Use of Highly Inbred Chickens in Research

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SUMMARY

The Regional Poultry Research Laboratory's (RPRL) inbred lines, developed by an intensive inbreeding program, represent populations of chickens that have immense possibilities in research programs. Each line and subline has been selected for 32 generations to develop genetic uniformity. As anticipated, the various lines of inbred chickens have diverged to the extent that each now has mutually exclusive characteristics. Body weight, egg weight and quality, egg production, fertility, and hatchability are discussed in detail to illustrate the changes that resulted in the production and reproduction traits of the lines as the level of inbreeding increased. Results of studies on disease susceptibility and resistance, blood types, and histocompatibility demonstrate the value of inbred lines in research programs. Some speculation is made of the future value of inbred lines in research on such factors as quantitative and qualitative genetics, mechanisms of disease resistance, transplantation, biochemistry, and developmental genetics.

INTRODUCTION

Many early studies of the effects of inbreeding and of outbreeding in plant and animal populations have been plagued by lack of control, inaccurate observations of breeding populations, and misconceptions concerning the inheritance laws (*23, 32*).[1] Subsequent to these reports, however, systematic breeding programs have been organized or revised by breeders to remedy these deficiencies and to specifically determine the effects of different systems of breeding upon artificial and natural selection processes (*24*). Understandably, the concept of inbreeding has played a prominent role in many of the programs.

East and Jones (*23*) and Lerner (*32*) considered both inbreeding and outbreeding as requirements for improvement of existing stocks. In practice, outbred or random-bred populations were subjected to inbreeding followed by differential selection to develop a number of different inbred lines. Each line became fixed or homozygous at a series of loci and each was evaluated for the traits of interest. These lines were systematically crossed to obtain a relatively uncomplicated evaluation of their genetic worth. Desirable crosses were then utilized to improve the stock. In essence, inbreeding isolated specific genetic factors for study and evaluation, but crossbreeding produced a variety of character combinations to which selection could be applied for breed improvement (*23*).

Although outbreeding with inbreeding has its merit in many breeding programs, other approaches also have been used advantageously. In 1939, the Regional Poultry Research Laboratory (RPRL) at East Lansing, Mich., originated with a breeding policy of inbreeding only. This program existed largely because the researcher in animal diseases, faced with the problem of proper appraisal of host factors and the disease-inducing agent, required experimental material with a known genetic background (*3*). Hence a plan was established to develop lines of chickens that were highly uniform within the line, but that were widely different among lines in response to exposure to the diverse etiological agents of the avian leukosis complex.

This publication discusses in detail the highly inbred lines of chickens maintained at the labo-

[1] Italic numbers in parentheses refer to Literature Cited, p. 20.

ratory and the application of such lines to research programs. Specific emphasis will be given to (1) the origin and development of the inbred lines and (2) their past, present, and future utilization in disease research as well as other research programs.

ORIGIN AND DEVELOPMENT OF INBRED LINES OF CHICKENS

The breeder developing inbred lines of chickens should select foundation stock that excels in as many productive and reproductive traits as possible. Preferably, choice of initial breeding stock should be from a breeder who has been selecting for desirable traits within his own flock for many generations. Probably the greatest emphasis should be placed on selection for genes that influence hatchability followed by selection for genes influencing viability, egg weight, egg quality, egg production, sexual maturity, and body weight (*51*).

Most breeders contemplating inbreeding are aware of the more obvious detrimental effects upon production and reproduction traits (*34*). However, inbreeding promotes homozygosity of both desirable and undesirable traits at more or less the same rate, and inbreeding with selection should cause some lines to diverge in desired directions. Thus, successful development of inbred lines depends greatly on the ability of the breeder to start with desirable breeding stock, to propagate the desirable traits, and to select against undesirable traits.

General plan of development

During 1939 Dr. Nelson F. Waters and coworkers at the RPRL initiated studies designed to provide effective control methods for the avian leukosis complex (ALC) (*53*). The genetic approach to this problem called for the formation of families inherently resistant or susceptible to the complex. Though susceptible families would be of little economic value, their genetic importance would be extensive. Without such families, the mode of inheritance of resistance and the influence of the environment would be difficult to determine. In addition, susceptible but disease-free stocks were necessary for pathological studies.

Sources of stock to initiate the experimental lines were carefully selected. A survey of breeding stock available in the United States indicated that it was possible to obtain, from widely separated geographical regions, many strains of Single-Comb White Leghorns whose viability (particularly to the ALC), production, and reproduction records were in part known.

In the spring of 1939, approximately 1,000 hatching eggs were obtained from each of 10 different White Leghorn flocks. All resulting chicks were of known ancestry except those of one flock, which were discarded. Upon hatching, the chicks were divided into two groups. One group, confined to strictly quarantined houses, represented the base or foundation population. The other group was exposed to suspensions of ALC tumor material and was placed in similar houses in a nonquarantined environment. The inoculated birds were to provide an estimate of the resistance or susceptibility of their unexposed sibs.

In the fall of 1939, the entire flock was closed to all outside breeding, and an inbreeding program was designed to segregate, as quickly as possible, those birds with the most or the least desirable characters. It was also thought to be somewhat of a risk to follow, at the start of the experiment, any one system of mating to the exclusion of all others. Therefore, careful selection accompanied by *inter*strain and *intra*strain crosses, together with line breeding, mild inbreeding, and occasionally intense inbreeding, was chosen as the initial approach. Later, progress of each system was used to choose the most promising. Thus, for the initial formation of inbred Lines 1 to 15, except Line 10, sires were selected from given strains and were mated with dams from one or more strains (table 1), thereby creating strain crosses for the progeny in the 1940 generation. Line 10 traces its ancestry to a single male and to a single female of the same original strain.

It seemed desirable to know much about a few birds rather than a little about many. Thus, as the 1940 hatching season progressed, the breeding population was reduced to 70 hens. Choice of parents to produce this population

TABLE 1.—*Strain composition of the Single-Comb White Leghorn inbred lines developed at the Regional Poultry Research Laboratory*

Inbred line	Strains involved ♂♂	♀♀
1	5, 7	3, 5, 7
2	1	2
3	2, 5	1, 2, 5, 10
4	1, 2	2
5	1	2
6[1]	1, 2, 5, 7	2, 3
7[1]	1, 7	2, 7
8	4	4, 6
9	5	1, 5
10	7	7
11	7	3
12	7	2, 7
13	5, 7	2, 7
14	1, 7, 8	2, 7, 8
15[1]	2, 7	2, 3
15I[1]	2, 7	2, 3

[1]Currently maintained.

was based mainly on family size because an incomplete disease history was available from the 1939 breeding population. Each hen was evaluated for disease resistance or susceptibility as in the 1939 season. Selected dams also produced at least 6 sires and 10 dams, which were held unexposed to the ALC for use as potential breeders in the next generation.

The management and history of the birds were described in detail by Waters (*43*). Each year chicks were hatched at 2-week intervals from about January to June. To conserve space, families less than 60 days old were selected on the basis of number of female progeny (10 or more if possible) average fertility, and average hatchability. No families more than 60 days of age were culled, and no individuals were culled from a selected family at any time. Each line was maintained in four pens, each containing 1 male and 25 females. Usually four sires and two to three dams per sire contributed progeny to the next generation. Brother-sister matings were not strictly adhered to in the early generations, but they occurred quite frequently. More often than not, closely related individuals (half sibs and first cousins) were mated.

Development of Line 15I

Early in the establishment of the inbred lines, a relatively high incidence of lymphomatosis, now known as lymphoid leukosis (LL), occurred in various inbred lines maintained in the quarantined areas. Because of the strict sanitation procedures and circumstantial evidence of egg transmission of the LL agents (*2, 42*), the birds were considered to be susceptible and probably infected by vertical or parent to offspring transmission. Because certain families had higher incidences of LL than did others, attempts were made to obtain dam families free of infection, and hence free of disease, through proper testing, selection, and isolation.

