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Duck Influenza Lacking Evidence of Disease Signs and Immune Response

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Influenza viruses A/duck/Hokkaido/5/77 (Hav7N2), A/budgerigar/Hokkaido/1/77 (Hav4Nav1), A/Kumamoto/22/76 (H3N2), A/Aichi/2/68 (H3N2), and A/New Jersey/8/76 (Hsw1N1) were experimentally inoculated into Pekin ducks. Of these, the influenza viruses of duck and budgerigar origin replicated in the intestinal tract of the ducks. The infected ducks shed the virus in the feces to high titers, but did not show clinical signs of disease and scarcely produced detectable serum antibodies. Using immunofluorescent staining, we demonstrated that the target cells of the duck virus in ducks were the simple columnar epithelial cells which form crypts in the large intestines, especially in the colon. After primary infection, the birds resisted reinfection with the duck virus at least for 28 days, but from 46 days onward they were susceptible to reinfection. These infections were quickly restricted by a brisk secondary immune response, reflected in the rapid appearance of high titers of antibody after reinoculation. In contrast to the avian influenza viruses, the remaining three influenza viruses of human origin did not replicate in the intestinal tract but did cause a serum antibody response.

Many influenza A viruses have been isolated from several species of apparently healthy, wild, free-flying birds (1, 2, 3, 8, 10, 14). Among these, more influenza A viruses have been obtained from ducks than from other avian species (4, 5, 8, 12). The role of these birds as reservoirs or vectors in the natural history of influenza in humans and animals remains obscure. Recent studies on the ecology of influenza viruses have shown that many more influenza viruses have been isolated from cloacal samples than from the respiratory tract in ducks (8, 12, 15). We previously reported (8) that in the serum of an apparently healthy pintail, from which an influenza virus was isolated, no hemagglutination inhibition (HI) antibodies to the homologous isolate were found. This calm and stable infection pattern with the lack of evidence of disease and the apparent absence of antibody response seems to be one aspect of the host-parasite relationship between wild ducks and their influenza viruses.

Webster et al. (16) and Slemons and Easterday (13) showed that influenza viruses isolated from the cloaca of naturally infected feral ducks replicated in the cells lining the intestinal tract of feral and domestic ducks. Kawano et al. (7), on the other hand, showed that an influenza virus which was isolated from a budgerigar replicated in the respiratory tract of budgerigars.

The present study was an attempt to clarify the nature of the host-parasite relationships between ducks and influenza viruses through observation of the course of experimental infections caused by influenza viruses of duck, budgerigar, and human origin.

MATERIALS AND METHODS

Viruses. The following influenza viruses were used: A/duck/Hokkaido/5/77 (Hav7N2), which was isolated from a feral teal (8); A/budgerigar/Hokkaido/1/77 (Hav4Nav1), which was isolated from a budgerigar (9) and for which it was shown experimentally that the site of replication was in the upper respiratory tract of budgerigars (7); A/Kumamoto/22/76 (H3N2); A/Aichi/2/68 (H3N2); and A/New Jersey/8/76 (Hsw1N1). The viruses were propagated in the allantoic cavities of 11-day-old chicken embryos. Infectious allantoic fluids were used. A/duck/Hokkaido/5/77 inactivated with 0.01% Formalin for 4 days at 4°C was also used.

Ducks. The fertile eggs of white Pekin ducks (*Anas platyrhynchos domesticus*) were supplied by the Department of Anatomy, Faculty of Veterinary Medicine, Hokkaido University, Sapporo, Japan. The ducks used in the present study were exclusively those hatched and carefully reared in our laboratory to avoid natural infection with influenza viruses. They were used at 1 to 4 months of age.

Experimental infection of ducks with influenza viruses. The groups of ducks were inoculated with 0.2 ml of the allantoic fluid containing $10^{6.7}$ to $10^{8.0}$ EID_{50} (50% egg infective doses) of each virus intranasally, orally, by injection into a vein, or by

catheterization into the colon. The normal allantoic fluid of the chicken embryos was inoculated into the control ducks. The ducks were housed individually in separate cages and observed clinically until they were killed.

