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SPECIFIC IMMUNITY TO INFLUENZA VIRUS IN FERRET ORGAN CULTURES

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Summary.—Ferret tracheal organ cultures prepared from animals previously infected intranasally with influenza A virus required approximately 130 times more homologous virus (A/PR/8/34(HON1) or A/Port Chalmers/1/73 (H3N2)) to become infected *in vitro* than similar cultures from normal ferrets. Also, these cultures from convalescent ferrets required 9 times more heterologous virus (A/PR/8/34(HON1) or Sendai) to become infected *in vitro* than similar cultures from normal animals challenged *in vitro* with the heterologous virus. We conclude that these tracheal rings are specifically immune.

Once tracheal rings are infected, they continue to shed viruses for at least 60 days, the longest period any cultures were kept. Virus shedding in the intact ferret lasts normally 5–7 days. Thus recovery in the intact ferret seems to be dependent upon factors which are not present, or at least not functional, in the tracheal explant. This is consistent with the hypothesis that recovery is dependent upon systemic rather than local phenomena.

Bladder tissue from normal and previously infected ferrets was also cultured and challenged with homologous and heterologous virus. The bladder from previously infected ferrets exhibited specific immunity, although the immunity was more variable.

MANY questions remain unanswered regarding the mechanism of immunity to and recovery from influenza virus infection. Further, factors responsible for immunity are not necessarily the same as those responsible for recovery. Some authors perceive specific serum antibody and therefore systemic immunity to be protective against infection. Stuart-Harris (1973) reported that serum antibody was protective in man. Both Allan, Madeley and Kendal (1971) and Rott, Becht and Orlich (1974) reported serum antibody to be protective in chickens. More recently Virelizier (1975) concluded that serum antibody was most important for protection against reinfection by influenza in mice. McLaren, Potter and Jennings (1974), using ferrets in challenge experiments, found serum antibody against the haemagglutinin to correlate best with protection against infection.

In contrast to these observations, Morris *et al.* (1966) demonstrated that influenza infection in humans is not prevented by high levels of serum antibody. Small *et al.* (1976) passively immunized ferrets so that their haemagglutinin inhibition titres (HAI) were 1 : 1000 or greater but were unable to prevent influenza infection. They concluded that serum antibody played no role in preventing infection in ferrets.

Other investigations support the idea that antibody produced locally in the respiratory tract and distributed on the mucous surfaces is the primary mode of protection. Beare *et al.* (1969) concluded

that a factor, probably local antibody, exerted a considerable influence on human resistance to infection with influenza virus. Waldman and Coggins (1972) concluded that a vaccine's ability to stimulate nasal secretory antibody was the deciding factor as to whether it could protect humans against influenza infection. Potter *et al.* (1972) showed production of nasal antibody after nasal infection of ferrets but not after immunization with killed virus. They postulated this was the reason for better protection of these animals upon subsequent challenge with virus.

Part of this debate may be due to species differences. In the mouse there seems to be agreement that serum antibody will prevent disease. In human and ferret, however, protection experiments have not been in agreement as to which, serum Ab or local Ab, is most important in resistance.

Influenza infection in ferrets appears to resemble influenza infection in humans and thus has been deemed a useful model to study human influenza infections. Hoorn and Tyrrell (1965) showed that organ cultures could be used to study viral infections of the respiratory tract. Many studies have used this technique to answer questions concerning pathogenesis (Klein and Collier, 1974), resistance (Schmidt and Maassab, 1974), detection (Neumann and Kaleta, 1975) and host range of viruses (Schmidt, Massah and Davenport, 1974). As was done in one of those studies (Schmidt and Maassab, 1974), we also used organ cultures to test tissue immunity of ferrets to challenge by influenza virus.

In this study, an attempt was made to demonstrate, *in vitro*, specific immunity to influenza virus in previously infected tissues of the ferret. Organ cultures of trachea from ferrets 21 days convalescent from influenza infection were shown to be resistant to reinfection when challenged by homologous virus. The resistance appeared to be specific. Bladder tissue from the same animals also exhibited specific resistance upon challenge *in vitro*.