To this end, an isolation house was made available as described by Waters and Prickett (*53*). Twenty-two dams of inbred Line 15 were selected from families that had shown no LL before they were used for breeding during the spring of 1941. The progeny were distributed by families into five different pens of the isolation house. Full-sib progeny were also produced and reared outside of isolation with the mixed population of inbred Lines 1 to 15. The isolated population when 300 days old had 6-percent LL mortality (table 2), whereas the full sibs of the same age in contact with the mixed populations had 25-percent LL mortality.

Certain families of the isolated population had no LL. Two of these families, represented by four males and four females, were chosen to repopulate the isolation house during the 1942 generation and produce representative sibs for rearing with the non-isolated stock. At 300 days of age no birds had died with LL in the isolation house, whereas LL mortality occurred in 14 of 51 birds, or 28 percent, housed with the mixed population (table 2). This isolated population, probably representing the first group of chickens to be grown free of LL infection, was identified as 15I (*53*). Thereafter, it was held in isolation and was reproduced similarly to the other 15 inbred lines.

Development of other lines

Table 3 shows the intended direction of selection for the inbred lines (*54*). As more knowledge of the different lines became available, certain of the least desirable inbred lines were

TABLE 2.—*Lymphomastosis mortality at 300 days for Single-Comb White Leghorn chickens*[1]

Year and type of pen	Birds	Lymphomatosis deaths	Percentage of mortality[2]
1941:	*Number*	*Number*	*Percent*
Isolated	197	12	6
Nonisolated	130	33	25
1942:			
Isolated	91	0	0
Nonisolated	51	14	28

[1]Chickens reared in isolation; their full sibs reared as controls in an exposed pen.
[2]Waters and Prickett (*53*).

eliminated (*47*, 52). By 1951 inbred Lines 1 to 5, 8, and 11 to 13 had been discarded because of poor productivity, lack of desirable traits for disease study, or both. Inbred Lines 6, 7, 9, 10, 14, 15, and 15I remained, each with individual bird inbreeding coefficients in excess of 0.95 (52). Lines 9, 10, and 14 were subsequently discontinued with the 1968, 1964, and 1961 generations.

TABLE 3.—*Intended selection to establish inbred lines with susceptibility and resistance to the avian leukosis complex*

Inbred line	Intended selection		Discontinuance
	Visceral form	Neural form	
1	[1]R	R	By 1951
2	[2]S	S	By 1951
3	R	R	By 1951
4	S	S	By 1951
5	R	R	By 1951
6	R	R	No
7	S	S	No
8			1941
9	S	S	[3]1968
10	R	R	1964
11	S	S	By 1951
12	R	R	By 1951
13	R	R	By 1951
14	S	S	1961
15	S	S	No
15I	S	S	No

[1]R = Resistant.
[2]S = Susceptible.
[3]Line 9 is maintained by Dr. Paul Sammelwitz, University of Delaware.

In 1962, Dr. Lyman B. Crittenden initiated a brother-sister mating program for all inbred lines. Each line was maintained by 8 to 12 sires and 7 to 10 dams per sire. Selection of brother-sister matings to re-populate the next generation was based upon egg production, percentage of fertility, percentage of hatchability of the sire families, early chick and brooding viability, and the number of chicks available per dam family. The initial brother-sister matings of the 1962 population represented the derivation point of newly formed individual sublines within a given inbred line.

Development of Line 100

Crittenden and associates (*21*) and Crittenden (*14*) reported that Line 6 was homozygous susceptible to subgroups A and B of the leukosis-sarcoma (L/S) group of the ALC (9), relatively resistant to subsequent tumor induction by viruses of these subgroups, and quite resistant to the neural form of the ALC, now known as Marek's disease (MD). They further reported that Line 7 was homozygous resistant to subgroup A and was segregated for resistance to subgroup B of the L/S group, had unknown resistance to tumor induction, and was quite susceptible to MD.

Efforts were made to establish a new line (100) with susceptibility to MD and with different combinations of susceptibility and resistance to the L/S subgroups by employing the breeding program shown in table 4. Four Line 6 males were originally crossed with six Line 7 females to produce an F_1. Females of the F_1 generation were backcrossed to Line 7 males in the 1963 generation. Females of the resulting backcross were tested for L/S subgroup A susceptibility by chorioallantoic membrane (CAM) inoculation with Rous sarcoma virus (*19*). Heterozygous females were backcrossed to Line 7 males.

With four consecutive backcrosses, birds were produced that were composed of mainly Line 7 genetic material (97 percent) and segregated for susceptibility to the A L/S subgroup. When subgroup B viruses became available, this line was also found segregating for resistance at the B locus. Double heterozygous adults of the 1966 generation were selected; brothers and sisters

TABLE 4.—*Mating system utilized to develop Line 100*

Year	Type	Mating system: Description	Selected offspring: Sex	Selected offspring: Percent Line 7	Selected offspring: Genotype[1]
1961	F_1	[2]6 ♂♂ x 7 ♀♀ ↓	—	—	—
1962	BC_1	7 ♂♂ x 6 x 7 ↓	♀♀	50.00	Aa.
1963	BC_2	7 ♂♂ x BC_1 ↓	♀♀	75.00	Aa.
1964	BC_3	7 ♂♂ x BC_2 ↓	♀♀	87.50	Aa.
1965	BC_4	7 ♂♂ x BC_3 ↓	♀♀	93.75	Aa.
1966	[3]B-S	BC_4 ↓	♂♂ + ♀♀	96.88	AaBb.
1967	B-S	Inbreds ↓	♂♂ + ♀♀	96.88	Variable.
1968	B-S	Inbreds ↓	♂♂ + ♀♀	96.88	Variable.
1969	B-S	Inbreds ↓	♂♂ + ♀♀	96.88	Variable.
1970	B-S	Inbreds ↓	♂♂ + ♀♀	96.88	Variable.
1971	B-S	Inbreds	♂♂ + ♀♀	96.88	Variable.

[1]Crittenden and associates (*21*).
[2]Refers to the line of the individual, for example, Line 6 or Line 7.
[3]Brother-sister matings.

were mated and used to develop sublines of Line 100 with selective cell susceptibility to the A and B L/S subgroups (table 5). This line has been maintained by brother-sister matings.

Specific pathogen-free program

To effectively study the avian leukosis viruses and the interrelation of other infectious agents to neoplastic diseases, experimental chickens and embryos free of leukosis viruses and other pathogens were necessary. Chickens free of specific diseases permit investigators to measure the effects of genetics, pathology, histology, immunology, and related disciplines of science uncomplicated by other unknown endemic disease variables.

TABLE 5.—*Avian leukosis complex cell susceptibility in the sublines of Line 100*

Subline	ALC subgroup: A	ALC subgroup: B	Marek's disease
C/O[1]	[2]S	S	S
C/A	[3]R	S	S
C/B	S	R	S
C/AB	R	R	S

[1]Genetic details of sublines in Crittenden and associates (*21*).
[2]Susceptible.
[3]Resistant.

