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INFLUENCE OF GENOTYPE, PROTEIN-CALORIE RESTRICTION AND THEIR INTERACTION UPON RSV-INDUCED TUMORS IN CHICKENS

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INFLUENCE OF GENOTYPE, PROTEIN-CALORIE RESTRICTION AND THEIR INTERACTION UPON RSV-INDUCED TUMORS IN CHICKENS

BY

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ABSTRACT

INFLUENCE OF GENOTYPE, PROTEIN-CALORIE RESTRICTION

AND THEIR INTERACTION

UPON RSV-INDUCED TUMORS IN CHICKENS

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Kathy K. Clark

University of New Hampshire, December, 1980

The major objective of this research was to investigate the relative contribution of genetics, nutritional restriction and the genetics by nutritional restriction interaction upon Rous sarcoma virus (RSV)-induced tumor development in chickens. Two genetic systems were used. The first involved an inbred line and a noninbred line of chickens. The second utilized F2 generation progeny from a cross of lines 6-1 and 15-1, highly inbred lines of White Leghorns from the Regional Poultry Research Laboratory of the United States Department of Agriculture at East Lansing, Michigan. These chickens had been blood typed for alloantigens coded for by genes of the major histocompatibility complex and were of two genotypes--B2B2 and B5B5. Four-week-old chickens were either full-fed or restricted to 60% of the feed consumed by full-fed chickens of the same age. Two weeks after being placed on experimental rations, chickens were inoculated with RSV-1.

Tumors were scored subjectively for size several times during a 10 week period.

Forty percent nutritional restriction delayed the appearance of tumor and reduced tumor size at 2 and 3 weeks post-inoculation (PI). E genotype profoundly influenced tumor size. E2B2 chickens had smaller tumors between 3 and 10 weeks PI than did corresponding B5B5 chickens. Similar to 40% restriction, 50% restriction delayed tumor formation and retarded early tumor growth.

Nutritional restriction may be retarding initial tumor growth by two possible mechanisms: (1) nutritional deprivation may inhibit antibody production, including blocking antibody, and enhance cell-mediated immunity, resulting in inhibited tumor growth, or (2) rapid tumor growth is restricted due to a limited supply of nutrients to the cancer cells. Forty percent restriction did not exert an effect on immunocompetence based upon antibody production to sheep erythrocytes and phytohemaglutinin-stimulated lymphocyte blastogenesis as measures of cell-mediated and humoral immunity, respectively. Thus a limited supply of nutrients may retard initial tumor growth.

I. INTRODUCTION

Each year in the United States, there are more than 675,000 new cases of cancer and about 375,00 deaths caused by it (Boyd, 1978). The more common cancers are largely due to environmental factors; however, there is increasing evidence that genetic characteristics predispose individuals to some forms of cancer, in particular rare childhood cancers (Emery, 1978).

The proposed etiopathogenesis of the major diseases afflicting industrialized humankind (arteriosclerosis, cancers, adult-onset diabetes) increasingly relate to dietary variables (Weindruch et al., 1979). Moreschi (1909) and Rous (1914) were the first to report a marked decrease in incidence and growth of spontaneous and transplanted malignancies in mice fed restricted diets. Nutritional deprivation has since been reported to increase resistance to tumor growth for many tumor types in mice (White and Andervont, 1943; Saxton et al., 1944), rats (Ross and Bras, 1965) and cattle (Anderson et al., 1970).

Rous sarcoma regression in chickens is influenced by strain (Cotter et al., 1973) and selection (Gyles and Brown, 1971; Carte et al., 1972). Collins et al. (1977) showed that a major gene(s) within or closely linked to the B blood

group-major histocompatibility complex had a major effect upon the ability of chickens to regress Rous sarcoma virus-induced sarcomas. In this system, among the F2 segregants, B2B2, B2B5 and B5B5, the percentage of chickens dying of tumor (by 10 weeks post-inoculation) was 5, 26 and 93, respectively. This system is ideally suited to investigate the effect of nutritional restriction and the interaction of genetics and nutritional restriction upon tumor development, including tumor regression.

Objectives

1. Investigate the relative contribution of genetics and nutritional restriction upon Rous sarcoma virus-induced tumor development.

2. Investigate the effect of level of nutritional restriction imposed and of duration of nutritional restriction prior to Rous sarcoma virus inoculation upon tumor development.

3. Investigate the effect of B genotype and of line upon the delayed wattle reaction as an *in vivo* measure of cell-mediated immunity.

4. Study the effect of nutritional restriction upon the level of humoral and cell-mediated immunity.

II. REVIEW OF THE LITERATURE

Nutrition and Cancer

There is increasing epidemiological evidence that nutrition plays a dominant role in the pathogenesis of several types of human cancer (Wynder, 1976). Of particular importance are data indicating that overnutrition significantly affects the development of certain cancers including cancers of the colon, pancreas, kidney, breast, ovary, endometrium and prostate. Except for cancer of the endometrium and kidney cancer in women, there is no significant relationship to obesity (Wynder *et al.*, 1966, 1974). Rather, the development of cancer in man appears to be related to an excessive intake of certain nutrients, rather than caloric excess *per se* (Wynder, 1976).

Many diverse types of neoplasms respond to protein and/or calorie deprivation by a reduction in tumor incidence and a delay in appearance: spontaneous mammary carcinoma (Tannenbaum, 1942, 1945b; Visscher *et al.*, 1942; White and White, 1944), skin tumors induced by carcinogenic hydrocarbons (Tannenbaum, 1942, 1945a) or ultraviolet light (Rusch *et al.*, 1945b), induced sarcoma (Tannenbaum, 1942; Rusch *et al.*, 1945a), spontaneous hepatomas (Tannenbaum and Silverstone, 1949b) and induced leukemia (White *et al.*,

1944)--all of the mouse; and lymphosarcoma and induced mammary carcinoma (Dunning et al., 1949) of rats.

However, the influence of protein and/or calorie restriction on incidence and growth of tumors in experimental animals appears to be dependent on the tissue origin, type and malignancy of the tumor as well as on the degree of restriction imposed and the composition of the restricted diet (Tannenbaum and Silverstone, 1953).

