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**Further Investigations
of the Transmission of Fowl Paralysis
(*Neurolymphomatosis*) by Direct Transfusion**

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SUMMARY

1. Fowl paralysis is readily produced in White Leghorn chicks by using intravenous technique.
2. Visibly affected birds near six months of age are suitable donors.
3. One cubic centimeter of virus-containing blood is sufficient to induce the disease in a high percentage of recipients.
4. This technique produces the disease early in suitable recipients (one in 18 days and four in 19 days).
5. White Leghorn chicks one to four days of age are quite receptive to the virus.
6. White Wyandotte chicks one to four days of age are very refractory to intravenous inoculation.
7. Soiled fronts indicate fowl paralysis.
8. A rather high percentage of apparently healthy birds show gross nerve involvement.
9. This technique of transmission may have application in other diseases of man, animals, and birds.

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Further Investigations of the Transmission of Fowl Paralysis (*Neurolymphomatosis*)* by Direct Transfusion

A. J. DURANT AND H. C. MCDUGLE

INTRODUCTION

During the course of investigations on fowl paralysis, beginning in 1936, an attempt was made to determine whether the disease may be carried by the egg. It was observed that up to 60% of chicks hatched from visibly affected hens developed fowl paralysis, when maintained under observation for ten or more months.

From this modest beginning the study of fowl paralysis was continued, and in Missouri Research Bulletin 304 the transmissibility of the virus was definitely shown. The results of these rather extensive experiments, involving over 1000 day-old chicks, not only seemed to show that the virus occurred in the circulating blood of chicks hatched from visibly affected hens, but furthermore, that the amount of virus appeared to fluctuate in donors until they were 4½ months old, the period during which the investigation was carried on. The fluctuation referred to indicated that 0.25 cubic centimeter of blood from the chicks of ages from 20 to 30 days, 50 to 60 days, and 110 to 120 days was capable of transmitting the disease to susceptible day-old chicks to a much higher degree than at any other time during the period of observation.

MANAGEMENT OF INOCULATED AND CONTROL BIRDS USED IN THESE EXPERIMENTS

In most of the experiments reported in this bulletin a control chick was maintained for each inoculated chick. The controls and the inoculated chicks were from the same source of White Leghorns, and both groups were confined in a single room 60 x 30 feet. No special effort was made to prevent exposure between the two groups.

The inoculated chicks were confined to the lower decks of an electrically heated brooder and the controls were confined to the upper decks. After the chicks had reached the age of four or five weeks they were transferred from the battery brooders into batteries divided

*The authors have chosen to continue to use the name "fowl paralysis" and the descriptive term "*neurolymphomatosis*", because they believe the disease involved is essentially a nerve type disease, and that it is commonly characterized by types of paralysis.

into 16 compartments each. (Fig. 1). From two to five birds were put in each compartment. The controls were placed in separate identical batteries and the birds were fed and cared for by the same attendant.



Fig. 1.—Batteries in which experimental birds were confined after they were removed from the battery brooders.

EXAMINATION OF EXPERIMENTAL BIRDS

The authors have made no attempt to demonstrate histologically the presence or absence of fowl paralysis. They have depended entirely on clinical and other manifestations of the disease, followed by a post-mortem examination and observation of gross lesions, either at death or when killed because of advanced stages of disease. The birds were examined daily for physical signs of any form of fowl paralysis, and were reported as suspected of having the disease the first day a symptom appeared. The final diagnosis was withheld, however, until confirmation by autopsy. All of the control birds were killed and examined in the same manner.

A VARIATION IN TRANSMISSIBILITY OF THE VIRUS IN DONORS 23, 33, and 50 DAYS OF AGE

Following the publication of the results of the virus transmission, experiments were set up to confirm the findings, which indicated that there was a fluctuation in the amount of virus occurring in infected chicks. Chicks of three ages (23, 33, and 50 days respectively) were selected for donors. They were hatched from visibly affected hens.

The first group was of an age which produced a high incidence of fowl paralysis; second, an age which produced only a very few cases of fowl paralysis, and the third, an older group which produced a high percentage of infected chicks (Table 1).

TABLE 1. - VARIATION IN THE ABILITY OF DONORS OF DIFFERENT AGES TO TRANSMIT FOWL PARALYSIS. THE THREE GROUPS WERE KEPT UNDER OBSERVATION FOR TEN MONTHS.

Group	Date In-oculated	Inoculated Birds			Control Birds		Total controls
		Total recipients	Developing fowl → paralysis		Developing fowl paralysis		
			Cases	%	%	Cases	
1	5-25-39	35	20	57.14	11.42	4	35
2	6-5-39	33	4	12.12	12.12	4	33
3	6-22-39	36	14	38.88	11.11	4	36

In Group 1 the donors were 23 days of age. The incidence of disease in the recipients was 57.14 per cent, whereas the controls for the same group showed a transmission of only 11.42 per cent, a difference of definite statistical significance. In Group 2 the donors were 33 days of age. The incidence of disease in the recipients showed only 12.12 per cent transmission of fowl paralysis, the same percentage as in the controls; therefore there apparently was little transmission of fowl paralysis in this group. In Group 3 the donors were 50 days of age, and 38.88 per cent of the recipients developed fowl paralysis, whereas there was only 11.11 per cent of transmission in the chicks used as controls. This experiment shows results somewhat similar to those previously secured, indicating that an amount as small as 0.25 of a cubic centimeter of the blood of infected chicks definitely varies in its ability to transmit fowl paralysis from donors of various ages.