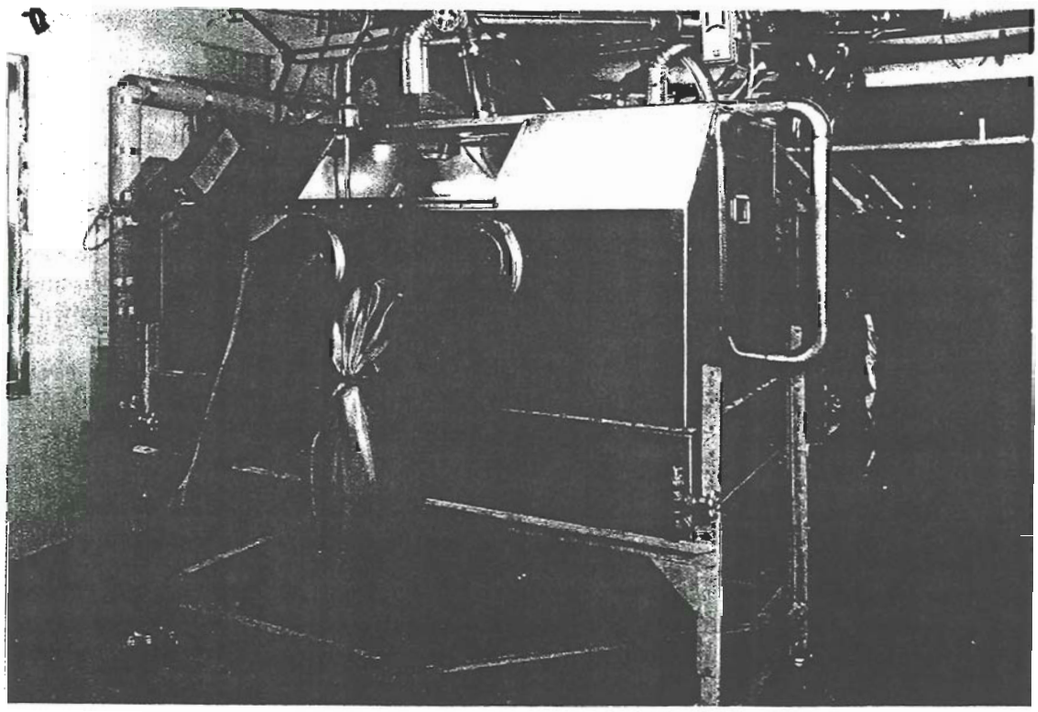
In 1965 the Specific Pathogen Free (SPF) program was initiated. The term SPF, as it applies here, was defined as freedom from the ALC (both the leukosis-sarcoma and Marek's disease viruses), infectious bronchitis, Newcastle disease, Salmonella pullorum, Salmonella gallinarium, Mycoplasma gallisepticum, avian encephalomyelitis, avian adenovirus, reticuloendotheliosis virus, laryngotracheitis, bursal agent, and chicken embryo lethal orphan (CELO) virus. The primary objective was to establish the laboratory genetic lines in isolation and utilize their offspring in disease research. This required an adequate number of prospective breeder parents (considering genetic worth

and possible disease freedom), a testing system for the infectious agents, adequate isolation facilities to rear and house the SPF flocks, and a reliable monitoring system to insure continued freedom from the specified agents.

Initially, individual birds of selected lines were tested for certain of these infectious agents, their respective antibodies, or both. Only those parents that were negative by these tests were selected to produce chicks to rear in isolation. Filtered air positive pressure (FAPP) brooding isolators were stainless steel (figs. 1 and 2), had glove ports, and a passthrough sleeve to move material into or out of the unit without seriously jeopardizing isolation. A phenolic disinfectant (O-syl) was used to treat the outside of all introduced material. Air was forced (fig. 3) through Dri-Pak filters (American Air Filter) with efficiency ratings of 93 to 97 percent for 10-micron particles and Astrocel filters (American Air Filter) with efficiency ratings of 99.9-percent for 0.3-micron particles.

TABLE 6.—*Lines and sublines maintained in specific pathogen-free environments*

Generation and line	Subline	Isolators	Parent stock	
			Sires	Dams
1970:				
100	all	4	12	59
6	1	2	8	29
7	2	4	11	43
15	1	1	2	13
15I	4	2	8	31
15I	5	3	7	45
Subtotal		16	48	220
1971:				
100	all	4	55	64
6	1	4	24	64
7	2	8	24	192
15	1	1	6	16
15I	4	2	12	32
15I	5	2	12	32
Subtotal		21	133	400
Total		37	181	620



PN-3936

FIGURE 1.—Filtered air positive pressure (FAPP) isolator for brooding specific pathogen-free chickens (single unit).

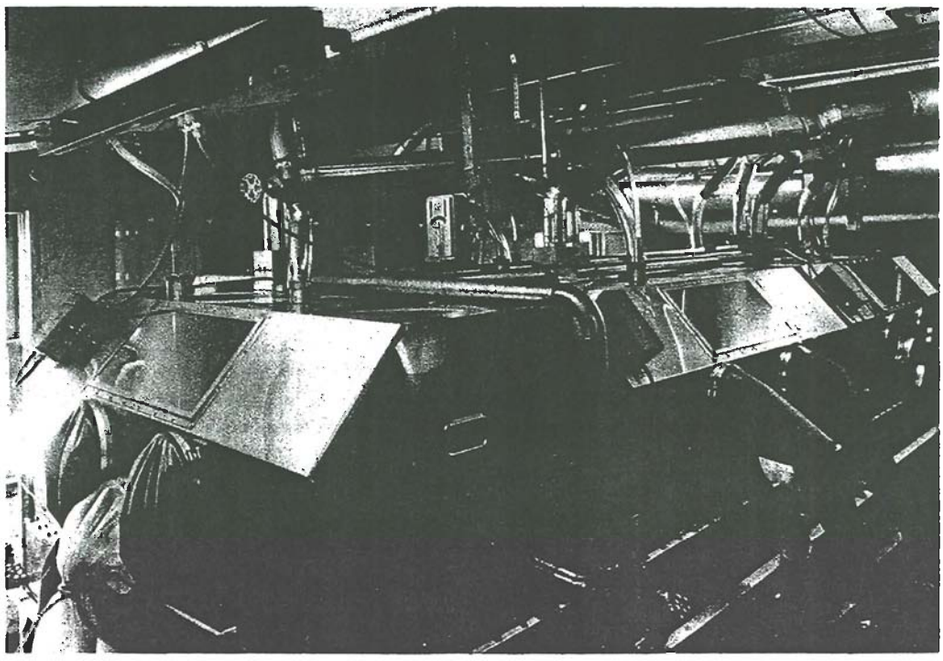


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FIGURE 2.—Filtered air positive pressure (FAPP) isolators for brooding specific pathogen-free chickens (group unit).

Six-week-old chicks were transferred in portable isolated carriers to large FAPP laying isolators (figs. 4 and 5) constructed with a stainless steel base and a flexible plastic canopy that were operated as described for the brooding isolators. Birds were monitored at approximately 5 weeks, 6 months, and at termination, usually at 18 months, for some or all of the agents previously listed. Whenever infection was detected, the entire isolator was retested and terminated if original tests were confirmed.

This program has progressed to the extent that subpopulations of most of the breeding populations at the laboratory are now maintained free of all agents in the program protocol. Specific lines and sublines in SPF conditions are shown in table 6. Plans are being developed to convert additional lines to this status and to increase the size of existing lines.

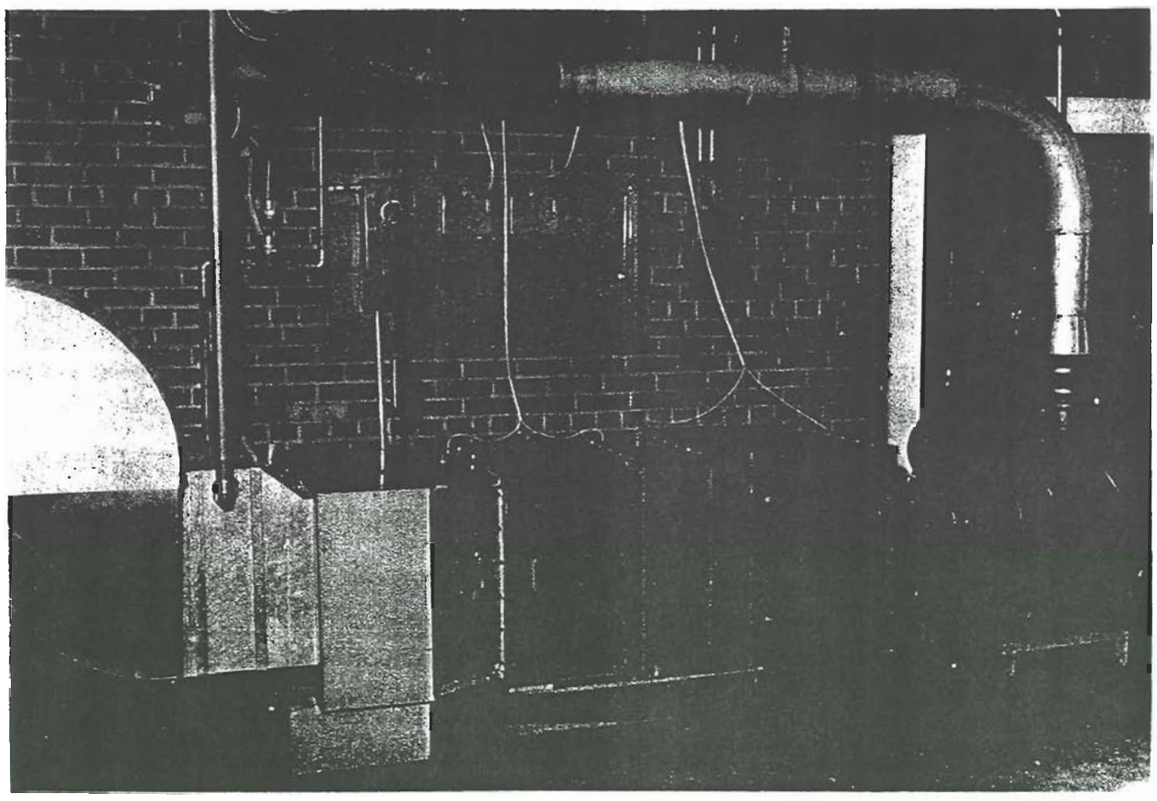
Productive and reproductive performance (non-SPF inbred lines)

