THE RESPONSE OF INBRED LINES OF CHICKENS TO LYMPHOID TUMOR TRANSPLANTS

By

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INTRODUCTION

At the time the present study was initiated there existed at the United States Regional Poultry Research Laboratory a number of inbred lines of White Leghorn chickens with various degrees of resistance to naturally occurring lymphomatosis. Likewise, there existed a tumor material which was obtained originally from a chicken infected with lymphomatosis which was contracted naturally. This tumor material after numerous and frequent passages *in vivo* was termed "Strain RPL 16" and at the time of this study had been carried approximately 61 passages.

When tumor material such as RPL 16 was transplanted into very young chicks of certain inbred lines there was, within a period of a few days, a lymphoid growth response at the site of inoculation and frequently metastasis to other parts of the body. Among the most susceptible chicks tumor injection resulted in death even before extensive lymphoid growth was evident.

Further, it was observed that when tumor material was transplanted into young chickens of other inbred lines there was little or no tumor growth response and no mortality, thus suggesting some degree of host resistance to a specific tumor transplant.

The object of the present study is an attempt to discover whether chickens within certain inbred lines that have been selected for resistance or susceptibility to naturally occurring lymphomatosis will show a similar degree of resistance or susceptibility to transplants of lymphoid tumors. An answer to this problem is of importance to the study of lymphomatosis because under the present method of testing chickens for resistance or susceptibility to the disease a long and costly holding period (600 days) is necessary. If a rapid test for resistance to lymphomatosis could be developed the breeding program would proceed more rapidly.

DESCRIPTION OF LYMPHOMATOSIS

Lymphomatosis, the disease concerned in this study, is a form of "The Avian Leukosis Complex" which is a tentative terminology suggested by Jungherr, Johnson, and Doyle (1941) to cover a group of diseases which are primarily characterized by autonomous proliferation of the essential blood forming cells.

Definition: Lymphomatosis may be defined as a naturally occurring malignant disease of chickens characterized by the formation of lymphoid tumors which may be found in nearly all tissues of the bird's body. There are four main forms of lymphomatosis: neural, ocular, visceral, and osteopetrotic, according to the tissue affected.

Neural lymphomatosis or fowl paralysis attacks, primarily, young birds and is clinically manifested by asymmetric paresis of the leg or wing.

Ocular lymphomatosis, commonly called gray eye, is manifested by irregularity in the pupil, gradual loss of light accommodation, and grey color of the iris.

Visceral lymphomatosis affects the large abdominal organs such as the liver, spleen, and kidneys, but any organ of the body, including the skin, may be involved at times. Unless this form of the disease is accompanied by systemic disturbance the outward sign of the disease is indefinite.

Osteopetrotic lymphomatosis, often called marble bone or thick-leg disease, affects the long bones of the skeleton and it can be detected by clinical inspection.

Etiology:

Critical experimental studies, suggest that lymphomatosis is caused by one or more virus or virus-like agents. This agent may be present in certain chick embryos, newly hatched chicks, and in the blood and tumor tissue of affected birds.

It is naturally transmitted either through the egg or by contact. Doyle (1927) was the first to suggest that this disease was transmitted by way of the egg. Later, numerous investigators, Warrack, and Dalling (1932), Biely et al. (1932), Seager (1933), Blakemore and Glover (1935), Gibbs (1936), Tower (1937), McClary and Upp (1939) and Lee and Wilcke (1941), presented the evidence that the egg and infected birds are carriers of lymphomatosis. Waters and Prickett (1944) emphasized that infected birds serve as a means of disease transmission. Waters (1945a) stated that lymphomatosis is transmitted both by way of the egg and by contact. The subject of egg transmission of lymphomatosis has recently been reviewed by Cole (1949) and by Cottral (1949b). Cole's (1949) interpretation of the results of his work was that if egg borne transmission of avian leukosis from infected breeders does occur it is not of great importance in determining the viability of the progeny. Recently, Cottral, Burmester, and Waters (1949a) have demonstrated that certain embryos are carriers of the agent of lymphomatosis. These experiments also indicate that certain normal-appearing hens transmit lymphomatosis to their progeny by way of the egg. It is equally evident that modifications of the environment (Hutt, et al., 1944) and genetic resistance (Hutt, et al. 1941, Taylor, et al. 1943, and Waters, 1945b), determine to a large extent the incidence of lymphomatosis. Waters (1947) and Waters and Bywaters (1949) concluded that the incidence of lymphomatosis in experimental birds is dependent to some extent on the degree of exposure, age at time of exposure, and genetic resistance.

The presence of the avian leukosis agents in the blood suggests that the disease may be transmitted by blood-sucking parasites. While most of the experiments were negative, Johnson (1937) showed that the infection is mechanically transmitted by common red mite and fowl tick. He also presented the idea that minor operations may have the same effect.

The disease has been reported from every major poultry country in the world. The losses from this disease have been estimated to be $60,000,000 annually in the United States. (U. S. Dept. Agric., Regional Poultry Res. Lab., East Lansing, Mich. 10th Annual Rept. 1949)

At present there are no known remedies or measures which will prevent or cure lymphomatosis. There is, however, enough information available to show that innate resistance or susceptibility to disease exists in breeds and varieties of animals. Actually, it has been demonstrated that selective breeding will produce chickens highly resistant to lymphomatosis.

<u>Inheritance in relation to disease</u>

Until quite recently the value of inherited qualities as a factor in disease phenomena was not fully appreciated. The evidence acquired in plant genetics suggested that the inherited qualities of the individual should be considered as an important host variable in various diseases and structural anomalies.

The yellow mouse lethal (Cuenot, 1908), lethal anemia of mice associated with white spotting (Little, 1915), achondroplasia in cattle (Crew, 1923), cryptorchidism in goats (Lush et al, 1930) and cryptorchidism in swine (McPhee and Buckley, 1934), are all significant examples of the effect of inheritance of non-infectious disease conditions in the animal kingdom.

Recently many workers have been concerned with studies on the inheritance of susceptibility or resistance to infectious and parasitic diseases. Roberts and Card (1926) and Roberts, Severens and Card (1939a.b.) demonstrated the natural resistance of fowl to Salmonella pullorum. Also, the evidence of genetic resistance of chickens to fowl typhoid was presented by Lambert and Knox (1932). The resistance of fowl to the round worm Ascaridia liniata has been studied by Ackert (1938) and he reported that certain breeds of chickens are less resistant to parasitic infection than others.

Inheritance in relation to tumors in mice.

Inbred lines of mice have been developed, which show definite tendencies toward resistance or susceptibility to spontaneous and transplantable tumors. The experimental results by Loeb (1901), Tyzzer (1907), Haaland (1911), Cuenot (1908), Murray (1911), Slye (1915) (1931), Lynch (1924, Little (1916, 1931, and 1941), Bittner (1938, and others, indicate beyond doubt that genetics plays an important part in influencing naturally occurring tumors in mice. It is also concluded from the work of Loeb (1902), Little (1915), Little and Tyzzer (1916a.b.), Little and Johnson (1922), Loeb and Wright (1927), Bittner (1939), and others that the incidence and development of transplantable tumors are primarily dependent upon the genetic constitution of the mice.

Naturally occurring lymphomatosis

Pappenheimer, Dunn, and Cone (1926) stated that their observations indicated a significant breed or variety difference in susceptibility to fowl paralysis. Doyle (1927), suggested that fowl paralysis may be transmitted through the egg. Asmundson and Biely (1932) furnished data indicating that resistant and susceptible birds differ by a single dominant gene. Additional evidence in support of this theory was furnished by Biely, Palmer, Lerner, and Asmundson (1933). It should be noted, however, that in both these experiments the number of birds involved was rather small.

It was noticed by Kennard and Chamberlin (1934) that the flock with which they had been working for several years was more resistant than birds which were purchased from non-affected flocks and reared with their own birds.

Gildow, Williams, and Lampman (1936) reported that the progeny of certain individual hens were found to be more resistant than the progeny of other hens. They also found that the disease developed more extensively among the progeny of affected parent stock than among the progeny of non-affected parents.

Upp and Tower (1936) observed inherent differences in resistance to fowl paralysis.

Wilcke, Lee, and Murray (1938) were able to develop by selection two groups of birds that proved to be different with respect to the incidence of fowl paralysis in their offspring.

Marble (1939) observed marked family differences in the extent to which resistance to fowl paralysis is developed by selection methods. Hutt, Cole and Bruckner (1941) came to the conclusion after three years of breeding to increase viability. A significant fact emerging from the work of Hutt et al. (1941, 1947) is that by careful selection of breeders, based upon progeny test, it was possible to develop lines of chickens that were resistant and others that were susceptible to lymphomatosis. After eight years of progeny test selection, Taylor et al. (1943) also developed relatively resistant and susceptible lines of chickens with respect to lymphomatosis.

Starting with 9 strains of White Leghorns, Waters and Prickett (1944) developed inbred lines that eventually showed significant differences in their resistance to lymphomatosis. The data in table 1 show that selections and inbreeding were very useful tools in segregating the lines into susceptible and resistant populations. The data also show that genetic selection with inbreeding has decreased the incidence of lymphomatosis in certain lines, while in other lines there has been an increase. Waters (1945 a) accounted for the progressive increase and decrease in the incidence of lymphomatosis as due to definite segregation of genes for resistance or for susceptibility to lymphomatosis.

Experimentally transplanted lymphomatosis

Although a great deal has been reported on the transmission of lymphomatosis, very little work has been done on the relation of inheritance to experimentally transplanted lymphomatosis. Cole (1941) conducted experiments to determine the extent to which resistance to transmissible sarcoma (Jungherr,1937) could be increased by genetic selection. In Cole's study the principle of progeny test was used and a total of 2676 pedigreed chicks were inoculated with the neoplasm. The results showed that breeding for susceptibility within his susceptible lines was not successful. On the other hand, he succeeded in increasing the resistance of his resistant lines by selection.

TABLE 1.

The Mortality Percentage from Naturally Occurring Lymphomatosis at 600 Days of Age. (1939-1948) Only Females were Recorded in these Data.

