inoculated with each serum-virus mixture. In the tables below the 50 per cent mortality end point will be given as the "standard neutralization titer." This quantity expresses that dilution of serum which is capable of neutralizing 3100 fifty per cent mortality doses of the PR8 strain of virus and was determined by means of the linear relationship between serum and virus which was reported in a previous communication (8). In the absence of satisfactory methods for the accurate determination of the titer of antibodies against distemper virus, it was not possible to study the various ferret sera for this capacity.

EXPERIMENTAL

Cross Immunity Tests in Ferrets with Influenza and Distemper Viruses.—Since it seemed not impossible that human influenza virus and canine distemper virus might contain a common antigenic component, it was essential to determine whether ferrets known to be solidly immune to one virus were susceptible to infection by the other.

To immunize normal ferrets against influenza virus the animals were given four separate intranasal inoculations of the virus, more than 1000 infectious doses each, at monthly intervals. The first inoculation resulted in a typical attack of experimental influenza but no reaction followed any of the three subsequent inoculations, indicating that the animals were solidly immune. Before and 10 days after each inoculation, blood serum was obtained and the neutralizing antibody titer determined. About 4 weeks after the last inoculation these ferrets were inoculated with more than 1000 infectious doses of distemper virus.

A parallel series of normal ferrets was immunized against distemper virus by vaccination. 2 cc. of a formalinized 10 per cent suspension of the spleen from a ferret moribund with distemper were used. Either two or three subcutaneous injections of this vaccine were given at intervals of about 1 month. Approximately 30 days after the last vaccina-

TABLE I

Results of Cross Immunity Tests with Human Influenza and Canine Distemper Viruses

Ferret	Immunization		Immunity test								
			Influenza virus				Distemper virus				
	Virus	Strains	Strain	Fever	Symptoms	Standard neutralization titer	Strain	Fever	Skin signs	Liver, spleen lesions	Remarks
1	Influenza	149 + PR8	PR8	0	0	1:364	X	+	+	+	Killed when moribund
2	"	149	149	0	0	1:128	X	+	+	+	" "
3	"	149 + PR8	PR8	0	0	1:404	X	+	+	+	" "
4	Normal control		PR8	+	+	1:128					
5	"	"					X	+	+	+	" "

TABLE II

Results of Cross Immunity Tests with Canine Distemper and Human Influenza Viruses

Ferret	Immunization		Immunity test							
			Distemper virus			Influenza virus				
	Virus	Strain	Strain	Fever	Skin signs	Strain	Fever	Symptoms	Lung consolidation	Standard neutralization titer
6	Distemper	X	X	0	0	PR8	+	+	+	1:102
7	"	X	X	0	0	PR8	+	+	+	1:102
8	"	X	X	0	0	PR8	+	+	+	1:40
9	"	X	Y	0	0					0
10	"	X	Y	0	0					0
11	Normal control		Y	+	+					0

tion these ferrets were tested for active immunity to distemper virus by the inoculation of more than 1000 infectious doses. Subsequently these same ferrets were tested for susceptibility to influenza virus by the intranasal inoculation of approximately 1000 infectious doses. The results of these experiments are presented in Tables I and II.

It will be seen that ferrets which were proved to be solidly immune to influenza virus and which possessed relatively high titers of neutralizing

antibodies against this virus were fully susceptible to infection by distemper virus. On the other hand, ferrets which were proved to be immune to distemper virus were subsequently found to be fully susceptible to infection by influenza virus. Also it will be noted that ferrets vaccinated with the X strain of distemper virus were found to be immune to the Y strain.

Cross Neutralization Tests in Ferrets with Influenza and Distemper Viruses.—To gain additional information regarding the presence or absence of a

TABLE III

Results of Cross Neutralization Tests with Human Influenza and Canine Distemper Viruses

Ferret	Antiserum		Virus	Strain	Results					
						Influenza		Distemper		
	Virus	Strain			Highest temperature	Symptoms	Standard neutralization titer	Skin signs	Liver, spleen lesions	Remarks
					°F.					
12	Influenza	PR8	Influenza	PR8	102.4	0	0			
13	"	PR8	Distemper	X	105.2		0	+	+	Killed when moribund
14	"	PR8	"	Y	104.6		0	+	+	" "
15	Distemper	Y	Influenza	PR8	105.1	+	1:128			
16	"	Y	Distemper	Y	103.2		0	0	−	Normal 3 mos.
17	Normal serum		Influenza	PR8	104.8	+	1:128			
18	" "		Distemper	X	105.5		0	+	+	Killed when moribund
19	" "		"	Y	106.1		0	+	+	" "

− = not examined.

common antigen in human influenza and canine distemper viruses, cross neutralization tests were carried out with ferret antisera against these viruses.