The influence of the laboratory's inbreeding program on such economic traits as egg weight (*43*), hatchability (*45*), sexual maturity (*46*), and body weight (*48*) has been reported. Since the 1962 generation, egg production, fertility, and hatchability records have been maintained on a subline basis.

Subline means of these traits are shown in tables 7, 8, and 9. Genetic interpretations, however, were impractical because lines, sublines, and full-sib families were housed in pedigree cage blocks, in a nonrandom system, and, therefore they do not lend themselves to reliable determinations of genetic or environmental influences from year to year. The tables do show the trait averages as they occurred and illustrate the relative levels of performance of the inbred lines.

Egg production (table 7) was determined on a hen-day basis from September 1 to May 31. Generally, subline production varied by approximately 15-percent among the eight generations. However, the production levels for each year were adequate for re-population and supply of experimental materials.

Percentage fertility (table 8) was based on

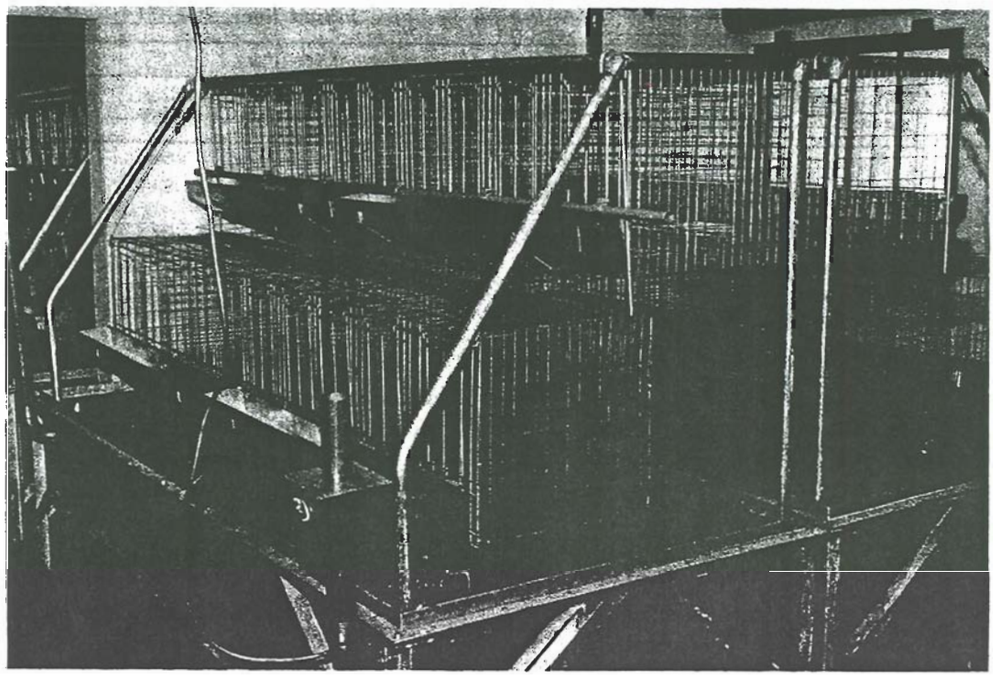


PN-3938

FIGURE 3.—Blower filter units to supply noninfectious air to specific pathogen-free chickens.

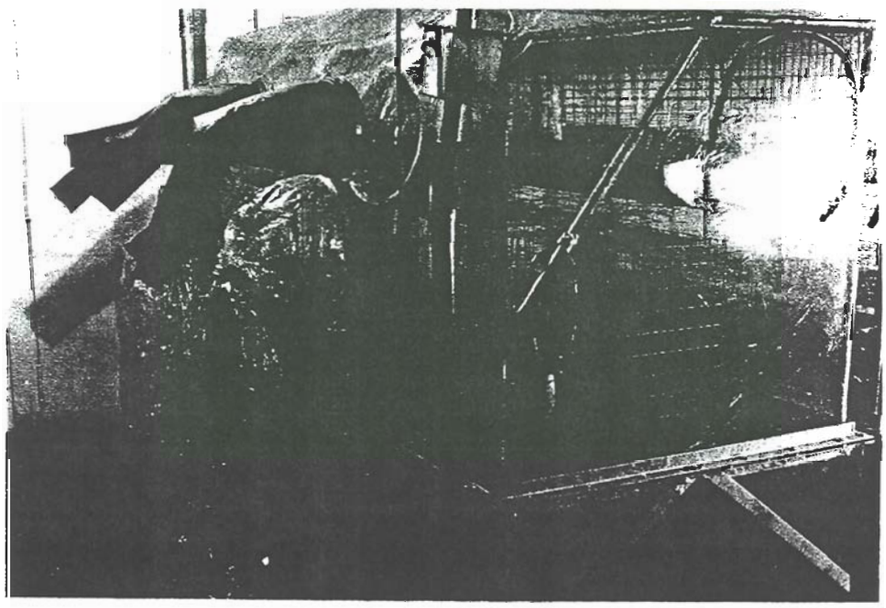
TABLE 7.—*Percentage of egg production for pedigree inbred lines, 1963-71*

Line and subline	Females per generation	Percentage of production							
		1963-64	1964-65	1965-66	1966-67	1967-68	1968-69	1969-70	1970-71
	Number	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
100, all	84	41	41	37	41	43	50	47	52
6:									
1	28	42	42	52	53	51	42	37	44
3	56	41	42	44	46	51	46	45	52
7:									
1	14	37	41	29	46	48	34	52	47
2	56	33	44	27	22	30	32	32	35
3	14	43	33	35	39	46	36	41	51
15:									
1	28	36	37	37	37	34	48	37	53
4	28	47	47	58	55	59	57	50	56
6	28	36	36	55	42	56	47	47	48
15I:									
4	30	27	34	27	17	27	32	32	34
5	30	22	29	32	51	37	44	46	37



PN-3939

FIGURE 4.—Internal view of filtered air positive pressure (FAPP) isolator to house mature specific pathogen-free chickens.



PN-3940

FIGURE 5.—External view of filtered air positive pressure (FAPP) isolator to house mature specific pathogen-free chickens.

TABLE 8.—*Percentage of fertility for pedigree inbred lines, 1963-71*

Line and subline	Females per generation	Percentage of fertility								
		1963	1964	1965	1966	1967	1968	1969	1970	1971
	Number	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
100, all	84	[1]51	[1]97	[1]87	91	73	80	83	88	86
6:										
1	28	64	80	76	77	66	76	73	78	59
3	56	87	87	93	91	91	90	84	85	92
7:										
1	14	89	89	87	76	87	78	70	53	72
2	56	88	89	87	84	68	75	85	76	69
3	14	46	98	72	93	88	88	60	66	76
15:										
1	28	91	91	82	83	75	84	82	80	82
4	28	84	81	82	86	86	92	86	79	90
6	28	61	79	83	97	86	90	90	85	82
15I:										
4	30	94	97	69	91	72	87	66	96	92
5	30	88	90	79	90	83	92	80	89	92

[1]Indicative of fertility of the Line 7 males and the backcross females.

data obtained during the re-population seasons. Most sublines had acceptable fertility. Percent hatchability (table 9) also referred to data collected during the re-population season and again, except for recent responses in Line 6 subline 1, Line 7 subline 3, and Line 15 subline 6, reflect acceptable hatchability.