MATERIALS AND METHODS

Animals.—Mature ferrets were obtained from Marshall Research Animals, Inc., North Rose, New York 14516, and housed in individual cages under conditions which prevent cross-infection. Males and females were used in approximately equal numbers.

Viruses.—Influenza viruses used were A/PR/8/34 (HON1) and A/Port Chalmers/1/73 (H3N2). Large stocks of both viruses were obtained by injecting virus into allantoic cavities of 10-day-old embryonated eggs which were then incubated for 3 days at 36°, at which time allantoic fluid was harvested, pooled and stored at −85° in 1-ml aliquots. The A/PR/8/34 HON1 virus had a chick erythrocyte haemagglutination (HA) titre of 1280, and contained $10^{7\cdot2}$ 50% egg infectious doses/ml (EID_{50}). The A/Port Chalmers/1/73 (H3N2) virus had an HA titre of 160, and contained $10^{8\cdot2}EID_{50}$/ml. Sendai virus was propagated in the same manner and had an HA titre of 512, containing $10^{9\cdot2}EID_{50}$/ml.

Intranasal innoculation of virus into ferrets.—Ferrets were anaesthetized with 0·5 ml of Ketaset (ketamine hydrochloride, Bristol Laboratories) and infected with 0·1 ml of undiluted virus in each naris.

Assays.—Virus was detected by inoculation of sample into the allantoic cavity of embryonated chicken eggs that were 10 days old, as previously described (Barber and Small, 1974). HA and HAI titres were performed with a microtitre kit using disposable microtitre plates (Cooke Engineering Co., Alexandria, Virginia) as described by Sever (1962). Sera used for HAI assays were first absorbed with kaolin and chick RBCs, and heated at 56° for 30 min as described previously (Barber and Small, 1974).

Tissue cultures.—Ferrets were anaesthetized and exsanguinated by cardiac puncture. Trachea and bladder were removed aseptically and placed into sterile 100 × 15 mm Petri dishes containing approximately 20 ml Hanks' ballanced salt solution with 100 u/ml penicillin and 100 μg/ml streptomycin. While in the Petri dish the trachea was cut into individual rings and the bladder was cut into pieces approximately 3 × 3 mm. Each individual piece of tissue was then placed into a 35 × 10 mm Petri dish containing 3 ml of nutrient medium (Eagle's minimal essential media with 10% fetal calf serum, gentamicin (50 μg/ml), streptomycin (100 μg/ml), penicllin (125 u/ml), and mycostatin (100 u/ml)). The following day (Day 1) the 3 ml of medium was removed and replaced with 3 ml of the same medium containing virus.