Inbred line number	1939	1940	1941	1942	1943	1944	1945	1946	1947	1948
	<u>Lines selected for susceptibility to naturally occurring lymphomatosis</u>									
4	22	0	-	23	50	57	46	47	27	-
7	25	13	-	14	42	21	24	20	43	33
9	22	29	55	36	44	48	47	43	44	40
11	24	33	57	51	46	40	27	16	20	-
14	23	19	18	38	49	53	44	33	24	21
15	25	32	30	37	43	26	48	40	56	52
	<u>Lines selected for resistance to naturally occurring lymphomatosis</u>									
6	22	21	50	15	25	20	8	8	9	18
10	20	21	28	23	17	2	16	14	25	12

The first series of transmission studies carried on at the U. S. Regional Poultry Research Laboratory were with lines of birds that had little or no inbreeding. These chickens were inoculated with blood from birds with lymphomatosis. An analysis of the gross pathological data from these different groups revealed that chickens from the various strains responded differently with respect to lymphomatosis when they were subjected to the different treatments and environments. The data further showed that the results obtained from inoculation of any one strain of birds was not correlated with the incidence of lymphomatosis occurring among chickens of that strain under natural conditions. In more recent experiments at the U. S. Regional Poultry Research Laboratory various lines of birds (lines 2, 3, 6, 7, 9, 10, 11, 14, and 15) were inoculated with cell suspensions of tumor tissue from strain RPL 18. Chickens of line 15, a susceptible line, were found to be more susceptible to the tumor transplant than were any of the other lines that were tested. The reaction of the other lines to the tumor transplant somewhat paralleled their response to the naturally occurring disease. De Ome (1943) inoculated chicks with lymphomatosis nerve tissues during three different years. In each year the inoculated birds suffered higher mortality than did the controls. In one year the difference in mortality between inoculated chicks of resistant and susceptible lines was significant and followed the trend of the respective lines in relation to the naturally occurring disease.

Heisdorf et al. (1947), recorded that subcutaneous inoculation with lymphomatous tissue did not differentiate between the lines which were selected under natural conditions for resistance or susceptibility to lymphomatosis. Neither was there any relationship between the incidence of lymphomatosis among the inoculated birds of the resistant line, and the losses shown by their sibs reared as controls under natural exposure. However, placing lymphomatous tissue in the crops, eyes, and nostrils of baby chicks from the resistant and susceptible lines, resulted in highly significant differences between the losses from lymphomatosis in the two lines.

In another series of experiments conducted at the Regional Poultry Research Laboratory, birds of lines 6, 7, 10, 11, and 15 were inoculated with a cell-free filtrate of tumor strain RPL 18. Line 15 was found to be the most susceptible. The other lines in order of their increased susceptibility to the inoculation were rated as follows: lines 6, 11, 10, and 7. Further work needs to be done before these results can be correlated to the naturally occurring disease.

MATERIALS AND METHODS

Source of material for inoculum

The tumor material used as an inoculum in this study was obtained from pectoral muscle lymphoid tumors that were grown on birds of susceptible inbred line 15 of the U. S. Regional Poultry Research Laboratory flock. This tumor strain has been designated as RPL 16. The tumor strain originated naturally from lymphomatous liver of the bird E1121A. This bird was not inoculated and had never been in contact with inoculated stock.

The original liver tissue was inoculated into chicks. When the tumor developed in the breast muscle of the inoculated chicks, suitable donors with large tumors were selected to furnish material to propagate the tumor in other susceptible line 15 chicks. (Burmester and Prickett, 1943). Thus, the tumor was carried approximately through 61 serial passages.

At the 61st passage a large number of chickens were inoculated with strain RPL 16 and the resulting tumors were harvested when they had reached a suitable size. The tumors were asceptically removed from these birds, scissors minced, and then pooled in a large container for thorough mixing. Samples of the mixed tumor tissue were then removed and placed in sterile pyrex glass and the tubes were flamed and sealed. The tubes containing the collected tumor tissue were then frozen slowly by immersing the tubes in 95 percent ethyl alcohol and the temperature was lowered approximately $1^{\circ}C$ per minute by the addition of pieces of dry ice (CO_2). After freezing, the tubes were transferred and stored in a CO_2 ice box having a temperature range of -65 to -76°C. This procedure provided a source of inocula that could be considered as nearly uniform.

Preparation of inoculum

The frozen tumor samples were thawed just before inoculation by placing the sealed tubes under running tap water. The tubes were broken and the material was minced in a tissue mincer to subdivide the tissue into a fine paste-like material. The minced material was weighed and then diluted with 0.85 percent NaCL solution in the proportion of 1:9. The bulk of the resulting cell suspension was filtered through a layer of sterilized cheese cloth.

Birds of line 15 were used for serial passage of the tumor strain because they were found to be highly susceptible to both natural infection with lymphomatosis and to transplanted lymphoid tumors. The tumors were harvested from a large number of birds, pooled and stored in the manner described to provide a more uniform source of inocula that could be used at various periods of time during the course of the experiments. Aseptic precautions were used to prevent contamination in obtaining the material and in preparing it for inoculation.

Administration of inoculum

The standard dose of inoculum in all of the experiments was 0.1 cc. The route which was used in all cases was the right pectoral muscle. The inoculation was made into the breast with a 20 gauge needle attached to a 1 cc. tuberculin syringe. The needle was inserted into the pectoral muscle at the anterior part of the breast. This technique lessened the danger of accidental intraperitoneal inoculation.

Recipients

The chickens used in this study were all White Leghorns and consisted of both males and females. Altogether, eight different inbred lines of chickens, representing two consecutive generations, designated as "J" and "K" were involved. The history of the eight inbred lines, designated as lines 4, 6, 7, 9, 10, 11, 14, and 15, used in this study is described by Waters (1945, b). Of the eight lines used, two have been classified as resistant and six as susceptible. Chicks were hatched approximately every two weeks starting in September, 1948, and continuing until January, 1949. All chicks were held in battery brooders until they were 21 days of age. As previously described all chickens, at 21 days of age, received 0.1 cc. of tumor material in the right pectoral muscle. All chickens were observed for a 28 day period after inoculation. On the 28th day all survivors were killed and examined. During the period the chickens were under observation they were held in special holding batteries and isolated from all other chickens. Dead birds were removed several times daily and all necessary information concerning the bird was entered on a card that accompanied the bird to the autopsy room. The dead birds were refrigerated until post mortem examinations were performed.

Table 2 shows the total number of chickens of both sexes used in this experiment as well as the number of birds from each line represented.

The lines represented in this experiment were developed in the laboratory through selective inbreeding for resistance or susceptibility to naturally occurring lymphomatosis. (Waters, 1945 a)

TABLE 2

	Generation J			Generation K			J and K
Line	Male	Female	Total	Male	Female	Total	Total
4	-	-	-	61	78	139	139
6	144	142	286	146	182	328	614
7	22	16	38	92	98	190	228
9	23	33	56	76	110	186	242
10	66	64	130	-	-	-	130
11	61	60	121	27	37	64	185
14	35	36	71	62	60	122	193
15	108	71	179	63	54	117	296
Total	459	422	881	527	619	1146	2027

Collection of data

Throughout the entire work each individual was examined for clinical manifestation of tumors at the site of inoculation on the sixth, tenth, fourteenth, seventeenth, twenty-first, and twenty-eighth day of survival. A method for evaluating the growth of the tumor in the breast muscle was devised in which six classes were designated to cover the reaction obtained in the breast. No visible tumor was scored in Class O, tumors that were not more than 1 cm., 2 cm., 3 cm., 4 cm., and more than 4 cm. in diameter were graded in Classes 1, 2, 3, 4, and 5 respectively. The liver was not classified numerically, but was classified as O, A, or B. No visible metastasis was indicated by O, A indicated a diffused type of involvement with no focal tumors, and B indicated a focal type of involvement. Frequent metastasis was observed in each of the following organs: heart, spleen, kidney, pancreas, proventriculus, gonad, and peritoneum, at the time of autopsy. The data collected was grouped under two classes. No visible metastatic formation in each organ was scored in Class O. Any metastatic formation for each organ was classified in Class R.

The above methods of grading are based on purely arbitrary consideration and represent an evaluation of the interplay between the tumor transplant and resistance of the host.

Duration of experiments

Each experiment lasted 7 weeks after the hatching of the chickens. The chickens that survived were killed on the 28th day after their inoculation and they were 42 days of age at the termination of the experiment.

EXPERIMENTAL RESULTS

Differentiation of lines

Altogether 2027 pedigreed White Leghorn chicks were inoculated with tumor material from strain RPL 16. Eight different inbred lines of chickens representing two consecutive generations designated as "J" and "K" were involved in these studies. All chickens were hatched during the latter part of 1948.