Recovery of viruses. The ducks were killed for virus isolation from organs by bleeding through heart puncture at various times after inoculation with the viruses. The sinus, larynx, trachea, lung, esophagus, crop, proventriculus, gizzard, duodenum, jejunum, ileum, cecum, colon, rectum, bursa, cloaca, brain, eye, thymus, spleen, heart, pancreas, liver, gonad, and air sac were obtained aseptically and then ground with sterile sand to yield suspensions of 10 to 20% in broth containing antibiotics. After centrifugation, the supernatants were inoculated into the allantoic cavities of either two or four 11-day-old embryonated eggs. When influenza virus was recovered from the sample, each serial 10-fold dilution of the samples was injected into four eggs. The virus titers were calculated by the method of Reed and Muench and expressed as EID_{50} per gram of tissue. Tracheal and cloacal swabs of cotton and feces and blood were taken daily or hourly and assayed similarly. The viruses recovered from each sample were identified with specific antisera (8, 9) by the HI tests (11). The sera of ducks were assayed for specific antibody production by the HI tests after treatment with receptor-destroying enzyme (Takeda Chemical Industries Co., Osaka, Japan) and checking for the absence of nonspecific hemagglutinin. Neutralization tests were also done for the sera and globulin fractions of feces obtained from two ducks.

Globulin preparation from duck feces. The feces were suspended in saline at a concentration of 25%. The suspension was centrifuged at 9,000 × *g* for 30 min at 4°C, and the supernatant was filtered through no. 2 filter paper twice. Globulin fraction was obtained first by precipitation with 50% saturated ammonium sulfate, pH 7.0, then kept in the supernatant after addition of saturated ammonium sulfate to 20%, and by precipitation again with 50% saturated ammonium sulfate. The resultant precipitate was dissolved in phosphate-buffered saline (pH 7.2), followed by dialysis against the same solution. Each globulin fraction obtained was approximately 0.1 volume of feces.

Immunofluorescent staining. After the chicken antiserum to A/duck/Hokkaido/5/77 (8) was fractionated with ammonium sulfate, the crude immunoglobulin was labeled with fluorescein isothiocyanate by Kawamura's method (6). The fluorescein-to-protein molar ratio of the final product was 1:1, and the staining titer was 1:16 when examined with primary chicken embryo fibroblast cells infected with A/duck/Hokkaido/5/77. The 1:4 dilutions of the conjugate were used. Cryostat sections (4 μm thick) of the duck organs were air dried at room temperature and then fixed with acetone for 15 min in an ice bath. Each tissue section was stained directly with the conjugate in a moist chamber at room temperature for 1 h, washed in phosphate-buffered saline, and then mounted in buffered glycerol (pH 9.0) for observation. The sections were examined with a fluorescence microscope (BH-RFL, Olympus Optics, Tokyo, Japan) with a dark-field condenser and by ultraviolet excitation. Organs of control ducks were examined simultaneously for nonspecific fluorescence. Tissue sections other than those used for immunofluorescent staining were stained with a stain for frozen sections (Paragon C. & C. Co., New York, N. Y.) to study the histological details in the same area.

RESULTS

Replication site of A/duck/Hokkaido/5/77 in ducks. Eight ducks were inoculated with $10^{8.0}$ EID_{50} of A/duck/Hokkaido/5/77 (Hav7N2) either orally or intranasally. Two other ducks were inoculated orally or intranasally with normal allantoic fluid from chicken embryos.

The virus was recovered from the feces of all of the eight ducks inoculated with the virus from the first day postinoculation until day 6 or 7 postinoculation or until the day of sacrifice (day 3 or 4 postinoculation). Throughout the experiment, the birds showed neither clinical signs of disease nor pathological lesions upon necropsy.

Five of the eight ducks were killed 3 or 4 days after inoculation, and Table 1 shows the organs of these ducks from which virus was isolated. The virus was not recovered from any of the organs of the remaining three ducks, which were killed 10, 21, or 39 days after inoculation, or of the two control ducks, which were examined 4 days after inoculation.