Undiluted ferret antisera were mixed with an equal volume of the virus dilution containing more than 1000 infectious doses, and the mixtures were held at room temperature for 4 hours and at 4°C. for an additional 18 hours. Normal ferrets were then inoculated intranasally with the various mixtures. Mixtures of normal ferret serum and identical quantities of virus which had been treated in a similar manner were included as controls.

The results of these experiments are shown in Table III. It will be noted that the antiserum against influenza virus completely neutralized the

homologous virus but failed to effect any demonstrable neutralization of either strain of distemper virus. Similarly the antiserum against distemper virus completely neutralized the homologous virus but failed entirely to neutralize the PR8 strain of influenza virus.

The results of both the cross immunity and the cross neutralization tests afford clear evidence that there was no antigenic relationship between the strains of influenza virus and distemper virus used in this study. Eichhorn and Pyle (9), on the basis of preliminary experiments, suggested that such a relationship might exist between these two different viruses. However, their initial observations have neither been confirmed nor extended, and there does not appear to be any other published evidence which would indicate that such an antigenic relationship exists.

Course of the Infectious Process in Ferrets Inoculated with Distemper Virus.—To facilitate the interpretation of results obtained in ferrets infected with both influenza and distemper viruses concurrently, it was necessary to determine, as accurately as possible, the course of the infectious process in ferrets infected with each virus separately. Although Dunkin and Laidlaw (10) have described in detail most of the pertinent facts relating to the infection produced in ferrets by canine distemper virus, it was considered important to know whether the two strains used in this study differed in any respects from those recorded in the literature.

The X strain of distemper virus used in these investigations regularly produced a very pronounced infection in ferrets. None of a large number of ferrets infected with this virus recovered; the majority died within 2 or 3 weeks after inoculation. The disease was characterized by a relatively short incubation period, as judged by a significant febrile response, and in twenty inoculated ferrets the incubation period averaged 6 days with a range of from 3 to 9 days. Erythematous patches usually appeared on the skin, and were commonly noted about the lips, in the groins, on the skin of the abdomen, or on the foot pads. Frequently small vesicles also appeared on the lips and about the anus. Occasionally the vesicles progressed to the stage of pustulation. In about one-fourth of the inoculated animals either unilateral or bilateral purulent conjunctivitis developed. In twenty inoculated ferrets these external signs appeared at an average of 8 days after inoculation with a range of 7 to 10 days. At autopsy small grayish areas suggesting lobular pneumonia were found in ten of twenty inoculated ferrets. These irregularly distributed patches of pulmonary consolidation were very different from those produced by influenza virus. The liver was usually soft, friable, and of a light yellowish brown color, and lobular markings were often prominent. The spleen was dark purple, enlarged, tense, and rounded at the margins. On section, the cut surfaces bulged and were notably soft; the malpighian corpuscles were numerous and prominent. Spleens weighing two or three times as much as those of normal animals were frequently encountered. Sera obtained from ferrets at various intervals from 5 to 28 days after inoculation with distemper virus were found in every instance to be devoid of neutralizing antibodies against influenza virus.

The X strain of distemper virus was inoculated intracerebrally in mice and guinea pigs and subcutaneously in mice, guinea pigs, rabbits, Syrian hamsters, and monkeys. In no instance did any evidence of infection develop in these species even after serial passage. This evidence confirms the absence of pathogenicity of this virus for some of these species as found by Dunkin and Laidlaw (10). It also excludes the possibility that the distemper virus used in this study was accidentally contaminated with lymphocytic choriomeningitis virus, as was found to be the case in one instance by Dalldorf (11).

Normal ferrets were inoculated with more than 10,000 infectious doses of the X strain of distemper virus and killed at varying intervals thereafter. The presence of distemper virus in the lungs and the spleens of these animals was determined by the subinoculation of other normal ferrets. The results of these experiments are summarized in Table IV.

