Genetic Control of Lymphomatosis in the Fowl

F. B. Hutt and R. K. Cole

Department of Poultry Husbandry, Cornell University

AVIAN LYMPHOMATOSIS IS A DISEASE characterized by the accumulation of undifferentiated lymphocytes in the nerves, in the viscera, or in the iris. If the resultant tumors are in the sciatic or brachial nerves, the leg or wing is paralyzed, and the bird is incapacitated even though otherwise in good health. In many cases nerves of the autonomic system are affected. Involvement of the ovary, liver, spleen, and kidney is common.

The disease is known—in its various forms, and by different people—as lymphomatosis, neurolymphomatosis, leukemia, the avian leucosis complex, iritis, fowl paralysis, range paralysis (because it often afflicts young birds on the rearing ranges), and big-liver disease. In Europe, it is sometimes called Marek's disease after the Hungarian pathologist who first described it in 1907. It is prevalent in many parts of the world.

Because under certain conditions lymphomatosis can be transmitted from bird to bird by inoculation with a cell-free filtrate from diseased fowls, it is generally considered to be caused by a virus. It is also spread by contact among young chickens.

The disease usually does not cause death before 6 weeks of age, but most of the susceptible birds die within 18 months of hatching. In the United States it is responsible (*4*, *17*) for about 40 per cent of the high mortality now prevailing in growing stock and during the first year of life. Since the first detailed study of the disease in this country by Pappenheimer, Dunn, and Cone (*13*), many investigators have sought to discover its cause, the paths of infection, the basis for the diverse manifestations, and possible methods of control. That progress in these directions has been slow is disappointing to the poultry industry, but not surprising to anyone familiar with the difficulties besetting any study of neoplasia.

A special laboratory for research on avian lymphomatosis was established by the U. S. Department of Agriculture in 1939 at East Lansing, Michigan. The director of that laboratory, Berley Winton, has estimated (*17*) that the disease causes in this country alone an annual loss exceeding $50,000,000.

That the disease may be effectively controlled by raising the chicks from hatching to about 5 months of age in complete isolation from older birds was proved experimentally by Kennard and Chamberlin (*10*) and by Johnston and Wilson (*9*). After about 5 months of age, exposure to the disease seems not to matter. However, it is not possible for many poultrymen to provide separate attendants and equipment for their growing stock; hence, complete isolation is somewhat impracticable. Losses from lymphomatosis can still be reduced with lesser and more feasible degrees of isolation, particularly if the chicks be kept at some distance from older stock during the first few weeks after hatching, which is apparently the critical period (*6*).

The Experiment

Object. Beginning with an unselected population hatched in 1935, an experiment has been carried out at Cornell University to determine the feasibility of breeding strains of fowls resistant to lymphomatosis. It was well known then that families differed in susceptibility (*1*). The question was whether or not, by selection, genetic resistance could be established at a level high enough to constitute control of the disease in large populations that are thoroughly exposed, *i.e.* in commercial poultry flocks. Furthermore, resistant strains would be of little economic value unless superior (or satisfactory, at least) in body size, in egg production, and in egg size. It was desirable, therefore, to determine whether or not increased resistance to lymphomatosis could be attained without sacrificing these other indispensable desiderata.

Procedure. Reduced to its simplest terms, the procedure for 12 years has been (a) to have all chicks pedigreed, so that the sire and dam are known for each, (b) to ensure a natural exposure to lymphomatosis, (c) to maintain, as nearly as possible, uniform environment for all birds at every age from the egg on, and (d) to select for breeding the sires and dams whose production of the most resistant families has been proved, together with promising untested birds from those same families.

Some idea of the scope of the experiment is given by the fact that, in all selected generations after the fourth (which was hatched in 1939), from 2,100 to 2,600 females of the White Leghorn breed have been started at 6 weeks of age in tests of their viability and have been maintained under test to the age of 500 days. Complete records have been kept of their ages at commencement of laying, of their egg production, size of egg, and body size.

Every bird that died was autopsied to ascertain the cause of death. The usual thorough macroscopic examination was supplemented, when necessary, with bacteriological study, or with histological examination to determine the type of tumor involved. Most of these diagnoses were made by one of us (R.K.C.), except for a period of 46 months when he was absent in military service. During that time the examinations were made by R. F. Ball, whose assistance is gratefully acknowledged.

