AN INVESTIGATION OF FACTORS INFLUENCING THE SPONTANEOUS REGRESSION OF ROUS SARCOMAS OF CHICKENS

PAUL FRANCIS COTTER
AN INVESTIGATION OF FACTORS INFLUENCING THE SPONTANEOUS REGRESSION OF ROUS SARCOMAS OF CHICKENS

Keywords
Biology, General

This dissertation is available at University of New Hampshire Scholars' Repository: https://scholars.unh.edu/dissertation/1018
AN INVESTIGATION OF FACTORS INFLUENCING THE SPONTANEOUS REGRESSION OF ROUS SARCOMAS OF CHICKENS

by

PAUL FRANCIS COTTER
A.B., Suffolk University, 1966
M.S., Northeastern University, 1968

A THESIS

Submitted to the University of New Hampshire
In Partial Fulfillment of
The Requirements for the Degree of

Doctor of Philosophy
Graduate School
Genetics Program (Animal Sciences)
June, 1973
This thesis has been examined and approved.

W.M. Collins
W.M. Collins, Professor of Poultry Science and
Genetics, Chairman

W.R. Dunlop
W.R. Dunlop, Professor of Poultry Science

F.K. Hoornbeek
F.K. Hoornbeek, Associate Professor of
Zoology and Genetics

D.M. Green
D.M. Green, Professor of Biochemistry and
Genetics

L.W. Shierman
L.W. Shierman, Associate Professor of Pathology.
New York Medical College, Vahalla, NY.

W.C. Skoglund
W.C. Skoglund, Professor of Poultry Science

May 14, 1973
ACKNOWLEDGEMENTS

The author wishes to express his appreciation to Dr. W.M. Collins, his advisor, and to Dr. W.R. Dunlop, for their guidance and advice throughout this program. To Drs. F.K. Hoornbeek and D.M. Green for their willingness to serve on his thesis committee. To Dr. L.W. Schierman of the New York Medical College for serving as a member of the thesis committee and for his advice during this study. To Dr. W.C. Skoglund, Chairman of the Animal Sciences Department, for his encouragement.

A special thanks is extended to Dr. A.C. Corbett for helpful discussions of poultry pathology throughout this study.

The author wishes to express his gratitude to Mrs. K. Moore and Mrs. G. Amazeen for their cooperation and assistance. To all members of the Animal Sciences Department, and especially to the Poultry Farm workers for their assistance.

A further acknowledgement is made to Hubbard Farms Inc., Walpole, N.H. for providing the Hubbard Farms Graduate Fellowship, and to the Graduate School for providing a Dissertation Fellowship for Semester II, 1973.
RESULTS AND DISCUSSION ........................................ 26

Incidence of Regression in Lines 105 and 6
Inoculated at Various Ages with RSV-1, RSV-2,
and RSV-49 .................................................. 26

The Incidence of Regression as a Function of
the Age of the Host at Inoculation ...................... 32

The Effect of Bursectomy on the Incidence of
Regression in Line 105 and Line 6 ......................... 37

The Role of the Thymus ...................................... 40

The In Vitro Detection of Cellular Immunity to
Tumor Cells by Use of the Migration Inhibition
Reaction ....................................................... 43

Histological Examination of Two Rous Tumors ......... 48

GENERAL DISCUSSION .......................................... 50
CONCLUSIONS .................................................... 54
BIBLIOGRAPHY .................................................. 86
APPENDIX ....................................................... 91
BIOGRAPHICAL DATA .......................................... 102
LIST OF TABLES

1. Results of inoculation of Line 105 chicks with different dilutions of RSV-1, at one and 1½ days of age ........ 56

2. Results of inoculation of Line 105 chicks with different dilutions of RSV-1, at 3 and 5 weeks of age ........ 57

3. Results of inoculation of Line 105 chicks with different dilutions of RSV-1, at 3 and 6 weeks of age ........ 58

4. Percentage of Line 105 chicks having a score greater than 1 at one week post-inoculation .................. 59

5. Results of two later trials with Line 105 chicks inoculated with RSV-1 at 6 weeks of age ......................... 60

6. Results of inoculation of Line 6 chicks with RSV-1 and RSV-2 at 6 weeks of age .............................. 61

7. Incidence of regression of RSV-1 tumors in chicks from matings of Line 6 regressor males x Line 6 regressor females ............................................. 62

8. The incidence of regression in Line 105 and Line 6 chicks inoculated at 6 weeks of age with RSV-49 ........ 63

9. The results of inoculation of Line 6 chicks with RSV-1 at 1, 1½, and 28 days of age .............................. 64

9a. The analysis of variance of the data of Table 9 .............. 65

10. The incidence of regression of RSV-1 induced tumors in Line 105 chickens surgically bursectomized at hatching ... 66

11. The incidence of regression of RSV-49 induced tumors in Line 105 chicks bursectomized in ovo by testosterone propionate .................................................. 67

12. The incidence of regression and metastases in Line 6 chickens thymectomized at hatching ........................ 68

13. The incidence of tumor regression and metastases in Line 6 chickens thymectomized at hatching and restored at two weeks of age ........................................ 69

14. The incidence of regression and metastases in Line 6 (subline 1) chickens thymectomized and x-irradiated at hatching ......................................................... 70
15. The incidence of regression and metastases in Line 6 (subline 1) chickens thymectomized and X-irradiated at hatching and restored at two weeks of age ........... 71

16. The results of the migration inhibition test of chickens of Line 6 ................................................. 72

16a. Analysis of variance of the data of Table 12 ............... 73
LIST OF FIGURES

Figure 1. Growth rates of RSV-1 induced tumors of Line 6 inoculated at 1, 14 and 28 days of age, respectively .................................... 75
Figure 2. Thymus graft from Line 6 chick "restored" at two weeks of age ................................ 77
Figure 3. Size 1, RSV-1 induced tumor of Line 6 ............... 79
Figure 4. Size 2, RSV-1 induced tumor of Line 6 ............... 81
Figure 5. Liver of Line 6 chicken showing metastases .......... 83
Figure 6. Line 6 chick inoculated at hatching with RSV-1 ...... 85
Spontaneous regression of tumors induced by Rous sarcoma virus (RSV) was studied in two lines of chickens. Chickens were inoculated in the wing-web with purified preparations of RSV-1, RSV-2, and RSV-49. The effect of host age at inoculation was studied at various ages at inoculation from one day to 6 weeks. Bursectomy, thymectomy, and the macrophage migration inhibition reaction were used to investigate immunological aspects of tumor regression.

The incidence of spontaneous regression was approximately 6 percent for RSV-1 induced tumors of UNH Line 105, and approximately 60 percent for RSV-1 and RSV-2 induced tumors of RPRL Line 6 when a $10^{-3}$ dilution of stock virus was given at 6 weeks of age. The incidence of regression of RSV-49 induced tumors was approximately 10 and 86 percent for Line 105 and Line 6 chicks, respectively, when inoculated at 6 weeks of age.

The incidence of regression was lower when chicks of either line were inoculated prior to 6 weeks of age. There was a higher incidence of metastases in Line 6 chicks inoculated prior to 6 weeks of age. The incidence of metastases was found to be 100, 85, and 35 percent, respectively, in Line 6 chicks inoculated at one, fourteen, and twenty-eight days of age.

Bursectomy, whether performed chemically in ovo by testosterone propionate or surgically at hatching, was ineffective in altering the incidence of regression in Line 6 chicks inoculated at 6 weeks of age.
Neonatal thymectomy, on the other hand, was associated with a decrease in the incidence of regression, and an increase in the incidence of metastases in Line 6 chicks inoculated with RSV-1 at 6 weeks of age.

A delayed hypersensitivity reaction was demonstrated in regressor chicks in vitro, which was reduced or absent in chicks with progressively growing tumors.
Rous sarcoma virus-induced tumors of chickens generally either grow progressively ultimately killing the host, or, after a short period of growth regress completely. When the latter occurs the host remains alive and usually becomes immune to further virus challenge (Freire et al., 1953). Since tumor regression is not a consistent occurrence it is imperative that those factors which determine whether a tumor will grow progressively, or grow for a time and then regress be understood.

Since it is already known that cellular susceptibility to infection by Rous virus is genetically controlled in chickens (Crittenden et al., 1967) it would be of interest to know if tumor regression is also influenced by host genotype. If regression is under genetic control does the mechanism involved represent a specific response to Rous virus induced tumors, or rather, is it an example of a much broader type of disease resistance. Moreover, is tumor regression the result of action by one or several genes and are there pleiotropic effects associated with the gene(s)? These questions are of the utmost importance to investigators using the chicken as a research tool in cancer work.

Some aspects of tumor regression seem to suggest that this phenomenon is a type of delayed hypersensitivity reaction. For example, one might compare tumor regression to an allograft rejection reaction. The tumor, like an allograft, possesses antigens not found on host tissue (Huebner, 1971). These are recognized as "foreign" by the immunologic surveillance system of the host (Burnet, 1970). Thus, the host
is stimulated to produce the necessary immunologic response which results in rejection (regression). This analogy may be an oversimplification but if the comparison were legitimate, one could then proceed to investigate tumor regression using an experimental approach similar to that used in investigating other types of delayed hypersensitivity reactions.

If the analogy just described were extended, those genetic and immunological parameters that are important in allograft reactions could be important phenomena underlying tumor regression. Several fundamental questions arise. Do regressions occur more frequently in one breed, strain or line of chickens than in another? Do tumors produced by virus of one subgroup regress as often as those produced by virus of another subgroup? Does the age of the host at inoculation influence the regression incidence? What is the nature of the immunologic response involved? Is the immunologic response thymus dependent or bursa of Fabricius dependent or may both systems be involved?

These questions, and others, deal for the most part with a specific case, i.e. the regression of an artificially induced tumor. A tacit assumption underlying this specific case is that it is related in some way to events that can, or do, occur in naturally induced (field cases) of neoplastic diseases. One may argue that the regression of a tumor induced by artificial exposure (injection) of a laboratory strain of virus is not a useful model in an analysis of naturally occurring events. There may be some truth to this argument. Nevertheless, there does exist in some chickens the genetic, thus the immunologic, capability for tumor regression. It may be that the same gene(s) responsible for regression of artificially induced tumors are responsible for a "second line" of defense against naturally occurring diseases. This
would be the type of defense mechanism operating after the host is infected by virus. The defense process might involve the elimination of neoplastic tissue once it has appeared, or the restriction of metastases. It is conceivable, and probable, that the genetic properties of a host chicken that enable it to regress an artificially induced tumor, on the one hand, would at the same time play a role in the defense against naturally occurring disease on the other.

With these thoughts in mind I have undertaken this study of factors underlying tumor regression in the chicken. Hopefully, the results of my experimentation and conclusions drawn therefrom will make some contribution, however small, to an understanding of this important but complex problem in cancer research. Moreover, I hope that this research will help bridge the gap between purely genetic and purely immunologic approaches to cancer research.
REVIEW OF LITERATURE

Early Reports of Regression of Rous Sarcomas of Chickens

An early observation of a regression of a chicken tumor was made by Rous (1910) who reported the "retrogression" of a spindle cell sarcoma in two of three market bought Plymouth Rock chickens less than three months old. Although his primary interest was in the transplantability of these tumors to other chickens, not in their regression, he concluded that "the resistance which in some individuals prevents the growth of the implanted tumor is a resistance directed against the graft as a strange tissue and is unconnected with the neoplastic qualities which this happens to possess". In a later report Rous and Murphy (1912) noted inflammatory reactions occurring in situ about tumor grafts. They reported that retrogression of well developed sarcomas was infrequent, but when occurring produced resistance to subsequent tumor grafts. Rous (1913) concluded that there were two sorts of resistance to avian tumors, one directed against the implanted tumor cells, the other directed against the etiologic agent causing the tumor. He stated that these two types of resistance seemed to be independent in that none, one, or both types may be present in an individual chicken.

Roussy et al., (1932) reported the occurrence of regression in 15 of 55 chickens receiving pectoral muscle grafts of a tumor which resembled histologically the Rous tumor. They also reported the regular occurrence of metastases during normal tumor development.

Banting and Gairns (1934) reported several instances of
regression of chicken tumors. In one instance, a chicken which had received tumor transplants in both breasts developed small tumors which later regressed. A regression occurred in another chicken leaving a small cyst at the tumor site. This bird received five more transplants at one month intervals. Tiny nodules developed on two occasions and these later regressed. These workers concluded that regression varies with the type of tumor. The slowly-growing, hard, fibrous tumor regresses slowly and disappears completely leaving a soft normal muscle. A rapidly-growing, soft tumor first develops a line of demarcation between itself and the muscle, begins to shrink, and the skin over it becomes normal in appearance. The tumor gradually separates from the muscle and a cyst is formed which may persist for sometime.