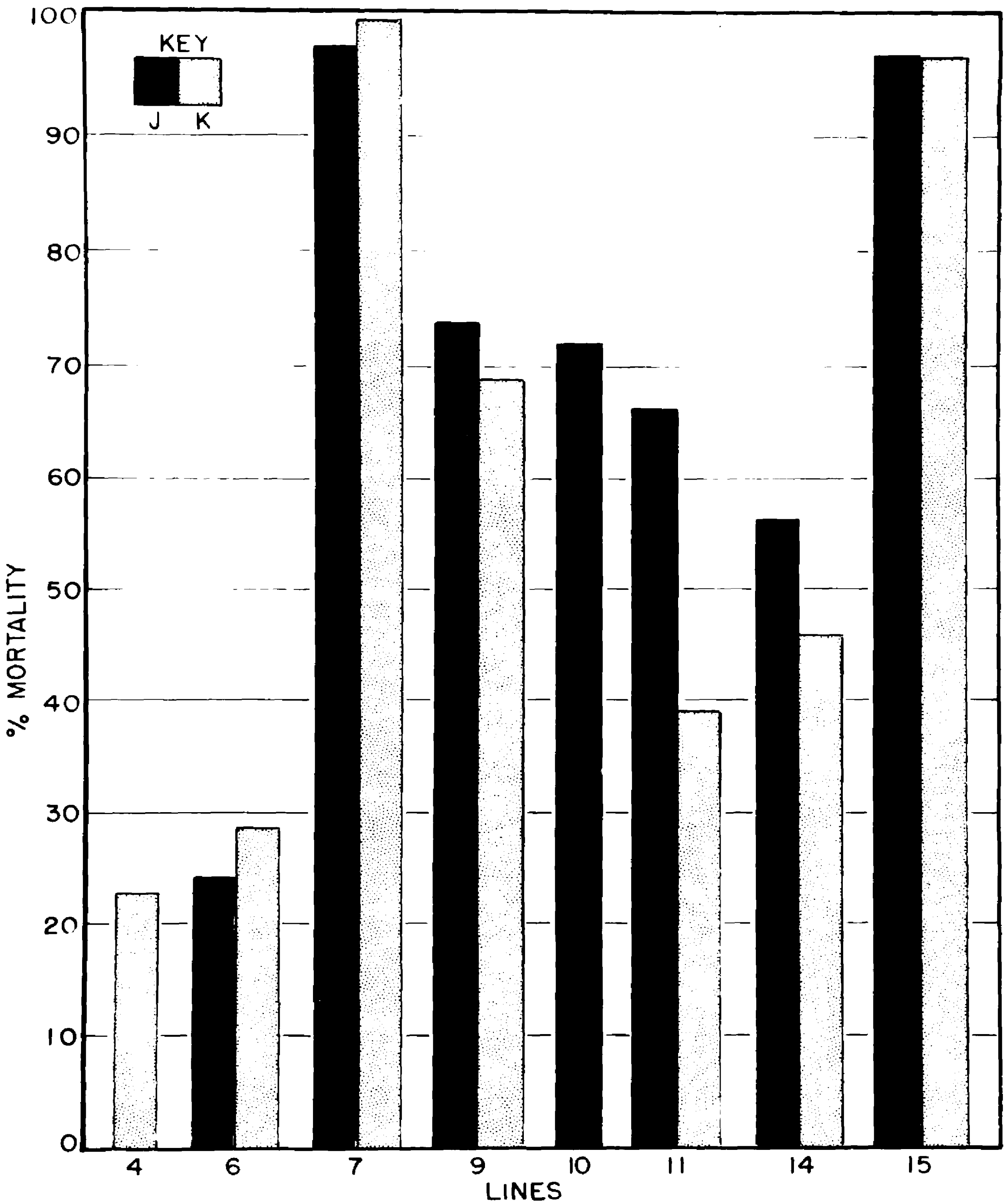
The average percentage of mortality for each of the inbred lines of chickens and for each generation is shown in table 3. Among the "J" generation chickens the lowest mortality was in line 6, while the highest mortality was found among the chickens of lines 7 and 15. For the "K" population the lowest mortality was in lines 4 and 6 while the highest mortality was among the chickens of line 7 and 15. There were no inbred line 4 chicks available for study in the "J" generation and no inbred line 10 chicks available in the "K" generation.

A graphic presentation of the results shown in table 3 is given in figure 1.

TABLE 3

Percentage of Mortality 28 Days after Inoculation among Two Different Generations of Inbred Chickens Inoculated with Tumor Strain RPL 16.

Inbred line	"J" Generation			"K" Generation		
	No. of birds	No. dead	Percent dead	No. of birds	No. dead	Percent dead
4	-	-	-	139	32	23.0
6	286	69	24.1	328	98	29.8
7	38	37	97.4	190	189	99.5
9	56	41	73.2	186	128	68.8
10	130	93	71.5	-	-	-
11	121	81	66.9	64	25	39.0
14	71	40	56.3	122	57	46.7
15	179	173	96.6	117	113	96.5



Comparison of the total mortality rates for both generations in the various lines.

Mortality in Generation "J"

The data in table 3 show that in generation "J" the lines follow the order of 7, 15, 9, 10, 11, 14, 6 respectively, ranging from the susceptible to the resistant line. As can be noticed from the data in table 4, there is a variation between the lines for the distribution of the death rate during the period of the experiment. Extending over a period of 28 days the chickens of line 6 showed the lowest total mortality. Throughout the 28-day period a few chickens in line 6 died each day with the highest mortality occurring on the 12th and 20th day following inoculation.

The mortality of line 7 (table 4) shows two peaks which are on the 12-14th and on the 20-23rd days. At the first period 18.41 percent of the chickens died while in the latter period 52.59 percent died. Only one chicken in line 7 survived to the end of the experiment.

The maximum percentage of mortality reached in line 9 was 10.70 percent on the 15th day after inoculation. The highest percentage of deaths (43.52 percent) occurred between the 12th and 17th day and 17.84 percent of the chickens died between the 19th and 22nd days.

Of the 71.5 percent deaths in line 10, 23.06 percent and 29.21 percent of the deaths occurred on the 11th-14th and the 16th-20th days, respectively. The average mortality within these two periods was approximately the same for each day. In the first period the average mortality was 5.79 percent per day and in the second it was 5.99 percent.

The mortality in line 11 is characterized by a series of small peaks, none of which are significantly high when compared to the average mortality of 3.93 percent.

TABLE 4

Mortality Percentage for the Various Days after Inoculation in the Different Lines of Generation "J"

Lines	Days of Survival																		
	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
6	0.34	0.69	0.69	3.49	2.09	1.04	0.69	1.04	1.74	2.44	1.74	3.49	0.34	0.69	0.69	1.04	0.69	0.69	0.34
7	-	-	2.63	7.89	-	10.52	2.63	2.63	7.89	2.63	2.63	15.80	18.40	7.89	10.50	2.63	2.63	-	-
9	1.78	-	-	7.14	5.35	3.57	10.70	7.84	8.92	1.78	5.35	3.57	5.35	3.57	-	3.57	1.78	1.78	1.78
10	-	1.53	6.92	6.15	4.61	5.38	2.30	4.61	5.38	6.15	10.00	3.07	2.30	2.30	2.30	3.07	1.53	0.76	2.30
11	-	-	0.82	1.65	4.95	4.13	8.26	4.13	3.30	4.95	3.30	7.43	2.47	4.13	5.78	4.95	1.65	2.47	2.47
14	-	4.22	7.04	8.45	12.70	2.81	2.81	-	5.63	4.22	2.81	-	1.40	1.40	1.40	-	-	-	1.40
15	-	5.02	25.10	23.50	7.26	5.02	1.11	0.55	-	1.67	10.60	7.82	5.58	2.23	0.55	-	-	-	-

On the 10th day 4.22 percent died in line 14 and the death rate increased until it reached 12.70 percent on the 13th day. It decreased on the 14th and 15th and 16th days and then increased a little on the 17th and 18th and continued decreasing on the 21st, 22nd, and 23rd days.

Line 15 which is highly susceptible also shows two periods in which the mortality rate was high. On the 10th day 5.02 percent died, which is the highest death rate attained in any line on that day.

On the 11th and 12th days 25.10 percent and 23.50 percent died. By the 15th day a total of 67 percent had died. Mortality dropped sharply to 0.55 percent on the 16th day then increased to 10.60 percent on the 19th day and then decreased to 7.82, 5.58, 2.23, and 0.55 percent on the 20th, 21st, 22nd, and 23rd days respectively. The total mortality in line 15 was 96.01 percent.

Comparisons of Mortality in Generation "J"

The first deaths in the "J" generation occurred on the 9th day. Line 6 and line 9 each showed more mortality. On the 10th day deaths occurred among the chickens of line 6, 10, 14, and 15.

On the 11th day considerable variation occurred in the percentage of deaths of the chickens in the various lines. Line 15 had the highest mortality, showing 25.1 percent. An increasing percentage of mortality occurred in the other lines except for line 9 which showed no deaths.

Starting with the 12th day all lines showed some mortality with the highest percentage again present in line 15.

On the 14th day all lines showed a mortality of less than 5 percent. During the first 14 days line 15 showed a mortality of approximately 66 percent while line 6 had less than 9 percent mortality.

A comparison of the mortality for all lines in the "J" generation during the 28-day period indicates that lines 7 and 15 were the most susceptible while line 6 was the most resistant. In line 15 most of the chickens died prior to 15 days of age whereas in line 7 most of the deaths occurred after the 15-day period.

Mortality in Generation "K"

As is shown in table 5, lines 4 and 6 proved to be the most resistant. Death from the tumors were 99.5 percent and 96.6 percent in lines 7 and 15 respectively. Starting from the most susceptible to the most resistant, the lines can be arranged as 7, 15, 9, 14, 11, 6, 4.

Line 4 showed 3.59 percent mortality on the 12th day, then it dropped and again increased on the 16th day. Between the 16th and 21st day, 13.91 percent of the birds died.

In line 6 the highest mortality (5.79 percent) was reached on the 12th day, and 19.49 percent died between the 11th and 14th days. Mortality was less than 1 percent during the remaining days of the experiment except on the 15th and 20th when it was 1.52 percent.

Among the chickens of line 7 which were highly susceptible to tumor inoculation, 99.5 percent died prior to the 25th day after inoculation. The greatest losses occurred between the 11th and 17th day and accounted for 94 percent of the total deaths.

Line 9 showed a 55.84 percent mortality between the 11th and 17th days. After the 17th day the percentage of mortality ranged between 3.2 percent and 0.5 percent.

Line 11 of the "K" generation performed similarly to what it did in the "J" generation. The mortality again was characterized by a series of small peaks none of which were significantly high when compared to the overall average death rate of 3.2 percent per day for this line.

Line 14 reached its highest mortality on the 11th, 12th, and 13th days and accounted for 28.19 percent.

Most of the mortality in line 15 occurred before the 15th day after inoculation and during this period 93.1 percent of the birds died.

Line 15 in the "K" generation differs from line 15 in the "J" generation in that the mortality was higher (33.3 percent) in the former. This peak mortality occurred on the 14th day, while in the "J" generation the mortality was higher on the 11th day (25 percent). Furthermore, no secondary rise in mortality occurred during the latter period in the "K" generation.