The virus content of the organs was titrated in an orally inoculated duck (no. 4) and in an intranasally inoculated duck (no. 5). The virus

TABLE 1. *Virus recovery from ducks 3 or 4 days after inoculation with A/duck/Hokkaido/5/77 (Hav7N2)*[a]

Organ	Duck no. and age				
	No. 1, 1 mo	No. 2, 3 mo	No. 3, 3 mo	No. 4, 4 mo	No. 5, 1 mo
Sinus	+	+	−	2.8[b]	2.4
Larynx	−	−	−	1.8	−
Esophagus	−	+	−	−	−
Proventriculus	−	+	−	−	−
Jejunum	−	+	−	−	−
Ileum	+	+	+	3.5	4.9
Cecum	+	+	+	5.0	5.4
Colon	+	+	+	8.0	8.4
Rectum	+	+	+	7.5	7.0
Bursa	+	+	+	6.0	6.4
Cloaca	+	+	+	4.0	4.9
Feces	+	+	+	7.5	7.4
Kidney	−	−	−	−	3.4
Other organs[c]	−	−	−	−	−

[a] The ducks were inoculated with $10^{8.0}$ EID_{50} orally (ducks no. 1–4) or intranasally (duck no. 5–8). The birds were killed 3 days (duck no. 1) or 4 days (ducks no. 2–5) after inoculation, and their organs were examined for virus isolation as described in the text.

[b] Virus titer, log EID_{50} per gram of tissue.

[c] Brain, eye, trachea, lung, gizzard, duodenum, thymus, pancreas, liver, spleen, gonad, air sac, heart, and blood.

titers were high in the lower intestines and very low in the upper respiratory tracts in both birds.

The organs of these two ducks were examined by immunofluorescent staining. Only the colon and the rectum were positive; the other organs from which the virus was recovered were negative in both birds. Figure 1 shows specific fluorescence on the simple columnar epithelial cells which formed crypts in the colon of duck no. 4. Figure 2 shows specific fluorescence which was stronger in the lumen than in the epithelial tissue of a crypt in the colon of duck no. 5. Specific fluorescence was also observed in the epithelial cells of the crypts in the rectum; however, fewer positive crypts were observed than in the colon.

Serum HI antibody was not detected in any of the eight birds inoculated with the virus until the time of sacrifice (titer of less than 1:16).

Course of infection of ducks with A/duck/Hokkaido/5/77. Two 3-month-old ducks were inoculated orally with $10^{8.0}$ EID_{50} of A/duck/Hokkaido/5/77. Tracheal and cloacal swabs, feces, and blood were collected from the ducks hourly until 12 h after inoculation and then at daily intervals. The virus was detected first in the tracheal swabs 4 h after inoculation and then in the cloacal swabs and feces 12 h or 1 day after inoculation (Table 2); however, it was not isolated from the blood. The virus titers in the feces were the highest at 3 to 4 days after inoculation in duck no. 11.

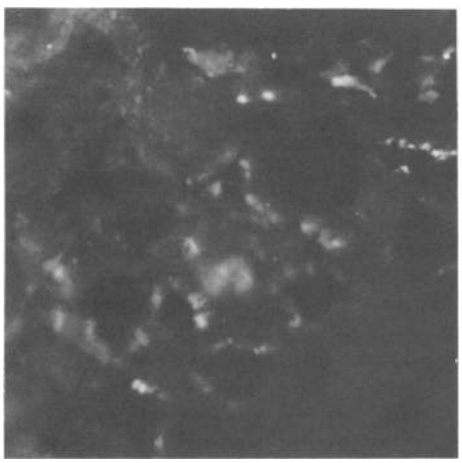

FIG. 1. *Fluorescent columnar epithelial cells which formed crypts in the colon of duck no. 4, examined 4 days after inoculation. ×300.*