RESULTS

Immunity of tracheal organ culture to homologous virus

The basic experimental design is shown

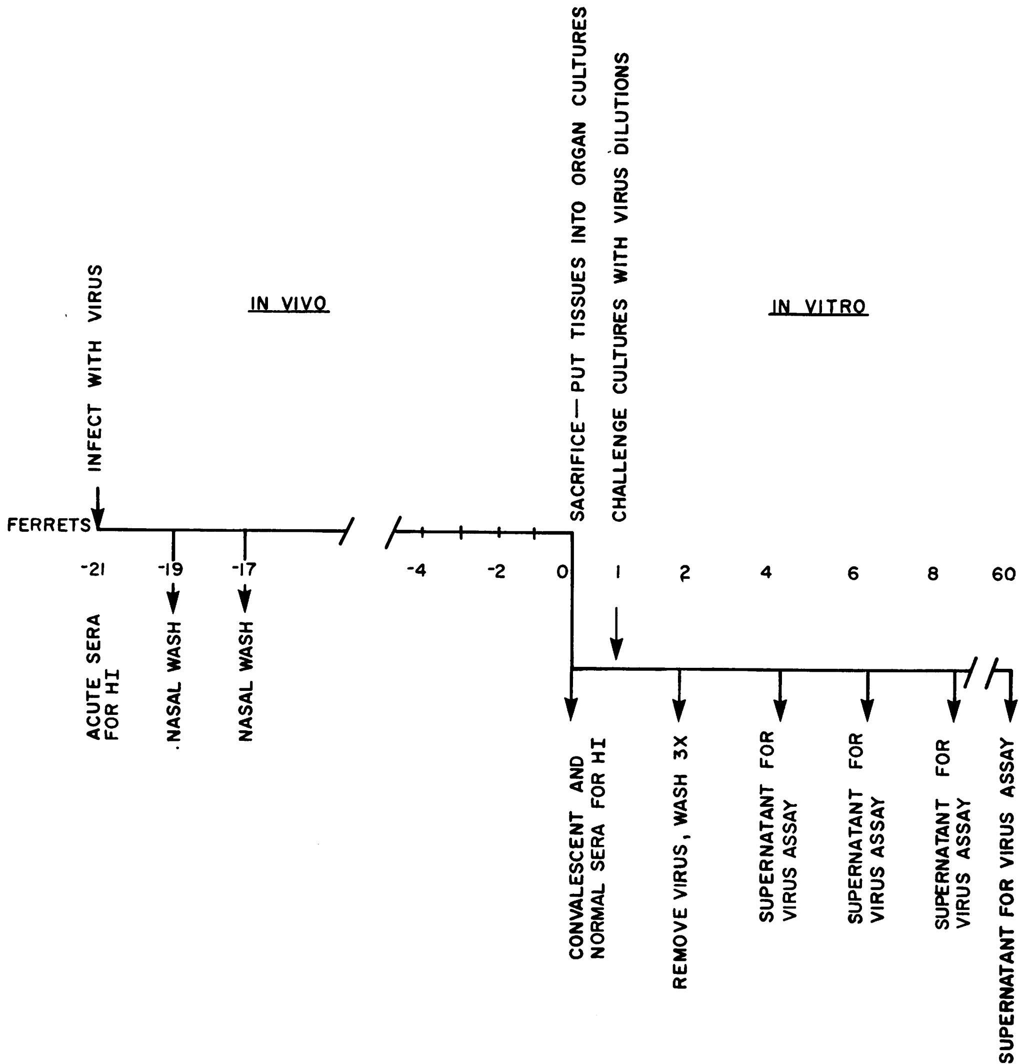

FIG.—Experimental Protocol—Experimental sequence in days.

in the figure. In all experiments ferrets, subsequently identified as convalescent, were infected 21 days before being killed. Nasal washes were taken 2 and 4 days after this infection. Serum was taken at the time of infection and of killing. Animals were proven to be infected by both virus isolation from nasal wash (one exception noted in table) and a 4-fold or greater rise in HAI antibody titre to the virus. At Day 0 normal and convalescent ferrets were killed and their tissues put into organ cultures. Serum taken from normal ferrets at the time of killing (Day 0) had no detectable HAI antibody (<1 : 8) to influenza virus. Cultures were challenged with varying dilutions of influenza virus on Day 1. The number of organ cultures/dilution varied in different experiments depending upon the number obtained from each animal. Fluids were completely drained and replaced every other day and supernatants were assayed for virus on Days 1, 4, 6 and 8 and in one experiment periodically up to 60 days.

Table I shows data from Experiment IV.