Underfeeding (all components of diet restricted) reduces tumor incidence and influences tumor growth. Moreschi (1909) found that grafts of mouse sarcoma grew less frequently and more slowly in animals on a restricted diet and losing weight. Other neoplasms which respond to underfeeding in the mouse include spontaneous mammary carcinoma (Tannenbaum, 1940), spontaneous hepatomas (Tannenbaum and Silverstone, 1949b), lung adenoma (Tannenbaum, 1940, 1942; Larsen and Hester, 1945), spontaneous leukemia (Saxton et al., 1944) and skin tumors induced by ultraviolet light (Tannenbaum, 1940, 1942). Rous (1914) reported that several transplanted rat and mouse tumors grew slower in underfed hosts than in controls. After surgical removal of primary tumor it was possible to delay the development and growth of tumors, in most cases, by underfeeding. Rous also noted, however, that the transplantable Flexner-Jobling rat carcinoma and a few spontaneous tumors were unaffected by underfeeding.

Altering the proportion of dietary protein influences the genesis of some tumor types but not that of others. Slonaker (1931) reported that rats fed isocaloric diets containing 22 or 26% protein appeared to be less subject to tumors of the mammary glands and ovaries in the females and of the skin in the males than those fed diets containing 10, 14 and 18% protein. Using a different tumor system, Tannenbaum and Silverstone (1949a) found that neither the incidence nor metastasis of spontaneous mammary carcinoma were altered when adult mice were fed ad libitum or restricted amounts of an isocaloric diet varying in the proportion of casein from 9 to 36%. In contrast, the incidence of hepatomas was considerably lower in adult male mice fed a diet containing 9% casein than in mice fed a diet containing 18 or 45% casein (Silverstone and Tannenbaum, 1951).

Ross and Eras (1965) also indicated that the proportion and intake of dietary protein evoked different effects, depending upon tumor type. The morbidity due to malignant lymphomas for male rats fed a low protein diet ad libitum, was 50% less than that of moderately restricted rats provided a diet, in isocaloric amounts, containing an adequate level of protein. In addition, pancreatic and primary lung tumors were found among rats with ample intake of protein but rarely when the intake of protein was low.

Ross and Eras (1973) undertock a study involving large pcpulations cf rats to determine whether chrcnic marginal protein undernutriticr and protein overnutrition under isccaloric conditions modified the tumcr-type spectrum of the populatien. Chrcnic marginal protein undernutrition predisposed the rats to an early occurrence and high morbidity due to tumcrs of lymphoreticular and hematopoietic tissues. Protein overnutrition, in contrast, increased the susceptibility tc urinary bladder papillomas. For other types of tumors of epithelial origin, principally those occurring in the pituitary, thyroid and pancreas, the highest mcrbidities cccurred when rats were fed a diet adequate in protein content. The morbidities due to these tumors were markedly decreased when the level of protein was either marginally low or excessively high.

In chickers nutritien has been shown to influence susceptibility to virus infection and tumorigenesis and development. Rcus (1911) observed that young, healthy, well-nourished chickens were more susceptible to transmissible chicken sarcoma than unthrifty chickens.

Biley and March (1959) studied the effect of two planes of nutrition on the incidence of the avian leukosis complex in two generatiens of fcur strains of White Leghorns. The lcw plane of nutritien provided sub-optimal levels of protein, vitamins and calories. The diets fcrmulated to promote the high plane cf nutritien ccntained ample levels of all nutrients without containing excessive amounts of any

cne nutrient. Growth rate was somewhat retarded in chickens cn the low plane, egg production was similar with the low and high plane and hatchability was depressed on the low plane of nutrition. The incidence of mortality frcm leukcsis varied between the strains but with all strains the incidence of leukosis was greater when the chickens were on the high level cf nutriticn.

In the previously cited research, infection was the result of natural exposure in an environment in which the disease was endemic. Biley and March (1965), usirg the same two planes cf nutriticn, studied the effect of nutrition on the incidence cf leukcsis fcllcwing inoculation with avian leukcsis virus. The high plane of nutriticn favored the development cf lymphcid leukosis fcllcwing inoculation with tumor virus.

Proudfoot and Aitken (1969) fed a 10% protein and a 16% protein rearing diet between 56 and 147 days of age to five ccmmercial leghorn strains. Mortality frcm a natural outbreak cf Marek's disease was nct only significantly different among strains but the chickens grcwn cn the higher protein rearing diet exhibited significantly higher mcrtality.

In summary, dietary restrictien of calories reduces the incidence and delays first appearance of many diverse types of neoplasms. Altering the prcpcrtion of dietary protein, within limits that support relatively normal growth and weight of the animal, influences the genesis of some tumor

types but not that of others. Protein and/or caloric restriction influence the growth of neoplasms but less impressively than they affect the genesis of them.

Immunity and Nutrition

Two types of immunity protect the body from the hazards of infection and cancer. A cell-mediated immune response combats fungi and viruses and initiates the rejection of foreign tissues, such as transplanted organs and tumors (Leclerc et al., 1972; Leclerc and Cantor, 1980). Humoral immunity is effective against bacterial infections and viral reinfection. Although the two mechanisms are not entirely independent, and cooperation between them is important, they are distinct (Golub, 1977).

The ultimate basis for this division of labor in the immune system lies in two populations of cells. These are the bone marrow or, in avian species, bursa-derived (B) lymphocytes which are direct precursors of antibody-producing cells and the thymus-derived (T) lymphocytes. Subpopulations of T-lymphocytes function as killer or effector cells, helper cells for induction of maximal antibody production (Miller and Mitchell, 1969) and suppressor cells with the capacity to inhibit antibody production (Katz and Benacerraf, 1972). As T-lymphocytes develop into these functional subpopulations, they pass through a series of steps in differentiation, and their surfaces display an extraordinary variety of surface

alloantigens (Boyse, 1972).