An essential part of the program was that no birds were culled from the flock because of small size, low egg production, or ill health. Some whole families were eliminated when the numbers of female chicks therein were too small to permit evaluation of the family's genetic potentialities, but this was done early, before the test had progressed very far. Once started, all birds were given an equal chance to demonstrate their ability to survive to 500 days of age. This does not mean that every hen still able to stagger about at that age was counted as a survivor. On the contrary, all birds then obviously sick were killed and examined, and their records were included with those of birds deceased earlier.

Full details of the procedure and management have been given in interim reports (*5–8*). Anyone comparing the data in these reports with figures in the present paper should remember that, whereas the former analyses considered a test period beginning at 160 days of age, records for the early years have now been extended to include the period from 6 weeks to 500 days.

Strains involved. The flock of White Leghorns with which the experiment was begun had been bred at Cornell University for many years for increased egg production, but no selection had been made for resistance to disease, except in so far as the least fit had eliminated themselves from the breeding pens by premature death. From this stock, three differing strains have been developed. One of these, the C Resistant line, has had no new blood introduced. Another, the K Resistant line, comes from an outcross of the original Leghorns to an entirely unrelated line in 1936. The third strain, the Susceptible line, has come from the original stock but has been bred for susceptibility to lymphomatosis.

Exposure. The birds under test have been exposed naturally, not inoculated with pathogenic material. When the experiment was started, little was known of the transmission of the disease or of the channels of infection. However, from its prevalence in this flock and in others, it was assumed that natural exposure adequate for differentiation of resistant and susceptible families (and hence for selection) was inevitable. It seemed preferable to breed for resistance to that type of infection rather than to some aberrant pathological condition that might result from artificial inoculation. This decision was fortunate, for it has subsequently been shown that genetic resistance to natural infection is not revealed by subcutaneous inoculation (*3*) or by inoculation in some manner not specified (*18*), although oral inoculation does permit differentiation of genetically resistant and susceptible stock (*3*).

To ensure adequate natural exposure, sanitation of the kind so generally advocated was practically dispensed with. Brooder houses, rearing quarters, and laying pens were cleaned when necessary but were not disinfected. Trucks, equipment, feed, and attendants moved freely from buildings housing adult birds to the rearing range. Chicks were managed during the critical first two weeks after hatching by an attendant who also cared for adult birds and whose work was so arranged that he walked many times daily from hen-pens to chick-pens.

Proof that natural exposure was ample in this experiment was provided by the high incidence of lymphomatosis among birds of the Susceptible strain. Since these were hatched along with those of the C and K Resistant strains, and mixed with them right from the incubators onward, it is obvious that all must have been equally exposed. In similar selection experiments conducted by others, failure to maintain such a susceptible strain has led to the erroneous belief that strains resistant to lymphomatosis had been quickly established when (as the experience in later years showed) they had merely been temporarily relieved from severe exposure.

Results

(A) *Neoplasms.* As is shown in Fig. 1, the selection practiced was effective in differentiating two lines highly

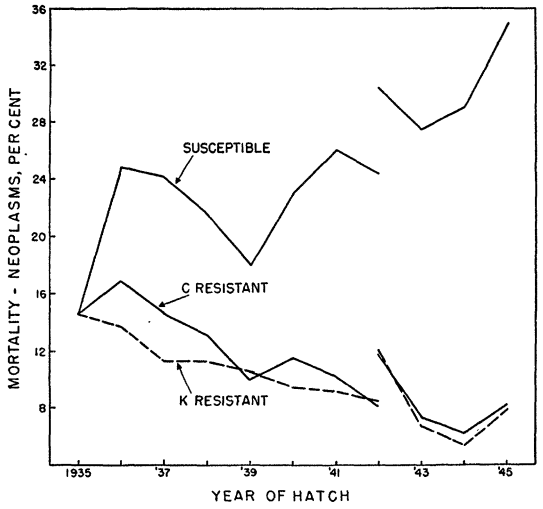

FIG. 1. Reduction in deaths from neoplasms in the C and K Resistant lines and increase in the Susceptible line during 10 generations of selection. The graphs are smoothed by using a 3-year moving average (except for terminal points). The break in 1942 is explained in the text.

resistant to neoplasms and one that was very susceptible. Of the neoplasms, 90–95 per cent were cases of lymphomatosis, but, because there was at one time disagreement among pathologists concerning the different manifestations of this disease, the all-inclusive term, neoplasms, was used from the very beginning of the experiment.