TABLE I.—*Virus Isolation from Tracheal Rings*

Virus isolation* on day

Virus challenge†	Rings	0	4	6	8	10	12	14	17	19	21	25	31	37	60	Infected rings (Day 8) / Total rings	OCID₅₀
		Normal ferret															
Control	1	0	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	0		
	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5	
	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
10^{-3}	1	0	+	+	+	+	+	+	+	+	ND	+	0	+	+		$10^{-0.8}$EID$_{50}$s/ml
	2	0	+	+	+	+	+	+	+	+	ND	+	0	+	+		
	3	0	+	+	+	+	+	+	+	+	ND	+	+	0	+	5/5	
	4	0	+	+	+	+	+	+	+	+	ND	+	+	+	+		
	5	0	+	+	+	+	+	+	+	+	ND	+	+	+	+		
10^{-4}	1	0	+	+	+	+	+	+	+	+	+	+	+	+	+		
	2	0	+	0	+	+	+	+	+	+	+	+	0	+	+		
	3	0	+	+	+	+	+	+	+	+	+	+	+	+	+		
	4	0	+	+	+	+	+	+	+	+	+	+				5/5	
	5	0	+	+	+	+	+	+	+	+	+	+	+	+	+		
10^{-5}	1	0	+	+	+	+	+	+	+	+	+	+	+	+	+		
	2	0	+	+	+	+	+	+	+	+	+	+	0	+	+	5/5	
	3	0	+	+	+	+	+	+	+	+	+	+	0	+	+		
	4	0	+	+	+	+	+	+	+	+	+	+	+	0	+		
	5	0	+	+	+	+	+	+	+	+	+	+	+	+	+		
10^{-6}	1	0	+	+	+	+	+	+	+	+	+	+	0	+	+		
	2	0	+	+	+	+	+	+	+	+	+	+	0	+	+		
	3	0	+	+	+	+	+	+	+	+	+	0	+	+	+	4/5	
	4	0	+	+	+	+	+	+	+	+	+	+	0	+	+		
	5	0	0	0	0	0	0	0	0	0	0	0					
10^{-7}	1	0	0	0	0	0	0	0	0	0	0	0					
	2	0	0	0	0	0	0	0	+	0	0	0					
	3	0	+	+	+	+	+	+	+	+	+	+	0	+	+	3/5	
	4	0	+	+	+	+	+	+	+	+	+	+	0	+	+		
	5‡	0	0	+	+	+	+	+	0	0	+	+	+	+	+		

TABLE I.—(*continued*)

Virus challenge†	Rings	Virus isolation* on day 0	4	6	8	10	12	14	17	19	21	25	31	37	60	Infected rings (Day 8) / Total rings	$OCID_{50}$
		Convalescent ferret															
10^{-2}	1	0	+	+	+	+	+	+	+	+	+	0	+	+	+		
	2	0	+	+	+	+	+	+	+	+	+	+	+	+	+	4/4	
	3	0	+	0	+	+	+	+	+	+	+	+	+	+	+		
	4	0	+	+	+	+	+	+	+	+	+	+	0	+	+		
10^{-3}	1	0	+	+	+	+	+	+	+	+	+	+	+	+	+		$10^{1.5}EID_{50}s$/ml.
	2	0	+	+	+	+	+	+	+	+	+	+	+	+	+	4/4	
	3	0	+	+	+	+	+	+	+	+	+	+	0	0	+		
	4	0	+	+	+	+	+	+	+	+	+	+	+	+	+		
10^{-4}	1	0	0	0	0	0	0	0	0	0	0	0					
	2	0	+	+	+	+	+	+	+	+	+	+	+	+	+	3/4	
	3	0	+	+	+	+	+	+	0	+	+	+					
	4	0	+	+	+	+	+	+	+	+	+	+	0	+	+		
10^{-5}	1	0	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					
	3	0	0	0	0	0	0	0	0	0	0	0				1/4	
	4	0	+	+	+	+	+	+	+	0	+	0	+	+	+		
10^{-6}	1	0	+	0	0	0	0	0	0	0	0	0	0	0	0		
	2	0	+	+	+	+	+	+	+	+	+	+	+	+	+		
	3‡	0	0	0	0	0	0	0	0	0	+	+	+	+	+	1/4	
	4‡	0	0	0	0	+	+	+	+	+	+	+	+	+	+		
10^{-7}	1	0	0	0	0	0	0	0	0	0	0	0					
	2	0	0	0	0	0	0	0	0	0	0	0					
	3	0	0	0	0	0	0	0	0	0	0	0				0/5	
	4	0	0	0	0	0	0	0	0	0	0	0					
	5	0	0	0	0	0	0	0	0	0	0	0					

* + = at least one of the duplicate eggs show HA 3 days after inoculation with sample.
† Dilution of stock virus containing $10^{7.2}$ EID_{50}/ml of A/PR/8/34 (HONI).
‡ Ring which changed from not infected to infected.
ND = Not done.