Recent studies in the inbred lines include egg weight, egg quality, and body weight. The data, limited to the current generation (1970), are shown in table 10. Fall and spring egg weights from hens 28 to 35 weeks old and 50 to 58 weeks of age, respectively, were significantly influenced by line and subline. A distinct decrease in

TABLE 9.—*Percentage of hatchability of fertile eggs for pedigree inbred lines, 1963-71*

Line and subline	Approximate number per generation	Percentage of hatchability								
		1963	1964	1965	1966	1967	1968	1969	1970	1971
		Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
100, all	84	79	82	79	81	70	75	70	66	64
6:										
1	28	[1]31	51	78	69	65	63	65	52	47
3	56	71	80	79	81	81	74	75	81	84
7:										
1	14	73	73	66	60	86	68	66	77	72
2	56	77	71	72	73	75	70	67	66	65
3	14	73	84	74	65	71	53	56	43	38
15:										
1	28	84	92	69	67	79	71	79	77	66
4	28	58	69	53	55	59	59	56	58	65
6	28	70	68	57	64	62	58	46	30	32
15I:										
4	30	70	77	53	48	74	56	45	64	34
5	30	75	77	76	72	49	21	30	45	37

[1]1 family only.

TABLE 10.—*Egg weight, shell thickness, and body weight of the 1970-71 generation of pedigree inbred lines*

Line and subline	Sire families	Dam families	Egg weight		Shell thickness	Body weight			
						6 weeks		20 weeks	
			28-35 weeks	50-58 weeks	(X10⁻³ inch)	♂♂	♀♀	♂♂	♀♀
			Grams	*Grams*		*Grams*	*Grams*	*Grams*	*Grams*
100, all	12	84	45	51	11.56	—	—	—	—
6:									
1	4	28	41	45	12.29	356	334	1502	1175
3	8	56	42	46	12.18	439	401	1756	1333
7:									
1	2	14	48	51	11.42	515	430	1711	1286
2	8	56	44	48	11.90	507	410	1781	1275
3	2	14	43	48	11.09	455	382	1708	1288
15:									
1	4	28	37	44	10.64	440	373	1668	1338
4	4	28	45	50	12.15	440	348	1553	1152
6	4	28	45	50	12.50	448	407	1842	1194
15I:									
[1]4									
[1]5									

[1]Maintained only in the SPF program. This study has not been extended to SPF populations.

egg weight occurred in Line 15 subline 1. Shell thickness, the trait initially used to investigate egg quality, and body weight of 6- and 20-week-old chickens were significantly influenced by lines, sublines, and dams. As before, the production traits were derived from populations maintained in a nonrandom management procedure and therefore may be biased.

UTILIZATION OF INBRED LINES

Past utilization

Waters (*49*) presented one of the earliest summaries of disease response for the RPRL inbred lines. Line 6 was reported to be relatively resistant to the visceral and neural forms of the ALC; Line 7 was quite resistant to the visceral form and susceptible to the neural form; and Lines 15 and 15I were quite susceptible to the visceral and neural forms. Considerable year variability occurred for each line. Most obvious was an extremely low mortality rate for all lines in the 1951 generation. Perhaps this can be explained by the drastic management procedures (depopulation) that occurred for that generation (*50*) to prevent the possible transmission of infectious bronchitis from infected adults to their progeny.

Crittenden and associates (*20*) studied the 1963-66 generations of Lines 6, 7, and 15I following inoculation or contact exposure to various L/S agents. For comparative purposes the results obtained with the 1964 generation are presented. Chicks, intravenously inoculated with RPL-12 or avian myeloblastosis virus (AMV) or naturally exposed to the RPRL farm conditions, produced results (table 11) that indicated that the intended direction of selection for susceptibility and resistance in these lines had been accomplished (table 3 and Waters (*49*)).

In addition to the overall disease response, much progress has been made in identifying the mechanisms of disease resistance. Two distinct mechanisms of resistance to the L/S group (*14*) are the primary level of defense or resistance to cellular penetration and the secondary level of defense or resistance to development of L/S tumors. Three distinct loci, Tv_a, Tv_b, and Tv_c, have been identified that control susceptibility or penetration of the L/S viruses (*21*, *22*, *38*), each controlling susceptibility to a different subgroup of viruses. Of these, only the loci controlling subgroups A and B L/S viruses show importance in disease control in the field. Genetic control at the secondary level of defense

TABLE 11.—*Least-squares mean (corrected) percentage mortality with lymphoid leukosis (LL) and avian myeloblastosis virus (AMV)*

Line	RPL-12		AMV[1]		Natural exposure	
	Number birds 101 days old	Lymphoid leukosis deaths	Number birds 31 days old	Myeloblastosis deaths	Number birds 101 days old	Lymphoid leukosis deaths
		Percent		*Percent*		*Percent*
6	74	0.7	60	12.5	73	0
7	73	0	59	67.1	65	3.3
15I	67	65.7	57	74.8	68	8.3

[1]Avian myeloblastosis virus (mostly B subgroup). Crittenden and associates (*20*).

has been suggested by Crittenden and associates (*19*).

The response of chickens of Lines 6, 7, and 15I (table 12) exposed to different L/S viruses illustrates the interrelation between the primary and secondary levels of defense. The Bryan standard Rous sarcoma virus, BS (RSV), antibody indicated the extent of growth of subgroup A virus in the lines, thereby serving as a guide for the extent of primary defense. This was also indicated by the development of neoplasms in Lines 6 and 15I and lack of neoplastic development in Line 7 after embryo inoculation with a high concentration of RPL-12 (subgroup A virus). In contrast, when Lines 6 and 15I, which were essentially equally susceptible to infection, were inoculated at 14 days of age with RPL-12 or AMV (a mixture of subgroup A and B viruses), Line 15I developed a much higher proportion of neoplasms than Line 6, therefore, seemingly exhibiting differences in secondary defenses. Perhaps Line 6 was more efficient than Line 15I in utilizing its secondary defense, or alternatively, it may be more efficient in producing type-specific antibody, thus enhancing the immunological aspects of the first line of defense.

Further evidence of different mechanisms of resistance to L/S tumors and mortality induced by subgroup A viruses, was observed in that crosses between Lines 6 and 7 often have a higher mortality than either parent (table 13) and (*19*). This finding may be explained because the crosses were fully susceptible at the primary level of defense and may be more susceptible at the secondary level of defense than pure Line 6.

Selection for resistance to penetration is relatively straightforward, and it can be accomplished directly by inoculation with appropriate virus preparations of the specific subgroups.

TABLE 12.—*Responses of chickens of various phenotypes to high doses of avian tumor viruses*[1]

Line	Phenotype	Antibody to BS-RSV[2] (A)[3]	Neoplasms: Embryo inoculation RPL-12 (A)	Neoplasms: Chick inoculation RPL-12 (A)	Neoplasms: Chick inoculation RPL-25 (A, B)	Neoplasms: Chick inoculation AMV[4] (B, A)
		Percent	*Percent*	*Percent*	*Percent*	*Percent*
6	C/O	92	83	11	31	21
7	C/A, C/AB	0	3	4	21	69
15I	C/O, C/A	67	88	81	90	72

[1]Crittenden and associates (*19*).
[2]Bryan standard Rous sarcoma virus.
[3]Subgroup specificity.
[4]Avian myeloblastosis virus (mostly B subgroup).

TABLE 13.—*Responses of chickens of various phenotypes to contact or natural exposure to avian tumor viruses*

Line and phenotype	Lymphoid leukosis		
	Contact RPL-12		Exposure to field isolates[2]
	Trial 1[1]	Trial 2[2]	
	Percent	*Percent*	*Percent*
6x6, C/O	1.0	0	0.2
7x7, C/A, C/AB	0	.3	.6
6x7, C/O	17.0	11.4	4.2

[1]Crittenden (*14*).
[2]Crittenden and associates (*20*).