El Dardiry et al., (1952) reported that 21-day-old chickens of inbred RPRL Line 6 showed considerable resistance to inocula derived from a lymphomatous liver. Additionally, certain chickens of this line inoculated in the pectoral muscle developed tumors which started to regress on the 14th day post-inoculation. If the bird lived, regression was nearly complete by the 28th day post-inoculation.

The regression of several Rous sarcomas was reported by Epstein (1952). Several chickens were injected with a low titer Rous virus. Two of four tumors produced by one virus preparation with a titer of \(10^{-0}\) (prepared from undiluted, disintegrated, Rous tumors) regressed. Only one tumor out of ten regressed, however, when a high titer \(10^{-4}\) virus preparation was used.

Munroe and Southam (1958) reported a regression incidence of approximately 2 percent in White Leghorn chickens inoculated in the wing web with a \(10^{-2}\) dilution of a Rous sarcoma virus preparation at
3 to 5 days of age. These workers were primarily interested in determining whether systemic virus distribution and viremia occurred during the incubation period (latent period) following local inoculation. They concluded that, indeed, in 5 day-old chicks systemic virus distribution and viremia did occur in association with localized inoculation of virus. The pattern of virus distribution in time and in various tissues suggested that visceral tumors may result from viral distribution as well as from cellular metastases, and that the virus may go through a transient non-recoverable phase in the wing web and viscera.

Dinowitz and Rabin (1966) reported regression in 17 of 42 RSV tumors induced in 4 to 8 week-old White Leghorns. Regressing and progressing tumors had mean latent periods of 10.2 and 8.8 days, respectively, this difference being statistically significant. Regressing tumors did not grow to the same size as progressive ones during a comparable period. Regressing tumors contained very little RSV in tumor homogenates or in tissue culture fluids assayed over a long period. No evidence of interferon was found in either tumor homogenates or in tissue culture fluids of 7 regressing tumors.

Two chickens with completely regressed tumors were inoculated with RSV in the wing opposite that of the original tumors. They developed progressive tumors at the site of the original tumor, but not at the site of the challenge inoculation. These authors suggested that this recurrence of tumors may have been caused by stimulation to RSV production of cells containing the virus genome.
Gyles et al., (1967,b) reported that size, score, and speed of development of progressive tumors, from subcutaneous inoculations with RSV, gave the same relative rankings of susceptibility as inoculations of RSV on the CAM's of embryos. They suggested that the criteria of tumor development (i.e. negative, progressive, regressive) may be used to gauge differences in degrees of susceptibility to RSV between individuals, families and strains of chickens.

The Development of Genetically Well-Charcterized Lines for Use In Avian Leukosis Research.

Early studies of the regression phenomenon were hampered by the unavailability of genetically well characterized lines. Moreover, in some instances birds with regressed tumors were used in development of leukemia-resistant lines. Negative birds (birds not developing tumors after virus challenge) were not always used for this purpose because it was not known that resistance versus susceptibility to Rous virus was genetically controlled. Thus, these birds were not considered to be important.

Chickens of known susceptibility and resistance to Rous virus became available, however, with the development of inbred lines of White Leghorns by N. F. Waters at the Regional Poultry Research Laboratory, East Lansing, Michigan, beginning in 1939 (Waters and Bywaters, 1940). A genetic approach to the study of the diseases of the avian leukemia complex, of which Rous virus induced tumors are a part, required the formation of families inherently resistant or susceptible to the complex. This was accomplished by selection and intensive inbreeding.
Another program designed for this same purpose was initiated by A. W. Greenwood of the Institute of Animal Genetics, Edinburgh, Scotland, (Greenwood et al., 1948). A Brown Leghorn flock was subdivided into a number of separate inbred lines each selected for some special trait influencing egg production. The flock was believed to be highly resistant to neoplastic diseases since the annual mortality rate resulting from these diseases was about 1.6 percent. A nonsusceptible (NS) line was established by mating birds which had regressed tumors. The progeny of this line continued to show, for the most part, either complete regressions or a small tumor.

Characterization of the Etiologic Agent of Rous Tumors.

The etiologic agent causing fowl tumors was demonstrated to be filterable by Rous and Murphy (1914). It was from this agent that the present day Rous sarcoma virus (RSV) was derived. It produced then, as now, spindle-cell tumors that appeared promptly in susceptible hosts, within 2 to 3 weeks, post-inoculation. Additionally, it was mentioned in this early report that the dosage of the agent was an important factor in determining whether the resulting tumor would be progressive or regressive.

Rous virus particles were seen regularly by electron microscopy and appeared identical in sarcomas and leukemias. They had a central core, surrounded by an inner and an outer membrane, and were called "C-type" virus particles (Bernhard, 1960).

Rous viruses consist of an RNA nucleoid surrounded by an inner membrane and an outer protein envelope containing two or more
glycoproteins. The envelope contains the viral subgroup-and type-specific antigens and also may contain some host-cell specified material (Temin, 1971).

The Histology of Rous Tumors.

A comprehensive cytological study of Rous sarcomas of chickens was made by Levine (1939). Histological sections of both progressing and regressing tumors were prepared and the types of cells observed in both were described. Chickens received injections of dessicates or filtrates of the Rous tumor. Tissues were removed and examined at various times ranging from a few minutes to 73 days post-inoculation. The presence of inflammatory-cells, monocytes, fibroblast-like cells, etc., was described. It was suggested that monocytes invaded the area immediately surrounding the injection site and that these cells became modified into fibroblast-like cells which made up the bulk of the tumor.

On the other hand, Loomis and Pratt (1956) studied large numbers of chickens that received inocula of partially purified Rous virus. They identified rows of altered subcutaneous fibroblasts within 72 hours post-inoculation. These disappeared concomitantly with the appearance of characteristic early tumor cells. They suggested that the subcutaneous fibroblast was the cellular component of normal tissue from which the tumor cell of Rous sarcoma is derived.

Histological examination of RSV tumors from Rous associated virus (RAV) tolerant birds was made by Rubin (1962). These tumors invariably grew progressively and consisted of spindle cells and round cells with highly basophilic cytoplasm. Later, round cells with
abundant cytoplasm were seen with increasing frequency. Rubin suggested that the cell-rounding represented a late stage of the infectious process. Lymphocytes were usually absent in the tumors, but when they occurred they were restricted to small discrete areas. This was in contrast to the general occurrence of infiltrating lymphocytes seen in tumors from control chickens.

Stenkvist and Ponten (1963) investigated the growth curves, the histology and the virus titers of both progressing and regressing Rous sarcomas using non-inbred White Leghorn cockerels 24 days old at the time of RSV injection. Progressing tumors contained more infective virus than regressing tumors. Neither the rate of growth, the histological appearance, nor the virus content of the tumors that grew progressively until the death of the animals, or of the tumors that eventually regressed, differed significantly until 25 days after virus inoculation. At that time the tumor either continued to progress, or regressed. Tumors in a regressing phase showed an increased infiltration of lymphocytes, hemorrhages, and necrosis, and in advanced stages, fibrosis. Progressive and regressive tumors were never found in the same bird. They suggested that sustained progressive growth of Rous tumors is normally only possible if normal cells are continuously "converted" into Rous cells by released virus.

The Effect of Host Age at Inoculation on Regression Incidence.

Freire et al., (1953,a) studied the growth and regression of Rous sarcomas as a function of the age of the host. Regression occurred in 25 of 165 adult Plymouth Rock chickens bearing primary Rous tumors induced either by tumor cell suspensions or by cell-free filtrates.
The age of the adults at the time of injection varied from 6 to 32 months with a predominance of birds approximately 10 months of age. No regressions occurred in 1,328 young chickens of the same breed, inoculated at 15 days of age with the same preparation.

Freire et al., (1953,a) reported that as the age of tumor material used for inoculation of young chickens increased, the incidence of metastases decreased.

The filterability of tumors (free virus) was found by Duran-Reynals and Freire (1953) to be inversely related to the age of the tumor and to the age of the host. Free virus was more frequently present in tumors induced by cell suspensions than in those induced by filtrates. As the age of the tumor increased, its filterability decreased. The occurrence of metastases was directly related to the filterability of the tumors and to their transmissability by cells. Thus, regression as well as the incidence of metastases was concluded to be the result of change in the virus, not in the host.

A most interesting phenomenon occurred (Duran-Reynals and Freire, 1953) when cells obtained from non-filterable tumors were passaged in other hosts, usually young chickens. All resulting tumors yielded active filtrates. This would appear to be an early observation of "genome rescue" (Katz and Kohn, 1971; Sarma, et al., 1966), a phenomenon which occurs when certain non-virus producing cells are co-cultivated with susceptible chick embryo fibroblasts in vitro in the presence of a helper virus of the avian leukosis-sarcoma group.
The Role of The Immune Response in Tumor Regression.

The antiviral immune response. Freire et al., (1953,b) reported that regression of Rous tumors usually was followed by immunity to further virus challenge. Vigier (1958) made a quantitative investigation of the growth of dermal (Rous) sarcomas and of the formation of neutralizing antibodies in White Leghorns. In one experiment, regression occurred in 4 of 17 chickens. He suggested that regression was induced by a particular mechanism, the intervention of anti-tissue, antisarcoma antibodies (distinct from antiviral antibodies) on the growth of the tumor.

Dougherty et al., (1960) attempted to quantify the relationship between infecting dose of Rous sarcoma virus, antiviral immune response, and tumor growth in White Leghorn chickens. With large infecting doses of RSV the relationship between the development of a "size 3" tumor (1 gram of tumor tissue) and the initial production of antibody was relatively linear, but less clear if low infecting doses of RSV were used to initiate the tumor. They had difficulty estimating the rate of tumor growth when low infecting doses were used because of frequent regressions. They found no apparent relationship between the rate of tumor growth and the rate or magnitude of the antiviral immune response or the final fate of the infected bird. Regressions did not appear to be related to the antiviral immune response.

Passive immunization experiments (Dougherty et al., 1960) demonstrated that high levels of circulating antibody can affect susceptibility of chickens to RSV. These effects were limited to a transient delay in appearance and a slight reduction in incidence of tumors when low infecting doses were used. They concluded that a
reduction in tumor growth rate coincident with the appearance of antibody depended on factors other than antibody per se. A change in growth rate of tumors in older birds, but not in very young chickens, suggested that some host defense mechanism other than antibody in older birds was influencing tumor growth rate (and regression).

The role of cell mediated immunity in tumor regression. Rous and Murphy (1912) noted that an inflammatory reaction occurred in the area of a tumor graft. Freire et al., (1953,b) observed an inflammatory reaction with conspicuous infiltration of lymphoid-like cells and pronounced muscle necrosis following the inoculation of tumor cells into immune chickens.

An extensive investigation of the immunological basis for "non-infective" (non-virus yielding) Rous sarcomas was conducted by Rubin (1962). The infective virus content of tumors was found to decline as they grew older. The correlation between the virus content of the homogenate and the virus producing potential of washed intact cells in any given tumor was high.

Tumors from birds infected at one week of age or younger remained highly infective even when harvested as late as 5 to 6 weeks after infection. That high virus yield was obtained even after the age of immunological competence of the host suggested that the chicken had to become tolerant to tumor antigens resulting from early and continuing exposure to high antigen concentrations.

Lymphocytic infiltration, evident even in the earliest tumors, became more marked with time and was accompanied by a connective tissue reaction which tended to separate the tumor into nodules. Heavily infiltrated tumors yielded little or no virus and contained
many swollen, highly vacuolated tumor cells. Rubin concluded that (1) the lymphocyte figures prominently in the infiltration of non-infective tumors and in tumor regression and (2) that lymphocytic infiltration of Rous sarcomas represented a cell-mediated immunological response to new antigens located in the tumor.

The role of the bursa of Fabricius. The bursa of Fabricius in chickens plays a role in the development of humoral immunity to certain antigens. Early removal of the bursa significantly impairs or eliminates future antibody production, Glick et al., (1956). Peterson et al., (1964) demonstrated that surgical removal of the bursa at hatching and at 29 days of age prevented the development of visceral lymphomatosis ordinarily induced by the RPRL-12 virus. Visceral lymphomatosis is a member of the avian leukemia-sarcoma complex. Peterson et al., (1966) demonstrated that visceral lymphomatosis is a malignancy arising exclusively from that component of the lymphoid tissue derived from and/or dependent upon the bursa for its development.