TABLE II.—*Immunity in Tracheal Organ Cultures*

Expt.	No. of animals*	No. of rings/animal/ dilution of virus	Virus	$OCID_{50}$ Log 10†, ‡ (mean ± SD)			P¶
				Normal	Convalescent §	Ratio of infectivity**	
IV	2	4 or 5	HON1	−0·8	1·5	200 ×	< 0·0005
V	10	2	HON1	0·3(± 0·4)	2·45(± 0·8)	140 ×	< 0·0005
						weighted avg. 150 ×	

MS

* Half the number of animals listed were normal and half were convalescent.
† $OCID_{50}$ obtained by using total infected and total uninfected cultures for an entire group of animals.
‡ $OCID_{50}$ = 50% organ culture infectious dose as determined by the Reed–Muench 50% endpoint method (1938).
§ 11 of 12 animals were convalescent by two criteria: (a) Virus shedding; (b) 4-fold increase in specific Ab titre after infection. (One convalescent animal in Expt. V was not tested for virus shedding.)
¶ Probability that difference occurred by chance calculated by Student's t test.
** Antilog of (Log 10 $OCID_{50}$ convalescent—Log 10 $OCID_{50}$ normal) homologous virus.

TABLE III.—*Specific Immunity in Ferret Tracheal Organ Culture*

			$OCID_{50}$ log 10 †, ‡ (mean±SD)						Ratio of infectivity¶		
			Virus		Normal		Convalescent				
Expt.	No. of* animals	Rings/ animal/ dilution of virus	Homologous	Heterologous	Homologous	Heterologous	Homologous	Heterologous	Homo-logous challenge	Hetero-logous challenge	P§
VII	4	4		HON1		1·3(±0·7)		0·9(±0·3)		0·4	NS
		4	H3N2		1·8(±0)		3·1(±0·2)		20		0·05
IX	6	2		HON1		1·4(±0·2)		2·2(±0·4)		6	<0·005
		2	H3N2		3·95(±0·4)		5·5(±0·3)		32		<0·005
XI	8	2		Sendai		0·6(±0·5)		1·8(±0·4)		16	<0·005
		2	H3N2		2·2(±0·7)		4·5(±0·3)		200		<0·005
						Weighted avg.			104	9	

* Half the number of animals listed were normal and half were convalescent.
† $OCID_{50}$ obtained by using total infected and total uninfected cultures for an entire group of animals.
‡ $OCID_{50}$=50% organ culture infectious dose as determined by the Reed–Muench 50% endpoint method (1938).
§ Probability that difference occurred by chance.
¶ Antilog of (Log 10 $OCID_{50}$ convalescent—Log 10 $OCID_{50}$ normal).

In general, once a culture was infected it remained so throughout the experiment. Three of the 55 cultures differed significantly from this pattern. Transient conversions from positive to negative occurred occasionally but were probably due to the egg assay system which apparently can have false negatives but not false positives. When a large number of false negatives appeared on one day it probably can be attributed to improper handling of samples (*e.g.* Day 31). Since very little variation occurred, Day 8 was selected to calculate an $OCID_{50}$ (50% organ culture infectious dose). $OCID_{50}$s were calculated by the method of Reed and Muench (1938). For the remaining experiments cultures were usually carried through Day 10 and $OCID_{50}$s calculated for Day 8.

Table II summarizes the results of Experiment IV (already shown in Table I) and Experiment V, in which tracheal organ cultures from 5 normal and 5 convalescent ferrets were compared for relative resistance to influenza infection. As can be seen, convalescent ferret trachea required between 140 and 200 times more virus to infect them than did normal cultures. This difference was statistically significant and hence the experiments demonstrate the presence of immunity.