The interrelationships between immunity and undernutrition are complex and involve multiple interacting components (Ritchie and Copeland, 1979). Much of our knowledge of dietary effects on the immune system is derived from clinical studies of severely malnourished human populations in whom one or a combination of calories, protein, vitamins, or other dietary essentials are deficient. Meaningful interpretations of most clinical data are impossible because of lack of controls of several critical variables, such as concomitant infection, dose of antigen, severity of nutritional deficiencies, simultaneous institution of nutritional therapy, liver functions and competitive microbes (Chandra, 1977). For these reasons, only experimental animal studies will be reviewed here.

The nature of the antigenic stimulus employed is an important variable in evaluating the effect of chronic protein deprivation on the immune system. The antibody response to T-independent antigens, such as Brucella abortus, appears generally to be unaffected by chronic protein restriction (Cooper et al., 1974; Law et al., 1974; Price and Bell, 1977). In contrast, both the number of antibody-producing cells and serum antibody titers are markedly reduced in chronically protein-deprived mice immunized with sheep erythrocytes, a T-dependent antigen (Cooper et al., 1974; Law et al., 1974; Price and Bell, 1977; Fernandes et al., 1976a).

In protein-deficient mice, cell-mediated immunocompetence has been evaluated using graft-versus-host reactivity. Spleen cells from chronic protein-deprived mice demonstrated enhanced graft-versus-host reactivity compared to spleen cells from regular chow-fed mice (Bell and Hazell, 1975; Cooper et al., 1974).

A number of other assays have provided evidence that T-cell immunocompetence is maintained or even enhanced under conditions of chronic moderate protein deprivation. Skin allograft rejection appears to be enhanced in chronic protein-deprived mice (Cooper et al., 1974; McFarlane and Hamid, 1973). The capacity of T-cells to proliferate in vitro in response to phytohemagglutinin (PHA), a T-cell mitogen, is another indication of cell-mediated immunological competence. Spleen cells from mice (Cooper et al., 1974; Fernandes et al., 1976a) and guinea pigs (Kramer et al., 1977) fed protein-deficient diets were found to respond to PHA as well as or better than spleen cells from normal animals. In contrast, Aschkenasy (1975) noted that rats fed protein-free diets manifested poor in vivo responses to PHA.

Nutritionally deprived animals have reduced resistance to bacterial infections but increased resistance to certain viruses and growth of malignant tumors (Schaedler and Dubos, 1956; Boyd and Edwards, 1963; Anderson et al., 1970; Ross and Bras, 1965). These observations may be related to previously described studies in which chronic

protein-deprivation depressed humoral immunity while improving cell-mediated immune function. Jose and Good (1971b) reported that lymphocytes from immune rats subjected to moderate protein or caloric restriction manifested normal cytotoxic T-cell activity against xenogeneic target cells. Serum from immune rats fed a normal diet prevented cell-mediated destruction against target tumor cells. Such blocking activity was absent from the serum of nutritionally deprived rats. In a subsequent study, mice placed on protein restricted diets maintained cell-mediated immunity to both alloqeneic and syngeneic tumor cells, while serum blocking activity was inhibited or eliminated (Jose and Good, 1973a).

More recently, Fernandes et al. (1976a) studied the effect of chronic protein-calorie restriction on development of spontaneous adenocarcinoma in C3H/UmC mice. Although the life span of mice receiving 10 total calories per day was not significantly prolonged over that of mice receiving the normal diet (16 calories), none of the former group developed tumors whereas 71% of the latter group developed adenocarcinoma. Fernandes et al. (1976a) suggested that protein-calorie restricted animals might lack the ability to develop suppressor cells which occur in animals during tumor growth (Gershon et al., 1974; Gorczynski, 1974; Kuperman et al., 1975; Fujimoto et al., 1976) and are implicated in the inhibition of cellular immune function. The elimination of the development of such suppressor-cell activity and/or

absence of serum blocking factors (Jose and Good, 1971a, 1973a, 1973b) by protein-calorie restriction might facilitate destruction of newly arising tumor cells by immunocompetent T-cells and macrophages.

More drastic reductions in protein and/or caloric intake undoubtedly have more devastating effects on the total immune response. Fernandes et al. (1976a) showed that mice maintained on a severely restricted 3% casein diet had a depression of both humoral and cellular anti-tumor immune responses.

In summary, it is clear that dietary manipulations may profoundly influence immune reactions in experimental animals. Certainly in mice, rats and guinea pigs, protein-calorie restriction inhibits antibody production and humoral immunity. Although cell-mediated immunity appears to be enhanced by protein-calorie restriction in all these species, it can be profoundly depressed by more severe dietary deprivation.

Genetic Control of Immune Responses

The major histocompatibility complex (MHC) is a linked series of genes which control a large variety of immunological phenomena. While an MHC has been detected in all mammalian species studied (Paul and Benacerraf, 1977), that of the mouse, called the H-2 complex, has been characterized most thoroughly.

Molecules that differ from individual to individual and are recognized in graft rejection, were first described in the mouse by Gorer (1937). Gorer et al. (1948) designated these molecules as histocompatibility antigens and gave them a serial number--2. The gene coding for these structures was designated H-2. Later research proved that H-2 was a multigene, multiallelic complex, and it became termed the H-2 complex (Klein, 1975). The H-2 complex is located on chromosome 17 (Klein, 1979).

Besides coding for alloantigens responsible for rejection of incompatible neoplastic and normal tissue grafts, the H-2 complex influences antibody synthesis, mixed leukocyte reactions, graft-versus-host reactions, anamnestic response, delayed hypersensitivity, serum complement levels and T-cell:B-cell interactions (reviewed by Shreffler and David, 1975). In addition, the susceptibility of mice to several tumor viruses is associated with the H-2 complex.

The current H-2 map is divided into six regions (K, I, S, G, D and T) with the I region divided into five subregions (A, B, J, E and C) (Klein, 1979). K and D regions code for serologically defined H-2 antigens and transplantation antigens. The marker gene for the S region controls the serum serological (Ss) protein and the sex-limited protein (Slp). The Ss and Slp proteins are associated with, or may actually constitute one of the complement components (Nec et al., 1975). The G region codes for the appearance of an antigen on erythrocytes

(Klein, 1979). Klein and Chiang (1978) postulated the existence of a T region which codes for a gene that controls antigenic specificity of cytotoxic effector cells.