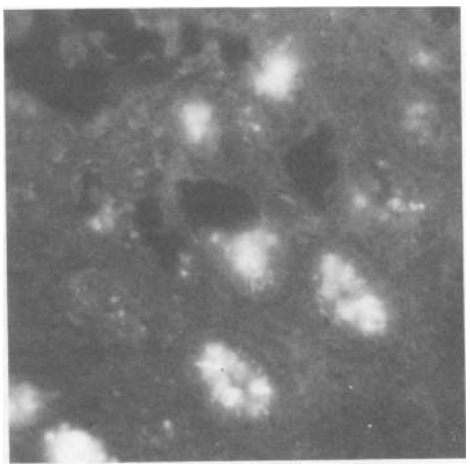

FIG. 2. *Fluorescence in the crypts in the colon of duck no. 5, examined 4 days after inoculation. ×300.*

TABLE 2. *Virus recovery from ducks after inoculation with A/duck/Hokkaido/5/77 (Hav7N2)*[a]

Time after inoculation	Duck no. 11			Duck no. 12		
	Tracheal swab	Cloacal swab	Feces	Tracheal swab	Cloacal swab	Feces
Before inoculation	−	−	−	−	−	−
1 h	−	−	−	+	−	−
2 h	−	−	−	−	−	−
4 h	+	−	−	+	−	−
7 h	+	−	−	+	−	−
12 h	+	−	−	+	+	+
1 day	+	+	4.9[b]	+	+	+
2 days	+	+	4.4	+	+	+
3 days	+	+	6.9	+	+	+
4 days	+	+	6.9	+	+	+
5 days	+	+	5.9	+	+	+
6 days	+	+	2.9	−	+	+
7–24 days	−	−	−	−	−	−

[a] The ducks were inoculated orally with $10^{8.0}$ EID_{50} and were examined for virus isolation from the tracheal and cloacal swabs, feces, and blood at various times after inoculation. All examinations of blood were negative.
[b] Virus titer, log EID_{50} per gram of tissue.

No clinical signs of disease or serum HI antibodies were detected in any of the birds (titer of less than 1:16).

Virus recovery from feces and serum antibody response after intravenous inoculation with A/duck/Hokkaido/5/77. Two 3-month-old ducks were inoculated with $10^{8.0}$ EID_{50} of A/duck/Hokkaido/5/77 intravenously, and another three ducks of the same age were inoculated orally. The virus was recovered from feces to high titers in the intravenously inoculated birds as well as in the orally inoculated birds (Table 3). The two birds which were intravenously inoculated shed the virus in feces until 10 or 21 days after inoculation. The birds which were orally inoculated shed the virus until 6, 14, or 21 days after inoculatio. .

In this experiment, both birds inoculated intravenously and two of three birds inoculated orally had low levels of serum antibody (HI titer of 1:16), and the appearance of the antibody was only transitory, 2 to 4 weeks after the inoculation.

Antibody response of ducks after reinoculation with A/duck/Hokkaido/5/77. Ten ducks were reinoculated orally with $10^{8.0}$ EID_{50} of A/duck/Hokkaido/5/77 on various days after the primary inoculation. After the primary inoculation, all of the birds had shed the virus in the feces for at least 7 days, had showed scarce antibody response, and had not exhibited clinical signs of disease. After reinoculation, the virus was not recovered from the feces of any of the birds upon daily examination for 14 days or until the day of sacrifice. Two of the birds which were reinoculated with the virus 21 or 56 days after the primary inoculation (ducks no. 20 and 21) were killed 4 days after the reinoculation. The virus was not recovered from any of the organs of these birds.

No serum HI antibodies were detected in the three birds which were reinoculated within 28 days after the primary inoculation. In contrast, a secondary immune response was obvious in the seven birds which were reinoculated with the virus 46 days after the primary inoculation or later (Table 4). None of the reinoculated birds showed clinical signs or pathological lesions upon necropsy.