SECOND TEST OF TRANSMISSION OF THE VIRUS FROM DONORS 1 TO 20 DAYS OF AGE

After the completion of the experiment described above it was decided to continue the investigation, particularly in regard to the daily virus content of the blood of chicks hatched from visibly affected hens. In the first experiment (1938-39, Research Bulletin 304), the chicks which furnished the blood for the transmission were bled every seven to ten days during the 4½-month period. In this second experiment donors were bled every day from the time they were 1 day old up until they were 20 days of age. The blood was transfused into day-old baby chicks to determine if there was a fluctuation of the virus in the circulating blood of the donors.

It should be pointed out that one of the difficulties in the study of fowl paralysis is the fact that in using baby chicks hatched from

visibly affected hens only 50 per cent approximately may be carrying the virus in the circulating blood, and certainly the blood of some of the donors would not transmit fowl paralysis. However, it was considered important to know just what the situation was from day to day, as far as the transmissibility of the blood was concerned. The number of chicks inoculated daily varied from 8 to 16. The following Table 2 and Fig. 2 show the results of this experiment. Although the percentage of infection does occur higher on the 7th, 8th, 14th, and 19th days, a statistical test of the recipients against the controls in regard to actual diseased birds in each group shows the 7th, 14th, 19th, and 4th days to be most effective. However, a statistical test for homogeneity indicates that such a difference in the percentages of cases could occur about 40 times out of 100. The authors wish to point out that although the results shown in this table may be insignificant, somewhat similar results were obtained in the experiments reported in Research Bulletin 304. The cause of the variation in the transmission of the disease at the different dates is not definitely known.

VIRUS SOURCE—ONE FLOCK

During the entire course of all of these studies, which extended from 1936 to 1944, one flock of White Leghorns furnished the infected fowl paralysis birds. This probably is of some importance, for the reason that the type of disease produced was more or less constant. Furthermore, it was of interest that dyscrasias were unusual, or practically did not occur in the recipients from this particular strain of virus. Specimens of this strain of virus submitted to the Regional Laboratory at East Lansing, Michigan, produced 15 per cent osteopetrosis, an even higher incidence of that form of the disease than occurred in the experiment at this station.

SUCCESSFUL TRANSMISSION OF FOWL PARALYSIS BY DIRECT TRANSFUSION

In the spring of 1944 it was decided to continue the study of the transmission of fowl paralysis by blood and an effort was made to obtain specimens from the same source as had been previously used. It was impossible to secure this strain either from the original source or from the Regional Laboratory at East Lansing, Michigan. It appeared, therefore, if experiments were to be continued on the blood transmission, that other sources would have to be sought.

In considering the problem during the summer, the idea of checking the blood of adult birds visibly affected with the ocular form of fowl paralysis was conceived. A study of the literature showed that no extensive experiments of this kind had ever been attempted. Furthermore, there were no definite data on birds past the age of four and one-half months. (See Research Bulletin 304.)

TABLE 2. - RESULTS OF INOCULATIONS, USING DONORS 1 THROUGH 20 DAYS OF AGE. THE DONORS WERE HATCHED FROM VISIBLY AFFECTED BIRDS.

Age of donor at time of inoculation	Total recipients	Recipients				Controls				Total controls
		Tumor	Leukemia	Fowl paralysis		Tumor	Leukemia	Fowl paralysis		
		Cases	Cases	Cases	Per cent	Cases	Cases	Cases	Per cent	
1	11	1	0	5	45.45	0	1	1	11.11	9
2	13	1	0	4	30.76	0	0	2	15.38	13
3	9	1	1	1	11.11	0	0	1	12.50	8
4	16	0	2	4	25.00	0	0	0	0.00	15
5	12	0	0	5	41.66	0	0	2	18.18	11
6	16	0	0	5	31.25	0	0	2	13.33	15
7	16	0	0	11	68.75	0	0	0	0.00	14
8	15	0	1	9	60.00	0	0	4	28.57	14
9	13	0	0	6	46.15	0	0	3	23.07	13
10	8	0	0	4	50.00	0	0	1	16.66	6
11	12	1	0	4	33.33	0	0	1	8.33	12
12	6	0	0	1	16.66	0	1	0	0.00	8
13	8	0	0	4	50.00	1	0	1	14.28	7
14	8	0	0	5	62.50	0	0	0	0.00	7
15	9	0	0	4	44.44	0	0	1	11.11	9
16	9	1	0	5	55.55	0	0	2	20.00	10
17	10	1	0	4	40.00	0	0	3	30.00	10
18	10	1	0	5	50.00	0	0	1	10.00	10
19	10	0	0	6	60.00	0	0	1	10.00	10
20	9	1	0	4	44.44	0	0	3	30.00	10
Total	220	8	4	96	43.63	1	2	32	15.16	211