Comparisons of Mortality Lines in Generation "K"

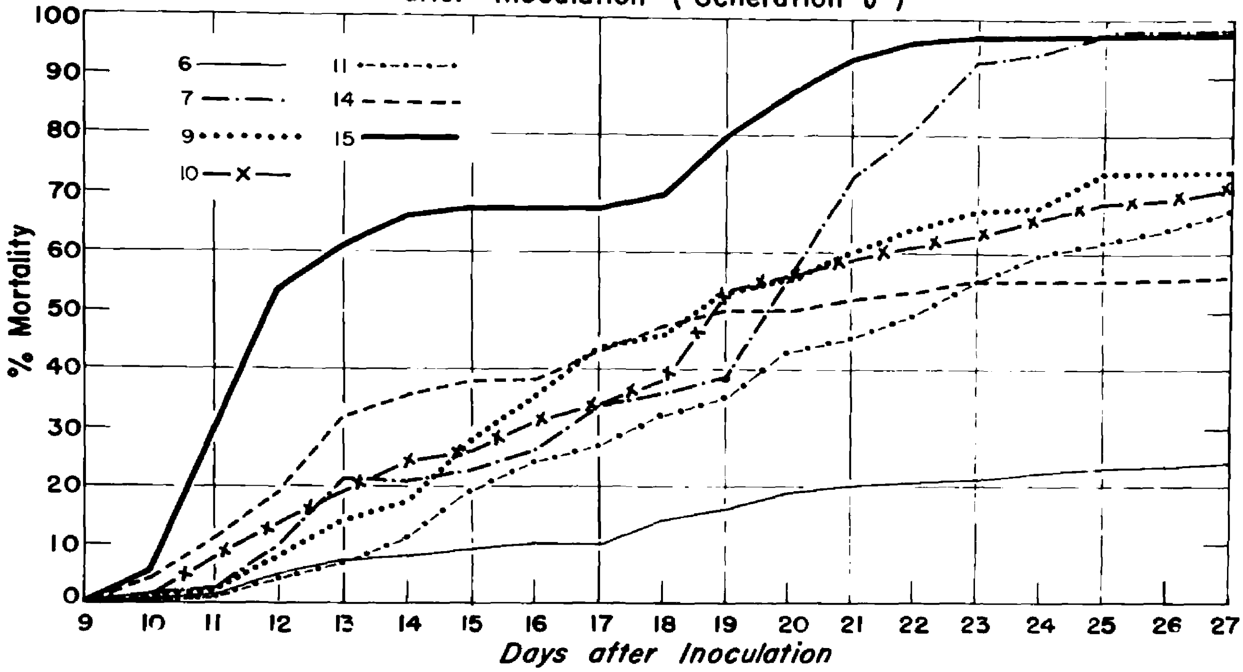
Following inoculation the first deaths in the "K" generation occurred on the 10th day. The highest percentage of deaths occurred among the birds of line 15 while lines 11 and 14 show no deaths. (Table 5)

On the 12th day after inoculation the mortality picture changes rapidly. In line 15, 23.9 percent of the birds were dead and all other lines except line 11 showed increases in mortality.

On the 13th day lines 7, 14, and 15 all showed a high mortality while lines 4, 6, and 9 showed a decrease in mortality over the previous day. No deaths had as yet occurred in line 11.

Starting with the 15th day nearly all lines showed a decrease in percentage of mortality over the high of previous periods. After the 15th day none of the lines exceeded a mortality of 8 percent and during this latter period line 11 usually had the highest percentage of mortality.

Cumulative Percentage Mortality for Succeeding Days after Inoculation (Generation J)



Cumulative Percentage Mortality for Succeeding Days after Inoculation (Generation K)

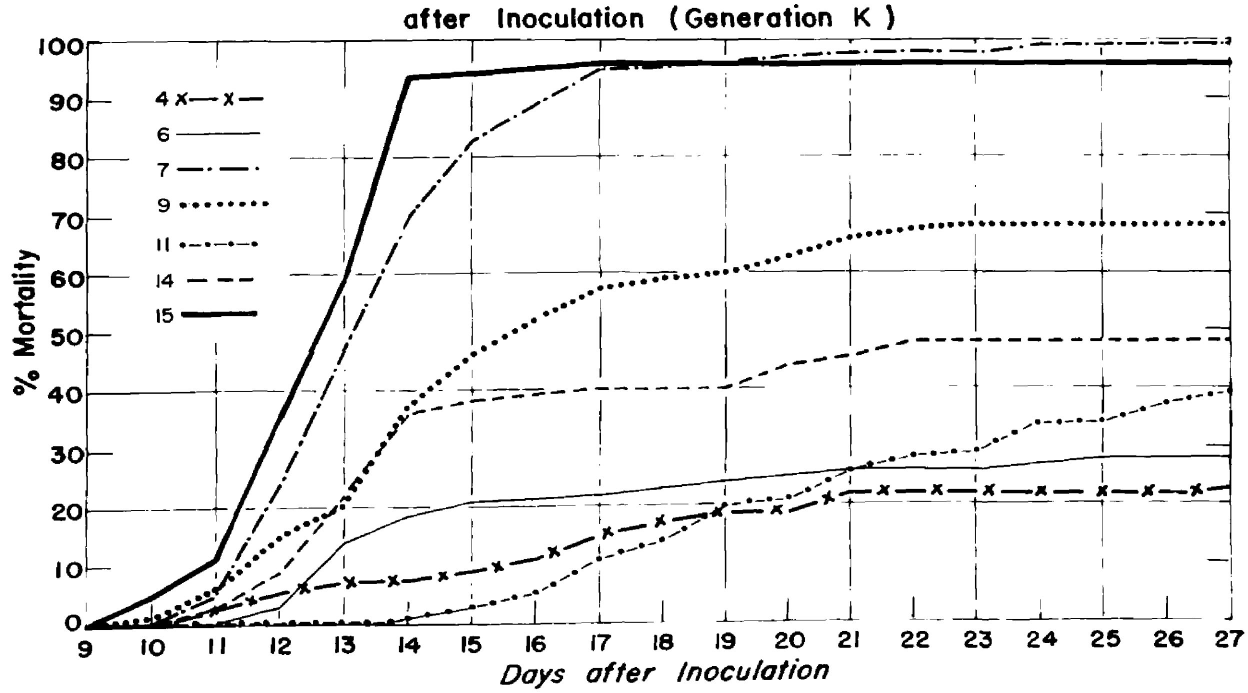


TABLE 5

Mortality Percentage for the Various Days after Inoculation in the Different Lines of Generation "K"

Line	Days of Survival																	
	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
4	1.43	0.71	3.59	1.43	0.71	1.43	2.15	2.15	3.59	3.15	-	2.87	-	-	-	-	-	0.71
6	0.30	3.65	5.79	4.57	5.48	1.52	0.60	0.91	0.60	0.60	1.52	0.60	0.30	0.30	0.60	0.91	0.30	-
7	0.52	4.73	17.40	24.70	23.20	12.60	6.30	5.78	1.05	0.52	0.52	0.52	1.05	-	0.52	-	-	-
9	1.61	4.83	9.13	5.37	16.10	7.52	7.52	5.37	1.61	1.61	3.22	2.15	1.07	1.07	0.53	-	-	-
11	-	-	-	-	1.56	-	1.56	7.81	3.13	6.25	1.56	4.68	1.56	1.56	4.68	-	3.12	1.56
14	-	3.27	5.73	13.10	13.90	2.45	0.81	-	1.63	-	3.27	1.63	0.81	-	-	-	-	-
15	5.12	5.98	23.90	24.80	33.30	0.85	1.70	-	-	-	-	0.85	-	-	-	-	-	-

A better picture of the susceptibility of the various lines in the two generations can be seen in figures 2 and 3 in which the mortality curve is plotted on a cumulative percentage basis. It is obvious from the chart that lines 15 and 7 were the most susceptible to the tumor transplant. Lines 9, 10, 11, and 14 assumed an intermediate position in regard to susceptibility and lines 6 and 4 were found to be the most resistant. In the "J" population the curves in all the lines are more gradual, but in the "K" generation the curves of the susceptible lines rise sharply.

In general, although a comparison of the daily mortality percentages for the various lines of the "K" and "J" generations showed considerable variation, the cumulative mortality percentage at the end of the experiment was remarkably similar.

<u>Analysis of Data</u>

The data presented in tables 4 and 5 and figures 1, 2, and 3 show that there were significant differences between the lines within each generation in regard to percentage of mortality (or percentage of survival). In order to determine whether or not differences between generations, between sexes, and between families within lines influenced the results, the following tests were made, using the percentage of survivors:

A "t" test was made using lines that had families common to both generations. As is shown in table 6 there was no significant difference between the generations, between the two sexes, between females in the two generations, and between males in the two generations.

As a result of not finding differences between the generations or sexes in similar lines (6, 7, 9, 11, 14, and 15) sexes and generations

TABLE 6

"t" Test for Survival Percentages

Sex	Line 6	Line 9	Line 14	Line 15
Males "J"	86.8	18.2	45.5	3.1
Males "K"	86.4	15.8	60.0	3.8
"t"	0.059	0.165	0.665	0.146
Females "J"	85.3	33.3	54.5	0
Females "K"	85.4	20.0	50.0	0
"t"	0.132	0.852	0.206	0
Males "J" and "K"	86.4	16.6	52.5	3.4
Females "J" and "K"	84.6	22.8	52.5	0
"t"	0.400	0.696	0	0
Males and Females "J"	86.1	23.8	50.0	2.2
Males and Females "K"	85.2	18.1	55.0	2.0
"t"	0.204	0.587	0.33	0.02

within families in each line were combined and a chi-square test was conducted to see if there were significant differences in mortality between families in the same line. The method used is shown in table 7. Data from line 9 are presented as an example. Lines that showed significant differences between families were lines 6, 9, 11, 14, and 15.

Figures 1 to 3 give the data on the death rate. From the data assembled, the average survival in days for each line was computed, using only the birds that died within the experimental period. Both the "t" test and the analysis of variance were employed to test the differences.

"t" Tests

Table 8 gives the results of a primary "t" test and the following observations were made:

1. There was a significant difference at the 1 percent level between the males of generation "J" and males of generation "K".