To examine whether such a secondary immune response is caused by a mere antigenic stimulation with the second inoculum, we inoculated Formalin-inactivated virus orally after the primary inoculation. Five 3-month-old ducks were inoculated orally with 0.2 ml of infectious allantoic fluid containing $10^{8.0}$ EID_{50} of A/duck/Hokkaido/5/77 and then were inoculated orally with 0.5 ml of the Formalin-treated allantoic fluid 14 days (one duck), 70 days (two ducks), or 84 days (two ducks) after the primary inoculation. None of the birds showed a secondary immune response after inoculation with the inactivated virus.

The antibody response in the feces and serum of one reinoculated duck was compared with that of a primary inoculated duck (Table 5). Duck no. 12 was reinoculated with the virus 46 days after the primary inoculation, and duck no. 13 was given a primary inoculation of the virus. Duck no. 12 did not show any detectable antibodies at 46 days after the primary inoculation, but 4 days after reinoculation, a serum HI antibody response was evident. Neutralizing activities were also demonstrated in the globulin fractions obtained from the feces and the sera of duck no. 12 at 7 and 21 days after the reinoculation. On the other hand, in duck no. 13 the virus was recovered from the feces up to 10 days after inoculation. No antibodies were detected in the sera and feces of duck no. 13 in the HI tests (titer of less than 1:16), but low titers of the neutralizing activities were detected in the feces and the serum at 21 days after inoculation.

Replication site of A/budgerigar/Hok-

TABLE 3. *Virus recovery from feces and antibody response of ducks after intravenous or oral inoculation with A/duck/Hokkaido/5/77 (Hav7N2)*[a]

Route and duck no.	Days after inoculation								
	0	4	6	7	10	14	21	28	32–56
Intravenous									
15	—	1.4[b]	3.9	7.4	5.4	—	1.4[c]	—[c]	—
16	—	—	3.4	—	5.9	—	—[c]	—	—
Oral									
17	—	5.4	7.4	—	—	1.4	—[c]	—[c]	—
18	—	3.9	5.4	3.4	4.9	—	1.4	—	—
19	—	7.9	3.9	—	—	—[c]	—	—	—

[a] Dashes indicate that no virus was recovered.
[b] Virus titer, log EID_{50} per gram of feces.
[c] Tests of these ducks at these times showed an HI titer of 1:16. All other tests showed HI titers of less than 1:16.

TABLE 4. *Antibody response of ducks after oral reinoculation with A/duck/Hokkaido/5/77 (Hav7N2)*[a]

Days after primary inoculation	Duck no.	Days after reinoculation				
		0	4	7	10	14-21
21	20[b]	—[c]	—			
24	11	—	—	16[d]	—	—
28	19	—	—	—	—	—
46	12	—	128	128	256	128
56	15	—	128	256	128	128
56	17	—	32	64	64	64
56	22	—	64	64	64	64
56	21[b]	—	64			
84	16	—	32	32	32	32
84	18	—	32	32	32	32

[a] The ducks were reinoculated orally with $10^{8.0}$ EID_{50} on the indicated day after primary inoculation. After the primary inoculation, all of the birds had shed the virus in their feces for at least 7 days, had showed scarcely any serum HI antibody response, and had not exhibited clinical signs of disease.

[b] Ducks no. 20 and 21 were killed 4 days after reinoculation for virus isolation from their organs.

[c] —, Serum HI titer of less than 1:16.

[d] Reciprocal of serum dilution.

kaido/1/77 in ducks. Two 3-month-old ducks were orally inoculated with $10^{6.7}$ EID_{50} of A/budgerigar/Hokkaido/1/77 (Hav4Nav1). The virus was detected in the tracheal and cloacal swabs and in the feces of the ducks, but it was not isolated from the blood at any time (Table 6). Duck no. 23 was killed 4 days after inoculation, and distribution and virus content were examined (Table 7). The virus titers were high in the lower intestines and very low in the other organs. No clinical signs of disease or pathological lesions were found in either bird, nor were serum HI antibodies detected (titer of less than 1:16).