The role of the thymus gland. The chicken lymphoid system is composed of two major cell systems, Cooper et al., (1966). The thymus is necessary for the development of a widespread cell population which consists mainly of small lymphocytes. The bursa of Fabricius, on the other hand, appears to be the site of origin for a cell system represented in peripheral tissues by larger lymphocytes found in germinal centers, and by plasma cells. The thymus and the system of lymphocytes dependent upon it play the same functional role in chickens and mammals. These thymus derived lymphocytes are effectors of delayed hypersensitivity, of graft-versus-host reactions, and are the major elements in homograft rejection.
Radzichovskaja (1967) reported that the latent period of RSV induced tumors was 3 to 4 days shorter in thymectomized than in control chicks. Thymectomized chicks, moreover, had a higher frequency of susceptibility to higher dilutions of virus and a higher incidence of metastases than controls.

**The Role of Genetics in the Regression of Rous Tumors.**

The heritable nature of non-susceptibility to Rous virus infection and regression of Rous sarcomas was indicated by Greenwood et al., (1948). The distribution of responses to Rous virus challenge of the progeny of a single sire mated to fourteen dams was given. This distribution clearly indicated some offspring to be non-susceptible to infection, some susceptible, and susceptible chickens to have either regressive or progressive tumor growth, depending upon the dam.

Gyles et al., (1967,a), investigated the response of Giant Jungle Fowl, White Leghorns, and their F₁ and F₂ generation crosses to subcutaneous inoculations of RSV at 5 weeks of age. The White Leghorns had a regression incidence of approximately 3 percent, the Giant Jungle Fowl approximately 12 percent. The incidence of regression in the F₁ generation was slightly over 22 percent and the F₂ generation 11 percent. The striking increase in regression incidence in the F₁ generation was interpreted as being due to overdominance.

Progressive tumors emerged more quickly, developed more rapidly and reached a larger maximum size than tumors which ultimately regressed (Gyles et al., 1967,b). This was interpreted as indicating the presence of a mechanism of resistance that delayed the emergence of a regressive tumor, continued resistance to its development, ultimately forcing it to regress. Since this mechanism appeared early in
tumor development, it seemed likely that it might have a genetic basis.

The sexes did not differ in the development of either progressive or regressive tumors during the periods of tumor growth. Tumors in males regressed more quickly than in females when measured by size and score at various times after inoculation and by speed of regression.

Gyles et al., (1968), concluded that if the dilution of the virus is sufficiently low to overcome the resistance to cell transformation to malignancy, a tumor is formed. At that time in tumor development, another genetic resistance mechanism becomes involved which subsequently may cause regression.

Gyles and Brown (1971) selected chickens for high incidence of regression of tumors induced by RSV. Breeders to produce the first, second, and third generations of selection were chosen entirely on individual performance with regard to tumor regression. Preference was given to those individuals with the larger tumors that regressed. Breeders to produce the fourth, fifth and sixth generations were chosen on a combination of full-sib family performance and individual performance within selected families. Full-sib families selected were those having the highest percentage of regressive tumors based on the number of birds inoculated. Individuals within these selected families were chosen on their ability to regress larger sized tumors. The percentage of tumor regressions increased by 45 percent over unselected controls over 6 generations of selection. This experiment indicated a significant genetic influence on regression of Rous sarcomas of chickens.

Carte et al., (1972) selected single comb White Leghorns for
increased incidence of regression of RSV-1 induced wing-web tumors. After 4 generations the incidence of regression in the selected line was 4 times higher than that of the unselected control line. In serum neutralization tests birds with regressive tumors had higher antiviral antibody titer than did progressors or birds that failed to develop a tumor. Evidently, the selected line lived 47 and 70 percent better, respectively, than the control line when challenged with Marek's disease virus. It was concluded that selection for regression of RSV-1 wing web tumors had concomitantly increased the ability of the line to produce specific antibodies and that this latter response was genetic.
OBJECTIVES

1. To determine the incidence of spontaneous regression of Rous sarcoma virus induced tumors in RPRL Line 6 and UNH Line 105 chickens.

2. To determine the effects of host age at inoculation on regression incidence.

3. To determine whether or not cell-mediated and/or humoral immunity have a role in tumor regression.
EXPERIMENTAL METHODS AND PROCEDURES

Description of Lines.

RPRL Line 6. A single comb White Leghorn line was developed from hatching eggs obtained by the Regional Poultry Research Laboratory, East Lansing, Michigan, in the spring of 1939 (Waters, 1940). This was one of fifteen lines developed to provide effective control methods for the study of the avian leukosis complex. The genetic approach to this problem called for the formation of families inherently resistant or susceptible to the complex. While susceptible families would be of little economic value, their genetic importance would be extensive, for 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 studies of pathology.

This line was maintained with four mating pens, each containing one male and 25 females. Usually four sires and 2 to 3 dams per sire contributed progeny to the next generation. Brother-sister matings were not strictly adhered to in early generations, but they occurred quite frequently. More often than not, closely related individuals (half sibs and first cousins) were mated (Waters, 1945).

By 1951 nine of the fifteen original lines were eliminated because of poor productivity, lack of desirable traits for disease study, or both. Inbred lines 6,7,9,10,14,15, and 151 remained, each with individual inbreeding coefficients in excess of 0.95 (Waters and Fontes, 1960).
In 1962 Dr. L. B. Crittenden initiated a brother-sister mating program for all inbred lines, including Line 6. Each line was maintained with from 8 to 12 sires and from 7 to 10 dams per sire. Selection of breeders for brother-sister matings to produce the next generation was based upon egg production, percent fertility, and percent hatchability of the sire families; early chick and brooding viability and the number of chicks available per dam family.

Crittenden et al., (1967) and Crittenden (1968) reported that Line 6 was homozygous susceptible to subgroups A and B of the leukosis-sarcoma group of the avian leukemia complex, relatively resistant to subsequent tumor induction by viruses of these subgroups and quite resistant to Marek's disease.

RPRL Line 6, subline 1 (6\textsubscript{1}). This line was derived from Line 6 in 1962 by Dr. L. B. Crittenden by individual brother-sister matings within inbred Line 6 (Stone, personal communication). The objective was 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. Histocompatibility was measured by acceptance or rejection of donor wattle tissues which were grafted onto the recipient's shank (Purchase 1967). Line 6\textsubscript{1}, had no rejection of tissue within or between sire families. This, in conjunction with 100 percent acceptance of grafts for the three previous generations, suggested that Line 6\textsubscript{1}, was indeed histocompatible. The theoretical individual inbreeding coefficient for this subline is in excess of 0.99 (Stone, personal communication).

UNH Line 105. This is an experimental line that has been maintained by a commercial breeder since 1930 when it was derived from
the Rhode Island Red breed (Savage, personal communication). A sample of this stock was obtained from the breeder in 1968 and has been maintained by the Department of Animal Sciences, University of New Hampshire, since that time. It is known to be highly susceptible to viruses of subgroup A of the avian leukosis-sarcoma complex, fairly resistant to viruses of subgroup B, and segregating for susceptibility to viruses of subgroup C (Collins, unpublished data and Table 7).

Virus Stocks.

Three highly purified virus stocks were kindly provided by Dr. L. B. Crittenden, Avian Physiology Laboratory, A.R.S., United States Department of Agriculture.

**BH-RSV (RAV-1).** This virus is a member of subgroup A of the avian leukosis-sarcoma complex. It was originally isolated from a preparation of the Bryan high titer strain of RSV by Vogt (1965). The Bryan high titer strain of RSV is defective and requires a helper virus to achieve the maturation of infectious particles. When Rous associated virus, RAV-1, a helper virus, is used to activate RSV from non-virus producing cells which have been transformed into sarcoma cells by infection with RSV, the RSV which emerges (BH-RSV (RAV-1)) possesses the same outer coat as the helper virus used in its activation. This new virus is referred to as a pseudotype of RSV because it has the same genome as RSV but is of a different antigenic type (Rubin, 1965). BH-RSV (RAV-1) is symbolized RSV-1.

**BH-RSV (RAV-2).** This virus is a member of subgroup B of the avian leukosis sarcoma complex. It is produced in a similar manner as RSV-1, but in this case Rous associated virus, RAV-2, is used as the
helper virus. It is antigenically distinct from RSV-1, but contains the same genome. BH-RSV (RAV-1) is symbolized, RSV-2.

**BH-RSV (RAV-49).** This is a member of subgroup C of the avian leukosis-sarcoma complex. This is antigenically distinct from viruses which are members of subgroups A and B as demonstrated by host range and viral interference properties (Duff and Vogt, 1969).

BH-RSV (RAV-49) is symbolized, RSV-49.

The virus stocks were stored under liquid-nitrogen until used. At that time the stock virus was diluted with Hank's balanced salt solution (HBSS) to a final concentration of $10^{-1}$, $10^{-2}$, etc., depending on the need for a particular experiment.

Inoculation of chicks.

The left wing web area of the chicks to be inoculated was moistened with 95 percent ethanol. A virus suspension of 0.1 ml. per chick was injected subcutaneously. Care was taken to ensure the formation of a "blister-like" swelling at the site of the injection. Leakage of the inoculum by this procedure was minimized.

Examination of Tumors.

In early experiments, when it was desirable to observe the tumor latent period, daily examinations of the wing web area were made. The date of the first visible appearance of the tumor was recorded. Later, observations were made at weekly intervals.

A subjective method of scoring tumor size was used. Scores ranged from 0 to 4 based on the size of the tumor as follows:
0 = No tumor present
1 = Small pimple-like protuberance in the skin, no discoloration
2 = Larger protuberance, with discoloration
3 = Wing-web area almost entirely filled with tumor
4 = Massive tumor, often with ruptured surface, completely filling wing web area

A tumor was considered regressed only after complete disappearance of any visible or palpable mass and after 3 consecutive zero scores. Some tumors showed partial regression. For example, a given tumor might reach a score of 3 to 4 and then regress to a score of one and remain at that classification for the duration of the experiment. This occurred more frequently in Line 6 than in Line 105. Such birds were not classified as regressors and therefore not included in the calculation of regression incidence.

Surgical Procedures.

In order to understand the role of the immune mechanism in tumor regression it was necessary to isolate the effects of either the bursal or the thymus system. This was accomplished by removal of the bursa or the thymus at hatching allowing study of the regression response in chicks with either, but not both, an intact bursal or an intact thymus system.

In one study of the role of the thymus, x-irradiation was combined with surgical thymectomy in order to more completely eliminate the immune response of this system. A study of the role of the thymus
was also made by restoration experiments in which thymus grafts were implanted in thymectomized chicks to determine whether the return of thymic function to thymectomized chicks was possible.

**Bursectomy.** Surgical bursectomy was performed at hatching by blunt dissection without anesthesia. The detailed procedure appears in the Appendix.

**Thymectomy.** This was performed at hatching by a technique described by Aspinall et al., (1963). The detailed procedure appears in the Appendix.

**Restoration of thymus.** Thymic lobes, obtained from intact birds (Line 6) were placed in a subcutaneous space made by inserting blunt forceps through an incision made in the skin covering the thoracic vertebrae. The detailed procedure appears in the Appendix.

**Chemical bursectomy.** This was performed by dipping eggs into a 1.5 gram percent solution of testosterone propionate (Calbiochem, #5817) for 5 seconds on the third day of incubation (Glick, 1961). The detailed procedure appears in the Appendix.

**X-irradiation.** Irradiation was given on the day after hatching and surgery. The chickens were placed in a wire cage (21x13x8.5 cm.) 85 cm. below the source. The x-rays were generated by a Westinghouse 150 KV x-ray machine. The conditions of irradiation were as follows: 110 KV, 15 milliamps. The dosage in each experiment was 500 roentgens (r) in air at the surface at a dose rate of 50 r per minute in air.

The Migration Inhibition Test.

This test was used to demonstrate the presence or absence of delayed hypersensitivity in chickens with progressing or regressing
tumors. The procedure used was a modification of the technique developed by David et al., (1964). The in vitro migration ability of buffy coat cells exposed to tumor extract obtained from chickens with progressing, or regressed, Rous sarcomas was compared with that of the same cells not exposed to antigen. The detailed procedure is given in the Appendix.
RESULTS AND DISCUSSION

Incidence of Regression in Lines 105 and 6 Injected at Various Ages With RSV-1, RSV-2, and RSV-49.

Line 105, RSV-1. The results of inoculation of day-old Line 105 chicks with RSV-1 are given in the top half of Table 1. All chicks presumably were homozygous susceptible at the tumor virus A (tva) locus (Crittenden et al., 1967) based upon a chorioallantoic membrane (CAM) test of full sib embryos. Nine of eleven inoculated chicks developed tumors which grew progressively, ultimately killing the hosts. The two remaining chicks did not develop tumors and remained alive until discarded 6 weeks later.