2. There was a significant difference at the 5 percent level between the females of the two generations.

3. There was no significant difference between sexes for generations "J" and "K" combined.

4. There was a significant difference at the 1 percent level between the two generations grouping the sexes in each generation together.

The same procedure was followed to find out if there was a difference between the sexes within each generation. Sexes within generation "J" did not show any significant difference while there was a significant difference at the 5 percent level between males and females in generation "K". Thus the sex differences noted between the two populations may be attributed to the influence of generation or the interaction between sexes and generations.

TABLE 7

X^2 Test of Families of Line 9
Generations J and K

Family	No. in Family	No. Living X	Probability of Survival P	Products PX
404	8	1	0.1250	0.1250
405	1 1	10	0.9090	9.0909
406	7	2	0.2856	0.5712
407	19	7	0.3679	2.5753
409	18	3	0.1666	0.5000
411	17	1	0.0588	0.0588
412	10	3	0.3000	0.9000
415	8	2	0.2500	0.5000
416	12	6	0.5000	3.0000
418	6	1	0.1666	0.1666
419	9	2	0.2222	0.4444
420	12	2	0.1666	0.3333
422	7	5	0.7143	3.5715
423	15	3	0.2000	0.6000
424	7	2	0.2856	0.5712
425	7	3	0.4284	1.2856
427	13	3	0.2307	0.6923
428	25	6	0.2400	1.4400
430	6	2	0.3333	0.6666
431	8	4	0.5000	2.0000
Total	225	68		2 9 .2927

$\bar{p} = \frac{68}{225} = 0.3022$

Degrees of freedom - 19

$\bar{q} = 1 - 0.3022 = 0.6978$

$X^2 = \frac{\Sigma PX - \bar{p}\Sigma X}{\bar{p}\,\bar{q}} = 41.45^{**}$

** Significant at 1 percent level.

TABLE 8

"t" Test on Common Families in "J" and "K" Generations Using the Average Survival Day

Males Generation "J" : Males Generation "K" :	t = 3.75	**
Females Generation "J": Females Generation "K":	t = 2.25	*
Males Generation "J" and "K": Females Generation "J" and "K":	t = 0.632	
Generation "J": Generation "K":	t = 3.54	**

** Significant at 1 percent level.

* Significant at 5 percent level.

Analysis of Variance

The method of "expected subclass numbers" (Snedecor and Cox, 1935) was found to be most appropriate for this analysis under the circumstances of these experiments. The data varied because there were disproportionate numbers of birds in the various families and an unequal sex distribution within the families which may be attributed to the accidents of sampling. It was assumed that the population from which this sample was drawn really had proportionate subclass numbers.

Tables 9 and 10 show the procedure of analysis and the results. In Generation "J" there was a highly significant difference between sexes and also a line difference. In generation "K" there was a highly significant difference between the lines. The analysis did not show any difference between sexes in this generation.

PATHOLOGY

Breast reaction

Out of a total of 2027 birds which were inoculated with the lymphoid tumor only three birds failed to develop breast tumors. Two of these birds were from resistant line 6 and one was from line 11 of generation "J". After the growth of the implant had become established in some birds the tumor regressed, in others it continued to grow, and still in others it continued to grow and metastasized to the visceral organs. The size of the tumor differed and apparently was related to the length of the survival period.

In all of the susceptible birds of the various lines the tumor developed in the breast and metastasis occurred to the liver, spleen, and kidneys. However, many of the birds, particularly of lines 7 and 15,

TABLE 9

Analysis of Variance Generation "J"

1. Primary analysis of variance of means of survival days (actual)

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square
Total	532	10,952.86	
Between subclasses (sex line)	13	671.86	
Within subclasses	519	10,280.60	19.80

2. Analysis of variance of means of survival days (method of expected subclass number)

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square
Between subclasses	13	2,071.06	
Between sex means	1	232.12	232.12**
Between means of lines	6	1,735.27	289.21**
Interaction	6	103.67	17.28
Within subclasses	519	10,280.60	19.80

** Significant at 1 percent level.

TABLE 10

Analysis of Variance Generation "K"

1. Primary analysis of variance of means of survival days (actual)

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square
Total	637	6,111.52	
Between subclasses (sex line)	13	1,191.88	
Within subclasses	624	5,920.64	9.48

2. Analysis of variance of means of survival days (method of expected subclass number)

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square
Between subclasses	13	1,389.75	
Between sex means	1	1.88	1.88
Between means of lines	6	1,239.54	206.59**
Interaction	6	148.33	24.72*
Within subclasses	624	5,920.64	9.48

** Significant at 1 percent level.

* Significant at 5 percent level.

died in a short time so that the tumors did not attain a great size. Thus, the size of the tumor differed and apparently was related to the length of the survival period. This is also true for the birds that lived longer from the susceptible lines 7 and 15 which usually developed larger tumor masses in the breast and visceral organs.

Examination of the birds began on the 6th day after inoculation because past experience had shown that no appreciable breast tumor would develop prior to that time.

The data on the degree of involvement of the breast were used to compare the various lines on the 6th and 10th days after inoculation (see tables 11 and 12). The percentage of birds that had breast reactions on these days of those that survived (tables 15 and 16) to the end of the experiment was compared to the percentage of the non-survivals (tables 13 and 14) that showed a breast reaction on these same days.

TABLE 11

The Percentage of Chickens that Showed Visible Breast Tumors in the Various Inbred Lines of Generation "J" on the 6th and 10th Day after Inoculation.

Line	Total No. of Chickens	Percent Showing Tumors on 6th Day	Percent Showing Tumors on 10th Day
6	286	29.9	89.8
7	38	7.9	86.9
9	56	35.8	92.9
10	130	33.9	95.4
11	121	19.1	78.6
14	71	36.7	93.0
15	179	19.6	91.1

Breast reaction on different days. Generation "J"

6th Day

A comparison of the lines (table 11) showed that there was no great difference either in the percentage of birds showing a breast tumor or in the degree of involvement in individual birds. In all the lines, birds that had breast reaction were in Class 2 (table 13).

10th Day

The data in table 11 showed that on the 10th day the majority of birds showed a tumor reaction for lines 6, 7, 9, 10, 11, 14, and 15. The increased percentage in the 1, 2, and 3 tumor classes (table 13) is evidence of the continued growth of the transplant in the birds in all the lines. It was noticed that the percentage of birds that showed a growth of Class 2 was greater in the survivors than in the non-survivors in all lines except line 7.

Thus, susceptibility or resistance cannot be determined from the breast reaction alone during the first ten days after inoculation.

Breast reaction on different days. Generation "K"

6th Day

The data in table 12 shows that less than 11 percent of the birds in lines 4, 6, 7, 9, 11, 14, and 15, respectively, had breast reactions on the 6th day.

10th Day

The reactor percentage (by the 10th day) increased in each line. It was 77.6, 81.7, 96.4, 95.2, 62.5, 91.0, and 85.5 percent in lines 4, 6, 7, 9, 11, 14, and 15, respectively.

As a result of the continued growth of the transplant the number of reactors increased in tumor classes 1, 2, and 3 (table14). The

TABLE 12

The Percentage of Chickens that Showed Visible Breast Tumors in the Various Inbred Lines of Generation "K" on the 6th and 10th Day after Inoculation.

Line	Total No. of Chickens	Percent Showing Tumors on 6th Day	Percent Showing Tumors on 10th Day
4	139	1.5	77.6
6	328	10.4	81.7
7	190	9.5	96.4
9	186	11.8	95.2
11	64	4.7	62.5
14	122	9.4	91.0
15	117	0.8	85.5

TABLE 13

Class Frequency of Breast Tumor Size on the 6th and 10th Day among Those Chickens in the Various Inbred Lines of the "J" Generation Which were Alive on the 10th Day, but Died Prior to 28 Days after Inoculation.

Line	Number of Chickens	Tumor Size Class	Percentage of Chickens Showing the Size of Tumor on the-- 6th Day	10th Day
6	69	0	81.1	11.6
		1	13.0	47.8
		2	5.7	31.8
		3	0.0	8.6
7	37	0	91.8	13.6
		1	8.1	32.4
		2	0.0	37.8
		3	0.0	16.2
9	41	0	63.3	7.3
		1	36.6	21.9
		2	0.0	43.9
		3	0.0	24.4
		4	0.0	2.4
10	93	0	66.6	4.3
		1	27.9	19.3
		2	5.4	50.5
		3	0.0	25.8
11	81	0	82.7	24.6
		1	17.2	35.8
		2	0.0	35.8
		3	0.0	3.7
14	40	0	60.0	10.0
		1	35.5	15.0
		2	5.0	42.5
		3	0.0	32.5
15	172	0	81.4	9.3
		1	15.6	41.2
		2	1.9	41.2
		3	0.0	8.2

TABLE 14

Class Frequency of Breast Tumor Size on the 6th and 10th Day Among Those Chickens in the Various Inbred Lines of the "K" Generation Which were Alive on the 10th Day, but Died Prior to 28 Days after Inoculation.

Line	Number of Chickens	Tumor Size Class	Percentage of Chickens Showing the Size of Tumor on the-- 6th Day	10th Day
4	32	0	100.0	21.8
		1	0.0	43.7
		2	0.0	31.2
		3	0.0	3.2
6	98	0	89.3	29.6
		1	10.6	52.1
		2	0.0	15.9
		3	0.0	2.1
7	189	0	91.0	3.2
		1	8.9	59.7
		2	0.0	36.5
		3	0.0	0.5
9	128	0	89.0	5.4
		1	10.9	58.5
		2	0.0	32.1
		3	0.0	3.9
11	25	0	100.0	36.0
		1	0.0	56.0
		2	0.0	8.0
14	37	0	91.3	14.1
		1	8.7	50.8
		2	0.0	3.5
15	113	0	99.1	15.0
		1	.8	69.9
		2	0.0	14.2
		3	0.0	0.8

percentage of reactors in the survivors of each class of reaction was not less than for the corresponding classes of non-survivors.