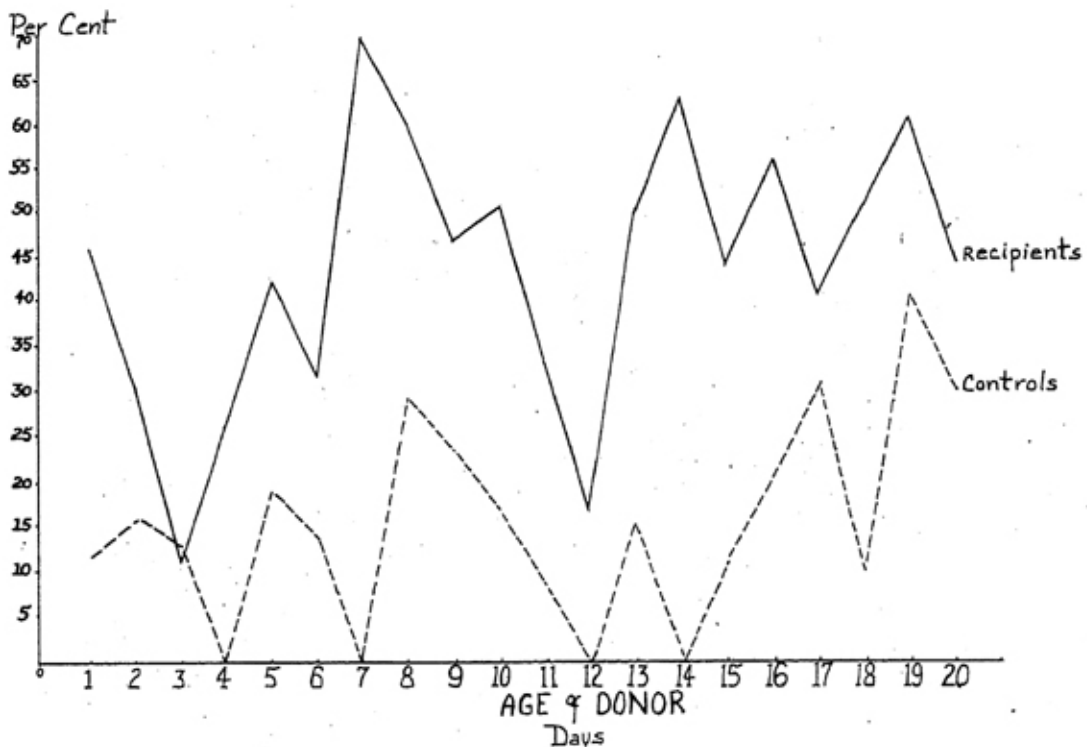


Fig. 2.—A chart illustrating the results of blood inoculation from donors 1 to 20 days of age. The upper or solid line shows the per cent of fowl paralysis developing in the recipients as a result of inoculation. The lower, broken line indicates the percentage of fowl paralysis that occurred in the controls during the same period.

Source of Donors

The question to be considered was, "Is the virus of fowl paralysis present in the circulating blood of chickens between the ages of four and one-half to seven months of age affected with the ocular form of fowl paralysis, and can it be transmitted?"

To secure suitable donors, or visibly affected adult birds, an appeal was made to three hatcheries in the state, asking that they ship to the Veterinary Department of the Experiment Station pullets visibly affected with fowl paralysis, obtained by the hatcherymen when culling their flocks. This appeal resulted in obtaining 50 such pullets near the age of six months. Thirty-seven of these birds were selected as suitable for donors for the experiments. All of these but one (*osteopetrosis*) showed typical changes of the eye. These ocular changes, indicative of fowl paralysis, varied from only moderate changes in the shape of the iris and pupil to extreme cases in which the birds were practically blind.

Source of Chicks Used as Recipients

The day-old chicks used as recipients were hatched from a selected flock of White Leghorn chickens. A study of this flock showed that there was no visible evidence of fowl paralysis in the adult birds from which the chicks were secured. The chicks varied in age from 1 day to 40 days. Most of the chicks inoculated, however, were from 1 to 4 days of age.

Chicks Tolerate Great Amount of Blood Intravenously

Reasoning that if the virus occurred in the circulating blood in these visibly affected pullets, the more blood which could be transfused into a baby chick at one time the more likely the recipient would be to develop fowl paralysis. Actual experiments by decapitation of chicks showed that about 1 c.c. of blood could be thus obtained. It was decided, therefore, to attempt to transfuse from the visibly affected hens at least 1 c.c. of blood into the circulation of day-old chicks. It was found that a day-old baby chick, when the transfusion was made slowly under proper conditions, would survive the immediate shock of approximately doubling the volume of the circulation.

When it was discovered that the chick could survive 1 c.c. intravenously it was decided to attempt to increase that amount, and 10 baby chicks were successfully given 2 c.c. each. Further experiments demonstrated that in chicks from 1 to 3 days of age, 1 to 4 c.c. of blood could usually be successfully transfused.

Forty Cubic Centimeters of Blood Obtainable from Each Donor

Investigations had already shown that it was possible to draw at one time from 30 to 50 c.c. of blood from an adult hen without fatal results, and that this procedure could be repeated about every ten days

TABLE 3. - A LIST OF DONORS USED IN THIS EXPERIMENT SHOWING BREED, THE TYPE OF VISIBLE LESIONS, AND ADDITIONAL LESIONS REVEALED ON AUTOPSY.