In the unselected population of 1935, 14.6 per cent died of neoplasms. In the 9th and 10th selected generations, hatched in 1944 and 1945, deaths from neoplasms in the K Resistant line were 5.4 and 7.9 per cent, respectively, and in the C Resistant line they were 6.3 and 8.3 per cent.

The reduction of neoplasms in these two lines is much more significant than the figures indicate. In 1942 it was discovered that one of the two brooder houses used was providing a much more severe exposure than the other,

possibly because of its closer proximity to adult fowls. Even though chicks were brooded in that house for only their first two weeks, they suffered over twice as many deaths from neoplasms after 5 months of age as did the chicks started in the other house (*6*). This effect is clearly shown in Fig. 1, in the data for 1942. In all three strains, the lower points for that year show, as for previous years, the deaths from neoplasms in all birds. Half of these were lightly exposed and half severely exposed. The upper points give the corresponding figures for the severely-exposed birds only. Since, in the two resistant lines, these latter figures are about 50 per cent higher than the average, it is evident that the other half of the birds was exposed rather lightly. This condition had prevailed, unknown, from the start of the experiment in 1935. After its discovery, all the chicks were started in the brooder house providing the severe exposure. That deaths from neoplasms in the two resistant lines were only 5–8 per cent in the 1944 and 1945 populations is more significant, therefore, than one might think at first glance.

Over the 10-year period of selection, deaths from neoplasms in the Susceptible line increased gradually. This is remarkable, because selection is less efficient in that line than in the others. Since the most susceptible birds die long before the normal breeding season, selection is limited to the use of the remaining birds (which are the more resistant ones) in the susceptible families. In spite of this, susceptibility was raised in the 10th generation to such a level that 34.9 per cent of the females in the Susceptible line died of neoplasms before 500 days of age. This figure was over four times those for the two resistant strains.

The foregoing data on mortality from neoplasms are based on the total populations in each line each year. Each of these groups can be divided into two classes: (a) daughters of proven sires, and (b) daughters of cockerels being tested for the first time. One might reasonably consider only the former class as the measure of the progress effected by selective breeding, as it is inevitable that some cockerels will fail to transmit in accord with the breeder's hopes and expectations. For this reason, the record for all daughters of proven sires is somewhat better than that for all daughters of cockerels and, hence, better than the averages for the strains shown in Fig. 1.

Considering now only the daughters of proven sires, selection has raised resistance to a level such that in the C and K lines deaths from neoplasms were only 2.0 and 4.4 per cent, respectively (during the 458-day test period), for the 9th generation and 6.6 and 7 per cent for the 10th generation. Exposure to the disease was severe enough to cause 34 and 38 per cent of the birds in the Susceptible line to die of neoplasms in those same years. These losses in the two resistant lines are so low as to be relatively unimportant.

(B) *Mortality from all causes.* The reduction of economic loss from mortality in the two genetically resistant strains is considerably greater than one would expect from the lowering of the death rate from neoplasms alone. In 1935, mortality from all causes during the test period was 66.8 per cent in the unselected population. In the 10th selected generation it had dropped to 22.4 per cent in the C Resistant line and 19.9 per cent in the K Resistant line (Fig. 2). These mortality rates mean a reduction of losses by about two-thirds.

Some of this reduction in mortality may properly be attributed to improvements in husbandry during the 10-year period, particularly to changes in management during the rearing period which reduced losses from coccidiosis and tapeworms. However, since total mortality in the Susceptible strain was still 53 per cent in the 10th generation, and as high as 68 per cent in the severely exposed pullets of the 7th generation (1942), it is clear that by far the greater part of the reduction of mortality in the resistant lines must be credited to breeding. Moreover, it is probable that potential deaths from lymphomatosis were actually higher in the earlier years of the

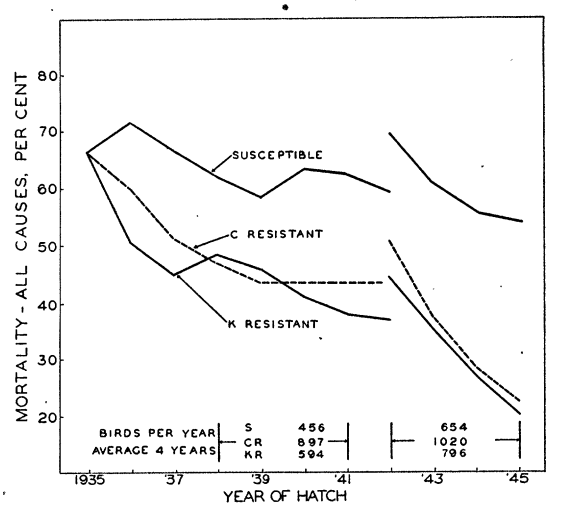

FIG. 2. Reduction in deaths from all causes in the resistant strains and maintenance of high mortality in the susceptible one. The lines show 3-year moving averages (except for terminal points).

experiment than the frequencies shown in Fig. 1. This is partly because many of the birds then died of intestinal parasites before an age at which they could show lymphomatosis. Moreover, some of the more obscure forms of the disease were probably not recognized in the earlier years.