TABLE IV

Presence of Canine Distemper Virus in Lungs and Spleens of Ferrets at Intervals after Inoculation

Ferret	Route of inoculation	Time after inoculation	Highest temperature	Skin signs	Liver, spleen lesions	Incubation period	Distemper virus demonstrated	
							Lung	Spleen
		days	*°F.*			*days*		
20	I.N.	3	106.7	0	0	3	+	−
21	"	4	105.2	0	0	4	+	−
22	"	5	106.5	0	0	3	+	−
23	S.C.	6	104.3	0	0	4	−	+
24	I.N.	10	104.4	+	+	7	+	−
25	"	10	104.5	+	+	6	−	+
26	"	12	105.8	+	+	6	+	+

I.N. = intranasal. S.C. = subcutaneous. − = not done.

It will be noted that as early as the 3rd day after inoculation distemper virus was present in the lungs of inoculated ferrets and persisted therein through at least the 12th day. It will also be seen that the virus was demonstrated in the spleen on the 6th, 10th, and 12th days after inoculation. Spleens obtained in the earlier stages of the disease were not tested.

Course of the Infectious Process in Ferrets Inoculated with Influenza Virus.—To obtain more exact data than have previously been recorded concerning the presence and concentration of influenza virus in the lungs of ferrets infected with influenza virus and the development of neutralizing antibodies at various intervals after infection, a series of experiments was performed.

Normal ferrets were inoculated intranasally with more than 1000 infectious doses of influenza virus and were killed at intervals thereafter. Serum was obtained by cardiac puncture, and the titer of neutralizing antibodies was determined as described above. Suspensions of both the lungs and the spleens were made and were tested by the intranasal inoculation of mice. The results of these experiments are presented in Table V.

It will be seen that all of the inoculated ferrets contracted a relatively severe influenza virus infection, as judged by the febrile response, the presence of typical nasal signs, and pulmonary consolidation. It will also be noted that despite the presence of extensive pulmonary consolidation, active

TABLE V

Comparison between Titers of Virus in Lung and Neutralizing Antibodies in Serum of Ferrets at Intervals after Infection with Human Influenza Virus

Ferret	Strain of virus	Time after inoculation	Highest temperature	Nasal signs	Lung consolidation	Standard neutralization titer	Virus titer	
							Lung	Spleen
		days	*°F.*					
27	PR8(m)	3	105.0	+	+	0	$10^{-3.4}$	0
28	PR8(m)	5	105.6	+	+	0	$10^{-4.0}$	0
29	PR8(m)	7	105.0	+	+	1:64	0	0
30	PR8(f)	8	104.9	+	+	—	0	—
31	PR8(f)	10	105.5	+	+	1:162	0	—
32	PR8(f)	10	105.5	+	0	1:128	0	—
33	W.S.(m)	3	106.0	+	+	0	$10^{-3.5}$	—
34	W.S.(m)	4	104.5	+	+	—	$10^{-4.5}$	—
35	W.S.(m)	6	104.8	+	+	0	$10^{-4.3}$	0
36	W.S.(m)	10	104.5	+	+	1:162	0	—

m = mouse-adapted strain. f = ferret passage strain. — = not done.

virus was demonstrable in the lungs of infected ferrets only through the 6th day after inoculation. In no instance was active influenza virus found in the lungs after the 6th day, and at no time during the course of the infection was active virus found in the spleens. Neutralizing antibodies against influenza virus appeared in the serum on the 7th day and increased rapidly to relatively high levels. Twenty-three normal ferrets inoculated intranasally with influenza virus were found to have an average standard neutralizing antibody titer of 1:110 on the 10th day after inoculation. In the light of these experiments it seems reasonable to suggest that the relatively rapid and vigorous production of antibodies in ferrets infected with influenza virus accounts for the equally rapid disappearance of active virus from the lungs.

Course of the Infectious Processes in Ferrets Inoculated with Both Influenza and Distemper Viruses.—With the aid of the information obtained by the study of ferrets inoculated either with distemper virus or with influenza virus, a comparative assessment of the infectious processes in ferrets inoculated with both viruses was undertaken.