Donor	Breed	Sex	Visible Lesions	Other Lesions on Autopsy
101	W. Leg.	F	Right iris 1/3 cloudy. Left iris 1/2 cloudy	
102	N. Hamp.	F	Right eye blind. Left pupil irregular	
103	N. Hamp.	F	Left eye "feline". Right iris irregular	
104	N. Hamp.	F	Right and left pupils "feline"	
105	N. Hamp.	F	Right pupil "caprine"	
106	N. Hamp.	F	Right pupil "caprine"	Healed scars in liver
107	N. Hamp.	F	Left pupil "caprine"	
108	W. Rock	F	Left iris little pigment left	
109	W. Leg.	F	Right iris broken	Mesenteric tumor
110	N. Hamp.	F	Right pupil "feline"	
111	W. Leg.	F	Right iris broken	Fowl paralysis - neural
112	W. Rock	F	Right and left iris affected	Fowl paralysis - visceral
113	N. Hamp.	F	Right pupil "feline"	Left pupil "caprine"
114	N. Hamp.	F	Left triangular pupil. Right iris broken	Left pupil almost closed
115	W. Leg.	F	Right iris broken	Left iris broken
116	W. Leg.	F	Right and left iris broken	
117	N. Hamp.	F	Right pupil "feline"	Fowl paralysis - neural
118	W. Leg.	F	Osteopetrosis	Fowl paralysis - osteopetrotic
119	W. Leg.	F	Right iris broken	Tumors
120	W. Leg.	F	Left iris broken	Left pupil "caprine"
121	W. Rock	F	Left pupil "caprine"	
122	W. Leg.	F	Right and left iris broken	
123	W. Leg.	F	Left iris broken	
814	N. Hamp.	F	Left iris broken	Left pupil "caprine"
804	W. Rock	F	Right and left iris cloudy	
805	W. Rock	F	One cloudy iris	
806	N. Hamp.	F	Right iris cloudy, "feline"	
807	N. Hamp.	F	Right and left iris cloudy	Tumors
808	N. Hamp.	F	Left iris broken. Right iris cloudy	
801	W. Rock	F	One distorted iris	
809	W. Rock	F	Right and left pupil cloudy	
810	W. Rock	F	Right and left pupil cloudy	
811	W. Rock	F	Right and left pupil cloudy	
815	W. Rock	F	Left iris cloudy	Visceral gout
816	N. Hamp.	F	Right and left iris cloudy	Blind - both eyes
812	W. Rock	F	Right pupil cloudy	Right pupil "caprine"
813	N. Hamp.	F	Right and left pupil "caprine"	
892-3	W. Leg.	M	Neural	
894-5	W. Leg.	M	Neural	
1094-5	W. Leg.	F	Neural	
1172-3	W. Leg.	M	Neural	
1186-7	W. Leg.	F	Neural	

to two weeks. This knowledge was important because it was thought that the same hen might again be needed, particularly to confirm the results of previous inoculations. Varying numbers of chicks were inoculated intravenously from each donor. In some, 1 c.c. of blood was transfused; in others, 2 c.c., and in others more. The injection was made into the large vein situated on the inner surface of the hock joint. A 26-gauge, $\frac{5}{8}$ -inch short-beveled hypodermic needle and a 2-c.c. glass syringe were used for inoculation. A larger needle, 20-gauge, $\frac{3}{4}$ -inch short-beveled, was put into the median vein on the inner surface of the wing of the donor and remained in the vein until the entire series of chicks were inoculated from that particular donor. The results of these inoculations are shown in the following tables and charts.

It was thought that if the blood of adult hens visibly affected with the ocular form of fowl paralysis carried the virus, large amounts, 1 c.c. or more of the blood, given to young chicks, would be more likely to transmit a high per cent of fowl paralysis than where smaller amounts were used as in the previous experiment.