It will be noted that between 1937 and 1942 mortality was not decreased much in the K strain and remained practically constant in the C strain. However, from 1942 onward it declined rapidly in both of these resistant lines. The reasons for this rather abrupt change in trend will be discussed later. The rise in mortality in all three lines in 1944 resulted from chronic respiratory disorders, which were unusually severe in that year.

(C) *Age at death.* Genetic differences in resistance to disease among these three strains are shown not only by the proportions that died during the test period but also

by the duration of life. Since most of the birds surviving to 500 days (all not retained for breeding) were then disposed of, a true figure for mean duration of life cannot be determined. However, among those of the 10th selected generation dying prior to 500 days of age, birds in the K and C Resistant strains lived 71 and 39 days longer, respectively, than those that died in the Susceptible strain. For these last the mean age at death was 233 days.

(D) *Differences among families.* Since the original demonstration by Asmundson and Biely (*1*) that sires differ in the degree of susceptibility to lymphomatosis transmitted to their daughters, similar differences have been commented upon by others. Some striking cases were cited in an earlier report (*7*). Without going into voluminous details, some idea of the genetic variability in this respect (which is the basis for selection) is given by the data in Table 1. This shows the distribution of 86 cockerels of the 8th, 9th, and 10th generations according to the mortality in their daughters from neoplasms and from all causes. (Lymphomatosis accounted for about 90 per cent of the neoplasms.) Only sires that had at least 30 daughters under test are included; three-quarters of them had over 40, and almost half had over 50.

TABLE 1

DISTRIBUTIONS OF 86 COCKERELS TESTED IN 1943, 1944, AND 1945 ACCORDING TO MORTALITY IN THEIR DAUGHTERS (a) FROM NEOPLASMS, AND (b) FROM ALL CAUSES, BETWEEN 42 AND 500 DAYS OF AGE

(a) Mortality from neoplasms among daughters (%)	Sires in: C and K Resistant lines (No.)	Sires in: Susceptible line (No.)	(b) Mortality from all causes among daughters (%)	Sires in: C and K Resistant lines (No.)	Sires in: Susceptible line (No.)
0	5				
0.1–3	12		5 –10	1	
3.1–6	13		10.1–15	6	
6.1–9	19	1	15.1–20	8	
9.1–12	9		20.1–25	13	
12.1–15	3	1	25.1–30	11	1
15.1–18	2	2	30.1–35	8	1
18.1–21	1	3	35.1–40	7	1
21.1–24	1	2	40.1–45	8	2
24.1–27		1	45.1–50	2	6
27.1–30		3	50.1–55		4
30.1–33		2	55.1–60	1	1
33.1–36		2	60.1–65		
36.1–39		1	65.1–70		2
39.1–42		1	70.1–75		3
42.1–45		2			
Total	65	21		65	21

Among the 65 sires of the Resistant lines, there were 5 that lost no daughters at all from neoplasms, and this in families of which the average size was 52. At the same time, 2 males of the Susceptible line each had 44 per cent of their daughters die of neoplasms. Three of the 21 cockerels in that strain lost over 70 per cent of their daughters from all causes before 500 days of age. These cases from opposite extremes, and the range shown in Table 1, give some idea of the variation available for selection and also of the differentiation between resistant and susceptible lines brought about by that process.

When a sire's genotype has once been evaluated by an adequate progeny test, fairly consistent performance of his offspring by comparable females can be expected thereafter. This is illustrated by the records for 2 males, both of which were used for 3 consecutive years. Though mated with different females each year, deaths of daughters from neoplasms in those years were 4.3, 1.5, and 0 per cent for one sire, but 33, 47, and 41 per cent for the other. While the difference must be attributed in part to the fact that the one was mated with hens of a resistant strain and the other with hens of the Susceptible one, there can be no doubt of the consistent influence of these 2 sires on the viability of their progeny.