The I region of the H-2 complex codes for several immunologically important traits. The Ir genes, located within the I region, regulate the humoral response to synthetic polypeptides and a number of natural antigens as well as cellular response as measured by delayed-type hypersensitivity or proliferation of T-cells in vitro (McDevitt and Benacerraf, 1969; Benacerraf and Germain, 1978). An Ir gene may either enhance or suppress the response. The enhancing genes map in the A, B, C or E subregions; the suppressor gene in the J subregion. In addition, the I region is associated with the mixed leukocyte reaction, graft rejection and cellular interactions including macrophage:T-cell and T-cell:B-cell (Benacerraf and Germain, 1978). The I region also codes for a series of serologically defined cell surface antigens, termed the I-associated or Ia antigens. Whether these Ia antigens actually represent the Ir gene product remains a controversial issue (Klein, 1979). One current hypothesis (Uhr et al., 1979) is that the Ia antigens interact with exogeneous antigen associated with macrophages and B-lymphocytes and thereby present an immunologenic complex to the T-lymphocytes. This results in the stimulation of T-cells essential in the triggering of B-cells to replicate and differentiate into antibody-secreting cells.

The gene(s) controlling susceptibility of mice to several tumor viruses, parasites and to autoimmune diseases is localized in the I region of the H-2 complex. This association was initially shown with susceptibility of mice to leukomogenesis with the Gross virus (Lilly, 1964, 1966) and since has been shown with mammary tumors (Muhlbock and Dux, 1974) and spontaneous lung tumors (Faraldo et al., 1979). An Ir gene controls susceptibility of mice to experimental autoimmune thyroiditis (Vladiutiv and Rose, 1971). Genes within the H-2 complex influence susceptibility to infection with the parasite Trichinella spiralis (Wassom et al., 1979).

The B complex in the chicken is the counterpart of the MHC of other species (Frelinger and Shreffler, 1975). The B blood group system in the chicken was discovered by Briles et al. (1950) and was shown to be a marker for the MHC by Schierman and Nordskog (1961). Like the H-2 complex, the B system is characterized by extensive multiple allelism (Briles et al., 1950). Hala and associates (1977) have suggested that the chicken MHC consists of three regions and three corresponding antigens can be assumed: B-F region codes for antigens common to both erythrocytes and leukocytes, B-L region for antigens present on leukocytes and absent on erythrocytes and B-G region for antigens present on erythrocytes and not detected on leukocytes.

The B complex, like the H-2 complex, controls many immunological functions. B complex influences skin graft survival (Schierman and Nordskog, 1961), graft-versus-host reaction (Jaffe and McDermid, 1962; Schiermann and Nordskog, 1963), lymphoagglutination (Schierman and Nordskog, 1962), mixed leukocyte reaction (Miggiano et al., 1974) and serum hemolytic complement levels (Chanh et al., 1976). Resistance to a herpesvirus-induced lymphoma (Marek's disease) has been found to be associated with the B complex (Hansen et al., 1967; Longnecker et al., 1976; Briles et al., 1977).

Evidence for an association of the B complex with immune responsiveness to well-defined antigens has been obtained in chickens. Balcarova et al. (1973) reported differences in immune responsiveness in different inbred lines of chickens to the dinitrophenol group and to human serum albumin. Pevzner and coworkers (1973, 1975) have found the B complex to influence immunological response to Salmonella pullorum. Similar associations between the B complex and immune responsiveness have been found for the synthetic polypeptides, (T,G)-A--L (Gunther et al., 1974; Balcarova et al., 1975), GT (Koch and Simonsen, 1977) and GAT-10 (Benedict et al., 1975, 1977; Pevzner et al., 1978) and for tuberculin (Karakoz et al., 1974). Ewert and Cooper (1978) isolated Ia-like alloantigens in several highly inbred lines of chickens.

Gyles et al. (1968, 1971) studied the inheritance of Rous virus-induced tumor regression and showed that the incidence can be modified significantly by selection. Carte et al. (1972) have effectively increased the incidence of tumor regression in a Leghorn strain through selection. Two independent studies demonstrated that genetic control of the fate of Rous sarcoma virus (RSV)-induced tumor is within or closely linked to the B complex. Collins et al. (1977) studied the influence of B genotype on the outcome of sarcomas induced by Bryan high titer RSV (subgroup A) in the F2 generation of a cross of lines 6-1 and 15-1. Among the F2 segregants, B2B2, B2B5 and B5B5, the percentage of chickens dying of tumor (by 10 weeks post-inoculation) was 5, 26 and 93, respectively. Schierman et al. (1977) used strain G-B2, which is capable of regressing Rous tumors, and strain G-B1, which is susceptible to progressive tumor development. Each strain was homozygous for a different B blood group-histocompatibility antigen. Results from inoculating line G-B1, G-B2, F1, and backcross progeny with Schmidt-Ruppin strain of RSV (subgroup B) suggested dominance of tumor regression over tumor progression. Gebriel et al. (1979) found that genes coding for control of the fate of RSV-induced tumors evidently are closely linked to an immune response gene which controls antibody production to the amino acid polymer GAT-10 (Pevzner et al., 1978).

Several immunological functions in the chicken known to be genetically controlled are not associated with the B complex. Palladino et al. (1977) showed that the immune response of two inbred lines identical for the B complex, measured by the delayed hypersensitivity reaction, differed for bovine serum albumin (BSA), dodecanoic-BSA, ferritin and oxazolone. A gene controlling the ability of leukocytes to respond to concanavalin A was found not to be associated with the B complex (Miggiano et al., 1976).

In summary, the B complex of the chicken, like the H-2 complex of the mouse, controls many immunological phenomena including response to synthetic polypeptides, cell-mediated functions such as graft-versus-host reactions and mixed leukocyte reaction, and hemolytic complement levels. Incidence of RSV-induced tumor regression is under genetic control and recently has been shown to to be influenced by B genotype.