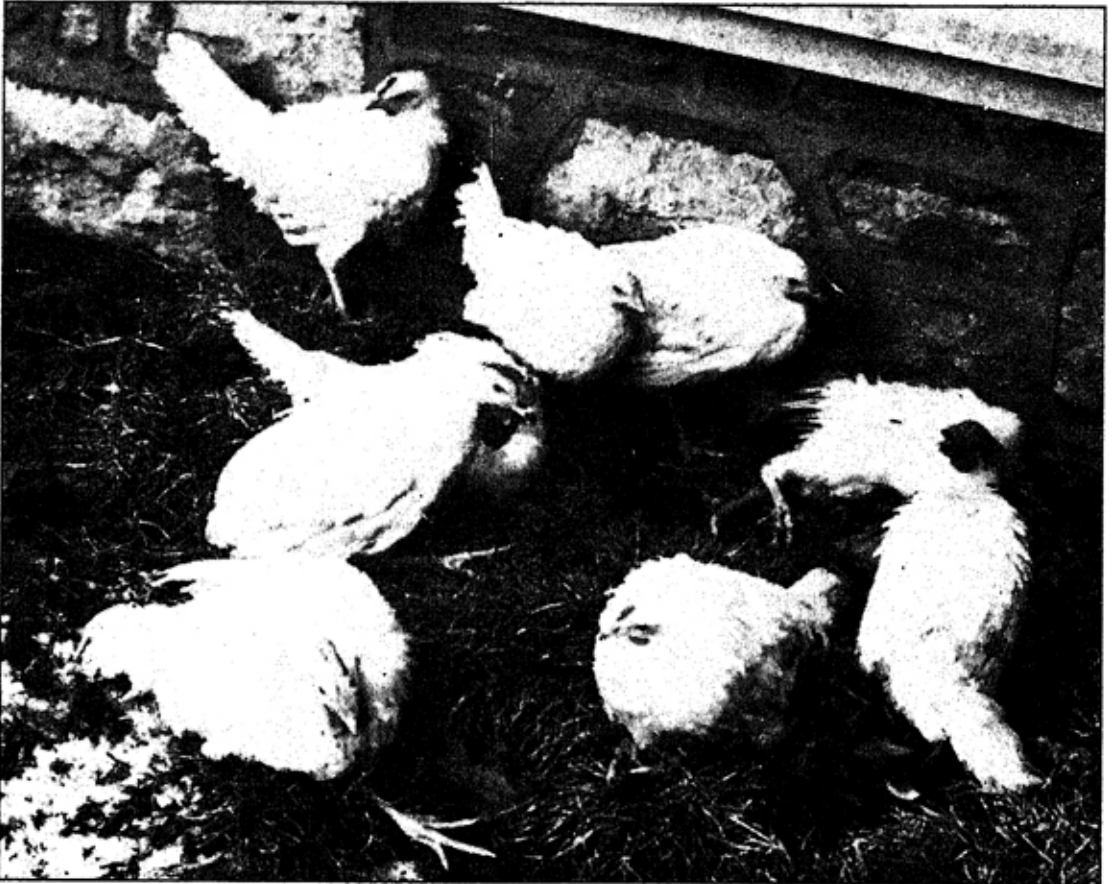


Fig. 3.—A group of nine inoculated birds 84 days old, showing various symptoms of fowl paralysis. All of these birds developed symptoms within 58 days and showed definite gross lesions of fowl paralysis upon autopsy.

Table 4 shows the results of the transfusions, from September 25, 1944, to January 12, 1945, or 3 months and 17 days, when all of the inoculations were made. The left side of the table is a record of all of the inoculated birds (398), and three-hundred and four (304) or 76.38 per cent developed some form of fowl paralysis within an average period of 89 days. Fifty-two birds are still alive and under observation. At the time this experiment was closed three to eight months had elapsed since the first and last groups of birds were inoculated. It is reasonable to assume, therefore, basing the assumption on the present data, that the majority of 52 birds still alive will develop fowl paralysis within a total period of ten months after inoculation.

Table 5 is a summation of the data showing the number of birds, recipients and controls, grouped into classes based on the percent developing the disease.

TABLE 4. - SUMMARY OF THE RESULTS OF THE TRANSFUSIONS IN WHITE LEGHORN CHICKS.

Donor			Recipients						Controls					
No.	Age Days	Total	Age When Inocu. Days	Cubic Centi-meters	Alive	Developed fowl paralysis			Total	Age Days	Alive	Developed fowl paralysis		
						Cases	Per Cent	Avg. Days				Cases	Per Cent	Avg. Days
801	173	2	25	2	0	2	100.00	44	2	25	0	1	50.00	178
804	168	7	20	5-10	3	3	42.85	111	7	20	2	1	14.28	112
805	168	3	20	5	0	3	100.00	51	3	20	0	2	66.66	108
805	197	14	1	1	0	14	100.00	82	14	20	0	2	14.44	82
805	296	18	4	2	7	10	55.55	85	18	4	5	3	16.66	88
806	154	1	20	10	0	1	100.00	100	1	20	0	1	100.00	206
806	183	6	1	1	0	6	100.00	70	6	1	0	0	00.00	00
807	154	3	20	5-10	0	3	100.00	102	3	1	0	1	33.33	168
808	154	1	20	10	0	1	100.00	139	1	20	0	0	00.00	00
808	184	18	2	1	0	16	88.88	97	18	2	0	4	22.22	187
809	189	4	41	5	0	3	75.00	60	4	41	0	1	25.00	126
809	199	22	3	1	2	18	81.81	55	22	3	0	8	27.50	105
809	294	8	2	2	0	7	87.50	79	8	2	0	4	50.00	118
810	189	5	41	5	0	3	60.00	40	5	41	0	0	00.00	00
810	197	1	1	1	0	1	100.00	90	1	1	0	0	00.00	00
810	233	4	3	1-2	0	3	75.00	71	4	3	0	3	75.00	103
810	251	4	2	2	1	3	75.00	98	4	2	0	0	00.00	00
811	190	3	42	5-20	1	1	33.33	28	3	42	1	2	66.66	169
812	191	1	43	10	0	1	100.00	208	1	43	0	0	00.00	00
812	199	10	3	2	0	9	90.00	91	10	3	0	1	10.00	59
812	296	19	5	2	4	11	57.89	97	19	5	1	10	52.63	127
813	179	1	45	25	1	0	00.00	00	1	45	1	0	00.00	00
813	185	20	3	1	4	16	80.00	83	20	3	0	7	35.00	173
814	179	2	45	6-10	0	2	100.00	36	2	45	0	2	100.00	95
815	193	1	45	20	0	1	100.00	22	1	45	0	1	100.00	166
816	179	1	45	5	0	1	100.00	59	1	45	0	1	100.00	143
816	185	10	3	1	2	6	60.00	54	10	3	0	4	40.00	118
104	231	3	12 hrs.	0.5-1	0	2	66.66	134	3	12 hrs.	0	2	66.66	88
105	232	9	1	1-2	2	5	55.55	94	9	1	2	3	33.33	120
105	254	2	2	2-3	0	2	100.00	88	2	2	0	1	50.00	121
106	232	5	2	1-2	0	5	100.00	96	5	2	0	2	40.00	140
106	254	19	2	2	5	13	72.22	88	19	2	0	8	44.44	148
107	232	6	1	1	1	5	83.33	138	6	1	1	2	33.33	96
107	254	6	2	4	0	5	83.33	67	6	2	0	2	33.33	117
108	233	5	2	1	0	5	100.00	103	5	2	0	1	20.00	140
109	217	9	2	1-2	2	7	77.77	90	9	2	1	2	22.22	104
110	233	9	2	1-2	0	8	88.88	112	9	2	0	7	77.77	97
111	217	7	2	1-2	0	6	85.71	134	7	2	0	0	00.00	00
112	217	10	2	1-2	2	7	70.00	87	10	2	1	5	50.00	159
113	255	11	3	5	2	8	72.72	112	11	3	1	4	36.36	162
114	255	10	3	4	1	7	70.00	115	10	3	0	5	50.00	105
115	240	10	4	4	3	6	60.00	81	10	4	3	1	10.00	177
116	240	6	4	2-4	0	6	100.00	68	6	4	0	1	16.66	39
117	256	5	4	4	0	3	60.00	76	5	4	0	1	20.00	171
118	253	9	3	2	1	8	88.88	97	9	3	0	6	66.66	113
119	253	9	3	1-2	2	6	66.66	88	9	3	0	4	44.44	90
120	253	10	3	1-2	0	10	100.00	82	10	3	0	3	30.00	95
121	269	9	3	2	1	6	66.66	83	9	3	0	4	44.44	71
122	253	8	3	2	0	8	100.00	92	8	3	0	3	37.50	125
892-3	98	9	2	2	0	7	77.77	78	9	2	0	3	33.33	107
894-5	98	3	2	2	0	1	33.33	130	3	2	0	0	00.00	00
1094-5	35	2	1	0.5-1	0	2	100.00	51	2	1	0	0	00.00	00
1172-3	72	6	3	2	4	2	33.33	51	6	3	3	1	16.66	170
1186-7	32	4	1	1	0	4	100.00	117	4	1	0	1	25.00	100
1186-7	69	8	3	2	1	5	62.50	102	8	3	0	4	50.00	118
Total --		398			52	304	76.38	89	398		22	135	34.00	128