TABLE 2

COMPARISONS OF BODY WEIGHT, EGG WEIGHT, AND EGG PRODUCTION IN THE ORIGINAL POPULATION WITH THAT IN THE 10TH SELECTED GENERATION

Population	Adult body weight (grams)	Adult egg size (grams)	Mean eggs/bird to 500 days of age: Per hen finishing (No.)	Mean eggs/bird to 500 days of age: Per hen starting (No.)
Original unselected (1935)	1,690	54.1	177	90
10th generation (1945)				
C Resistant strain	2,027	59.7	207	186
K Resistant strain	2,102	60.0	186	169
Susceptible strain	1,935	58.5	161	117

(E) *Economic characters.* To satisfy the poultryman, hens must be able not only to withstand disease but also to lay many eggs—eggs that weigh 2 ounces each or more—and to turn in at the end of the productive period a carcass large enough to be desirable for meat. While genetic resistance to lymphomatosis was being established, the two resistant strains were also steadily improved in egg production, size of egg, and body weight.

The full extent of that improvement is best shown by contrasting the performance of the 10th selected generation with that of the original population from which the resistant and susceptible lines were developed (Table 2). The original stock, though unselected with respect to viability, had been bred for high egg production for over 20 years prior to 1935 and was then considered a superior strain.

In spite of that, mean egg production in the C and K Resistant lines to 500 days for hens living to that age was increased in the decade covered in this report by 17 and 5 per cent, respectively. (The averages of 207 for one line and 186 for the other must not be belittled by comparing them with figures often cited by others for average egg production during the *first laying year*, a period that is about 55 days longer than the one used in this study.) However, the mean egg production of birds still alive at 500 days of age can be a misleading measure of value, as

it does not consider what proportion of the pullets that started were able to finish. In 1935, that proportion was only 33 per cent of the females alive at 6 weeks of age, but in 1945 it was 78 per cent in one line and 80 per cent in the other.

The better measure, therefore, is the mean egg production of the pullets that were housed in winter quarters at about 160 days (thus disregarding those that died before reaching the age of laying). This figure, sometimes called a "production index," is given in the extreme right column of Table 2 for each group of birds. The indices for the C and K Resistant lines are, respectively, 2.1 and 1.9 times that for the original population. On this basis, the productivity of these strains has been doubled during the decade of selection. In 1942, mean egg production of survivors in the C and K strains was from 10 to 50 eggs per bird higher than for pullets brought in as chicks from four leading breeders of White Leghorns and reared for these comparisons on the local premises (*8*).

Adult body weight and egg weight were recorded annually in March when the birds were about one year old. The former was increased in the decade by 20 and 24 per cent. Egg weight was raised by the 10 or 11 per cent necessary to bring it to the desired level of 58–60 grams, beyond which further increase was unwarranted.

In the Susceptible strain, body size and egg size were improved somewhat, but egg production declined. This was chiefly because high mortality left comparatively few birds for selection as breeders, but also because incipient disease in survivors still alive at 500 days of age lowered the mean production of that group.

General Considerations

In view of (a) the reduction of deaths from lymphomatosis and other neoplasms to 2–7 per cent in the offspring of proven sires and (b) the reduction of mortality from all causes by two-thirds, as well as (c) the improvement in economic characters considered in the previous paragraphs, it would seem that to the original problem one may now write *quod erat demonstrandum*. Two strains highly resistant to lymphomatosis have been developed. Both are more than satisfactory with respect to egg production, egg size, and body size.

Some say that it has taken too long to accomplish these objectives. The answer is that with the experience of the past decade the same result could now be achieved in less than 10 years. Limiting factors were (a) light exposure of half the chicks up to 1943, which lessened the effectiveness of selection in the first 6 generations, and (b) the difficulty of finding proven sires good enough to use for more than one year. These two limitations retarded progress in the K Resistant strain and completely prevented it in the C Resistant line from 1939 to 1943 (Fig. 2). After they were removed, mortality was quickly lowered in three years.

Uniformly severe exposure was ensured by starting all chicks hatched after 1942 in the brooder house that provided the more severe exposure.