As in generation "J", the breast reaction did not distinguish resistant from susceptible lines at either 6 or 10 days.

Breast reaction of survivors from the 14th to 28th days. Tables 15 and 16.

A study of the breast reaction in the birds that survived until the end of the experiment showed that by the 14th day after inoculation, the breast reaction in each line had taken a more definite pattern. In some lines the birds showed tumor regression so that the number of non-reactors greatly increased. At the same time as a result of continued growth of tumor in some birds the number of reactors increased in classes 3, 4, and 5 of tumor size.

Breast reaction in different lines. Generation "J".

Line 6: The regression in the size of the tumor began on the 14th day. The number of non-reactors increased noticeably until on the 28th day it reached 83.4 percent of the total survivors.

Line 7: Only one bird did not die from the tumor and it showed a growth of class 2 on the 14th day. There was no evidence of tumor in the breast when the bird was examined on the 17th, 21st, and 28th day.

Line 9: Generally regression in the size of the tumor started by the 14th day and by the 17th day for 20 percent of the birds that survived until the end of the experiment at which time no tumors were evident. The number of birds that showed complete regression increased 73.3 percent on the final day of the experiment. There were no birds showing a 3, 4, or 5 class tumor growth at 28 days after inoculation.

Line 10: Of the birds that remained on the final day of the experiment, 94.5 percent did not show any localized tumor, however, the rest of the birds showed a tumor reaction of Class 5. With the number of reactors continually decreasing, there were no birds that showed Class 5 tumors, except on the final day of the experiment. Up to the 21st day none of the birds in this line had a breast reaction greater than 4 degrees.

Line 11: The data in table 12 for the 14th day indicate that all of the birds had breast tumors and 45 percent of the birds were of Class 4 tumor size. On the 17th day the percentage of birds that were in 1, 2, 3, and 4 class of tumor size was approximately equal.

Apparently the birds in this line reacted similarly to those of line 10 in that a comparatively high number of birds in Class 5 tumor size were present on the last day of the experiment.

Line 14: The size of the tumors in this line regressed after the 14th day. On the 21st day 3.2 percent of the survivors were of Class 5 tumors, but these tumors regressed to such an extent that by the 28th day none were larger than a Class 2 reaction. Eighty-seven percent showed no breast lesion on the final day.

Line 15: The tumor continued to grow in the birds that survived during the period of experiment. After the tumor had reached a growth of Class 4 in some birds, regression started and progressed continually. By the end of the experiment 71.4 percent of the survivors did not show tumor growth, while the rest showed Class 1 tumor growth.

Breast reaction in different lines. Generation "K"

Line 4: No birds in this line developed a Class 5 growth during this experiment. On the 17th day 8.4 percent of the birds had a tumor of Class 4. This was the highest percentage of reactors in line 4 that reached this class during the period of the experiment. Starting from the 14th day the number in the upper classes of tumor reaction decreased continually and by the 28th day 95.4 percent of the survivors did not show any growth. A very small percentage of the birds showed a local reaction of 1, 2, and 3 on the final day of the experiment.

Line 6: On the 14th day the number of 0 Class in this line increased in comparison with the same percentages on the 10th day. None of the birds were in Class 1. The number of birds in Class 2 decreased and the number of birds in Classes 3, 4, and 5 increased. On the 17th, 21st and 28th day the number of birds in each class of growth decreased continually. This shows that the tumors in the birds of this line reached the point of maximum growth by the 14th day.

Line 7: Similar to the same line in generation "J", one bird survived. This bird showed a tumor growth of Class 1 on the 14th day. The tumor had regressed completely when the bird was examined on the 17th day.

Line 9: The number of birds that did not show tumors began to increase by the 14th day. This shows that most of the tumors started to regress by the 14th day. However, in a few birds the tumor continued to grow.

TABLE 15

Class Frequency of Breast Tumor Size among those Chickens in the Various Inbred Lines of the "J" Generation which Survived until 28 Days after Inoculation.

Line	No. of Chickens	Tumor Size Class	Percentage of Chickens Showing the Size of Tumor on the-- 6th Day	10th Day	14th Day	17th Day	21st Day	28th Day
6	217	0	67.7	9.6	10.5	37.5	61.7	83.4
		1	29.0	43.3	12.9	15.6	11.0	12.4
		2	3.2	36.8	35.4	17.9	12.5	2.7
		3	0.0	10.2	30.5	15.2	6.5	0.5
		4	0.0	0.0	10.6	13.3	7.8	0.4
		5	0.0	0.0	0.0	0.4	0.4	0.4
7	1	0	100.0	0.0	0.0	100.0	100.0	100.0
		1	0.0	100.0	0.0	0.0	0.0	0.0
		2	0.0	0.0	100.0	0.0	0.0	0.0
9	15	0	66.6	6.7	0.0	20.0	60.0	73.3
		1	20.0	13.3	13.3	20.0	20.0	20.0
		2	13.3	66.6	26.6	33.3	13.3	6.6
		3	0.0	13.3	20.0	20.0	6.6	0.0
		4	0.0	0.0	14.0	6.6	0.0	0.0
10	37	0	64.8	5.4	0.0	16.2	59.4	94.5
		1	29.7	18.9	5.4	32.4	24.3	0.0
		2	5.4	48.6	21.6	21.6	5.4	0.0
		3	0.0	24.3	54.0	16.2	8.1	0.0
		4	0.0	2.7	18.9	13.5	2.7	0.0
		5	0.0	0.0	0.0	0.0	0.0	5.4
11	40	0	77.5	15.0	0.0	12.5	40.0	70.0
		1	22.6	35.0	2.5	22.5	20.0	5.0
		2	0.0	45.0	12.5	17.5	7.5	12.5
		3	0.0	5.0	20.0	22.5	10.0	2.5
		4	0.0	0.0	45.0	22.5	17.5	0.0
		5	0.0	0.0	20.0	2.5	5.0	10.2
14	31	0	67.7	3.3	0.0	9.6	38.7	87.0
		1	29.0	19.3	6.5	19.5	22.5	9.7
		2	3.2	58.0	19.3	22.5	19.3	3.2
		3	0.0	19.3	48.3	22.5	9.7	0.0
		4	0.0	0.0	25.8	25.8	6.5	0.0
		5	0.0	0.0	0.0	0.0	3.2	0.0
15	7	0	56.1	0.0	0.0	28.5	28.5	71.4
		1	28.5	14.3	0.0	0.0	14.3	28.5
		2	14.2	42.8	42.9	14.4	42.8	0.0
		3	0.0	42.8	28.5	28.5	14.3	0.0
		4	0.0	0.0	28.5	28.5	0.0	0.0

TABLE 16

Class Frequency of Breast Tumor Size among those Chickens in the Various Inbred Lines of the "K" Generation which Survived until 28 Days after Inoculation.

Line	No. of Chickens	Tumor Size Class	Percentage of Chickens Showing the Size of Tumor on the-- 6th Day	10th Day	14th Day	17th Day	21st Day	28th Day
4	107	0	98.1	22.4	24.3	47.7	78.5	95.4
		1	1.8	51.4	23.4	15.8	12.1	1.8
		2	0.0	25.2	37.3	19.6	0.9	1.8
		3	0.0	0.9	14.0	8.4	5.6	0.9
		4	0.0	0.0	0.9	8.4	2.8	0.0
6	230	0	89.7	13.7	20.0	47.8	66.7	91.4
		1	9.8	47.8	0.0	0.0	16.7	5.5
		2	0.4	36.7	20.5	19.2	10.7	1.7
		3	0.0	1.7	41.5	18.8	2.9	0.0
		4	0.0	0.0	17.5	11.5	2.9	1.3
		5	0.0	0.0	0.4	0.25	0.0	0.0
7	1	0	0.0	100.0	0.0	100.0	100.0	100.0
		1	100.0	0.0	100.0	0.0	0.0	0.0
9	58	0	86.2	3.4	12.0	34.4	68.9	96.5
		1	13.7	48.4	17.4	20.6	13.8	1.7
		2	0.0	44.8	55.1	29.3	12.0	0.0
		3	0.0	4.3	13.7	12.0	5.2	0.0
		4	0.0	0.0	1.7	3.4	0.0	1.7
11	39	0	92.3	38.5	15.3	33.3	51.3	82.0
		1	7.6	43.5	25.6	2.5	10.3	7.8
		2	0.0	17.9	43.5	30.8	17.9	2.5
		3	0.0	0.0	10.3	23.0	7.6	2.5
		4	0.0	0.0	5.2	10.3	12.8	5.1
14	75	0	90.7	4.6	16.9	40.0	75.3	89.2
		1	9.2	60.0	12.4	12.4	4.7	9.2
		2	0.0	35.4	53.8	16.9	10.7	0.0
		3	0.0	0.0	15.4	23.0	4.7	0.0
		4	0.0	0.0	1.4	7.6	3.0	1.6
		5	0.0	0.0	0.0	0.0	1.5	0.0
15	4	0	100.0	0.0	0.0	100.0	100.0	100.0
		1	0.0	75.0	25.0	0.0	0.0	0.0
		2	0.0	25.0	75.0	0.0	0.0	0.0

Line 11: In this line some tumor regressions occurred by the 17th day. The birds showed the same growth trend as those of line 9 except that on the 28th day reactors occurred in all classes except 5. Five and one-tenth percent of the birds were in Class 4. This is a fairly high percentage when compared to the percentage in this class in the other lines.

Line 14: The data show that the percentage in all classes of growth, except 5, decreased after it reached its maximum on the 17th day. On the last day of the experiment one bird out of 75 survivors showed a Class 4 tumor size. This was the only bird with a larger than Class 1 tumor.

Line 15: Only 3.4 percent of these birds (4 out of 117) lived to the end of the experiment. On the 10th and 14th day 25 percent and 75 percent of these birds showed classes 1 and 2 breast reactions. On the 17th day the tumors had completely regressed in size.

In general, it was noticed that there was a period in which the transplant started and continued growing followed by a regression in size. There were also noticeable variations between the different lines in regard to tumor growth and regression. Birds in some lines had an earlier start in growth of tumor than others. It was also observed that the distribution of birds in Class 5 fluctuated more in some lines than in others.