TABLE 5. - SUMMARY OF THE RESULTS OF 398 INOCULATED WHITE LEGHORNS, AND 398 WHITE LEGHORN CONTROLS.

Classes (Per Cent Infection)	Recipients				Controls			
	Total Inoculated	Cases	Alive	Actual Per Cent	Total	Cases	Alive	Actual Per Cent
100	78	78	0	100.00	78	23	0	29.48
90.00-99.99	10	9	0	90.00	10	1	0	10.00
80.00-89.99	105	89	8	84.76	105	40	1	38.10
70.00-79.99	80	58	13	72.50	80	31	3	38.75
60.00-69.99	59	37	9	62.71	59	20	3	33.90
50.00-59.99	46	26	13	56.52	46	16	8	34.78
40.00-49.99	7	3	3	42.86	7	1	2	14.29
30.00-39.99	12	4	5	33.33	12	3	4	25.00
Zero	1	0	1	00.00	1	0	1	00.00
Totals	398	304	52	76.38	398	135	22	33.92

**WHITE WYANDOTTE CHICKS DIFFERED FROM LEGHORNS IN
THEIR REACTION TO DIRECT TRANSFUSION OF BLOOD
FROM BIRDS VISIBLY AFFECTED WITH
FOWL PARALYSIS**

Forty-seven White Wyandotte chicks from one to three days of age received 1 to 2 c.c. of blood by direct transfusion. Only 10 of the 47, or 21.27% of the recipients, developed fowl paralysis, in an average of 150 days. Of the 47 recipients 24 are still alive and apparently healthy. There were not sufficient birds in this experiment to draw definite conclusions, but there was a marked difference in the number of cases produced in this strain of White Wyandottes as compared with the strain of White Leghorns used in these experiments. Of the 47 control birds, 3, or 6.38% have developed fowl paralysis in an average period of 176 days after inoculation. Of the 47 controls 40 are still living and apparently healthy.

For comparison one group of 46 inoculated White Leghorn chicks was selected, which was of approximately the same age and which received the same amount of blood as the White Wyandottes. Of the 46 recipients in this group 41, or 89.13%, developed fowl paralysis in an average of 95 days. Of the 46 controls for this inoculated group, 15, or 32.66% developed fowl paralysis, in an average of 140 days. By comparing the data compiled on the White Leghorns with that on the White Wyandottes, it can be seen that there is a wide difference in the two groups. (Table 6.) White Leghorns appear to be far more susceptible to fowl paralysis than the White Wyandottes used in this experiment. The Chi-square test shows over seven times the required 6.635 for a probability of .01.

There is a possibility that these White Wyandottes were more resistant than other birds of this breed. However, they were purchased at random from a reputable hatcheryman. The flock from which they originated was a farm flock used as a source of hatching eggs.

TABLE 6. - A COMPARISON OF THE DIFFERENCE BETWEEN WHITE WYANDOTTES AND WHITE LEGHORN CHICKS IN THE TRANSMISSION OF FOWL PARALYSIS BY DIRECT TRANSFUSION

DONOR		WHITE WYANDOTTES							CONTROLS					
No.	Age Days	Total	Age When Inocu. Days	cc's	Alive	Developed Fowl Paralysis			Total	Age Days	Alive	Developed Fowl Paralysis		
						Cases	Per Cent	Avg. Days				Cases	Per Cent	Avg. Days
101	211	7	3	1-2	1	3	42.85	153	7	3	5	1	14.28	126
102	227	10	3	1-2	8	2	20.00	134	10	3	8	2	20.00	202
103	227	10	3	1-2	6	2	20.00	208	10	3	9	0	00.00	00
808	207	20	1	1	9	3	15.00	148	20	1	18	0	00.00	00
Total--		47			24	10	21.27	150	47	40	40	3	6.38	176