The other limitation—too few proven sires—resulted from the fact that several objectives had to be considered in the selection of breeders. With a single measure of desirability, such as low mortality, half the cockerels tested will prove superior to the average. With any three additional objectives, *e.g.* egg production, body size, and efficient reproduction, half the males are again superior in each, but only about 1 in 16 is superior in all four desiderata. Study of this problem revealed that adequate tests for viability of progeny could be made with families of 30–50 daughters (*12*), these numbers being much less than had previously been considered desirable. This meant that enough daughters for a test could usually be hatched from three weeks' eggs from each breeding pen. Whereas previously each cockerel had sired chicks for the whole hatching season of 10 weeks, a "second shift" and even a "third shift" of cockerels are now used. As a result, 10 pens available for such tests now provide tests of 30 cockerels instead of 10, thus tripling the chance of having superior proven sires to use for two or three years. This has been a big factor in the rapid improvement shown from 1943 to 1945.

Other friendly critics suggest that the C and K Resistant lines differ from the Susceptible one, not in genes, but merely in lacking the causative virus. This, they suggest, is present in the Susceptible line and is regularly transmitted through the egg to each successive generation of that strain. The fact that the virus causing mammary carcinoma in mice can be transmitted in the milk of nursing mothers, or foster mothers, makes plausible the hypothesis of cytoplasmic transmission of the agent, or agents, responsible for lymphomatosis in the fowl. However, such a channel of infection has not yet been demonstrated with respect to avian lymphomatosis. To test the validity of this theory, reciprocal crosses have been made between the C and K Resistant lines and the Susceptible one. The results of these, which will be reported in detail elsewhere, provide no support for the theory, although they do not necessarily disapprove it. They show that the fate of the chick depends as much upon the genes received from the sire as on genes and any cytoplasmic agent transmitted by the dam.

In similar studies, Taylor, *et al.* (*15*) found it easier to lower resistance by selection than to raise it. Some progress was apparently made toward establishing resistance in the first three generations, but this was later lost. From their data it seems possible that insufficient numbers of proven sires may have been a limiting factor, as it was in the Cornell experiments until the "double shifts" and "triple shifts" were introduced. At the Regional Poultry Laboratory, East Lansing, deaths in the 6th selected generation from lymphomatosis alone were already up to 11.4 per cent in their resistant stock by 300 days of age and

about twice as high in the susceptible lines (*19*). However, since investigators there are attempting to combine breeding for resistance to lymphomatosis with a program of close inbreeding, it is not surprising that progress has been slow.

Utilization of Resistant Stock

The object of this experiment was to determine the feasibility of controlling genetically the most serious disease besetting the poultry industry and of improving productivity at the same time. We feel that this has been done. The extent to which poultry breeders not supported by public funds would be justified in undertaking the same sort of work remains to be determined. The desirability of maintaining a susceptible line to ensure exposure may be a deterrent, as such a stock can hardly be profitable. At the same time, more and more breeders and hatcherymen are recognizing that stock sold from flocks not exposed to disease may be the cause of intense dissatisfaction when it is so exposed in the hands of customers. As one of them (*16*) expressed it: "I am convinced . . . that no breeder can afford to have a disease-free farm, or even a relatively disease-free farm." So far as lymphomatosis is concerned, the disease is enzoötic and ubiquitous. Exposure is almost inevitable except in the smaller and more isolated flocks. Experience may show that breeders can develop resistant stock by exposing only enough of their chicks each year to provide tests of sires, while keeping the major part of the birds isolated, in so far as this is possible, and hence comparatively free from disease so that their productivity will not be impaired.

Even without any deliberate exposure to disease, poultrymen can raise the viability of their stock by selective breeding. While this has been demonstrated by Sturkie (*14*), by Bostian and Dearstyne (*2*), and by others, one must be prepared to find such stock highly susceptible to lymphomatosis when conditions are right for its reappearance (*11*).

Another possibility is that resistant strains may be developed at the agricultural colleges and experiment stations and distributed as have been the rust-resistant varieties of wheat and disease-resistant strains of other plants. However, since fowls are dioecious organisms, unless the strains resistant to lymphomatosis are inbred to the stage of almost complete homozygosity (which is unlikely if they are to be economically profitable), continued selection would be necessary to maintain the original resistance.

It may prove more practicable to utilize the resistant strains by sending out their desirable genes in thousands of cockerels to head flocks that supply the hatcheries. Such tests as we have been able to make thus far indicate that cockerels of the resistant stock, when mated with birds unselected for resistance to lymphomatosis, induce a remarkable increase in viability and even in egg production. If these results are substantiated in further tests, it would seem desirable to continue selection, and to have "multipliers" produce sufficiently large numbers of the resistant birds to supply the hatcheries with thousands of males. Since about 90 per cent of the country's chicks are now produced by commercial hatcheries, such a scheme would provide the maximum possible utilization of the genetic resistance that has been established by breeding.

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