The relatively short latent period suggested that the virus preparation used was quite potent even at dilutions of $10^{-2}$ and $10^{-3}$. The titer of the original virus stock was $10^{-4}$ based on a CAM test of susceptible embryos (Collins et al., unpublished data). The lack of tumor production in two of the four chicks in the $10^{-2}$ group may have resulted from any one, or combinations of the following factors: (1) the presumptive genotype (aa) may have been incorrect, (2) there may have been leakage of the inoculum from the chick prior to absorption of the virus by susceptible cells, (3) the presence of a high titer of maternal antibody to RSV may have prevented infection (Dougherty et al., 1960), and (4) the possible presence of resistance inducing factor (RIF) prevented virus infection (Rubin, 1960).

The results of inoculation of 2-week-old Line 105 chicks are given in the lower half of Table 1. The average latent period was
8.7 days, slightly longer than that for day-old chicks. The lack of tumor production in all birds may have been due to one or several of the reasons mentioned above. However, it should be noted that none of the six birds receiving an inoculum diluted to $10^{-5}$ developed tumors. This was most probably because the virus titer had been exceeded. One regression was observed which indicated that at least some chicks of this line could have regressive tumors.

The results of inoculation of Line 105 chicks presumed to be heterozygous ($a^s a^r$) at the tva locus are given in Table 2. The chicks used in this experiment were 3 and 5 weeks old, respectively, at inoculation. The relatively longer mean latent period for chicks inoculated with a $10^{-4}$ dilution at both 3 and 5 weeks of age compared with those inoculated with inoculum of the same dilution, and at 2 weeks (8.7 days), in Table 1, indicate a trend toward a longer latent period in older birds. One chicken inoculated with a $10^{-4}$ dilution at 3 weeks of age regressed its tumor.

No drastic increase in the incidence of tumor regressions was observed when the infected chicks were heterozygous ($a^s a^r$) at the tva locus (compare Tables 1 and 2 and see Table 3). This suggested that the type of resistance demonstrated by "regressor chicks" is different from that possessed by "negative chicks" ($a^r a^r$). The tva locus is known to control events in the earliest steps of viral replication in chick embryo fibroblasts "in vitro" (Crittenden and Briles, 1971). Tumor production in heterozygotes would indicate that susceptibility is at least partially dominant to resistance. However, it has been demonstrated that resistant as well as susceptible cells take up virus by pinocytosis and phagocytosis "in vitro" (Dyadkova et al., 1972).
This would imply that regression is not the result of a partial blockage of a cell's ability to be penetrated by virus. On the contrary, it is clear that cells of regressor chicks are penetrated and transformed by virus and that regression involves events that occur much later than those presently known to be under the control of the tv-a locus. Since regressions also occur in chicks homozygous susceptible at the tv-a locus (Tables 1 and 3) it is highly improbable that regression is the result of some type of intermediate susceptibility (or resistance) found in tv-a locus heterozygotes.

The tv-a locus could control such early events as deproteinization of virus particles (Dyadkova et al., 1972) or integration of the viral genome with that of the host genome. Genes controlling tumor regression, on the other hand, would probably be more concerned with such late events as cell transformation and production of tumor specific antigens.

The results of inoculation of 3 and 6 week-old (presumed a^s a^s) Line 105 chicks with RSV-1 are given in Table 3. Three dilutions of virus, 10^{-2}, 10^{-3}, and 10^{-4}, were used in each age group. None of the 77 chicks in the 3 week group regressed while 3 regressions occurred in the 6 week group, suggesting a possible age effect. Table 4 shows for the same experiment, the percentage of chicks having a tumor score greater than 1 at one week post-inoculation. These data give some indication of the speed of tumor development as related to both age at inoculation and strength of inoculum. Chicks receiving the highest concentration of virus (10^{-2}) in both age groups, had a higher proportion (77 percent) of tumors with scores greater than one at one week post-inoculation than did those injected with inoculum having
lower concentration of virus. These results are consistent with those of Dougherty et al. (1960) who found that chickens injected with 2000 pock forming units (PFU) developed tumors more rapidly than those injected with 200, 20, or 2 PFU.

The results of two later trials with presumed homozygous ss Line 105 inoculated with RSV-1 at a $10^{-3}$ dilution are given in Table 5. The incidence of regression was 6 percent in both trials. This suggested that 6 percent would have some value as an estimate of the expected regression incidence of Line 105 chicks inoculated with a $10^{-3}$ dilution of RSV-1 at 6 weeks of age.

Line 6, RSV-1 and RSV-2. The results of inoculation of Line 6 chicks with RSV-1 and RSV-2 are given in Table 6. This line was approximately 100 percent susceptible to each of these viruses (see Table 6). The regression incidence was similar for both viruses, being approximately 55 percent.

Cellular susceptibility in Line 6 to these two viruses is subgroup specific, and known to be controlled by autosomal dominant genes distinct for each viral subgroup and inherited independently (Crittenden et al., 1967). The similarity of the regression incidences of RSV-1 and RSV-2 induced tumors in Line 6 suggested, however, that regression as opposed to virus susceptibility, may be a group-specific rather than a subgroup specific phenomenon. This would seem to be a reasonable possibility since cellular susceptibility depends upon the type of protein coat on the virus during the early stages of infection (Crittenden and Briles, 1971; Dyadkova, 1972). Regression involves relatively later events such as cellular transformation, appearance of
tumor specific antigen(s), and tumor production itself. Both RSV-1 and RSV-2 possess the same nucleoid, that of the prototype virus, RSV (Duff and Vogt, 1969). Thus, once uncoating and other early events have taken place, cells of Line 6 infected by either RSV-1 or RSV-2 would probably have the same "neoplastic" characteristics because of the same infecting nucleoid. It is not surprising, therefore, to find a similar regression incidence in this line when either of these viruses is used as the inoculum.

Table 7 gives the results of inoculation at 6 weeks of age with RSV-1 of the progeny of Line 6 regressor males x Line 6 regressor females. The mean regression incidence was 19 percent, a substantial decrease in regression incidence when compared with approximately 55 percent found in chicks taken at random from Line 6 (Table 6). This reduction may have resulted from immunologic tolerance to the infective virus because of maternal transmission of virus via the egg to progeny of regressor parents. This explanation would appear reasonable since viruses of the leukosis group can infect and multiply in tissues of the female reproductive tract (Burmester, 1957). Moreover, tumors induced in chicks made tolerant to Rous associated virus (RAV) as embryos, developed progressively growing tumors almost exclusively, while tumors induced in control chicks usually regressed (Rubin, 1962).

Lines 105 and 6, RSV-49. The incidence of regression in chickens of Line 105 and Line 6 inoculated at 6 weeks of age with a $10^{-2}$ dilution of RSV-49 is given in Table 8. A $10^{-2}$ dilution was used in preference to the customary $10^{-3}$ used with RSV-1 and RSV-2 because the stock virus had a lower titer (approximately $10^{-3}$) when tested on the CAM's of susceptible embryos. Judging from the number of
chickens which developed tumors as a fraction of the number inoculated 
it would appear that Line 105 was segregating for cellular suscepti­
bility and resistance at the two locus. Line 6, on the other hand, 
appeared to be homozygous susceptible at this locus.

In Line 105 the average incidence of regression with RSV-49 
(10 percent) was slightly higher than that observed using RSV-1 
(6 percent) as given in Table 5. An increase in regression incidence 
was also observed in Line 6 when RSV-49 was used as the inoculum 
rather than RSV-1 (Table 8 vs. Table 6). This could have been due to 
the lower titer of the RSV-49 stock virus or perhaps to a peculiarity 
of RSV-49 itself. Gyles et al., (1968) found a peak regression inci­
dence of 34 percent at a virus dilution of 10^-3, while that for the 
dilutions of 10^-2 and 10^-4 was 20 and 16 percent, respectively. On the 
other hand, Yamanouchi et al., (1968) found that the incidence of 
regression in Japanese quail injected over a range of 1 to 10,000 
focus forming units (FFU) was inversely proportional to the strength of 
the inoculum.
The Incidence of Regression of Rous Tumors as a Function of the Age of
The Host at Inoculation.

The results obtained with Line 105 chicks suggested that the incidence of tumor regression might well be a function of the age of the host at inoculation and of the strength of the inoculum (Tables 1, 2 and 3). The regression incidence of Line 105, however, was relatively low even in those chicks inoculated at six weeks of age. Thus, it was decided that Line 6 with its higher regression incidence (Table 6) would be more useful in detecting possible age effects. Three ages were chosen for study— one, fourteen, and twenty-eight days of age. Accordingly, the chicks of these age groups were inoculated with a $10^{-3}$ dilution of RSV-1. The results are given in Table 9.

Nineteen of 20 chicks inoculated at one day of age developed tumors all of which grew progressively. One chick remained tumor free until discarded 5 weeks later. All chicks which developed tumors also developed visceral metastases. No regressions were observed. Survival time, post-inoculation, averaged 19.1 days with a maximum of 23 days.

Each of twenty chicks inoculated at 14 days of age developed tumors none of which regressed. The incidence of visceral metastases was 85 percent and survival time average 40.0 days.

Each of twenty chicks inoculated at 28 days developed tumors. Tumors in 10 of these birds eventually regressed. In arriving at average survival time only chicks with progressive tumors were included in the calculation. Mean survival time was 60.0 days and the incidence of visceral metastases was 35 percent. The regressor chicks lived until discarded at 24 weeks of age.
Analysis of variance of the data of Table 9, given in Table 9a, indicated a significant difference in survival time between age groups (P .05). Based upon a means separation test (Duncan, 1955), the differences between all possible combinations of age group means were statistically significant (P .05).

Two additional observations which occurred during the course of this experiment merit mention. In both instances, the chickens were inoculated at 28 days of age. First, one chicken had complete regression of a wing web tumor and simultaneously the presence of a metastasis on one leg. Second, another chick had an incomplete regression of the wing web tumor and the presence of visceral metastases. Occurrences of this sort were not observed in Line 6 chicks inoculated at 6 weeks of age. When regression of a wing web tumor occurred in such Line 6 chicks, metastases were invariably absent. Metastases did not always accompany progressively growing wing web tumors in Line 6 chickens, in fact, the simultaneous presence of a progressively growing wing web tumor and visceral metastases was the exception rather than the rule in chicks of this line inoculated at six weeks of age.

The growth of the tumors in this experiment proceeded quite rapidly as can be seen in Figure 1. The criterion of growth rate is the average score of the tumors of all birds in a given age group. Tumor growth proceeded most rapidly in chicks inoculated at one day of age and least rapidly in chicks inoculated at 28 days of age. The growth rate of the two youngest groups was quite similar. Tumors were detectable at one week post-inoculation and grew progressively until 4 weeks post-inoculation, at which time all chicks of the youngest group and 75 percent of the 14 day group had died. Each of the remaining
5 chicks in the latter group had died by 14 weeks post-inoculation. On the other hand, in ten of the twenty chicks in the 28-day group, the tumors grew progressively while the tumors of the ten remaining chickens began to regress about 3 weeks post-inoculation. Since from 3 weeks post-inoculation this group consisted of a mixture of chicks with progressing and regressing tumors, the average tumor score was approximately two.

Metastases were more frequent in the two youngest groups which also had the most rapidly growing tumors (Table 9 and Figure 1). These results are in agreement with those of Rous (1910) who found that metastases grew best in chicks with slowly growing tumors, but were more frequent, and when present more numerous, in chicks with rapidly growing tumors. Metastases were found in most visceral organs, but were most prominent in the liver (Figure 5 and 6). Metastases were often accompanied by hemorrhagic lesions (Figure 6) similar to those thought by Duran – Reynals (1940) to be the result of necrotising action of the virus on the endothelial cells of the vascular system in those organs showing these lesions. However, Carr (1962) attributed such hemorrhagic lesions to a special susceptibility to the virus of areas of extramedullary haematopoiesis.

That systemic virus could indeed be present in young chicks given subcutaneous inoculations of RSV was shown by Munroe and Southam (1958). Thus, in this instance metastases were most probably caused by viral infection of the cells of the viscera. Freire and Duran – Reynals (1953) suggested that the metastasizing power of a tumor was related to a host age-dependent change in the causative virus itself. Also, Duran – Reynals and Freire (1953) found that the filterability of the
Rous sarcoma is inversely related to the age of the host at the time of implantation, and to the age of the transplanted tumors themselves.

Perhaps tumors produced in young chicks can continually produce infective virus while those produced in older chicks are unable to continue sustained production of infective virus. The cells of tumors (induced in older chicks) could remain in a neoplastic (transformed) state due to the presence of some virus specific product(s) which is a non-structural component of the virion (Temin, 1971).