WHITE LEGHORNS

DONORS		RECIPIENTS							CONTROLS					
No.	Age Days	Total	Age When Inocu. Days	cc's	Alive	Developed Fowl Paralysis			Total	Age Days	Alive	Developed Fowl Paralysis		
						Cases	Per Cent	Avg. Days				Cases	Per Cent	Avg. Days
08	233	5	2	1	0	5	100.00	103	5	2	0	1	20.00	140
09	217	5	2	1	0	5	100.00	104	5	2	0	2	40.00	104
110	233	5	2	1	0	4	80.00	90	5	2	0	4	80.00	87
111	217	4	2	1	0	3	75.00	131	4	2	0	0	00.00	00
112	217	5	2	1	1	4	80.00	60	5	2	1	2	40.00	190
106	232	4	2	1	0	4	100.00	83	4	2	0	2	50.00	140
808	184	18	2	1	0	16	88.88	97	18	2	0	4	22.22	187
Total--		46	2	1	1	41	89.13	95	46	2	1	15	32.60	140

One Cubic Centimeter of Blood Was Sufficient to Produce Fowl Paralysis

It seemed desirable, if possible from the data presented, to determine the amount of blood used which gave the best results in producing fowl paralysis. Since the greatest number of chicks received 1 and 2 c.c., Table 7 shows the results of a comparison of these two amounts. Of 141 chicks which received 1 c.c., 120 or 85.10% developed the disease in an average of 84 days. Of 177 chicks which received 2 c.c., 127 or 71.75% developed fowl paralysis within an average period of 93 days.

TABLE 7.- RESULTS OF A COMPARISON BETWEEN THE CHICKS RECEIVING 1 C.C. AND THOSE RECEIVING 2 C.C. OF BLOOD.

Total	Cubic Centimeters	Alive	Cases	Per Cent	Avg. Days
141	1	10	120	85.10	84
177	2	31	127	71.75	93

There is the possibility that the difference in results might be influenced to a slight extent by the difference in age of the recipients. Nevertheless, it would appear from the results of this comparison that 1 c.c. of blood is quite as efficient in producing fowl paralysis as is 2 c.c.

Naturally, since 1 c.c. would be more easily and quickly used, with less danger to the recipient, it would be the more desirable amount to use.

This is probably the first time that such a large amount of blood containing a virus was ever transfused into so small an experimental animal. It would seem to indicate that in a very young recipient the blood vessels are capable of great expansion without fatal results. In a few cases death resulted from rupture of blood vessels. In one chick the jugular vein ruptured.

Direct Maximum Transfusions Induce Early Development of the Disease

As shown in Research Bulletin 304, the earliest case of fowl paralysis (neural) occurred 36 days after the inoculation and no other cases occurred under 50 days. In the experiment here reported many cases of fowl paralysis developed in the inoculated birds under 30 days of age; one bird developed the disease in 18 days and four in 19 days after receiving the blood of infected adult birds. This is probably the shortest length of time that fowl paralysis (neural) has ever been artificially produced in chickens.

Incidence of Fowl Paralysis in the Control Chicks

As explained, the control chicks were exposed to the inoculated chicks by being confined in the same room. In view of the fact that the parent stock from which the experimental chicks were hatched was apparently healthy, the relatively high incidence of fowl paralysis in the controls seems to indicate considerable indirect transmission. It would be expected in this case because of the great amount of fowl paralysis in the inoculated birds. In no previous experiments has the disease exceeded 13% in the control birds.

Method Dependable for Transmission

From the results of these experiments it seems possible to predict with considerable assurance that a high percentage of properly inoculated White Leghorn recipients (1 to 3 days of age) will develop fowl paralysis. It is also desirable that a minimum of 1 c.c. of blood be transfused, and that the donor be a visibly affected bird near six months of age. In the past the study of fowl paralysis has been retarded because it was not always possible to produce a high percentage of experimental cases.

Transmission Technique May Have Adaptation in Other Diseases

The successful transmission of the virus of fowl paralysis by the direct transfusion of large amounts of blood may have applications in the study of other bacterial and virus diseases, of both animals and man, that are not readily transmitted. Certainly, it is a technique which should be of aid in the solution of the fowl paralysis problem.

SOME INTERESTING OBSERVATIONS

In the course of this investigation of blood transmission of fowl paralysis some interesting observations were made which may be of value in future investigations of this disease. Certain of these are recorded in the following paragraphs.

Results of Direct Transfusion from a Donor Affected With Osteopetrosis

One, No. 118, of the 37 donors used in this experiment, was affected with the osteopetrotic type of fowl paralysis. Nine 3-day-old chicks were inoculated with 3 c.c. of blood from this donor. At the end of a five months observation period, two of the recipients developed typical osteopetrosis of both legs, and other extremities. One of these developed two months after inoculation; the other in a little more than five months. Eight of the nine birds inoculated, or 88.88%, have developed some form of fowl paralysis. One bird is still alive.

Of the 389 other birds that received transfusions of blood from hens affected with the ocular form of fowl paralysis, 5 or 1.28% have developed osteopetrosis. Though nine experimental birds are not enough from which to draw definite conclusions, it would appear to indicate that it is possible to actually produce osteopetrosis by using a donor bird showing this form of fowl paralysis.

Carriers of Fowl Paralysis

It has long been suspected that birds may have the disease and be capable of transmitting it through the egg without showing physical outward evidence of the disease. Data on the control of birds of this experiment may contribute some evidence to help clarify this question. Of 75 control birds which showed no outward evidence of disease upon autopsy, 5 or 6.66% showed gross evidences of fowl paralysis. In two the sacral plexus was greatly enlarged and in one the sciatic plexus was involved. In the two remaining birds the left vagus in both cases was greatly enlarged.