TABLE VI

Comparison between Presence of Human Influenza Virus in Lungs and Titer of Neutralizing Antibodies in Serum of Ferrets at Intervals after Mixed Infection with Human Influenza Virus and Canine Distemper Virus

Ferret	Influenza virus strain	Distemper virus strain	Time after inoculation	Highest temperature	Influenza					Distemper	
					Nasal signs	Lung consolidation	Standard neutralization titer	Virus titer		Skin signs	Liver, spleen lesions
								Lung	Spleen		
			days	°F.							
37	PR8(m)	X	3	105.2	+	+	0	$10^{-3.5}$	0	0	0
38	PR8(m)	X	5	105.6	+	+	1:16	$10^{-2.5}$	0	0	0
39	PR8(m)	X	7	104.4	+	+	1:25	$10^{-3.5}$	0	0	+
40	PR8(f)	X	8	105.6	+	+	1:4	10^{-1}*	−	0	+
41	PR8(f)	X	11	105.8	+	+	1:25	10^{-1}*	0	0	+
42	PR8(f)	X	11	104.8	+	+	1:8	10^{-1}*	0	+	+
43	W.S.(m)	X	5		+	+	0	$10^{-5.5}$	0	+	+
44	W.S.(m)	X	6	104.7	+	+	0	10^{-1}*	0	0	+
45	W.S.(m)	X	8	104.8	+	+	1:11	$10^{-4.5}$	0	0	+
46	W.S.(m)	X	11	104.9	+	+	1:8	$10^{-3.5}$	0	0	+
47	W.S.(m)	Y	8	104.3	+	+	1:55	0	0	0	+
48	W.S.(m)	Y	11	104.8	+	+	1:256	0	0	0	+

m = mouse-adapted strain. f = ferret passage strain. − = not done.
* Lung suspensions in dilutions higher than 10^{-1} were not tested.

Normal ferrets were given more than 1000 doses of both influenza virus and of distemper virus intranasally from the same syringe and were killed at varying intervals thereafter. Serum was obtained by cardiac puncture immediately prior to the autopsy. Suspensions were made of the lungs and spleens of these ferrets and were tested for the presence of influenza virus by the inoculation of mice as described above. The titer of neutralizing antibodies was also determined as previously indicated. The results of these experiments are summarized in Table VI.

It will be observed that, as judged by a significant febrile response, the development of typical nasal signs, and the presence of extensive and typical pulmonary consolidation at autopsy, all the ferrets contracted a relatively pronounced infection by influenza virus. It will also be seen that in those ferrets

which were observed for a sufficiently long period there was definite evidence that they had also suffered from infection by distemper virus. Most significant, however, is the fact that influenza virus persisted in at least undiminished titer in the lungs of these ferrets throughout the interval covered by the study. Because of the severity of the infection produced by the massive dose of distemper virus, it was not possible to study these ferrets for longer than 11 days after inoculation since at that time they were moribund. As was found in the case of ferrets infected only with influenza virus, the spleens of ferrets infected with both distemper and influenza viruses did not contain the latter virus in a single instance. It will be noted also that although neutralizing antibodies against influenza virus appeared at approximately the same time after the infection as was observed in ferrets infected with influenza virus alone, the antibody titer did not increase significantly but persisted at a relatively low level. It was found that the average standard neutralizing antibody titer of seven ferrets infected concurrently with influenza and distemper viruses and bled between the 10th and 12th day after inoculation was only 1:12. This was a standard titer approximately ten times lower than that given above for ferrets inoculated with influenza virus alone. In terms of the neutralizing capacity of the undiluted serum, it can be shown by application of the linear relationship (8) that the serum of the doubly infected ferrets was capable of neutralizing only one-thirtieth as much influenza virus as the undiluted serum of ferrets infected with influenza virus alone. It will be noted further that persistence of influenza virus in the lungs after the 6th day and the diminished antibody response were demonstrable only when the X strain was mixed with influenza virus but were not demonstrable when the Y strain was used. Comparing these results with those obtained in ferrets infected with influenza virus alone, it appears that in the doubly infected ferrets the antibody response to influenza virus was quantitatively depressed as a result of the severe and generalized distemper infection and that it was insufficient to cause inactivation of influenza virus in the lungs.

DISCUSSION

The inoculation of two different viruses simultaneously or almost simultaneously into a single host is known in some instances to produce unusual results. The best known examples of this kind, however, have resulted in the inhibition of the usual effects produced by one virus or the other. This inhibition has been termed "interference," and was first demonstrated with neurotropic and viscerotropic strains of yellow fever virus by Hoskins (12).