III. MATERIALS AND METHODS

Experiments

The present study is made up of 13 experiments. The following list gives the experiment numbers according to specific objective:

Specific Objective	Experiment Numbers
Effect of genotype and of 40 percent protein-calorie restriction and their interaction on tumor development	1,2,3,4,5
Comparison of 25 and 50 percent protein-calorie restriction on tumor development	6
Effect of age at RSV-inoculation on tumor development	7,8
Effect of length of 40 percent protein-calorie restriction prior to RSV-inoculation on tumor development	9,10
The delayed wattle reaction test as an *in vivo* measure of cell-mediated immune response	11
Effect of genotype and 40 percent protein-calorie restriction and their interaction on specific immunological tests in uninoculated chickens	12
Effect of genotype and 40 percent protein-calorie restriction on tumor size	13

Stocks

Two genetic systems were used to study the effect of protein-calorie restriction and their interaction upon Rous sarcoma development in chickens over a total of five experiments. In the first two experiments two genetically different lines of chickens were utilized. Line six subline three (6-3), a highly inbred (F > 0.99) single combed White Leghorn chicken, was developed and is maintained at the Regional Poultry Research Laboratory (RPRL) of the United States Department of Agriculture, East Lansing, Michigan. Line 105, a noninbred line of New Hampshires, which is maintained at the University of New Hampshire was used. The second genetic system, used in experiments 3, 4 and 5, involved comparisons between two homozygous B genotypes from the F2 generation of crosses of RPRL White Leghorn line six subline one (6-1) and line 15 subline one (15-1), each with an inbreeding coefficient in excess of 0.99 (Stone, 1975). Chickens for these three experiments were blood typed for B alloantigens and only genotypes B2B2 and B5B5 used.

Line 6K chickens were used in experiments 6 through 10, in which the effect of 25 and 50% protein-calorie restriction, the effect of age at Rous Sarcoma Virus (RSV)-inoculation, and the effect of length of protein-calorie restriction prior to RSV-inoculation upon tumor development were studied. Line 6-3, with 68.9% incidence of regression in 400 chickens RSV-inoculated at 6 weeks of age (Collins et al., 1980), was to be used in these

experiments. However, this line was later found to be genetically contaminated with an unknown stock; thus, the contaminated stock was designated line 6K. Since line 6K, like line 6-3, had a high incidence of tumor regression in chickens inoculated at 6 weeks of age (approximately 60%), it was used in these five experiments.

Several stocks of chickens were used in the delayed wattle reaction tests (experiment 11) including F2 and F3 generations of a cross of RPRL lines 6-1 and 15-1, and lines 6-3, seven subline two (7-2) and 105. After the experiment was completed lines 6-3 and 7-2 were found to be genetically contaminated, but this problem was not considered serious enough to warrant eliminating the experiment.

For studying the effect of 40% protein-calorie restriction upon immunological tests and tumor size (experiments 12 and 13, respectively), chickens from the F5 generation of a cross of lines 6-1 and 15-1 were used. Chickens for these two experiments were blood typed for B alloantigens and genotypes B2B2 and B5B5 only were used.

Brooding and Rearing

Chicks were brooded from hatching in conventional, electrically-heated brooding batteries located in windowless houses at the University of New Hampshire Poultry Research Farm. In protein-calorie restriction experiments chicks were transferred to a semi-isolated facility at 4 weeks of age at which time feed restriction began and the chickens

were maintained in holding batteries until termination of the experiments. In experiment 3, chicks were transferred to holding batteries at six, rather than at four, weeks of age at which time feed restriction began.

Chicks used in experiments 7, 8 and 11 were brooded from hatching in conventional, electrically-heated brooding batteries, transferred to semi-isolated facilities at 6 weeks of age and maintained in hanging cages until termination of the experiments.

Chicks were vaccinated at hatching with Marek's disease vaccine (live turkey Herpesvirus, chicken tissue culture origin, cell-free, Sterwin Laboratories Inc., Millsboro, Delaware) and at 10 days of age with Newcastle-bronchitis vaccine (live virus, chick embryo origin, Sterwin Laboratories Inc., Millsboro, Delaware) beginning with experiment 6. In experiments 12 and 13, each chick also received 0.2 mg gentamicin sulfate (Garasol, American Scientific Laboratories, Madison, Wisconsin) mixed with Marek's disease vaccine in a 0.2 ml dose subcutaneously at hatching to decrease chick mortality due to a recurrent respiratory problem at the University of New Hampshire Poultry Research Farm.

Feed

A commercially mixed chick starter feed (medicated with 0.004% amprolium and bacitracin methylene disalicylate to aid in the development of active immunity to coccidiosis

under conditions of slight exposure) was used in experiments 1, 2, 3, 4, 5, 12 and 13. With the exception of experiment 3, 4-week-old chickens were either full-fed or restricted-fed to provide 60% of the protein and calories consumed by full-fed chickens of the same age. In experiment 3 the same procedure was followed except that chicks were placed on experimental diets at 6 weeks of age. The amount of feed consumed by full-fed chickens was calculated twice weekly. For restricted-fed chickens, some vitamins and minerals were increased to compensate for the lower intake resulting from feed restriction (see Appendix I). Mortality was recorded daily and feed consumption recalculated on a per chicken basis on the day of death. With the exception of experiment 12, chickens remained on these rations for 12 weeks (2 weeks before RSV-inoculation and 10 weeks thereafter) at which time the experiment was terminated. In experiment 12, chickens were placed on experimental rations at 4 weeks of age and remained on these rations for 4 weeks at which time the experiment was terminated.