The nature of the tumor in the breast muscle on the 28th day was different than the tumors of similar size in the birds that died earlier. Tumors showed more necrotic tissue with the progress of time. Those of Class 1 and Class 2 on the 28th day were mostly a capsule containing necrotic tissue.

No gross alteration was found at the site of inoculation in the birds in which the transplant completely regressed. A variable amount of necrosis was commonly found in the tumors of birds that died at later ages. In some instances large tumors were almost entirely involved by necrosis and in such cases the birds were found to be dull, listless, and they refused to eat.

METASTATIC INVOLVEMENT OF THE INTERNAL ORGANS

Aside from local growth of the implant in the breast muscles, a diffuse type of metastasis was found in the viscera. The organs most frequently involved were the liver, kidney, spleen, heart, pancreas, proventriculus, and gonads. The organs are ranked in their order of frequency of involvement.

Liver: In the liver the metastasis manifested itself in two types, a "diffuse" (type A) and a "nodular" (type B). In the former type, which was the more common, the liver was enlarged to various degrees. In the birds that died a short time after inoculation, the liver showed considerable congestion and in some instances it reached twice the normal size. Birds that died during the later period of the experiment had a diffuse or focal involvement of the liver with little increase in the size of this organ.

Kidneys: These organs were primarily affected by diffuse greyish enlargement of some of the three lobes. In some cases they were noticeably puffy and enlarged. In general the involvement of the kidneys paralleled the severity of liver changes.

Spleen: In birds that died within the first week after inoculation this organ was enlarged. It had a uniform cherry-red color and did not contain tumor nodules. In the birds that died later, this organ was usually less congested, grayish-brown in color, soft in texture, and showed some degree of atrophy.

Heart: In the birds that died early, a diffuse myocarditis occurred and the organ was slightly enlarged. The birds that died in the later period of the experiment showed prominent myocardial tumors which were sometimes accompanied by pericarditis. These tumors usually were focal and often showed coalescence.

Pancreas: This organ was often affected with focal tumors. The metastatic foci were usually circumscribed and easily detected upon gross examination. In such cases the pancreas was thickened and firm. Some involvements of the pancreas may have been overlooked because the color of this organ is similar to the color of the tumor tissue. In the birds that died during later stages the tumors in the pancreas were quite small in size and gave the impression that they had undergone regression.

Proventriculus: Diffuse infiltration of the neoplastic lymphoid tissue was a characteristic finding in the wall of the proventriculus, however, this condition was not noticed except in birds that died in the later period of the experiment. The wall of the proventriculus was greatly thickened and necrotic at times.

Gonads: The immature ovary showed an increase in size. In some instances they were three times their normal size. The male glands were sometimes enlarged and were very firm. The involvement of these organs usually occurred in birds that died in the later stages of the experiment.

Peritoneum: In very few cases was the disease manifested in this structure. The tumors that existed were diffuse in nature.

Lungs: There were no significant changes in the lungs. In some cases the carcass was markedly emaciated.

Liver metastasis: (Tables 17 and 18)

In generation "J" the various lines of birds can be ranked as follows in order of their susceptibility to liver involvement: 7, 15, 10, 11, 9, 14, and 6. The lines fell into three groups. The most susceptible group was composed of lines 7 and 15, which had approximately the same percentage of involvement (96 percent). The second susceptible group included lines 10, 11, and 9 with approximately 70 percent involvement. The third group contained resistant line 14 with approximately 57 percent and line 6 with approximately 23 percent. In general, type "B"liver involvement was found in those birds that tended toward resistance to the tumor.

In generation "K" lines 7 and 15 were again the most susceptible lines having an incidence of liver involvement of 99.4 percent and 96.5 percent. Line 9 had the same incidence in both generations. Lines 11 and 14 showed less involvement of the liver in generation "K" than in generation "J". Line 4 was represented in the "K" generation only and line 10 was absent from this generation. The liver involvement in line 6 was slightly but not significantly higher (28 percent) than in generation "J". Thus, in the "K" generation the lines ranked for liver involvement as follows: 7, 15, 9, 14, 11, 6, and 4.

Metastatic incidence of the internal organs: The percentage of birds in various lines that had tumor involvement of the kidney, spleen heart, pancreas, proventriculus, gonads, and peritoneum is listed in table 19 for generation "J" and table 20 for generation "K".

TABLE 17

Types of Liver Involvement in Generation "J"

Line	Type	Percentage of Birds Living 28 Days	Percentage of Birds Living Less than 28 Days	Total Percentage
6	O	99.0	5.7	76.5
	A	0.9	88.5	22.0
	B	-	5.7	1.4
7	O	100.0	-	2.6
	A	-	97.2	94.7
	B	-	2.7	2.6
9	O	100.0	4.8	30.4
	A	-	90.3	66.0
	B	-	4.8	3.5
10	O	94.5	2.1	28.4
	A	5.4	95.7	70.0
	B	-	2.1	1.5
11	O	87.5	2.4	30.5
	A	12.5	93.8	66.9
	B	-	3.7	2.5
14	O	96.7	-	42.2
	A	3.2	100.0	57.7
	B	-	-	-
15	O	100.0	-	3.9
	A	-	98.8	94.9
	B	-	1.1	1.1

O = No reaction

TABLE 18

Types of Liver Involvement in Generation "K"

Line	Type	Percentage of Birds Living 28 Days	Percentage of Birds Living Less than 28 Days	Total Percentage
6	O	99.1	4.3	71.9
	A	0.8	86.2	25.3
	B	-	9.5	2.7
7	O	100.0	-	0.5
	A	-	97.8	97.3
	B	-	2.1	2.1
9	O	100.0	0.7	31.7
	A	-	96.8	66.6
	B	-	2.4	1.6
4	O	97.1	-	74.8
	A	1.9	90.6	22.3
	B	0.9	9.3	2.8
11	O	92.3	-	56.2
	A	7.6	92.0	40.6
	B	-	8.0	3.1
14	O	96.9	-	51.6
	A	3.0	100.0	48.3
	B	-	-	-
15	O	100.0	-	3.4
	A	-	100.0	96.5
	B	-	-	-

O = No reaction

In general in generations "J" and "K" there was close agreement on the percentage of birds within the various lines which had involvement of the kidney, spleen, and heart. Likewise, these percentages were in close agreement with the percentages that were observed for liver involvement.

It was observed that the pancreas, proventriculus, gonads, and peritoneum were less frequently involved than the other organs and the percentage of birds that had tumors of these organs was extremely variable and no relation to susceptibility or resistance could be detected.

These organs (pancreas, proventriculus, and gonads) were usually more severely involved in the birds that succumbed to the tumor but lived longer before dying.

In general the percentage of birds that showed involvement of these organs was higher in generation "J" than in generation "K".

DISCUSSION

In these experiments, the results of intramuscular transplantation with non-filtered tumor strain RPL 16, indicate a similarity in the reaction of the various lines to that of the naturally occurring disease. The data in table 3 on the response of the chickens in the several lines to tumor transplants of RPL 16 show that there is a great difference in resistance between lines. The lines which had been selected under natural exposure for resistance or susceptibility to lymphomatosis, in general, followed the same trend in regard to resistance or susceptibility to the experimentally induced tumors. Line 6 which was relatively resistant to the natural infection was likewise quite resistant to inoculation with tumor transplants. However, line 10 which had shown considerable resistance to naturally occurring lymphomatosis, was quite susceptible to tumor transplants.

Lines 7, 9, and 15 were the most susceptible lines. Data collected on natural infection over a period of 600 days (table 21) for sibs of the inoculated birds in generation "J" indicated that these lines reacted similarly to both types of infection. The same comparison for sibs in generation "K" was not available at 600 days of age. The sibs of birds selected for susceptibility to the natural disease fell in the same category when inoculated with the transplant. However, the lines did not follow exactly the same order in the two cases.

With the exception of line 6, the death rate of the inoculated birds was about twice as high as the death rate of their respective sibs exposed to the naturally-occurring disease. It has been reported by Johnson (1937), Jungherr (1937, Lee and Wilcke (1941) and DeOme (1943)

TABLE 19

Percentage of Lesions in Birds for Generation "J"

Line	Kidney		Spleen		Heart		Pancreas		Prov.		Gonad.		Perit.		Total
	0	R	0	R	0	R	0	R	0	R	0	R	0	R	No.
6	222	64	224	62	223	63	261	25	246	40	274	12	284	2	286
	77.6	22.3	78.3	21.6	77.9	22.0	91.2	8.7	86.0	13.9	95.8	4.1	99.3	.6	
7	1	37	1	37	3	35	20	18	9	29	28	10	36	2	38
	2.6	97.3	2.6	97.3	7.8	92.1	52.6	47.3	23.6	76.3	73.6	26.3	94.7	5.2	
9	19	37	21	35	19	37	31	25	27	29	51	5	55	1	56
	33.9	66.0	37.5	62.5	33.9	66.0	55.3	44.6	48.2	51.7	91.0	8.9	98.2	1.7	
10	39	91	38	92	40	90	75	55	77	53	110	20	124	6	130
	30.0	70.0	29.2	70.7	30.7	69.2	57.6	42.3	59.2	40.7	84.6	15.3	95.3	4.6	
11	38.	83	41	80	38	83	59	62	54	67	86	35	119	2	121
	31.4	68.5	33.8	66.1	31.4	68.5	48.7	51.2	44.6	55.3	71.0	28.9	98.3	.6	
14	31	40	32	39	32	39	57	14	56	15	70	1	70	1	71
	43.6	56.3	45.0	54.9	45.0	54.9	80.2	19.7	78.8	21.1	98.5	1.4	98.5	1.4	
15	9	170	13	166	17	162	147	32	118	61	175	4	179	0	179
	5.0	94.9	7.2	92.7	9.4	90.5	82.1	17.8	65.9	34.0	97.7	2.2	100	0	

0 - No visible tumor

R - Visible tumor

In each line first set of figures indicates number of birds and second set of figures indicates percentage of birds.