This effect was confirmed subsequently by Findlay and MacCallum (13) who also demonstrated that the phenomenon could be produced by combined inoculations of Rift Valley fever and yellow fever viruses. A somewhat less definite but probably related effect has been described by Dalldorf (14) in the case of the sparing action of lymphocytic choriomeningitis on poliomyelitis. Finally, an example of local interference has been described with fibroma virus and virus III by Andrewes (15).

The concurrent infection of ferrets with human influenza and canine distemper viruses resulted in a very different effect. The clinical course of neither of the two infections appeared to be significantly altered, as will be seen by comparing graph 3 with graphs 1 and 2 in Fig. 1; nor were the pathological effects induced by either of these two different viruses inhibited by the presence of the other. Instead, it was found that active influenza virus persisted in the lungs of such doubly infected ferrets for at least twice as long as it did in the lungs of ferrets infected with influenza virus alone. The prolonged persistence of influenza virus in apparently undiminished titer in the lungs of doubly infected ferrets seems most probably to be the result of the reduced ability of these ferrets to respond normally to the presence of influenza virus and to produce the usual amounts of neutralizing antibody against this virus. No direct explanation for this effect is at hand, although it seems reasonable to suggest that the concurrent, severe, and generalized infection with distemper virus sufficiently altered the usual responses of the animal so as to reduce the amount of antibody which was produced against influenza virus.

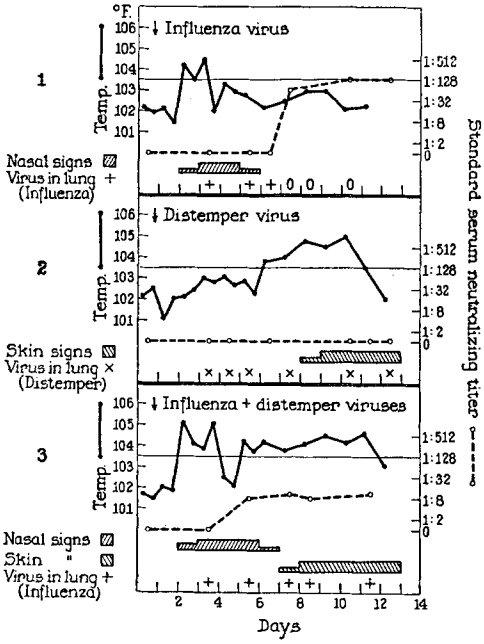

FIG. 1. Temperature curves, clinical symptoms, presence of virus in lung, and antibody response against influenza virus of experimentally infected ferrets.

Graph 1. Influenza virus infection.

Graph 2. Distemper virus infection.

Graph 3. Influenza virus and distemper virus infection.

No other instance of similar character appears to have been recorded.

It is well known that numerous viruses are capable of persisting in neoplasms. Following the discovery of this phenomenon by Levaditi and Nicolau (16) a number of papers on the subject have appeared. The literature concerning the prolonged persistence of various viruses in tumors has been reviewed recently by Rous and Kidd (17). While the end result in these cases has at least one feature in common with that reported herein, it would appear that the mechanisms responsible for the persistence of active viruses in tumors are not basically the same as the cause of the persistence of influenza virus in the presence of distemper.

The finding that active influenza virus persists for an unusually prolonged period in the lungs of ferrets suffering from both influenza and distemper offers a possible explanation for the effectiveness of the complex vaccine prepared from the tissues of such ferrets. However, if the antigenicity of this vaccine were due merely to the presence in it of unneutralized influenza virus, vaccines prepared from infected ferret tissues in the first few days after infection with influenza virus alone should be equally effective. The fact that they were not raises the possibility that some qualitative alteration is produced in the antigenic structure of influenza virus under these conditions since the quantity of active virus which persists does not seem to be sufficiently great to account for the observed differences in antigenicity. The elucidation of this hypothetical alteration in antigenic structure will require additional experimentation.

SUMMARY

The infections produced in ferrets by human influenza virus and canine distemper virus were studied. Cross immunity and cross neutralization tests showed that these two viruses were not related antigenically. Ferrets infected with influenza virus alone rapidly produced considerable quantities of neutralizing antibodies, and after the 6th day virus was not demonstrable in their lungs. Ferrets infected with both influenza and distemper viruses simultaneously produced but small amounts of neutralizing antibody, and influenza virus persisted in undiminished concentration in their lungs throughout the course of the infection.

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