A commercially mixed grower (rather than starter) feed (medicated with 0.0125% amprolium and 0.0004% ethopabate) was used in experiment 6. Restricted chickens received the same feed except that the mineral and vitamin content was augmented to compensate for feed restriction (see Appendix II). The amount of feed consumed by the full-fed chickens was calculated twice weekly. The restricted groups received

75% and 50%, respectively, of the feed consumed by the full-fed chickens rather than 60% in previous experiments. Mortality was recorded in all decks on a daily basis and feed consumption recalculated on a per chicken basis on the day of death. Feed restriction began when the chicks were 6 weeks of age (with RSV-inoculation at 8 weeks of age) and continued until termination of the experiment at 70 days post-inoculation (PI) (18 weeks of age).

Throughout experiments 7, 8 and 11, chickens were fed commercially mixed starter (medicated with 0.0044% amprolium and bacitracin methylene disalicylate) feed ad libitum.

In experiments 9 and 10, a commercially mixed grower feed (medicated with 0.0125% amprolium and 0,004% ethopabate) was used since these chickens were 18 and 20 weeks of age, respectively, at the termination of the experiment. Minerals and vitamins were not added to the feed. The restricted groups received 60% of the feed consumed by the full-fed chickens. Mortality was recorded in all decks on a daily basis on the day of death. There were two restricted groups in experiments 9 and 10. One group of chickens was feed restricted at 4 weeks of age and the second at 6 weeks of age. Feed restriction continued until termination of the experiment at 70 days PI (18 and 20 weeks of age in experiments 9 and 10, respectively).

Body Weights

In all protein-calorie restriction experiments, all chickens were weighed individually every 2 weeks from the time they were placed on the feed treatments until termination of the experiment. Data on the effect of ration on body weight, unconfounded by the effect of tumor, was provided by one replicate of full-fed and one replicate of each group of restricted-fed uninoculated chickens in experiments 4, 5, 6, 9 and 13.

Virus and Virus Inoculation Procedure

A highly purified pseudotype of Bryan high titer Rous sarcoma virus, subgroup A, designated BH-RSV(RAV-1) and abbreviated RSV-1, was used. The virus was supplied by Dr. L. B. Crittenden, RPRL, of the U. S. Department of Agriculture, East Lansing, Michigan, and was stored in liquid nitrogen. The stock virus was diluted in Hanks' balanced salts solution containing 5% fetal calf serum plus 100 units penicillin, 100 ug streptomycin (GIBCO) and 10 ug hyaluronidase (Sigma Chemical) per ml. All chicks in RSV-induced tumor studies were injected subcutaneously in the left wing-web with 0.05 ml of a 10^{-3} dilution of the stock virus. The virus dose was equivalent to approximately 10 pock-forming units when measured on the chorioallantoic membranes of susceptible embryos.

Tumor Measurements

Beginning at 6 days PI the wing-web of each chick was examined for the presence of a primary tumor daily until no new tumors appeared. Tumors were examined for size at 2, 3, 4, 6, 8 and 10 weeks after RSV-inoculation. Tumor score (subjective) was based upon the following criteria (Collins et al., 1977):

Score	Criterion
0	No palpable tumor
1	Small tumor (>0 and up to 0.5 cm diameter)
2	Tumor (>0.5 cm up to 1.2 cm diameter)
3	Tumor (>1.2 cm up to half wing-web area)
4	Tumor (>half wing-web area, but <complete wing-web)
5	Tumor (filled wing-web area completely)
6	Tumor (massive; extended beyond wing-web)

In experiment 13, tumors were subjectively scored for size and in addition, the two largest dimensions were measured with vernier calipers to the nearest 0.01 cm. The area of the tumor was calculated using the formula for an ellipse (Schierman et al., 1977).

Tumor Profile Index (TPI)

The tumor scores of each chicken were plotted against the day the tumor was subjectively scored. Based upon the criteria below (Collins et al., 1977), a tumor profile index (TPI) was assigned.

TPI	Criterion
1	Complete regression by 28 days, or earlier
2	Complete regression by 42 or 56 days
3	Complete regression by 70 days, or a decreasing slope, or complete regression by 42 or 56 days followed by recurrence
4	General upward trend, or plateau; slight regression after 56 days
5	Terminal tumor prior to 70 days

Blood Collection

Blood was obtained for blood typing by lancing the brachial vein with a scalpel and collecting several drops of blood in a screw cap test tube containing 2 ml of 10% sodium citrate solution. Chicks were blood typed for B alloantigens at 2.5 weeks of age in the laboratory of Dr. W. E. Briles, Northern Illinois University, DeKalb, Illinois.

Delayed Wattle Reaction (DWR) Test

The DWR test, experiment 11, was used to study the cell-mediated immune response to diphtheria toxoid (DT) in uninoculated chickens from the F2 and F3 generations of a cross of EPRL lines 6-1 and 15-1, and lines 6-3, 7-2 and 105. The procedure was that of Klesius et al. (1977), with modifications, and is described below.

Immunization. DT was obtained from Dr. Frank McCarthy, Wyeth Laboratories, Marrietta, Pennsylvania. DT was emulsified in complete Freund's adjuvant to give a final concentration of 10 Lf DT/ml. Each chicken was injected subcutaneously at three sites with a total of 1 ml of this emulsion. All immunized chickens were at least 12 weeks old.

Test. A DWR test was made on each chicken three times at 2 week intervals starting 3 weeks after initial immunization. Control chickens were included in the experiment, but not immunized, and were tested 3 weeks after the test chickens along with previously immunized chickens.

All immunized and control chickens were challenged by intradermal injection of 1 Lf DT in 0.1 ml of 0.015 M phosphate buffered saline (PBS) containing 1% normal chick serum (NCS) into the right wattle. The left wattle, serving as the control, was injected with 0.1 ml of NCS-PBS only. A 27-gauge needle was used. The thickness of each wattle at the site of injection was measured with a vernier caliper to the nearest 0.01 cm at 48 hours after each challenge.

Phytohemagglutinin (PHA) Test

The PHA test was used to determine the effect of 40% protein-calorie restriction upon cell-mediated immunological capacity of uninoculated chickens in experiment 12. The method for producing chick spleen cell suspensions and the protocol for the PHA assay were those of Guyre (1979), with modifications.