Bacterial Infection in the Blood of Some Donors

Five of the thirty-seven donor hens which furnished the blood for this experiment were carriers of an organism in the blood, pathogenic for the baby chicks. This infection was fatal to 50 one-day-old chicks

when inoculated with the blood of any of the five donors. In the case of donor No. 810 this infection caused the death of 29 chicks from 7 to 16 days after the chicks received the blood. A careful record was kept and the results will be published separately. This occurrence is of interest in connection with the fowl paralysis study, because of the fact that it was at first thought this might be a very acute form of fowl paralysis. It was determined, however, early in the investigations that death of the chicks had no connection with fowl paralysis, but was caused by this organism occurring in the blood of a few donors.

Soiled Front Feathers Indicate the Disease

During the course of the first comprehensive study of the transmission of fowl paralysis by blood transfusion, Research Bulletin 304, it was observed that in a number of birds the feathers just under the



Fig. 4.—White Leghorn cockerel affected with soiled front. Photograph taken in July, 1939.

lower beak along the throat, cervical and crop region appeared damp, darkened, and permanently discolored. It was suspected at the time that this might indicate a form of fowl paralysis yet unreported. Pictures were taken in July 1939 of a bird typically affected with the peculiar condition (Fig. 4). However, an autopsy did not reveal any

correlation between the soiled condition of the throat and nerve changes indicative of the neural form of fowl paralysis.

During the course of the present experiment a number of cases of such soiling of the feathers were observed (Fig. 5). A renewed and careful study of birds so affected revealed several nerves involved, which probably caused the soiling of the front feathers of the fowl.

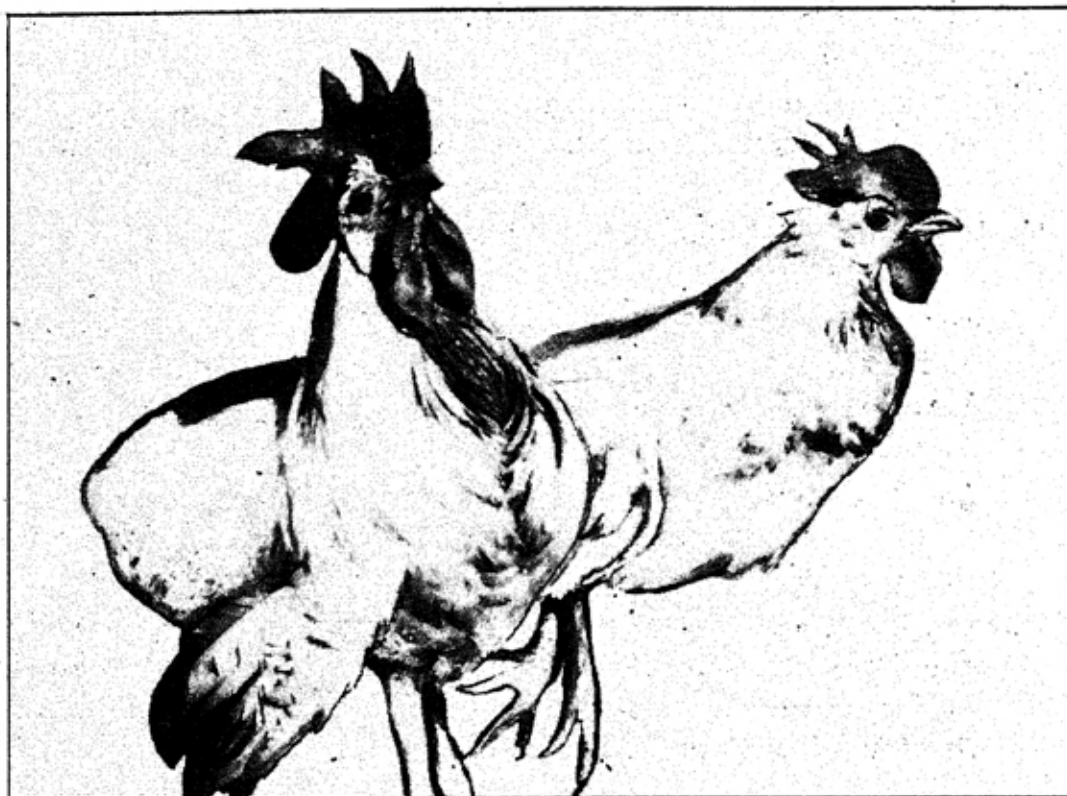


Fig. 5.—Two inoculated birds showing advanced symptoms of soiled fronts. Gross nerve changes were observed in each bird at the time of autopsy.

In birds with this symptom, the glosso-pharangeal, hypoglossal, trigeminal, mandibular trunk of trigeminal, third cervical, trochlear, and vagus nerves particularly have been found to be involved.

In some cases it has not been possible to demonstrate nerve changes responsible for the soiling. However, a sufficient number has been confirmed by gross changes in the nerves to definitely associate this soiled condition with fowl paralysis. Furthermore, 20 birds affected with severe cases of soiled fronts were selected from the inoculated birds, and 6 from the control birds. The 20 represented 5.02% of the 398 inoculated birds, and the 6 controls with the badly soiled fronts represented 1.5% of the 398 control birds. Thirteen, or 65% of the 20 inoculated birds with soiled fronts showed, on autopsy, definite lesions of fowl paralysis. Of the 6 control birds with soiled fronts, 4, or 66.66% showed fowl paralysis on autopsy. The ratio is practically the same for control and inoculated birds. Of the total of 26 selected birds with soiled fronts, 17, or 65.38% showed definite evidence of fowl paralysis, on autopsy.

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