Immunity in ferret tracheal organ cultures with homologous and heterologous virus

To determine if the immunity demonstrated in tracheal organ cultures was specific for the virus which had been used to infect the animal (homologous virus), half of the cultures were challenged with the homologous virus and the other half with a different virus (heterologous virus). The results are shown in Table III. Rings challenged with homologous virus (H3N2) were between 20 and 200 times more resistant to infection in culture than normal ferret tracheal organ cultures. Cultures challenged with the heterologous virus (HON1 or Sendai) showed no significant difference in one experiment and only 6–16 times more resistant in two experiments when the $OCID_{50}$s were compared with those from normal rings. The immunity therefore appears to be largely specific, although a variable amount of nonspecific immunity is sometimes observed.

Bladder immunity

To get an indication of whether specific immunity was restricted to the respiratory tract, a second anatomically distinct site was tested. Basarab and Smith (1970) had shown that ferret bladder tissue was susceptible to *in vitro* infection by influenza virus. Therefore, cultures of trachea (results shown in Table II and III) and bladder were taken from ferrets in some experiments. Table IV shows the $OCID_{50}$s for the normal and convalescent bladder in four experiments. In Experiment V bladder immunity is demonstrated since convalescent bladder tissue required approximately 200 times more virus to infect than did bladder from normal ferrets (statistically significant at $P<0{\cdot}10$). From Experiment VII it appears that nonspecific immunity exists since it took 20 and 16 times more virus respectively to infect convalescent tissues than to infect normal tissues with heterologous and homologous virus. Levels of significance varied with a P value of 0·10 for heterologous and a value of $<0{\cdot}10$ for homologous, hence this is not a conclusive experiment. The last two experiments (IX and XI) show specific immunity. In experiments which include tissues from a total of 14 animals it took 500 and 40 times more homologous virus to infect convalescent tissues than to infect normal tissues (significance $P=0{\cdot}025$ and $P=<0{\cdot}005$). At the same time, it took 10 and 0·25 times more heterologous virus to infect convalescent cultures than it took to infect normal cultures (significance level of $P=0{\cdot}10$ and $P=<0{\cdot}005$). Thus 3 of the 4 experiments suggest specific immunity in bladder to homologous virus.

DISCUSSION

Organ cultures were used to study immunity in tissues of ferrets exposed to

TABLE IV.—*Immunity in Ferret Bladder Organ Cultures*

Expt.	No. of Animals*	Pieces of tissue/ animal/ dilution of virus	OCID$_{50}$ log 10 †, ‡ (mean ±SD)						Ratio of infectivity**		P¶
			Virus		Normal		Convalescent §				
			Homologous	Heterologous	Homologous	Heterologous	Homologous	Heterologous	Homo-logous challenge	Hetero-logous challenge	
V	4	2 or 3	HON1		0·2(±0·8)		2·52(±0·5)		200		<0·10
VII	4	2		HON1		0·2(±0)		1·5(±0·35)		20	0·10
			H3N2		2·8(±0·4)		4·0(±0)		16		<0·10
IX	6	1		HON1		0·7(±0)		1·7(±0·7)		10	0·10
			H3N2		2·3(±0·8)		5·0(±1·0)		500		<0·025
XI	8	2		Sendai		2·3(±0·25)		1·7(±0·2)		25	<0·005
			H3N2		3·0(±0·5)		4·6(±0·9)		40		<0·005
							weighted avg.		190	8	

* Half the number of animals listed were normal and half were convalescent.
† OCID$_{50}$ obtained by using total infected and total uninfected cultures for an entire group of animals.
‡ OCID$_{50}$=50% organ culture infectious dose as determined by the Reed–Muench 50% endpoint method (1938).
§ 21 or 22 animals shown to be convalescent by both criteria: (a) Virus shedding as determined by assay of nasal wash; (b) 4-fold increase in specific Ab titre after infection. (One convalescent animal in Expt. V was not tested for (a) due to lost samples).
¶ Probability that difference occurred by chance.
** Antilog of (Log 10 OCID$_{50}$ convalescent—Log 10 OCID$_{50}$ normal).

influenza A. Tracheal organ cultures from ferrets convalescent from influenza infection required about 130 times more homologous virus to become infected than cultures from normal ferrets. It took only about 9 times more heterologous virus to infect convalescent cultures than to infect normal cultures. Immunity was therefore largely specific. Bang and Niven (1958) briefly reported that ferret mucosal tissue from convalescent animals was not resistant to infection. It is not clear why their results differed from those presented here.