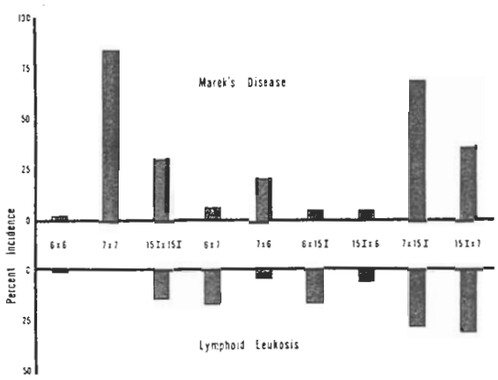


FIGURE 6.—Comparative incidence of Marek's disease after inoculation with JM strain cells and lymphoid leukosis after contact exposure to RPL-12 virus in three inbred lines and their reciprocal crosses.

Selection for resistance at the secondary level of defense is more complex because no method is available to measure neoplastic development in chickens which are resistant to viral penetration. Therefore, selection to improve this level of defense must be conducted in lines that are susceptible to penetration of their cells by the virus.

Most of the research effort at this laboratory has been on Marek's disease. Early reports of this disease for the inbred lines were published by Waters (*49*). Crittenden (*18*) studied the 1967 generation of Lines 6, 7, and 15I following inoculation and contact exposure to the JM and Ga Marek's disease strains. The results (table 14) of this study indicate the marked resistance of Line 6, the high level of susceptibility of Line 7, and the intermediate response of Line 15I.

Figure 6 gives the relative incidence of LL and MD in a full diallel-cross experiment (*14*). There seems to be little correlation of resistance to the two diseases except, perhaps, in Line 6, suggesting the possibility of a common mechanism of resistance in the secondary level of defense. Because little correlation of resistance was found in these lines, breeding for resistance to the ALC involves breeding for resistance to at least two independent diseases.

Present utilization

The Marek's disease-resistant Line 6 and susceptible Line 7 were considered excellent lines (table 14 and figure 6) to investigate the genetic control of MD. Therefore, the 1967 generation of these lines and their reciprocal F_1's were studied to determine their influence on MD mortality following inoculation with JM infected whole blood (0.01 ml) or contact exposure at 1 day of age. The results (table 15, experiments 1 and 2) indicate the high levels of resistance

TABLE 14.—*Least-squares mean percentage mortality with Marek's disease*[1]

Line	Strain of Marek's disease							
	Ga				JM			
	Inoculated		Contact		Inoculated		Contact	
	Number[2]	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*
6	49	2.7	49	0.1	48	11.6	50	0.7
7	49	86.3	49	75.9	46	96.0	50	80.0
15I ...	50	68.1	48	55.2	49	55.1	49	29.5

[1]Crittenden and associates (*18*).
[2]Number of 11-day-old birds present.

TABLE 15.—*Marek's disease mortality as influenced by type of chicken and method of exposure*

Line ♂♂ x ♀♀		Experiment 1 inoculated		Experiment 2 inoculated		Experiment 2 contact		Experiment 3 inoculated		Experiment 4 contact	
		Number	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*
6	6	18	6	44	5	47	0	15	0	77	0
7	7	21	90	26	73	22	55	13	85	62	58
7	6	23	13	49	8	50	2	14	7	107	2
6	7	23	70	50	44	48	6	16	44	96	2
7	(7x6)							14	29	103	13
7	(6x7)							16	44	105	21
6	(7x6)							16	6	108	1
6	(6x7)							15	0	97	0
(7x6)	6							16	0	107	1
(7x6)	7							14	64	94	24
(6x7)	6							15	13	109	0
(6x7)	7							15	67	93	18
(7x6)	(7x6)							16	19	106	6
(7x6)	(6x7)							16	25	106	6
(6x7)	(7x6)							14	36	107	8
(6x7)	(6x7)							16	0	104	6

in Line 6 and the high level of susceptibility in Line 7 for both inoculated and contact-exposed birds. However, the reciprocal F_1's differ according to maternal parent and to method of exposure. For example, the inoculated 6×7 F_1 was more alined with the maternal Line 7 parent response and the inoculated 7×6 F_1 with that of Line 6, whereas, there was no significant difference in the F_1's with contact exposure. These results led to experiments utilizing a mating system that compared pure lines with their reciprocal combinations of F_1's, F_2's, and backcrosses.

In two sequential experiments (table 15, experiments 3 and 4) chicks were either inoculated, as in experiment 1, or contact exposed to inoculated chicks. The results of these experiments confirm the data of experiments 1 and 2 for the pure lines and reciprocal F_1's. The response of the reciprocal F_2's suggests that the genetic control of MD may be effected by a relatively few number of loci. The response of the reciprocal backcrosses suggest that resistance may be dominant to susceptibility. Resistance, however, as a dominant factor seems related to severity of exposure and the relative susceptibility of the two lines (*18*). The apparent dominance observed may reflect only that the severity of exposure was below the susceptibility threshold for the particular crosses involved. Additional confusion results when a given inoculum is used to expose a host system that may be confounded by potential maternal effects. Thus, definite mechanisms of resistance of MD have not been identified, nor have single genes been identified which control resistance and susceptibility to this disease.

To eliminate the maternal effects associated with MD antibody (*11* and Burgoyne and Witter, unpublished data) and to obtain a clearer understanding of the genetic control of MD, chicks of Lines 6 and 7 and their reciprocal F_1's were obtained from SPF parents (SPF parents have had no exposure to MD; hence, they have no antibody). The chicks were compared with chicks produced from parents of outside populations that have MD antibody. One-day-old chicks of each source of parents were either inoculated with JM infected whole blood or contact exposed by hatchmates.

The results of these experiments (table 16) show distinct increases in mortality for the inoculated SPF lines and crosses and indicate some increase in mortality among the contact exposed SPF reciprocal F_1's. These results also suggest that while most chickens were sus-

ceptible to JM, MD infection, the differences in mortality among lines, crosses, and parent source were indicative of differences in the host secondary mechanisms of defense. In contrast to experiments with the L/S viruses, however, subgroup specificity for the JM inoculum is virtually unknown. The inoculum may be composed of a mixture of virus types of differing host specificity, thereby concealing resistance at the primary level of defense.

Immunogenetics

For the past 20 years or more the immunogenetics of the chicken has been researched (25). The major impetus was to understand the basic aspects of blood groups and to use this knowledge to rapidly improve poultry. Reviews by Briles (4), Nordskog (37), and Gilmour (28) indicate that methods were established to solve problems of parentage, misidentifications, and mispedigreeing. Limited use, however, was made to improve poultry flocks, and the anticipated levels of improvements were not realized. Even though immunogenetics is not a simple nor rapid method to improve poultry and its value remains controversial, its use as a tool to obtain greater insight of operating genetic mechanisms is valid (5).

At least 10, and perhaps 11, different blood group systems associated with the erythrocyte of chickens exist: A, B, C, D, E, H, I, J, K, L, and P (5); each is controlled by a separate genetic locus (26, 6). Possible other systems exist (25, 35), but their relationship is obscure because reagents developed at different laboratories have not been standardized. Letter designations were established by priority of reports.

An immunogenetic program was initiated at the RPRL in 1958 by L. Warren Johnson of Auburn University and was used to monitor inbreeding progress. Table 17 presents the results for the A, B, C, D, E, I, and P loci of the 1964 generation (the last generation with complete data on all lines) and illustrates the variation among lines and sublines. The extent of segregation of alleles at certain loci, particularly the B locus in Lines 15 and 15I, was not expected for chickens with inbreeding coefficients in excess of 0.96. Similar findings, however, reported by Briles and associates (7) and Gilmour (27), suggest a heterozygous advantage (36, 29).