TABLE 20

Percentage of Lesions in Birds for Generation "K"

Line	Kidney		Spleen		Heart		Panc.		Prov.		Gonad		Perit.		Total
	0	R	0	R	0	R	0	R	0	R	0	R	0	R	No.
4	107 76.9	32 23.0	106 67.2	33 23.7	106 67.2	33 23.7	129 92.8	10 7.1	122 87.7	17 12.2	138 99.2	1 0.8	139 1.0	0 0	139
6	241 73.4	87 26.5	243 74.0	85 25.9	242 73.7	86 26.2	314 95.7	14 4.2	306 93.2	22 2.7	321 97.8	7 2.1	327 99.6	1 0.4	328
7	2 1.0	188 98.9	2 1.0	188 98.9	2 1.0	188 98.9	178 93.6	12 6.3	136 71.5	54 28.4	178 93.6	12 6.3	190 1.0	0 100	190
9	62 33.3	124 66.6	66 35.4	120 64.5	63 33.8	123 66.1	169 90.8	17 9.1	146 78.4	40 21.5	177 95.2	9 4.7	186 100.0	0 0	186
11	38 59.3	26 40.6	39 60.9	25 39.0	37 57.8	27 42.1	46 71.8	18 28.1	41 64.0	23 35.9	52 81.2	12 18.7	63 98.4	1 1.5	64
14	63 51.6	59 48.3	63 51.6	59 48.3	62 50.8	60 49.1	110 90.1	12 9.8	106 86.8	16 13.1	120 98.3	2 1.6	122 100.0	0 0	122
15	5 4.2	112 95.7	5 4.2	112 95.7	12 10.2	105 89.7	114 97.4	3 2.5	97 82.9	20 17.0	117 100.0	0.0 0.0	117 100.0	0 0	117

0 - No visible tumor

R - Visible tumor

In each line first set of figures indicates number of birds and second set of figures indicates percentage of birds.

TABLE 21

Comparison of Percentage Mortality for Naturally Occurring and Transplanted Lymphomatosis.

Naturally Occurring Lymphomatosis	Generations		Transplanted Tumor Generations	
Lines	"I"	"J"	"J"	"K"
6	9.3	17.8	24.1	29.8
10	24.7	12.1	71.5	-
11	19.8	-	66.9	39.0
14	24.4	20.9	56.3	46.7
4	26.6	-	-	23.0
7	42.9	33.1	97.4	99.5
9	43.5	40.0	73.2	68.8
15	56.1	51.7	96.6	96.5

that the incidence of tumor following inoculation was more than in the case of natural infection. It will be observed in table 22 that from year to year considerable variation in mortality from naturally occurring lymphomatosis was present in all lines. In general there was less fluctuation in both generations "J" and "K" for the same lines when inoculated with tumor transplants.

The difference in the disease incidence from year to year under the natural conditions may in part be attributed to the difference in the infective dose within and between years.

Apparent differences between the lines suggest a degree of genetic identity within each line. The total and daily death rate differed for each line. The susceptible lines were characterized by a short incubation period. Less susceptible lines showed a more uniform distribution of mortality over the entire period.

Although the "J" and "K" generations are not identical, the comparison of the percentage of survivors in similar lines that had common families within the two generations showed that there was no significant difference between the two generations in regard to line and sex. However, when all the birds in a given family within the two generations were grouped together and compared to other families within their respective lines, differences were found between families in some lines. This may be expected as inbred lines have not as yet reached the degree of homogeneity where all the families within the line react identically.

The main difference between the "J" and "K" generations was evident when the average length of survival for each line was compared for the two generations. The "t" tests showed that there was a significant difference between sexes when the two generations were compared, using the average survival time as the basis for comparison. This seems to be the main factor which brought out the significant difference between the two generations when sexes were grouped together. The analysis of variance (table 9) for generation "J" showed a highly significant difference between the sexes and between the lines for average survival time. In generation "K" (table 10) there was a highly significant difference between the lines. There was also a significant difference in the interaction which means sex differences were not the same for all the lines. The analysis for generation "J" did not show a significant difference in the interaction between sex and lines. A definite conclusion cannot be reached to account for this significant difference in the interaction in "K" generation.

The results of the data collected in the gross pathological reaction of the breast showed that the transplant had grown in the breast of all the birds except three. Some of the birds recovered while others died after variable incubation periods. The reactions of the hosts bearing these lymphoid tumors seem to fall into a certain pattern which can be interpreted as a result of the interplay between the activity of the implant and the resistance of the host. Thus, the respective responses of no growth, degree of growth, regression, and degree of metastasis represent either the resistance in the host, activity of the tumor transplant, or combination of both. The difference in the percentage of mortality between birds exposed to the naturally occurring disease and those given the tumor transplant probably is in the standardization of the dose intake under the experimental conditions which under natural conditions is practically uncontrolled. In these experiments the infective dose was relatively stable, but the infective units contained in the inoculumwere not known.

It took the transplant some time to grow to a size that could be detected. A period of six days was not enough for the tumor to be detected in most of the birds. The total percentages of the birds that did not show tumor growth on the sixth day after transplantation in generation "J" were 70.1, 92.1, 64.2, 66.1, 80.9, 63.3 and 80.4 in lines 6, 7, 9, 10, 11, 14, and 15 respectively. The same percentages in generation "K" were 98.5, 89.6, 90.5, 88.2, 95.3, 90.9, and 99.2 in lines 4, 6, 7, 9, 11, 14, and 15 respectively.

The tenth day proved to be the optimum day for tumor observation because more than 70 percent of the birds in generations "J" and "K" showed tumor growth at this time. The amount of tumor growth in the breast showed a wide variation between different lines on the same day. The percentage of reactors in each class of tumor growth was not indicative of susceptibility or resistance. A susceptible and a resistant individual did in some cases show the same degree of tumor reaction on the same day.

In general, the birds that lived the longest, either resistant or susceptible, had the largest tumors and considerable necrotic material was frequently present. For the most part when the tumors reached maximum size at about 17 to 21 days there was either a gradual regression of the tumors or the birds having such enlarged tumors died. The most susceptible chickens, notably line 15, which died soon after inoculation, showed only a moderate growth of tumor and had little or no necrotic material. In line 7, similarly to line 15, mortality was very high except that there was a greater variation in age at death.

In general the internal metastatic reaction was parallel to the breast reaction. A general hyperemia of the internal organs was present in birds that died before the second week after inoculation. This was followed by the formation of a definite tumor tissue. The lymphoid tissue showed a tendency to coalesce as the bird lived longer. This may have been the result of the natural development of tumor tissue or a criterion of the defensive power of the body. The internal organs that showed the most metastatic reaction were liver, kidney, spleen, heart, proventriculus, pancreas, and gonads. Data on lesion incidence of the tumor in other organs were not collected, but the adrenals were involved in some cases.

These experimental results should be encouraging to the breeders interested in reducing the incidence of this disease by means of selection and possibly line breeding. The fact that there is a parallelism between susceptibility or resistance under the natural disease occurrence and induced disease may make the long task of selection for resistance to lymphomatosis easier.

A reasonable plan of selection and breeding within flocks subjected to the disease, may result in relatively resistant flocks. This may be the best answer on hand to reduce the losses from the disease until definite prophylactic and medical measures are provided.

SUMMARY

1. Inbred White Leghorn chickens resistant to and susceptible to naturally occurring lymphomatosis were inoculated intramuscularly with material from tumor strain RPL 16.

2. A total of 2027 chickens belonging to two different generations, "J" and "K", was used in these studies. In generation "J" random samples of chickens from inbred lines 6, 7, 9, 10, 11, 14, and 15 were used. In generation "K" chickens from lines 4, 6, 7, 9, 10, 14, and 15 were involved.

3. Standard inoculum of 0.1 cc of non-filtered tumor cell suspension from strain RPL 16 was transplanted in the breast muscle of 21-day old chickens.

4. Observations for tumor reaction were made on each bird daily through the 28-day experimental period following inoculation.

5. Percentage of survival and average age at survival following inoculation with tumor transplants were the criteria used to measure the resistance and susceptibility of the chickens in the various lines.

6. Under the naturally occurring disease lines 6 and 10 were selected for resistance and lines 7, 9, 11, 14, and 15 were selected for susceptibility.

7. Under the condition of these experiments line 10 was susceptible to tumor transplants. The rest of the lines in both generations were similar in their reaction to tumor transplants and naturally occurring lymphomatosis.

8. Death rate of the inoculated birds was about twice as high as the death rate of their respective sibs exposed to the naturally occurring disease with the exception of line 6.

9. In general there was no significant difference between the two generations when the percentage of survivors was used as a measure. There was a significant difference between the two generations when the average survival time was used as the criterion.

10. Using lines that had families common to both generations a "t" test showed that there was no significant difference between the generations, between the two sexes, between females in the two generations, and between males in the two generations.

11. Sexes and generations within families in lines 6, 7, 9, 11, 14, and 15 were combined. A chi-square test showed that there were significant differences in mortality between families in lines 6, 9, 11, 14, and 15.

12. Using the average day of survival, a "t" test showed that there was a significant difference between sexes in both generations.

13. The method of expected subclass numbers was used for analysis of the data. The average survival time in days was used as the criterion for comparison. In generation "J" there was a significant difference between sex means and line means. In generation "K" there was a significant difference between line means and in the sex-line interaction.

14. Breast reactions occurred in 2024 birds out of a total of 2027.

15. Size of tumor growth in the breast or degree of metastasis in each of liver, kidney, spleen, heart, proventriculus, pancreas, and gonads, did not indicate resistance or susceptibility of the individual bird.

16. The liver, kidney, spleen, heart, proventriculus, pancreas, and gonads were the organs that showed most of the internal metastasis.

17. The liver showed two types of involvement, a diffuse type, and a combination of both diffuse and focal tumors. The percentage of birds that showed the diffuse type was very high.

18. The severity of metastasis was in general parallel with breast reaction.

19. The results of these experiments tend to indicate that it may be possible to hasten the selection of inbred birds toward resistance by using results of inoculation of sibs with tumor cells as a criterion for selection.

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