Bladder tissue cultures were used to test if the specific immunity was localized in the respiratory tract or was more widespread. Experiments show that bladders from convalescent ferrets were about 190 times more resistant to challenge with homologous virus than normal bladder, and that convalescent tissues were only about 8 times more resistant to heterologous challenge. Bladder specific immunity could be explained in at least 2 ways: (a) the specific immunity is caused by systemic factors, or (b) it is a local response caused by either antigenaemia or homing to bladder mucosal tissue of specific lymphocytes stimulated in the respiratory tract. Basarab and Smith (1970) did show that influenza virus could replicate *in vivo* in bladders of ferrets. And it has been shown that during severe influenza infection that virus can be recovered from urine of patients (Naficy, 1963).

Since the immunity in both trachea and bladder appears to be specific, it could be mediated by antibody and/or lymphocytes. However, it has recently been demonstrated in cytotoxic studies using influenza-infected target cells (Effros *et al.*, 1977; Zweerink *et al.*, 1977) that CMI may be less specific than is required to account for the specific protection measured in challenge experiments. Therefore, antibody seems to be the more likely mechanism for prevention of influenza in ferrets.

Possible mechanisms of this hypothetical antibody-mediated immunity in these organ cultures are: (1) systemic serum antibody could have been responsible and simply be trapped in the mucous secretions and/or in the tissue itself or (2) the immunity could be locally produced in submucosal immunocompetent cells.

Turning from prevention of infection to recovery from infection, recent studies showed that mice with deficient CMI (nude mice; Sullivan *et al.*, 1976) or mice treated with ALS (Suzuki, Ohya and Ishida, 1974) shed virus over longer periods than did normal mice, suggesting CMI may play a critical role in recovery. In Experiment IV we showed that once a ferret tracheal organ culture was infected it remained so, that is, it did not recover. If CMI is responsible for recovery, it would follow that CMI was not functional in the tracheal organ culture.

Irrespective of the mechanism of prevention or recovery from influenza, it seems that the ferret tracheal organ culture enables one to separate the two mechanisms. Immunity to reinfection can be demonstrated in the same piece of tissue that lacks the ability to recover. Hence it seems that prevention and recovery are mediated by different mechanisms.

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REFERENCES

Allan, W. H., Madeley, C. R. & Kendal, A. P. (1971) Studies with Avian Influenza A Viruses: Cross Protection Experiments in Chickens. *J. gen. Virol.*, **12**, 79.

Bang, F. B. & Niven, J. S. F. (1958) A Study of Infection in Organized Tissue Cultures. *Brit. J. exp. Path.*, **39**, 317.

Barber, W. H. & Small, P. A., Jr (1974) Dissemination of Influenza Virus between Anatomically Isolated Sites in Ferrets. *Infect. Immun.*, **9**, 530.

Basarab, O. & Smith, H. (1970) Growth Patterns of Influenza Virus in Cultures of Ferret Organs. *Brit. J. exp. Path.*, **51**, 1.

Beare, A. S., Tyrrell, D. A. J., Hobson, D., Howells, C. H. L., Pereira, M. S., Pollock, T. M. & Tyler, L. E. (1969) Live Influenza B Vaccine in Volunteers. *J. Hyg., Camb.*, **67**, 1.

Effros, R. M., Doherty, P. C., Gerhard, W. & Bennink, J. (1977) Generation of Both Cross-reactive and Virus-specific T-cell Populations after

Immunization with Serologically Distinct Influenza A Viruses. *J. exp. Med.*, **145**, 557.