The results of this experiment suggest that regression of RSV tumors is an age-dependent phenomenon, since it occurred more frequently in chicks inoculated at six weeks than it did in chicks inoculated at earlier ages. The results are in agreement with those of Freire et al., (1953) who observed a regression incidence of 15 percent in chickens inoculated as adults while no regressions were observed in chickens of the same strain inoculated at 15 days of age.

Several phenomena occurred in chicks inoculated prior to four weeks of age: tumors grew rapidly, host survival time was short, incidence of regression was low, and incidence of metastases was high. Conversely, in chicks inoculated at four or six weeks of age, tumors grew relatively more slowly, host survival time was relatively longer, incidence of regression was relatively higher, and incidence of metastases was relatively lower. These associations are likely more than coincidental and probably represent different manifestations of a single phenomenon. They may be manifestations of a multi-faceted, cell-mediated, immunologic response to RSV induced tumors (Rubin, 1962).

Gyles et al., (1967) reported that regressive tumors took longer to emerge than progressive tumors. They suggested that there
was a "genetic mechanism" in regressive chicks which appeared early and continued to suppress tumor development. Presumably, this mechanism was a cell-mediated immune reaction induced by the presence of a recognizable foreign antigen, i.e., one not protected by "blocking factors" (Hellstrom et al., 1969), and probably related to the other phenomena, noted above, associated with regressor chickens.

The bursa of Fabricius has been shown to have a role in the development of humoral immunity to certain antigens. Early removal of the bursa significantly impaired or eliminated future antibody production (Glick et al., 1956). Peterson et al., 1964, demonstrated that surgical removal of the bursa at hatching prevented the development of visceral lymphomatosis ordinarily induced by the RPRL-12 virus. Furthermore, Peterson et al., 1966, demonstrated that visceral lymphomatosis was a malignancy exclusively of that component of the lymphoid tissue derived from and/or dependent upon the bursa for its development.

Tumor growth enhancing antibodies, or blocking factors, have been found in the sera of animals of several species possessing progressively growing neoplasms. These are thought to facilitate tumor growth by interference with the immune mechanism of the host (Hellstrom et al., 1961). Therefore, it was postulated that the bursa, because of its known association with antibody production and visceral lymphomatosis, may have some role in tumor regression. To test this hypothesis, three experiments were undertaken to compare the incidence of tumor regression in bursectomized chickens with that in intact controls. Bursectomy was performed either surgically at hatching or by dipping eggs in testosterone propionate at three days of incubation (see Appendix for details).

The results of inoculation of Line 105 chicks surgically bursectomized at hatching and inoculated with a $10^{-3}$ dilution of RSV-1
at 6 weeks of age appear in Table 10. Two of twenty-four bursectomized chicks and one of 30 controls regressed. Contingency table chi-square analysis of the data of Table 10 (Snedecor, 1956) indicated no significant difference between the two groups (chi-square = 0.24) in the incidence of regression.

The results of Line 105 chicks bursectomized in ovo by treatment with testosterone propionate and inoculated with a 10^{-2} dilution of RSV-49 at 6 weeks of age are given in Table 11. The regression incidence in the bursectomized group was 12 percent while none of the controls which developed tumors regressed. These data were not analyzed statistically because of the presence of a zero in one cell. The regression incidence of chicks of this study appeared to be similar to that of intact controls (Table 8).

Regression occurred in 12 of 13 (92 percent) Line 6 chicks surgically bursectomized at hatching and inoculated with a 10^{-2} dilution of RSV-49 at 6 weeks of age. Uninoculated controls were not included in this experiment but the results were comparable to those obtained with intact chicks of the same line given the same quantity of RSV-49 (Table 8).

In these experiments in which bursectomy was accomplished either surgically or chemically, no effect of bursectomy on regression incidence was detected. Cooper et al., (1966) found it necessary to combine surgical bursectomy at hatching with x-irradiation to completely eliminate antibody response. Glick et al., (1956), on the other hand, observed elimination or reduction of antibody response without x-irradiation. Lerner et al., (1971), in non-irradiated chicks found that surgical bursectomy at hatching caused a slight lowering in
the IgG level and a moderate drop in IgG specific antibody, but caused an increase in the level of IgM and normal levels of IgM specific antibody. Bursectomy performed with testosterone propionate in ovo on the third day of incubation resulted in marked lowering of both IgG and IgG-anti-sheep-red blood cell antibody.

Chicks bursectomized by the methods used in this study may have produced blocking factors, despite the absence of a bursa, since Mueller et al., (1971) found evidence for the existence of non-bursal-non-thymus antibody producing cells.
The Role of the Thymus in Tumor regression.

Cooper et al., (1966) showed that the thymus and the system of lymphocytes dependent upon it play the same functional role in the chicken as in mammals. They are the effectors of delayed hypersensitivity and graft versus host reactions and the major elements in homograft rejection.

Radzichovskaja (1967) reported that the latent period of RSV induced tumors in thymectomized chicks was 3 to 4 days shorter than in control chicks. Moreover, thymectomized chicks compared to controls had a higher percentage of susceptibility and a higher incidence of metastases to higher dilutions of virus.

Since tumor regression resembles a homograft reaction it may be the result of a thymus dependent immunologic reaction directed against a "foreign" antigen, i.e. a tumor specific transplantation antigen (TSTA) present on the tumor cells (Huebner, et al., 1971). To test this hypothesis, Line 6 chicks were thymectomized at hatching and inoculated with RSV-1 at 6 weeks of age. The results are given in Table 12. Since in most chickens thymectomy was incomplete (as evidenced by the presence of varying numbers of thymic lobes at autopsy) the chicks were classified into three groups according to the numbers of thymic lobes found at autopsy. In general, the greater the number of thymic lobes observed in a given bird at autopsy the greater the likelihood that tumor regression had occurred in that bird. Conversely, the fewer the number of thymic lobes observed at autopsy the greater the likelihood of finding metastases in that bird. Contingency table chi-square analysis of the data of Table 12 indicated
a highly significant association (chi-square = 10.05; \( p \leq 0.01 \)) between thymic lobe number and tumor regression.

Metastases were not observed in chickens which had regressed wing-web tumors. The incidence of metastases in thymectomized chickens of this line was 26 percent (Table 12) while in intact chicks of this line it was less than 10 percent.

In a second experiment, 13 Line 6 chicks were thymectomized at hatching. At two weeks of age each chick received from one to nine thymic grafts from two week-old intact chicks of the same line. The donated thymi were placed in a subcutaneous space made by inserting forceps through a small incision made through the skin covering the thoracic vertebrae. Each chick was injected with a \( 10^{-3} \) dilution of RSV-1 at six weeks of age. Chicks were classified according to the total thymus number (number of original thymi remaining plus grafted thymi) found at autopsy (Figure 2). The results are given in Table 13. Again the greater the number of thymic lobes found at autopsy the greater the likelihood a regression had occurred. Similarly, the fewer the number of thymi found at autopsy the greater the chance of the presence of a progressive tumor and of finding metastases. Chi-square analysis was not applied to these data because of the presence of zeros in some cells.

In a third experiment chicks of Line 6 (subline 1) were used. This line is highly inbred (coefficient of inbreeding, \( > 0.99 \)) and is histocompatible (Purchase, 1967). Thymectomy was performed at hatching as usual, following which the chicks were exposed to an X-ray dose of 500 roentgens (see Appendix). Restoration was performed as previously using chicks of Line 6 (subline 1) as donors. The results
are given in Tables 14 and 15. Generally, the results with Line 6 (subline 1) were similar to those obtained with Line 6 chicks. Regressions were not observed if the chick was completely thymectomized (Table 14). Restoration of syngeneic thymus material appeared to be effective (Table 15) and chicks with greater numbers of thymi (original plus grafts) had regressions more often than those with lesser numbers. They also had fewer metastases.
The *In Vitro* Detection of Cellular Immunity to Tumor Cells By Use of the Migration Inhibition Reaction.

David *et al.*, (1964) demonstrated that the migration of peritoneal exudate cells from guinea pigs with delayed hypersensitivity to tuberculin purified protein derivative, ovalbumin and diphtheria toxoid was markedly inhibited by the respective antigen, and that such inhibition was specific. Tumor specific antigens have been detected by the inhibition of migration of specifically sensitized macrophages in guinea pigs (Bloom *et al.*, 1969; Kronman *et al.*, 1969) and in mice (Halliday and Webb, 1969). Zwilling *et al.*, (1972) reported that the migration inhibition reaction could be demonstrated in avian delayed type hypersensitivity.

Regression of wing-web tumors in chickens resembles a delayed hypersensitivity reaction. Since the *in vitro* macrophage migration inhibition reaction has been correlated with delayed hypersensitivity it was postulated that this reaction could be used to demonstrate delayed hypersensitivity in regressor chicks. To test this hypothesis it was necessary to obtain suitable cells capable of migration *in vitro*. Buffy coat cells obtained from heparinized centrifuged cardiac blood were chosen as the source of the migrating cells. Use of buffy coat cells, rather than the more commonly used peritoneal exudate cells, eliminated the necessity of sacrificing chickens. The antigen used was derived from a homogenate obtained from minced RSV-1 induced tumors of Line 6 chickens (see Appendix for details of preparation).

The results of this experiment are given in Table 16 which compares the average area of migration of buffy coat cells of regressive, progressive, and uninoculated control chickens, in the presence
and absence, respectively, of tumor extract. Three observations on macrophage migration were made where the antigen was present and three where the antigen was absent. The difference between the means for these two groups is given in the right hand column as percent inhibition (see the Appendix for the calculation of percent inhibition). Positive migration inhibition appeared to be greater for R than for P or C chickens.

An analysis of variance of the data of Table 16 appears in Table 16a and involves unequal subclass numbers (Steel and Torrie, 1960). In this analysis the error mean square was generated from a preliminary analysis of variance unadjusted for unequal subclass numbers. The two main effects in the analysis (Antigen Absent vs. Antigen Present, and Type of Bird) were not statistically significant. Since the interaction term was significant a means separation test for the main effects was not valid. This means that no real difference in macrophage migration inhibition was detected which was related to whether antigen was present or absent or to type of bird.

In the face of a significant A x T interaction effect one may not draw conclusions about the main effects per se. That is, the significant interaction term draws one's attention to the fact that the difference in macrophage migration inhibition in the absence of antigen vs. in the presence of antigen was not the same for R birds as for P birds. This may be illustrated with the means in the 2 x 2 interaction table (next page) based upon the data of Table 16a. Since for the C birds the differences in migration inhibition without and with
antigen was negligible, control means were not shown and will be ignored in the illustration.

<table>
<thead>
<tr>
<th>Type bird</th>
<th>Absent</th>
<th>Present</th>
</tr>
</thead>
<tbody>
<tr>
<td>R</td>
<td>4.13</td>
<td>2.33</td>
</tr>
<tr>
<td>P</td>
<td>2.93</td>
<td>3.06</td>
</tr>
</tbody>
</table>

For R birds the mean difference in macrophage migration inhibition for cells without and with antigen (4.13 - 2.33) was +1.80. For P birds the corresponding mean difference in macrophage migration inhibition for cells without and with antigen (2.93 - 3.06) was -0.13. The difference between these two differences (1.80 - (-)0.13) equals +1.93, an arithmetic estimate of the interaction effect in this 2 x 2 table.

Since the difference between the controls in migration inhibition was negligible, it may be inferred that the significant A x T interaction effect (Table 16a) was generated primarily from the data for the R and P birds. Based upon this explanation of the A x T interaction the results of the macrophage migration inhibition test are interpreted as follows.

Regressor type chickens possessed lymphocytes sensitized by the presence of a tumor antigen(s). These were induced to synthesize a migration inhibition factor (MIF) (Bloom et al., 1969) in the presence of tumor extract. MIF inhibited the migration of macrophages in vitro.

On the other hand, lymphocytes obtained from chickens with progressively growing (p) tumors were not induced to synthesize MIF in
the presence of tumor extract, thus, macrophage migration was not inhibited. This could have been due to the presence of blocking antibodies (Halliday, 1971) present in vivo which protected tumor cells by interfering with sensitization of lymphocytes to tumor antigen(s).

Lymphocytes obtained from uninoculated control (C) chickens were not sensitized because no tumor was present in the cell donor. These chicks were tested periodically to monitor the possible presence of non-specific toxicity (apparent migration inhibition) of the tumor extract. Non-specific inhibition was not observed, but cells obtained from such chickens were sometimes stimulated to show an increase in the area of migration in the presence of tumor extract (Table 16, SPF-1 and SPF-2).