Recently, cooperative research with Drs. Crittenden and Briles led to the discovery that blood types may be used to identify chicken tumor

TABLE 16.—*Marek's disease mortality by type and source of chicks and method of exposure*

		Source of chicks							
		Conventional housing				SPF housing			
Line		Inoculation		Contact		Inoculation		Contact	
♂♂ x ♀♀		*Number*	*Percent*[1]	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*
6	6	22	0	18	0	112	[2]48	120	0
7	7	23	96	23	52	75	[3]77	72	76
7	6	49	2	50	2	28	100	37	30
6	7	41	8	42	2	40	[3]92	46	34

[1]Based on gross necropsy only.

[2]Lesions at necropsy were atypical. Total mortality was 98 percent. Histological examination may have indicated higher Marek's disease mortality.

[3]Total mortality (Marek's disease and nonspecific) was 100 percent. Histological examination may have indicated higher Marek's disease mortality.

TABLE 17.—*Blood group alleles in the 1964 generation of the RPRL inbred lines*[1]

Line and subline[2]	AE[3]	B	C	D	I	L	P
6:							
1	[4]x	13	1	2	[4]x	([5])	3
3	x	13	1	2, d	x	([5])	3
7:							
1	3	15	2	2	1, 2	1	3
2	3, 4	15	1	2	1, 2	1	3
3	4	15	1	2	1, 2	1, 0	3
15:							
1	8	10	2	d	1	([5])	3, 5
4	8	9, 11	2	1	1	([5])	3, 5
6	8	9, 11	2	1	1	([5])	3, 5
15I:							
4	14	9, 10, 11	1	1, 3	1, 5	([5])	3
5	13, 14	9, 11	1	1, 3	1, 5	([5])	3

[1]Unpublished data obtained from Dr. L. Warren Johnson, Auburn University.

[2]Refers to individuals traced back to the same male and female parents of the 1962 generation.

[3]A and E loci are linked.

[4]No segregation at this locus detected; no number given to the allele.

[5]Data not available.

virus susceptibility. The ability of the leukosis/sarcoma viral genome to penetrate the host cell appears to depend on the specific interaction between genetically controlled properties of the virus envelope and the cell membrane (*15*). Dominance of susceptibility suggests the presence of a specific receptor site for attachment or penetration (*40*) which may be inherited as a cell surface isoantigen and detected by immunological methods (*8*).

Attempts to associate susceptibility to L/S viruses, subgroups A and B, with the 11 known blood systems have failed. However, recently Briles and Crittenden (*8*) identified a new agglutinin that reacted with the erythrocyte antigen controlled by the R locus (Rl, r). Crittenden and associates (*16*) utilizing Line 100, known to be segregating at the loci controlling susceptibility to L/S subgroups A and B, presented evidence (table 18) for a close association between the occurrence of an erythrocyte isoantigen and susceptibility to subgroup B L/S viruses. All 35 birds with the b^s susceptible allele were Rl positive, and the five birds of the resistant genotype (b^rb^r) were Rl negative (rr).

A more direct method was also used to study the association. Embryos, produced from heterozygous susceptible (b^sb^r), Rl antigen positive, Line 100 males and homozygous resistant (b^rb^r), Rl antigen negative, Line 7 subline 2 females, were tested for virus susceptibility (*21*) and agglutination by the R agglutinogens (*8*). The results, table 18, show that 191 susceptible embryos were Rl positive, while 194 resistant embryos were Rl negative. Two resistant embryos that were Rl positive could have resulted from recombination or technical error.

Either of two hypotheses could explain these data: the Rl antigen and subgroup B virus susceptibility in Line 100 were controlled by the same locus, or they were controlled by two closely linked loci. If subsequent data validate the first hypothesis, this will be the first demonstration of a single gene controlling a cell membrane receptor site for an oncogenic virus and an erythrocyte isoantigen.

TABLE 18.—*Relationship between susceptibility to subgroups A and B leukosis-sarcoma viruses and red blood cell agglutination by Rl antiserum*

Material classified and R blood group	Leukosis-sarcoma virus susceptibility					
	Subgroup A			Subgroup B		
	a^sa^s	a^sa^r	a^ra^r	b^sb^s	b^sb^r	b^rb^r
Trial 1 adults:						
Rl	2	10	9	19	16	0
rr	0	1	2	0	0	5
Trial 2 embryos:						
Rl					191	2
rr					0	194

Histocompatibility

Histocompatibility between two individuals depends upon their genetic and immunologic relationship. Acceptance of such tissue grafts as skin, by one individual from another in a noninbred population, is an extremely rare event, and this suggests that a large number of histocompatibility loci exist (*1*, *41*). Usually, rejection results from an immunologic reaction induced by the presence of at least one antigen in the donor graft that is different from that found in the recipient. These antigens, controlled by histocompatibility genes, vary in their degree of importance and must be identical or similar for graft acceptance to occur.

Crittenden and associates (*17*) suggested that studies of the graft versus host reaction and the homograft reaction would predict the extent of similarities among individuals of an inbred line. Their results, obtained from inbred Lines 6, 7, 9, 10, and 15, showed that donor-host differences at the B blood group locus led to spleen enlargement in embryos and prompt rejection of homografts from adult males. In addition, genetic loci other than those studied, influenced homograft rejection but not the graft versus host reaction, suggesting the former may be more sensitive than the latter in identifying minor histocompatible differences.

Chai and Chiang (*10*) have reported the skin homograft reaction to be sensitive to slight genetic differences. Early attempts to obtain persistent homografts of normal chicken tissues using lines with low coefficients of inbreeding have failed (33) or been only slightly successful (*31*). Cock and Clough (*12*) reported that the Reaseheath I Line (F = 98.75) accepted a high

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FIGURE 7.—Source of wattle tissue for histocompatibility studies.

TABLE 19.—*Skin homograft survival in inbred lines of chickens*[1]

Line and subline	Number of grafts	Homograft condition		
		Equal to autograft	Poor or rejecting	Rejected
6:				
1	18	16	2	0
3	45	20	2	23
7:				
1	6	4	2	0
2	9	3	0	6
3	10	5	1	4
15:				
1	27	17	1	9
4	21	1	1	19

[1]Crittenden (unpublished data).

proportion (93 percent) of within-line transplants. Hăsek (*30*) has established, through genetic selection, histocompatibility in the Reaseheath C Line.

Histocompatibility studies were initiated by Dr. Crittenden in 1962 to monitor inbreeding progress, to develop histocompatible lines and sublines, to study highly specific immunologic reactions in a genetically compatible background, and to study experimentally transplanted tissues and organs. Early procedures (*13*) modified by Purchase (*39*) follow. Donor wattle tissues (fig. 7) were obtained from groups of four birds and reciprocally were transplanted onto recipient shanks of each bird. Transplantations, held in place for 7 days with porous tape, were observed at approximately weekly intervals for 38 to 40 days and finally before bird termination, usually 265 to 280 days post transplantation. Early results, based on graft classifications of (1) equivalent to the autograft (fig. 8), (2) discolored or rejecting, or (3) rejected (fig. 9) are shown in table 19. These results suggest that each of the line-subline classifications studied could be developed into histocompatible groups with additional work.

Subsequent studies to develop histocompatible groups have continued for Line 6 sublines 1

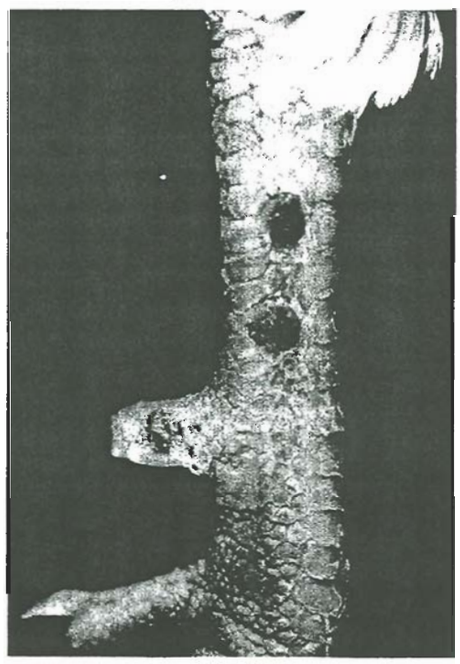

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FIGURE 8.—Autograft of histocompatible graft of Line 6 subline 1.