Antibody response of ducks inoculated with influenza viruses of human origin. Three 3-month-old ducks, two 3-month-old ducks, and three 4-month-old ducks were inoculated with $10^{8.0}$ EID_{50} of A/Kumamoto/22/76 (H3N2) orally, intranasally, and intracolonically, respectively. The birds were examined daily for virus isolation from the tracheal swabs and the feces and for antibody response in the sera on days 4 to 28 postinoculation. The virus was recovered from the tracheal swabs of two of the three ducks which were inoculated orally, on days 1 and 2 postinoculation from one duck and only on day 1 postinoculation from the other bird. The virus was not isolated from the tracheal swabs of any of the ducks inoculated intranasally or intracolonically, and it was not isolated from the feces of any of the birds. No virus was isolated from the organs of four ducks killed 4 days after inoculation or from those of the remaining ducks killed 28 days after inoculation. No clinical signs of disease or pathological lesions were found in any of the birds. However, serum HI antibodies were detected in the ducks which were inoculated orally (titers of 1:32 to 1:128) or colonically (titers of 1:16 to 1:64) from 7 days after inoculation and thereafter.

Three 3-month-old ducks were inoculated orally with $10^{8.0}$ EID_{50} of A/Aichi/2/68 (H3N2), and three were inoculated orally with $10^{7.0}$ EID_{50} of A/New Jersey/8/76 (Hsw1N1). The birds were examined daily for virus isolation from the tracheal swabs and feces and for serum HI antibodies. The virus was not isolated from any of the birds. Two of the ducks inoculated with A/Aichi/2/68 and one of the ducks inoculated with A/New Jersey/8/76 were killed after 4 days and examined for virus isolation from their organs. Virus was not isolated from any of the organs. All of the remaining ducks showed serum HI antibodies from days 7 to 28 (titers of 1:32 to 1:128 in the one remaining duck inoculated with A/Aichi/2/68 and titers of 1:16 to 1:128 in the two remaining ducks inoculated with A/New Jersey/8/76). No clinical signs of disease or pathological lesions were found in any of these ducks.

TABLE 5. *Virus recovery from feces and antibody response of ducks after reinoculation (duck no. 12) or primary inoculation (duck no. 13) with A/duck/Hokkaido/5/77 (Hav7N2)*[a]

Duck no.	Days after inoculation	Virus recovery from feces	Coproantibody		Serum antibody	
			HI	NT	HI	NT
12	0	—	—[b]	—[c]	—	—
	4	—	·[d]	·	128	·
	6	—	·	·	128	·
	7	—	16	128	128	256
	10	—	·	·	256	·
	14	—	·	·	128	·
	21	—	—	64	128	32
13						
	0	—	—	—	—	—
	4	8.4[e]	·	·	—	·
	6	6.4	·	·	—	·
	7	2.9	—	4	—	—
	10	2.9	·	·	—	·
	14	—	·	·	—	·
	21	—	—	8	—	4

[a] Duck no. 12 was reinoculated orally 46 days after the primary inoculation, and duck no. 13 was primarily inoculated. Antibody titers in feces and serum were examined by HI tests and neutralization tests (NT). Antibody titers are expressed as reciprocals of dilution.

[b] HI titer of less than 1:16.

[c] Neutralization titer of less than 1:2.

[d] ·, Not tested.

[e] Virus titer, log EID_{50} per gram of the feces.

TABLE 6. *Virus recovery from ducks after inoculation with A/budgerigar/Hokkaido/1/77 (Hav4Nav1)*[a]

Duck no.	Days after inoculation	Tracheal swab	Cloacal swab	Feces
23[b]	0	−	−	−
	1	+	−	−
	2	−	−	+
	3	−	+	+
	4	−	+	+
24	0	−	−	−
	1	+	−	−
	2	−	−	+
	3	−	+	+
	4	+	+	+
	5	−	+	+
	6	−	+	+
	7	−	−	+
	8–28	−	−	−

[a] The ducks were inoculated orally with $10^{6.7}$ EID_{50} and were examined for virus isolation from the tracheal and cloacal swabs, feces, and blood. The virus was not found in the blood of either duck.

[b] Duck no. 23 was killed 4 days after inoculation for virus isolation from the organs.