HOORN, B. & TYRRELL, D. A. J. (1965) On ;he Growth of Certain "Newer" Respiratory Viruses in Organ Cultures. *Brit. J. exp. Path.*, **46**, 109.

KLEIN, J. D. & COLLIER, A. M. (1974) Pathogenesis of Human Parainfluenza Type 3 Virus Infection in Hamster Tracheal Organ Culture. *Infect. Immun.*, **10**, 883.

McLAREN, C., POTTER, C. W. & JENNINGS, R. (1974) Immunity to Influenza in Ferrets: XIII Protection against Influenza Infection by Serum Antibody to Homologous Haemagglutinin or Neuraminidase Antigens. *Med. Microbiol. Immunol.*, **160**, 33.

MORRIS, J. A., KASEL, J. A., SAGLAM, M., KNIGHT, V., LODA, F. A. (1966) Immunity to Influenza as Related to Ab Levels. *New Engl. J. Med.*, **274**, 527.

NAFICY, K. (1963) Human Influenza Infection with Proven Viremia. *New Engl. J. Med.*, **269**, 964.

NEUMANN, U. & KALETA, E. F. (1975) Detection of Newcastle Disease Virus in Chicken Tracheal Organ Cultures by the Fluorescent Antibody Technique and by the Embryonated Egg Method. *Avian Path.*, **4**, 227.

POTTER, C. W., SHORE, S. L., McLAREN, C. & STUART-HARRIS, C. (1972) Immunity to Influenza in Ferrets: II Influence of Adjuvants on Immunization. *Br. J. exp. Path.*, **53**, 168.

REED, L. J. & MUENCH, H. (1938) A Simple Method of Estimating Fifty Per Cent Endpoints. *Am. J. Hyg.*, **27**, 493.

ROTT, R., BECHT, H. & ORLICH, M. (1974) The Significance of Influenza Virus Neuraminadase in Immunity. *J. gen. Virol.*, **22**, 35.

SCHMIDT, R. C. & MAASSAB, H. F. (1974) Local Immunity to Influenza Virus in Chicken Tracheal Organ Cultures. *J. inf. Dis.*, **129**, 637.

SCHMIDT, R. C., MAASSAB, H. F. & DAVENPORT, F. M. (1974) Infection by Influenza A Viruses of Tracheal Organ Cultures Derived from Homologous and Heterologous Hosts. *J. inf. Dis.*, **129**, 28.

SEVER, J. L. (1962) Application of a Microtechnique to Viral Serological Investigations. *J. Immunol.*, **88**, 320.

SMALL, P. A., WALDMAN, R. H., BRUNO, J. C. & GIFFORD, G. E. (1976) Influenza Infection in Ferrets: Role of Serum Ab in Protection and Recovery *Infect. Immun.*, **13**, 417.

STUART-HARRIS, C. (1973) Influenza—The Problems and the Future. *Med. J. Aust.*, **1** (Special Suppl.) 42.

SULLIVAN, J. L., MAYNER, R. E., BARRY, D. W. & ENNIS, F. A. (1976) Influenza Virus Infection in Nude Mice. *J. inf. Dis.*, **133**, 91.

SUZUKI, F., OYA, J. & ISHIDA, N. (1974) Effect of Antilymphocyte Serum on Influenza Virus Infection in Mice. *Proc. Soc. Exp. Biol. Med.*, **146**, 78.

VIRELIZIER, J.-L. (1975) Host Defenses Against Influenza Virus: The Role of Antihemagglutinin Antibody. *J. Immun.*, **115**, 434.

WALDMAN, R. H. & COGGINS, W. J. (1972) Influenza Immunization: Field Trial on a Univ. Campus. *J. inf. Dis.*, **126**, 242.

ZWEERINK, H. J., COURTNEIDGE, S. A., SKEHEL, J. J., CRUMPTON, M. J. & ASKONAS, B. A. (1977) Cytotoxic T Cells Kill Influenza Virus Infected Cells, but do not Disginuish between Serologically Distinct Type A Viruses. *Nature, Lond.*, **267**, 354.