Spleen Cell Suspensions. Spleens were removed and placed into approximately 15 ml of RPMI 1640 media (GIBCO) in a Petri dish. The capsule surrounding the spleen was removed and discarded. The spleen was teased into small fragments using forceps. A single cell suspension was made by drawing the fragments of spleen into a syringe and gently expressing them. This was repeated using an 18-gauge needle followed by a 22-gauge needle. Remaining large fragments were allowed to settle for about 5 minutes. The supernatant was centrifuged at 90 x g for 2 minutes. The supernatant was saved and centrifuged in a 12 ml conical centrifuge tube for 10 minutes at 150 x g. The buffy coat was carefully stirred into the fraction layer, the lymphocyte rich fraction was removed with a sterile Pasteur pipette and placed in a conical centrifuge tube. The lymphocyte rich fraction was centrifuged at 200 x g for 10 minutes. The pelleted cells were resuspended in Dulbecco's 1.11 X PBS and washed twice. The spleen cells were then resuspended in RPMI 1640 culture medium containing 2 mM L-glutamine (freshly added), 10% fetal calf serum, 100 ug streptomycin

and 100 units penicillin per ml of medium and immediately used in the PHA assay.

PHA Assay. Spleen cell density was adjusted to 3.33 X 10^6 cells/ml and 150 ul were added to each of 10 microtiter wells (Vanqard, U-bottom plate) for each spleen sample tested. Fifty microliters of a stock PHA (Sigma) solution (80 ug PHA/ml RPMI 1640 media) were added to each of five test wells. Fifty microliters of 1640 media were added to each of five control wells. The cultures were incubated for 72 hours in a humidified 5% CO2 incubator at 39C, then cultured for 16 hours with 1 uCi tritiated thymidine (New England Nuclear, 5.7 Ci/mmole) and harvested by suction onto glass fiber filters (Whatman 934) using a Mash II (Microbiological Associates) cell harvester. Filters were dried at room temperature overnight, placed in scintillation vials with 10 ml toluene (Baker) containing 0.4% 2,5 diphenyloxazole (PPO) and 0.005% 1,4-bis[2-(5-phenoxazolyl)]-benzene (POPOP) (Packard) and counted in a Packard Tri-carb scintillation counter (gain 51%, windows 50-1000).

A stimulatory index (SI) was calculated for each sample as follows:

$$SI = \frac{\text{Mean cpm PHA treated cells}}{\text{Mean cpm untreated cells}}$$

Assay for Antibody Production

An assay for antibody production against sheep erythrocytes was used to determine the effect of 40% protein-calorie restriction upon humoral immunological capacity of uninoculated chickens in experiment 12.

Immunization. Five chickens per replicate in experiment 12 were immunized with 1.0 ml of 1% suspension of washed sheep erythrocytes at 5 and 5.5 weeks of age. At 7 weeks of age each chick received a booster immunization of 1.0 ml of 1% suspension of washed sheep erythrocytes.

Serum Collection. A blood sample was obtained from each chicken at both 6 and 8 weeks of age by lancing the brachial vein with a scalpel and collecting the blood in sterile screw cap test tubes without heparin. The whole blood was allowed to coagulate at 37C for 1 hour and, upon syneresis, sera were collected with Pasteur pipettes and placed in sterile screw cap vials. The vials were heated in a 56C water bath for one hour to inactivate complement. The serum samples were then used in the hemagglutination test.

Hemagglutination Test. The protocol for the hemagglutination test was that of Herbert (1978), with modifications. Each serum sample was titered in microtiter plates (Vangard, U-bottom plate). In the first well, a dilution of 1:5 was made by adding 0.025 ml of serum to 0.100 ml of PBS. A two-fold dilution was made by transferring 0.050 ml of diluted serum into 0.050 ml of PBS, repeatedly. The final well served as a control with 0.050

ml PBS. Fifty microliters of 1% sheep erythrocytes were then added to each well. The microtiter plates were incubated at 37C for 2 hours at which time each well was examined for hemagglutination. The titer was determined as the inverse of the final dilution of serum in the last well showing hemagglutination.

Statistical Methods and Calculations

Data were subjected to analysis of variance (Snedecor and Cochran, 1967). In all these analyses statistical significance was determined at $P \leq 0.05$. A mean separation test (Duncan, 1955) was made regardless of significance of F (Steel and Torrie, 1960; Chew, 1977) in experiments with an equal number of experimental units. Because of unequal number of experimental units in experiments 7 and 8, the Bayes k-ratic t (LSD) test (Waller and Duncan, 1969) was made in these experiments.

In calculating mean tumor score for a given replicate, the tumor score of chickens dying with tumor prior to termination of the experiment entered into the determination in subsequent weeks. In the extreme case, for example, a replicate in which nine chickens have died with tumor (score 6) and one chicken completely regressed its tumor (score 0) is best represented with a mean tumor score of 5.4 rather than eliminate the dead chickens and give that replicate a mean score of 0.0.

IV. RESULTS

Effect of Genotype and of 40 Percent Protein-calorie Restriction and their Interaction on Tumor Development

Two genetic systems were used to study the effect of 40% protein-calorie restriction, and of the interaction of protein-calorie restriction and genotype, upon tumor development in chickens. Lines 6-3 and 105 were utilized in experiments 1 and 2 while B2B2 and B5B5 chickens from the F2 generation of a cross of lines 6-1 and 15-1 were used in experiments 3, 4 and 5. The results of these five experiments have been published (Clark et al., 1980).

Chickens of each line or of each B genotype were randomized to four replicates of approximately 10 chickens each with approximately equal numbers of each sex per replicate. Two replicates were then assigned at random to each dietary treatment (full-fed or restricted) within each line or B genotype. Each experiment was arranged factorially in a completely randomized block design.

Body Weight Controls. Figure 1 gives the effect of dietary restriction on body weight in uninoculated B2B5 chickens in experiments 4 and 5. After 12 weeks on dietary treatments (from 4 to 16 weeks of age), restricted-fed

chickens in experiments 4 and 5 weighed 58.7 and 59.4%, respectively, of that of full-fed chickens. Restricted-fed chickens continued to gain weight throughout the experiments.