TABLE 7. *Virus titers in the organs of duck no. 23 at 4 days after oral inoculation with A/budgerigar/Hokkaido/1/77 (Hav4Nav1)*

Organ	Titer[a]
Lung	1.5
Crop	1.5
Proventriculus	3.5
Ileum	3.0
Cecum	3.8
Colon	7.5
Rectum	5.8
Bursa	6.0
Feces	6.9
Other organs[b]	—

[a] Virus titer, log EID_{50} per gram of tissue.

[b] Brain, eye, sinus, larynx, trachea, esophagus, gizzard, duodenum, jejunum, pancreas, liver, thymus, spleen, kidney, gonad, heart, and blood.

DISCUSSION

The results of the present experiments indicate that in Pekin ducks an influenza virus of duck origin replicates in the lower intestinal tract, and the infected ducks shed the virus in feces to high titers. In addition, the fact that the intravenously inoculated ducks also shed the virus in feces to high titers supports the conclusion that the target organ of this virus in ducks is the intestinal tract. These findings confirm the results reported by Webster et al. (16) and the immunofluorescence studies by Slemons and Easterday (13). The present results show further that multiplication of the virus takes place in the simple columnar epithelial cells which form crypts in the large intestines, especially in the colon. The period of virus shedding in the feces was approximately 6 to 7 days in the experimentally infected ducks. It is, however, epidemiologically important that some of the infected birds shed the virus in the feces as long as 14 or 21 days after inoculation.

The fact that the infected ducks did not show any clinical signs of disease and produced scarcely detectable serum HI antibodies is in agreement with our previous findings in an apparently healthy feral pintail which was naturally infected with an influenza virus but did not possess detectable serum HI antibodies to the homologous isolate (8).

An influenza virus of budgerigar origin, which replicated in the respiratory tract of the budgerigar (7), also replicated in the lower intestinal tract of Pekin ducks without causing signs of disease or antibody response. The replication site of an influenza virus is, therefore, considered to vary according to the host species and the virus strain.

It was thus shown that the experimentally infected ducks which shed the viruses in the feces to high titers lacked evidence of disease and a detectable serum antibody response or had only low levels of transitory serum antibody. Kawano et al. (7) also found that HI antibodies were barely detectable in the budgerigars experimentally infected with the virus of budgerigar origin. It is conceivable, therefore, that there exist calm and stable host-parasite relationships between birds and their influenza viruses in nature. Our findings also suggest that the distribution of influenza viruses in avian species may not be estimated exactly from the results of seroepidemiological surveys.

The ducks which were orally reinoculated with the duck virus within 28 days after the primary inoculation did not show any antibody response; on the other hand, the ducks which were reinoculated with the same virus 46 days after the primary inoculation or later produced high titers of serum antibodies. Since the inoculation of 2.5 times the amount of Formalin-inactivated virus did not cause such a secondary immune response, the marked antibody response in the latter birds suggests that the virus replicated again in the birds after reinoculation. The negative results of virus isolation from any organs of the reinoculated birds may be due to a quick restriction of the infection by a brisk secondary immune response reflected in the rapid appearance of high titers of the antibody after reinoculation. The ducks which were reinoculated with the virus within 28 days after the primary inoculation appeared to resist rein-

fection despite the absence of detectable serum antibody. These observations might be explained if the protracted primary infection was associated with an antibody response obscured by formation of an immune complex. The mechanism which offers protective immunity against influenza virus infection in the avian species is not yet known. The fact that a low level of neutralizing activity was found in the globulin fraction of the feces of a duck 21 days after the primary inoculation suggests the possibility of a local immunity in the intestinal tracts which offers such protection. Cell-mediated immunity may also be important. Further investigations are necessary to clarify this point.

Of the influenza viruses used in the present experiments, the viruses of avian origin replicated in the intestinal tract and caused scarcely detectable antibody responses in the ducks. In contrast, the viruses of human origin did not replicate in the intestinal tract but caused antibody responses. The mechanisms responsible for such differences in the biological characters of these viruses have not yet been identified. Further studies in genetic analysis using recombinants are necessary to answer this question.

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