Two additional observations made during the course of this experiment merit further discussion. First, there was no migration inhibition in one regressor chick (No. 2378, Table 16). Four weeks had elapsed, however, from the time of the last visible presence of the tumor and the time of the migration inhibition test. Churchill et al., (1972) reported a decrease in the relative migration inhibition of guinea pig peritoneal exudate sensitized to Line-1 hepatoma cells between 4 and 10 days after the last intradermal immunization with hepatoma cells. Thus, the sustained presence of sufficient numbers of sensitized lymphocytes which reacted in vitro with tumor extract found in this experiment may have been due to the continuous presence of tumor antigen in vivo.

Second, chick No. 2385 (Table 16) showed a variable response. Although this chicken was an apparent progressor two of three times tested, some migration inhibition was detected. When migration was
inhibited the tumor score was 3. When migration was not inhibited the tumor score was 4. It would appear that some chicks with large progressive tumors may develop a weak or inefficient immune response. The final fate of tumors in these chickens would depend upon whether or not the immune mechanism ultimately overcame the progressive growth of the tumor.

In this experiment the macrophage migration inhibition reaction demonstrated that a delayed hypersensitive reaction may indeed occur in regressor chicks, that this is lacking in progressor chicks and in uninoculated controls.
Histological Examination of Two Rous Tumors.

A histological examination was made of a Line 6, size 1, progressive wing-web tumor induced by RSV-1 (Figure 3). This tumor, approximately the size of a small pea, was firm to the touch and was without discolorization. The tumor was sectioned, and upon microscopic examination was found to consist mainly of densely packed fibroblast-like cells (Figure 3, D) sometimes arranged in swirl-like patterns. Areas of more loosely arranged fibroblast-like cells (Figure 2, L) were occasionally seen. Dense areas of infiltrating lymphocytic foci were regularly observed (Figure 3, arrow).

Figure 4 is representative of the histological appearance of a size 2 progressing wing-web tumor induced by RSV-1. Typically these tumors were approximately the size of a cherry and were often much softer to touch than a size 1 tumor. Microscopic examination revealed the presence of fibroblast-like cells (Figure 4, F) much more loosely arranged than those found in a size 1 tumor. Large intercellular spaces (Figure 4, S) were regularly observed. Since a slimy fluid was easily expressed from such tumors, it was assumed that these large intercellular spaces contained a secretion product of the surrounding cells. Mucin production has been shown to have a characteristic early pattern of association with tumor cells. The role of mucin is not understood, even though it progressively occupies an increasing percentage of the total volume of the tumor (Loomis and Pratt, 1956).

Metastases were not often seen in progressor Line 6 chicks inoculated at 6 weeks of age, and were never observed in regressor
Line 6 chicks inoculated at the same age. They were seen regularly, however, in Line 6 chicks inoculated earlier than 6 weeks of age. Figure 5 shows one such metastasis found in the liver of a Line 6 chick inoculated with RSV-1 at two weeks of age. Microscopic examination revealed the presence of degenerating liver cords (Figure 5,H) which appeared to be replaced by loosely packed fibroblast-like cells (Figure 5,F).
GENERAL DISCUSSION

Regression of RSV induced wing-web tumors does indeed occur. The incidence of regression is a function of host dependent parameters both genetic and immunologic in origin, such as the genotype of the chicken, host age at inoculation, and presence or absence of RIF and maternal antibody. Regression incidence is also dependent upon parameters not controlled by the host as strain of virus (i.e., RSV-1, RSV-2, or RSV-49) and strength of inoculum. The latter are the domain of the experimenter. They are of critical importance and must be considered in the interpretation of experimental studies involving regression.

As host age at inoculation increased (from 1 day to 6 weeks of age), the incidence of regression increased and the incidence of metastases decreased. This suggested that the phenomenon under study is a complex mechanism expressing itself in diverse ways.

Since the tumors referred to throughout this study were artificially induced, one can hardly conceive of the existence of a gene(s) whose sole physiologic raison d'être is to effect the regression of such tumors. Thus, for this gene(s) to exist, it must have some other role. One possibility could be that such a gene(s) is responsible for the existence of a more efficient immunological surveillance system capable of eliminating certain neoplastic cells before they become established in the host. This would provide for a broader spectrum type of disease resistance, independent of such "purely genetic"
resistance as that possessed, for example, by chickens whose genotype is $a^r a^r b^r b^r$.

In general, chicks inoculated at 6 weeks of age had a longer latent period than those inoculated earlier. This suggested that whatever mechanism was responsible for the ultimate rejection (regression) of the tumor became functional soon after the host was inoculated – perhaps as early as the first encounter (sensitization) between a lymphocyte and a transformed cell. Progressive tumor growth might then occur, because this encounter was somehow delayed or completely prevented. One explanation for this might be that "blocking factors" enhance tumor growth, allowing it to outrun host defenses. This could occur at the level of the lymphocyte rendering it non-functional, or at the level of the transformed cell by covering tumor antigens.

The demonstration of the existence of a delayed hypersensitivity reaction \textit{in vitro} in regressor chicks and its absence in progressor chicks, suggested that the former do indeed possess sensitized lymphocytes capable of reaction with tumor antigens, while the latter lack these lymphocytes.

The phenomenon of regression may offer the breeder yet another trait to include in his selection program. It would appear that the progeny test could be used to evaluate the performance of sires and dams selected for breeders. A few suggestions are given below based upon observations made in this study: first, set up matings involving full pedigree of sires and dams; second, inoculate offspring of these matings at 6 weeks of age, and evaluate the parents on the basis of tumor size and incidence of regression of the offspring of that mating; third, select as breeders sires and dams whose progeny had a high
incidence of small tumors that eventually regressed. Male offspring which regressed might be used as breeders (if absolutely necessary), but female offspring which regressed would not, since they might produce offspring immunologically tolerant to RSV-like viruses (Table 7). Parents of offspring demonstrating regression of larger tumors should be discriminated against (see Gyles et al., 1971), as these individuals probably possess an inefficient immune response vis à vis the induced tumor.

If lines having a high incidence of regression have better livability under conditions of natural exposure to diseases of the avian leukosis-sarcoma complex (Carte et al., 1972), perhaps vaccination for these diseases (i.e. Marek's disease) would be unnecessary.

Some suggested experiments designed to further elucidate the basis for tumor regression are as follows: (1) Attempt to determine if a single major gene is involved in tumor regression. This might be accomplished by a cross of a high regressor line (developed as previously described) with a low regressor line (i.e. Line 105) and analysis of the F1, F2, and backcross generations. (2) Attempt to induce tumor regression in Line 6 chicks younger than 6 weeks at inoculation by means of grafts of syngeneic thymus material from older chicks. If this were successful, it might help to explain the higher incidence of regression in chicks inoculated at six weeks of age compared to that in chicks inoculated earlier. (3) Investigate the effect of spleenectomy on the incidence of regression of RSV tumors. Hayami et al., (1972) have suggested that "blocking factors" may be released from spleen cells of Japanese quail having progressively growing RSV induced tumors. It would be of interest to know if similar results could be obtained with chickens.
(4) Attempt to demonstrate the presence of "blocking factors" in the sera of progressors by means of a modified macrophage migration inhibition test (MMI), colony inhibition (CI), Hellstrom, (1967), or by leukocyte adherence inhibition (LAI), Halliday and Miller, (1972).
CONCLUSIONS

The results of these experiments indicated that chickens of Line 105 were highly susceptible to RSV-1 and were segregating for susceptibility and resistance to RSV-49. The incidence of regression of tumors induced by these viruses was dependent upon the inoculating dose and the age of the host at inoculation. Regressions were more frequently observed with inocula of lower dosage (titer). Regressions were more frequently observed in chicks inoculated at six weeks of age than in chicks inoculated earlier. The regression incidence in Line 105 was approximately 6 percent in chicks inoculated at six weeks of age.

Line 6 chickens were highly susceptible to RAV-1, RSV-2, and RSV-49. The incidence of regression was much higher (approximately 60 percent for RSV-1 and RSV-2 induced tumors, approximately 86 percent for RSV-49 induced tumors) than in Line 105. The effects of host age at inoculation on regression incidence were more apparent in Line 6 than in Line 105, because of the higher regression incidence in Line 6. The younger the chick at inoculation, the less likely tumor regression will occur, and the more likely metastases will develop.

Neither chemical nor surgical bursectomy appeared to alter the regression incidence in either line, but thymectomy reduced regression incidence in Line 6. Restoration of thymectomized chicks with syngeneic thymus tissue resulted in an increase in regression incidence over non-restored thymectomized Line 6 chicks. Metastases were found more frequently in thymectomized chicks than in intact chicks inoculated at the same age.
A delayed hypersensitivity reaction \textit{in vitro} was demonstrated by means of the macrophage migration inhibition reaction. This was done by showing that macrophage migration, in the presence of tumor extract, was inhibited to a greater extent in regressor chicks, than in pro-gressor chicks or in uninoculated controls. Thus, it was concluded that the actual effector mechanism of tumor regression was a thymus-dependent delayed hypersensitivity reaction of the host against the tumor.

The bursa of Fabricius may have a role in tumor regression, however, it is likely to be of secondary importance to that of the thymus.
Table 1. - Results of inoculation of Line 105 chicks with different dilutions of RSV-1, at one and 1½ days of age, respectively, presumptive genotype, a8a6

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>Virus dilution</th>
<th>No. of chickens developing tumors/no. inoculated</th>
<th>Mean latent period (days)</th>
<th>+S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10^-1</td>
<td>3/3 (0)a</td>
<td>6.3</td>
<td>2.50</td>
</tr>
<tr>
<td></td>
<td>10^-2</td>
<td>2/4 (0)</td>
<td>5.0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>10^-3</td>
<td>4/4 (0)</td>
<td>8.2</td>
<td>3.20</td>
</tr>
<tr>
<td>1½</td>
<td>10^-2</td>
<td>3/6 (1)</td>
<td>8.7</td>
<td>10.40</td>
</tr>
<tr>
<td></td>
<td>10^-3</td>
<td>4/6 (0)</td>
<td>8.7</td>
<td>2.45</td>
</tr>
<tr>
<td></td>
<td>10^-4</td>
<td>4/6 (0)</td>
<td>8.7</td>
<td>1.37</td>
</tr>
<tr>
<td></td>
<td>10^-5</td>
<td>0/6</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

a ( ) = Number of tumors regressed
Table 2. - Results of inoculation of Line 105 chicks with different dilutions of RSV-1, at 3 and 5 weeks of age, respectively, presumptive genotype, a

<table>
<thead>
<tr>
<th>Age (weeks)</th>
<th>Virus dilution</th>
<th>No. of chickens developing tumors/no. inoculated</th>
<th>Mean latent period (days)</th>
<th>±S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>$10^{-1}$</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>$10^{-2}$</td>
<td>8/9 (0)a</td>
<td>8.4</td>
<td>0.65</td>
</tr>
<tr>
<td></td>
<td>$10^{-4}$</td>
<td>4/8 (1)</td>
<td>11.0</td>
<td>1.00</td>
</tr>
<tr>
<td>5</td>
<td>$10^{-1}$</td>
<td>9/9 (0)</td>
<td>8.4</td>
<td>0.50</td>
</tr>
<tr>
<td></td>
<td>$10^{-2}$</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>$10^{-4}$</td>
<td>4/8 (0)</td>
<td>14.3</td>
<td>3.42</td>
</tr>
</tbody>
</table>

a( ) = Number of tumors regressed
Table 3. Results of inoculation of Line 105 chicks with different dilutions of RSV-1, at 3 and 6 weeks of age, respectively, presumptive genotype α α'.