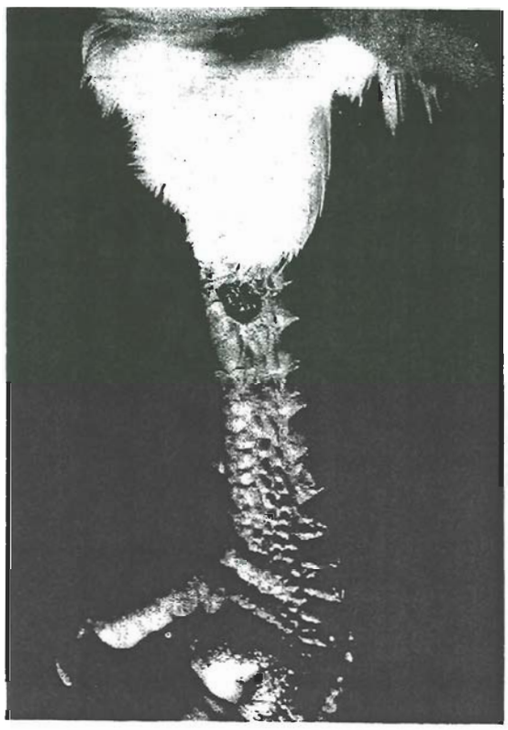

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FIGURE 9.—Autograft and rejected homograft of a nonhistocompatible line.

and 3 and Line 7 subline 2. In 1969, experiments were initiated for Line 100. Reciprocal transplantations were made. Birds were selected from either within or between sire families on a line-subline category. A few reciprocal transplantations were made between sublines 1 and 3 of Line 6. The results for the 1970 generation are shown in table 20.

Line 100 had 20-percent rejection of grafts within sire families. Line 6 subline 1 had no rejection within or between sire families. This, with the 100-percent acceptance of grafts for the three generations previous to this time, suggested that Line 6 subline 1 was indeed histocompatible. Line 6 subline 3 had 4 and 12-percent rejection of grafts within or between sire families, respectively. Line 7 subline 2 had no rejection of grafts within sire families. All grafts made between Line 6 sublines 1 and 3 were rejected. Continued work, utilizing established procedures, should further increase within subline histocompatibility.

Summary of line characteristics

Table 21 summarizes the characteristics studied in the RPRL inbred lines and sublines of chickens as they exist in the 1970 generation. Specific details for each subject area were discussed previously in this publication. All lines and sublines, except 15I, are maintained in conventional housing, and certain groups are maintained in limited numbers and in SPF housing.

TABLE 20.—*Histocompatibility in inbred lines of chickens*

Line and subline	Family exchange pattern	Number of grafts	Homograft condition		
			Equal to autograft	Poor	Rejected
100, C/O	Within	30	20	0	10
100, C/A	Within	36	26	0	10
100, C/B	Within	33	33	0	0
100, C/AB	Within	36	29	0	7
6, 1	Within	42	42	0	0
6, 1	Between	12	12	0	0
6, 3	Within	84	81	0	3
6, 3	Between	69	61	0	8
7, 2	Within	81	81	0	0
6:					
1 and 3	Between sublines	8	0	0	8
3 and 1	Between sublines	5	0	0	5

TABLE 21.—*Characteristics of the inbred lines of chickens maintained at the RPRL*[1]

Line and subline	Housing		Disease susceptibility				Inbreeding coefficient	Blood typed	Trying to develop histocompatibility
			Leukosis-sarcoma group			Marek's disease			
	Conventional	Specific pathogen free	Cell infection A	Cell infection B	Tumor development	Tumor development			
100:									
C/O	Yes	Yes	[2]S	S	?	S	0.78	[4]1	Yes.
C/A	Yes	Yes	[3]R	S	?	S	.78	1	Yes.
C/B	Yes	Yes	S	R	?	S	.78	[5]2	Yes.
C/AB	Yes	Yes	R	R	?	S	.78	2	Yes.
6:									
1	Yes	Yes	S	S	R	R	.99	2	Yes.
3	Yes		S	S	R	R	.99	2	Yes.
7:									
1	Yes		R	SR	?	S	.99	1	
2	Yes	Yes	R	R	?	S	.99	2	Yes.
3	Yes		R	SR	?	S	.99	2	
15:									
1	Yes	Yes	SR	SR	S	S	.99	1	
4	Yes		SR	SR	S	S	.99	1	
6	Yes		SR	SR	S	S	.99	1	
15I:									
4	Yes		SR	SR	S	S	.99	1	
5	Yes		SR	SR	S	S	.99	1	

[1]Supplements for productive and reproductive characteristics appear in tables 7-10.
[2]Susceptible.
[3]Resistant.
[4]Blood typing, by L. Warren Johnson, refers to data for the 1965 generation.
[5]Blood typing, by W. Elwood Briles, refers to data for the 1970 generation.

Susceptibility to the L/S viruses is relatively well characterized at the cell level for the A and B subgroups. Tumor response to the L/S viruses are known for Line 6, 15, and 15I but are unknown in Lines 100 and 7. Although no evidence exists that would suggest resistance to the Marek's disease agent at the cell level, susceptibility to MD tumor development is characterized for all lines.

Theoretical individual bird-inbreeding coefficients are 0.78 for the four sublines of Line 100 and in excess of 0.99 for all other line-subline classifications. Recent blood-typing data are available for certain chickens, though some information can be extrapolated from earlier test results for all other groups. Cooperative arrangements are currently being made to reactivate a comprehensive blood-typing program. Line 6 subline 1 is histocompatible while other lines and sublines are approaching histocompatibility.

Future utilization

Although discussions of this nature are often speculative, utilization of the RPRL's inbred lines in certain areas of research would be highly beneficial. Further characterization is needed for the genetic control of the mechanisms of disease resistance. Inbred lines would be especially amenable to the identification of single genes that mediate control at different levels of resistance to the L/S and MD virus groups. In addition, threshold levels of disease resistance as influenced by dose, route of inoculation, and age should be identified and characterized.

Further development of histocompatible lines and sublines would be extremely valuable. Currently, all estimates of histocompatibility are based on single sets of reciprocal graft exchanges in the adult chicken. Verification of these results should be made by reciprocally exchanging a duplicate set of grafts, second

sets, to eliminate the possibility of minor and undetected histocompatibility differences. In addition, the influence of age upon the expression of histocompatibility loci needs such investigation as do different histocompatibility loci operate at various stages of species development. Also, the structure of complex histocompatibility loci, similar to the H-2 locus in the mouse, needs investigation.

The attributes of quantitative genetics and additive genetic variance have been discussed in many textbooks. However, most of them have acknowledged the limitations of interpretation of these principles. Lerner (32), for example, states:

> It is possible, if not entirely probable, that selection in inbred lines is effective in improving the additive genotype. Inbreeding may under such a view impose a phenotypic ceiling on performance. If this is so, selected inbred lines should, upon release from consanguineous mating, show improved performance to the extent of expected gains under a strictly additive hypothesis of gene action. Such release may be accomplished by crossing good inbred lines. Much of the hybrid superiority would then be exhibited in the F_2 and following generations. Whatever the merits of this suggestion are, it is open to experimental proof.

The RPRL inbred lines, their reciprocal F_1's, F_2's, and backcrosses would be useful for comparative studies of the additive genetic variance at specific loci, acquiring specific information of genetic correlations, developing more detailed chromosome maps, and above all, assisting in obtaining a clearer understanding of the actual mechanisms of heterosis.

Relatively new areas of such research as biochemical and developmental genetics, which require refined tools and experimental materials, would be greatly benefited by the utilization of highly characterized inbred lines. Such materials would assist in the identification of genetically controlled biochemical markers that could be applied to the elucidation of the genetic control of quantitative and qualitative traits at the molecular level. Inbred lines would be useful in developmental genetics to identify biochemical sequences of reactions that occur during development. Findings at the embryonic, chick, and adult levels could be integrated to construct a detailed description of complex developmental process.

Undoubtedly, many other research areas could be mentioned where inbred lines are highly beneficial and their exclusion in this publication does not slight their importance. Apparently, as genetic research programs become more refined, the potential use of inbred lines increases.

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