<table>
<thead>
<tr>
<th>Age (weeks)</th>
<th>Virus dilution</th>
<th>No. of chickens developing tumors/no. inoculated</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>$10^{-2}$</td>
<td>26/26 (0)$^a$</td>
</tr>
<tr>
<td></td>
<td>$10^{-3}$</td>
<td>25/25 (0)</td>
</tr>
<tr>
<td></td>
<td>$10^{-4}$</td>
<td>26/26 (0)</td>
</tr>
<tr>
<td>6</td>
<td>$10^{-2}$</td>
<td>18/18 (2)</td>
</tr>
<tr>
<td></td>
<td>$10^{-3}$</td>
<td>17/17 (0)</td>
</tr>
<tr>
<td></td>
<td>$10^{-4}$</td>
<td>16/16 (1)</td>
</tr>
</tbody>
</table>

$^a$ ( ) = Number of tumors regressed
Table 4 - Percentage of Line 105 chicks having a score greater than 1 at one week post-inoculation, presumptive genotype a³a³

<table>
<thead>
<tr>
<th>Age (weeks)</th>
<th>Virus dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10⁻²</td>
</tr>
<tr>
<td>3</td>
<td>77</td>
</tr>
<tr>
<td>6</td>
<td>77</td>
</tr>
</tbody>
</table>
Table 5. - Results of two later trials with Line 105 chicks inoculated with RSV-1, $10^{-3}$ dilution, at 6 weeks of age, presumptive genotype a\textsuperscript{a}.a\textsuperscript{a}

<table>
<thead>
<tr>
<th>Trial</th>
<th>No. of chickens developing tumors/no. inoculated</th>
<th>Regression incidence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>35/36 (2)\textsuperscript{a}</td>
<td>6</td>
</tr>
<tr>
<td>2</td>
<td>17/22 (1)</td>
<td>6</td>
</tr>
</tbody>
</table>

\textsuperscript{a} ( ) = Number of tumors regressed
Table 6. - Results of inoculation of Line 6 chicks with RSV-1 and RSV-2, 10^{-3} dilution, at 6 weeks of age, presumptive genotype a^{a}s_{b}s_{b}^{s}

<table>
<thead>
<tr>
<th>Virus</th>
<th>Trial</th>
<th>No. of chickens developing tumors/no. inoculated</th>
<th>Regression incidence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RSV-1</td>
<td>1</td>
<td>49/49 (27)^a</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>13/13 (5)</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>23/23 (14)</td>
<td>61</td>
</tr>
<tr>
<td></td>
<td>1 - 3</td>
<td>85/85 (46)</td>
<td>54</td>
</tr>
<tr>
<td>RSV-2</td>
<td>1</td>
<td>52/53 (31)</td>
<td>58</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>14/14 (7)</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>1 - 2</td>
<td>66/67 (38)</td>
<td>58</td>
</tr>
</tbody>
</table>

^a ( ) = Number of tumors regressed
Table 7. - Incidence of regression of RSV-1 tumors in chicks from matings of Line 6 regressor males x Line 6 regressor females inoculated at 6 weeks of age, virus dilution $10^{-3}$

<table>
<thead>
<tr>
<th>Trial</th>
<th>No. of chickens developing tumors/no. inoculated</th>
<th>Regression Incidence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7/7 (2)$^a$</td>
<td>28</td>
</tr>
<tr>
<td>2</td>
<td>12/12 (3)</td>
<td>25</td>
</tr>
<tr>
<td>3</td>
<td>6/6 (2)</td>
<td>33</td>
</tr>
<tr>
<td>4</td>
<td>12/12 (0)</td>
<td>0</td>
</tr>
</tbody>
</table>

1 thru 4: 37/37 (7) 19

$^a$ ( ) = Number of tumors regressed
Table 8. - The incidence of regression in Line 105 and Line 6 chicks inoculated at 6 weeks of age with RSV-49, dilution $10^{-2}$

| Trial | No. of chickens developing tumors/no. inoculated | Regression incidence (%) |
|-------|------------------------------------------------|--|---|
|       |                                                | Line 105 |                 | Line 6 |                 |
| 1     | 25/43 (3)$^a$                                 | 12       |                | 14/14 (11) | 79          |
| 2     | 5/12 (0)                                      | 0        |                | 14/14 (13) | 93          |
| 1 and 2 | 30/55 (3)                                 | 10       |                | 28/28 (24) | 86          |

$^a$ ( ) = Number of tumors regressed
Table 9. - Results of inoculation of Line 6 chicks with RSV-1 at a dilution of $10^{-3}$ at 1, 14 and 28 days of age, respectively

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>No. of chickens developing tumors/ no. inoculated</th>
<th>Av. survival time post inoculation (days)</th>
<th>±S.E.</th>
<th>Incidence of metastases (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>19/20 (0)</td>
<td>19.1</td>
<td>0.99</td>
<td>100</td>
</tr>
<tr>
<td>14</td>
<td>20/20 (0)</td>
<td>40.0</td>
<td>5.03</td>
<td>85</td>
</tr>
<tr>
<td>28</td>
<td>20/20 (10)</td>
<td>60.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>15.37</td>
<td>35</td>
</tr>
</tbody>
</table>

<sup>a</sup> ( ) = Number of tumors regressed

<sup>b</sup> Includes progressors only
Table 9a. - Analysis of variance of the "survival time" data of Table 9

<table>
<thead>
<tr>
<th>Sources of variation</th>
<th>d.f.</th>
<th>Mean squares</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ages</td>
<td>2</td>
<td>5656.14</td>
<td>28.3*</td>
</tr>
<tr>
<td>Individuals/Ages</td>
<td>45</td>
<td>200.10</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>47</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Tabular F with 2 and 45 - d.f., @ 0.05 = 3.20
Table 10. - The incidence of regression of RSV-1 induced tumors in Line 105 chickens surgically bursectomized at hatching compared to that in intact controls.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of chickens which Developed tumors</th>
<th>Regressed tumors</th>
<th>Regression (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bursectomized</td>
<td>24/24</td>
<td>2/24</td>
<td>8.3</td>
</tr>
<tr>
<td>Controls</td>
<td>30/30</td>
<td>1/30</td>
<td>3.3</td>
</tr>
</tbody>
</table>
Table 11. The incidence of regression of RSV-1*9 induced tumors in Line 105 chicks bursectomized in ovo by testosterone propionate and inoculated at 6 weeks with RSV-1*9, $10^{-2}$ dilution.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of chicks which Developed tumors</th>
<th>Regressed Tumors</th>
<th>Regression (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bursectomized</td>
<td>25/43</td>
<td>3/43</td>
<td>12.0</td>
</tr>
<tr>
<td>Control</td>
<td>13/34</td>
<td>0/34</td>
<td>0.0</td>
</tr>
</tbody>
</table>
Table 12. - The incidence of regression and metastases in Line 6 chickens thymectomized at hatching and inoculated with a $10^{-3}$ dilution of RSV-1 at 6 weeks of age.

<table>
<thead>
<tr>
<th>Number of thymic lobes observed at autopsy</th>
<th>Incidence of Regression</th>
<th>Incidence of Metastases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>Percent of class</td>
</tr>
<tr>
<td>0 - 3</td>
<td>1/15(^a)</td>
<td>6</td>
</tr>
<tr>
<td>4 - 6</td>
<td>2/7</td>
<td>28</td>
</tr>
<tr>
<td>7 - 10+</td>
<td>6/9</td>
<td>66</td>
</tr>
</tbody>
</table>

\(^a\) Number regressions or metastases/total number of birds in the class.
Table 13. - The incidence of tumor regression and metastases in Line 6 chickens thymectomized at hatching and restored at two weeks of age and inoculated with a 10^{-3} dilution of RSV-1 at 6 weeks of age.

<table>
<thead>
<tr>
<th>Number of thymic lobes observed at autopsy</th>
<th>Incidence of Regression</th>
<th>Incidence of Metastases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>Percent of class</td>
</tr>
<tr>
<td>0 - 3</td>
<td>0/6^a</td>
<td>0</td>
</tr>
<tr>
<td>4 - 6</td>
<td>0/1</td>
<td>0</td>
</tr>
<tr>
<td>7 - 10+</td>
<td>5/6</td>
<td>93</td>
</tr>
</tbody>
</table>

^a Number regressions or metastases/total number of birds in the class.
Table 14. - Incidence of regression and metastases in RPRL Line 6 (subline 1) chickens thymectomized and X-irradiated at hatching and inoculated with a $10^{-3}$ dilution of RSV-1 at 6 weeks of age.

<table>
<thead>
<tr>
<th>Number of thymic lobes observed at autopsy</th>
<th>Incidence of Regression</th>
<th>Incidence of Metastases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>Percent of class</td>
</tr>
<tr>
<td>0 - 3</td>
<td>0/8a</td>
<td>0</td>
</tr>
<tr>
<td>4 - 6</td>
<td>4/4</td>
<td>100</td>
</tr>
</tbody>
</table>

* Number regressions or metastases/total number of birds in the class.
Table 15. - Incidence of regression and metastases in RPRL Line 6 (subline 1) chickens thymectomized and X-irradiated at hatching, restored at two weeks of age and inoculated with a $10^{-3}$ dilution of RSV-1 at 6 weeks of age.

<table>
<thead>
<tr>
<th>Number of thymic lobes observed at autopsy</th>
<th>Incidence of Regression</th>
<th>Incidence of Metastases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>Percent of class</td>
</tr>
<tr>
<td>0 - 3</td>
<td>1/2$^a$</td>
<td>50</td>
</tr>
<tr>
<td>4 - 6</td>
<td>1/1</td>
<td>100</td>
</tr>
<tr>
<td>7 - 10+</td>
<td>3/8</td>
<td>100</td>
</tr>
</tbody>
</table>

$^a$ Number regressions or metastases/total number of birds in the class.
Table 16. - The results of the migration inhibition test of chickens of Line 6 bearing progressive or regressive tumors and of uninoculated, specific pathogen free (SPF), controls

<table>
<thead>
<tr>
<th>Chick wing band No.</th>
<th>Bird type</th>
<th>Tumor Score at test</th>
<th>Mean area of migration (cm²) (antigen absent)</th>
<th>Mean area of migration (cm²) (antigen present)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mean area of migration (cm²) (antigen absent)</td>
<td>Mean area of migration (cm²) (antigen present)</td>
<td></td>
</tr>
<tr>
<td>2372</td>
<td>R</td>
<td>1</td>
<td>2.93 ± 0.26</td>
<td>1.60 ± 0.35</td>
<td>46</td>
</tr>
<tr>
<td>2381 (1)</td>
<td>R</td>
<td>1</td>
<td>3.57 ± 0.52</td>
<td>1.77 ± 0.18</td>
<td>51</td>
</tr>
<tr>
<td>2381 (2)</td>
<td>R</td>
<td>0</td>
<td>6.23 ± 0.03</td>
<td>3.37 ± 0.41</td>
<td>46</td>
</tr>
<tr>
<td>4174</td>
<td>R</td>
<td>1</td>
<td>7.90 ± 0.92</td>
<td>4.73 ± 0.38</td>
<td>41</td>
</tr>
<tr>
<td>4185</td>
<td>R</td>
<td>1</td>
<td>5.63 ± 0.12</td>
<td>2.63 ± 0.17</td>
<td>53</td>
</tr>
<tr>
<td>4186</td>
<td>R</td>
<td>1</td>
<td>0.87 ± 0.13</td>
<td>0.20 ± 0.00</td>
<td>77</td>
</tr>
<tr>
<td>4187</td>
<td>R</td>
<td>2</td>
<td>2.97 ± 0.55</td>
<td>1.60 ± 0.09</td>
<td>47</td>
</tr>
<tr>
<td>4200</td>
<td>R</td>
<td>0</td>
<td>2.17 ± 0.16</td>
<td>0.87 ± 0.36</td>
<td>65</td>
</tr>
<tr>
<td>4191</td>
<td>R</td>
<td>2</td>
<td>3.03 ± 0.29</td>
<td>1.10 ± 0.05</td>
<td>67</td>
</tr>
<tr>
<td>2374</td>
<td>R</td>
<td>0</td>
<td>1.97 ± 0.38</td>
<td>1.43 ± 0.12</td>
<td>27</td>
</tr>
<tr>
<td>2378</td>
<td>R</td>
<td>0</td>
<td>3.77 ± 0.35</td>
<td>4.67 ± 0.29</td>
<td>-24</td>
</tr>
<tr>
<td>none</td>
<td>R</td>
<td>0</td>
<td>8.16 ± 1.17</td>
<td>3.97 ± 0.37</td>
<td>51</td>
</tr>
<tr>
<td>2385 (1)</td>
<td>P</td>
<td>3</td>
<td>3.23 ± 0.12</td>
<td>2.40 ± 0.37</td>
<td>26</td>
</tr>
<tr>
<td>2385 (2)</td>
<td>P</td>
<td>3</td>
<td>5.10 ± 0.43</td>
<td>5.03 ± 0.08</td>
<td>2</td>
</tr>
<tr>
<td>2385 (3)</td>
<td>P</td>
<td>4</td>
<td>1.70 ± 0.33</td>
<td>1.00 ± 0.20</td>
<td>41</td>
</tr>
<tr>
<td>1403</td>
<td>P</td>
<td>4</td>
<td>4.26 ± 0.17</td>
<td>5.13 ± 0.49</td>
<td>-20</td>
</tr>
<tr>
<td>2377</td>
<td>P</td>
<td>4</td>
<td>4.23 ± 0.43</td>
<td>4.63 ± 0.46</td>
<td>9</td>
</tr>
<tr>
<td>1393</td>
<td>P</td>
<td>4</td>
<td>1.47 ± 0.26</td>
<td>2.72 ± 0.43</td>
<td>-85</td>
</tr>
<tr>
<td>4195</td>
<td>P</td>
<td>4</td>
<td>0.50 ± 0.00</td>
<td>0.50 ± 0.00</td>
<td>0</td>
</tr>
<tr>
<td>SPF-1</td>
<td>C</td>
<td></td>
<td>4.10 ± 0.58</td>
<td>5.93 ± 0.67</td>
<td>-44</td>
</tr>
<tr>
<td>SPF-2</td>
<td>C</td>
<td></td>
<td>5.30 ± 0.89</td>
<td>6.27 ± 0.35</td>
<td>-18</td>
</tr>
<tr>
<td>SPF-3</td>
<td>C</td>
<td></td>
<td>2.90 ± 0.20</td>
<td>2.43 ± 0.29</td>
<td>16</td>
</tr>
</tbody>
</table>

a R = regressor; P = progressor; C = control

b S.E. = standard error
Table 16a. - Analysis of variance of the data of Table 12.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antigen absent vs. Antigen present ( (A) )</td>
<td>1</td>
<td>7.79</td>
<td>7.79</td>
<td>2.42*</td>
</tr>
<tr>
<td>Type of bird ( (T) )</td>
<td>2</td>
<td>10.40</td>
<td>5.20</td>
<td>1.63</td>
</tr>
<tr>
<td>( A \times T )</td>
<td>2</td>
<td>28.65</td>
<td>14.32</td>
<td>4.53*</td>
</tr>
<tr>
<td>Error</td>
<td>38</td>
<td>120.16</td>
<td>3.16</td>
<td></td>
</tr>
</tbody>
</table>

* \( P < 0.05 \)
Figure 1. Growth rates of RSV-1 induced tumors of Line 6 inoculated at 1, 14, and 28 days of age, respectively.
Figure 2. Thymus graft taken from area over thoracic vertebrae at autopsy. D, dense area of thymocytes; H, Hassall's body; C, portion of connective tissue capsule. Magnification, 100X.
Figure 3. Size 1, RSV-l induced tumor of Line 6. D, densely packed fibroblast-like cells; L, loosely packed fibroblast-like cells; arrow, infiltrating lymphocytic focus. Magnification, 100X.
Figure 4. Size 2, RSV-1 induced progressive tumor of Line 6.

F, fibroblast-like cells; S, intercellular spaces.

Magnification, 100X.
Figure 5. Liver of Line 6 chicken showing metastases. H, degenerating liver cords; F, fibroblast-like cells. Magnification 100X.
Figure 6. Line 6 chick inoculated in the wing-web at hatching with a $10^{-3}$ dilution of RSV-1. Shown are the wing-web tumor, liver metastases (light areas), and hemorrhagic lesions (arrow).
BIBLIOGRAPHY


Savage, T.F., Personal Communication (1972).


LIST OF APPENDIX ITEMS

Procedure for surgical bursectomy ........................................ 93
Procedure for testosterone bursectomy .................................. 94
Procedure for thymectomy ....................................................... 95
Procedure for producing crude soluble antigen (CSA) ............... 96
Preparation of Bloom type cell migration chambers .................. 100
APPENDIX

Surgical Procedures

Procedure for Surgical Bursectomy

1. The down surrounding the vent was removed by plucking.
2. The area around the vent was moistened with 95% ethyl alcohol.
3. The chick was grasped firmly with the left hand, vent up.
4. An incision was made between the vent and the tail by means of a scalpel containing a No. 11 blade.
5. Subcutaneous tissue was dissected away with the scalpel until the bursa was clearly visible.
6. The bursa was grasped with forceps and pulled free.
7. The chick was returned to the brooding battery.

This procedure was used, because it could be performed by one individual in two to three minutes per bird. There was a minimum of bleeding and the incision closed by itself without sutures. Occasionally, the initial incision was made too deeply and the colon was severed. These birds were destroyed immediately.

Most chickens recovered completely within one week after surgery. There were some instances of "pasted-vents", but this was corrected by picking off the dried fecal material manually.
Procedure for Testosterone Propionate Bursectomy.

This procedure was modified from that of Glick and Sadler (1961) as follows:

1. A 1.5 gm. percent solution of testosterone propionate (TP) (Calbiochem No. 5817) was made by dissolving 1 gram of TP into 127 mls. of absolute ethyl alcohol.

2. Embryonated eggs were submerged in the above solution for 5 seconds on the third day of incubation.

3. The eggs were returned to the incubator.
Procedure for Thymectomy.

This was modified from a technique described by Aspinall et al., (1963).

1. The chick to be thymectomized was placed in a desiccator containing ether soaked cheese cloth.
2. The chick remained in the desiccator until it fell on its side and was unable to right itself.
3. Next, the chick was laid on its back and fastened to a dissecting board by inserting a thumb tack in the web of each foot. The bird was stretched slightly and held in place by inserting a common pin through the tip of the upper beak.
4. The neck area was moistened with 95% ethyl alcohol.
5. An incision was made with pointed scissors along the length of the neck.
6. The skin was held back by placing two hemostats on each side of the neck.
7. Connective tissue and fat were dissected away from the thymic lobes.
8. Each thymic lobe was pulled free by means of forceps.
9. A Pasteur pipette connected to a vacuum pump was used to take out fragments and lobes not previously removed.
10. The exposed neck area was rinsed twice with Hank's solution.
11. The wound was closed by means of silk sutures and steel wound clips.
12. The bird was then returned to the brooding battery.
In Vitro Procedures

Procedure for producing "crude soluble antigen" (CSA) for use in the migration inhibition test.

CSA was prepared by modifying the method of Halliday, (1971), as follows:

1. Approximately 6 to 7 grams (wet weight) of tumor tissue were obtained from a Line 6 chicken with a large progressively growing RSV-1 induced wing-web tumor.

2. Necrotic tissue was dissected away from healthy tumor tissue by means of forceps and a scalpel containing a No. 11 blade.

3. This yielded 5 grams of tissue which was minced.

4. The minced tissue was added to 15 ml. cold Hank's solution, making a 20 percent W/V suspension.

5. The tumor suspension was homogenized in a "Virtis 45" homogenizer for 2 to 3 minutes.

6. The homogenate was then centrifuged at 1000 x g for 30 minutes (6,000 RPM, Sorvall RC2-B centrifuge).

7. The resulting supernatant was then centrifuged at 100,000 x g for 1 hour (40,000 RPM, Beckman "Model L" ultracentrifuge).

8. The resulting supernatant was decanted and stored in liquid nitrogen at 1 ml. aliquots.
Procedure for the macrophage migration inhibition test.

1. 2-3 mls. of blood were drawn from the chicken to be tested by cardiac puncture.

2. The blood was transferred to screw-cap test tubes containing approximately 15 units of heparin sodium (Fisher No. H-19).

3. The tubes were rocked gently to ensure mixing of the heparin and blood.

4. Six sterile "Natelson type" blood collecting tubes (Fisher No. 2-668-15) were filled with the heparinized blood. These were sealed in a flame and placed in a 16 x 125 mm. screw-cap test tube.

5. The screw-cap tubes containing the Natelson tubes were centrifuged at 1000 RPM for 15 minutes (Sorvall, RC2-B centrifuge).

6. The Natelson tubes were scratched and broken at the buffy-coat-red cell interface.

7. The end containing the buffy-coat and plasma was tapped several times into a Falcon tissue culture dish (No. 3001) containing 1 ml. of medium M199. The buffy coats from the six Natelson tubes were pooled in this manner.

8. The M199 and cells were taken up several times in a 10 ml. pipette in order to break up clumps of cells.

9. The M199 and cells were transferred to screw-cap tubes containing 2 mls. M199. These were centrifuged in the RC2-B at 1000 RPM for 2 minutes.
10. The M199 was decanted and the remaining pellet of cells was resuspended in 2 mls. ACT (ammonium chloride-tris) (Kay and Kaelble, 1972) in order to remove excess red cells by hemolysis.

11. The ACT and cells were centrifuged at 1000 RPM for 2 minutes.

12. The ACT was decanted and the pellet was resuspended in 3 ml. M199.

13. 1.5 ml. of the above suspension was placed into each of two 12 ml. conical centrifuge tubes which were centrifuged at 1000 RPM for 2 minutes.

14. The cells in one conical tube were designated as control, and these were resuspended in 0.3 ml. M199. The cells of the other conical tube were designated as test, and these were resuspended in 0.1 ml. M199.

15. 0.2 ml. CSA was added to the test cell suspension. Thus, the control and the test conical centrifuge tubes contained approximately equal numbers of cells suspended in 0.3 ml. of liquid.

16. Four microhematocrit tubes (DADE No. B4415-1A) were filled with the contents of both the test and control conical centrifuge tubes. One end of each microhematocrit tube was sealed by a flame.

17. The microhematocrit tubes were placed in 10 x 100 mm. screw-cap test tubes and centrifuged at 1000 RPM for 2 minutes. This resulted in the suspended cells being packed at the sealed end of the microhematocrit tubes.
18. The microhematocrit tubes were scratched and broken at the cell liquid interface and placed in Bloom chambers (Berton Plastics, So. Hackensack, N.J.) prepared as described in the following section.
Preparation of Bloom type cell migration chambers.

1. Bloom chambers were placed in glass petri dishes and sterilized by autoclaving.

2. The edge of the wells on one side of the Bloom chamber was rimmed with stopcock grease (Dow Corning) dispensed through a 1 ml. tuberculin type syringe without a needle.

3. A round glass cover slip was placed over each well and pressed firmly onto the stopcock grease. This made a liquid-tight seal.

4. The Bloom chamber was inverted (cover slip side down) and 3 small spots of stopcock grease were placed on the floor of each well.

5. Packed buffy coat cells (see previous section) were pressed firmly into each spot. Thus, each well contained 3 packed buffy coat cell tubes firmly attached to the bottom floor of the well. One well contained 3 test buffy coat cell tubes and the other contained 3 control buffy coat cell tubes.

6. 0.4 ml. M199 was added to the control well.

7. 0.4 ml. CSA diluted 1:3 with M199 was added to the test well.

8. The upper edge of each well was rimmed with stopcock grease, and a cover slip was placed on top as before.

9. The remaining space in each well was filled with M199 containing 10 percent pooled avian serum, previously inactivated by heating at 56°C for 30 minutes.

10. The filling holes were plugged with stopcock grease and the Bloom chambers incubated at 37°C for 18-24 hours.
Measurement of migration inhibition test and calculation of the percent of inhibition.

1. The area of migration was measured by placing the Bloom chambers on an overhead projector (Porta. Scribe 1000, Charles Besler Co., East Orange, N.J.), and projecting the image on a piece of white paper used as a screen.

2. The magnification of the projected image was approximately 7.5 diameters.

3. Each image was traced with a pencil and the traced area was measured with a planimeter (Keuffel and Esser Co., Model No. 62 0005).

4. The percent of inhibition (\(\%I\)) was calculated by applying the formula: \((1-T/C) \times 100\) where \(T\) = the average area of migration of the packed cells of the test well; \(C\) = the average area of migration of the packed cells of the control well.
BIOGRAPHICAL DATA

Name: Paul Francis Cotter

Date of Birth: April 9, 1945

Place of Birth: Boston, Massachusetts

Secondary Education: Saint Columbkille High School
                      Boston, Massachusetts

Higher Education:

<table>
<thead>
<tr>
<th>Institution</th>
<th>Dates</th>
<th>Degree Received</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suffolk University</td>
<td>1962 - 66</td>
<td>A.B.</td>
</tr>
<tr>
<td>Northeastern University</td>
<td>1966 - 68</td>
<td>M.S.</td>
</tr>
<tr>
<td>University of New Hampshire</td>
<td>1969 - 73</td>
<td>Ph.D.</td>
</tr>
</tbody>
</table>

Honors or Awards:

- Hubbard Farms Research Fellowship, 1971 - 72
- U.N.H. Dissertation Fellowship, 1973

Publications:

Cotter, P.F., Collins, W.M., Corbett, A.C. and Dunlop, W.R.
Regression of Rous Sarcomas in Two Lines of Chickens, Poultry Science,

Positions Held:

- Graduate Research Assistant. Department of Animal Sciences,
  University of New Hampshire, 1969 - 73.
- Instructor. Science Department, Lasell Junior College,
  Auburndale, Massachusetts, 1968 - 69.
- Graduate Teaching Assistant. Department of Biology,
  Northeastern University, 1966 - 68.