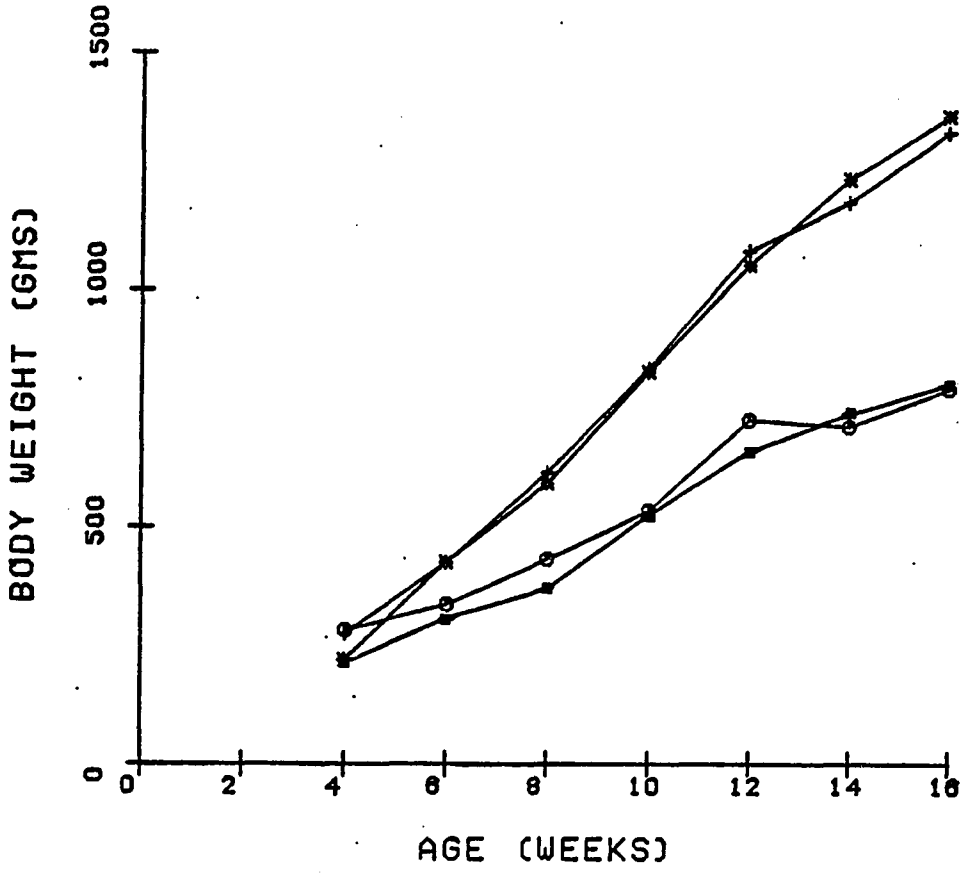

FIGURE 1. Mean body weight of uninoculated controls at various ages, experiments 4 and 5. Mean body weight was calculated for each of two replicates within a dietary treatment and the arithmetic mean of the two replicates was plotted for each experiment. + and *, Full-fed; ▪ and O, Restricted-fed.

Percentage of Chickens Developing Tumors. In line 6-3 76% of the restricted-fed and 63% of the full-fed chickens developed tumors. In line 105, however, 22% of the restricted-fed and 54% of the full-fed chickens developed tumors. An analysis of variance of these percentages, transformed to arcsins (Table 1), showed that line by dietary treatment interaction effect significantly influenced the percentage of chickens developing tumors.

In experiment 3, 73% of all chickens developed tumors. Considering both $\underline{B}^2\underline{B}^2$ and $\underline{B}^5\underline{B}^5$ genotypes, 56% of the restricted-fed and 90% of the full-fed chickens developed tumors (Table 2). An analysis of variance of the data underlying Table 2 showed that a significantly lower percentage of restricted-fed than full-fed chickens developed tumors.

Ninety-six percent of the chickens in experiment 4 and 100% of those in experiment 5 developed tumors. Therefore, in these experiments the data on percentage of chickens developing tumors were not subjected to statistical analysis.

TABLE 1

Analysis of variance testing effect of line, dietary treatment, and interaction upon tumor development, experiments 1 and 2

Source of variation	df	Mean squares for: Mean % of chickens developing tumors[1]	Mean length of latent period	Mean tumor score 2	3	4	6	8	10	Mean TPI
Experiment	1	261.9	0.37	0.01	0.81	0.71	6.80	4.83	5.88	1.89
Line (L)	1	1481.3*	0.02	0.24	0.44	1.36	2.46	0.64	0.57	0.02
Dietary treatment (D)	1	142.6	2.68	2.81*	2.29*	1.74	1.79	1.90	2.62	0.01
L X D	1	795.1*	0.05	0.07	0.33	1.45	0.41	1.28	2.31	0.12
Residual	11	75.7	1.92	0.18	0.32	0.73	1.53	2.10	2.40	0.48

[1]Percentages converted to arcsin before analysis

*$P \leq 0.05$

TABLE 2

Mean percentage of chickens developing tumors by genotype and dietary treatment, experiment 3

Genotype	Full-fed	Restricted
B2B2	95	56
B5B5	85	56

Latent Period. Time elapsing prior to first appearance of tumor was consistently longer in restricted-fed than in full-fed chickens in all five experiments. Analysis of variance of the data for experiments 1 and 2 (Table 1) and for experiments 3, 4 and 5 (Table 3), however, showed that restricted feeding significantly delayed the appearance of the tumor in experiments 3 through 5 only. The average delay due to feed restriction in the latter three experiments was 1.7 days compared to 0.8 day for experiments 1 and 2. The effects of line and B genotype on the time of first appearance of the tumor were not significant in any of the five experiments.

Tumor Score. Mean tumor score by line and dietary treatment, averaged across experiments 1 and 2, is plotted in Figure 2. For each of the six periods that tumors were scored for size the mean tumor score of replicates was subjected to analysis of variance (Table 1). Restricted-feeding reduced tumor score significantly relative to that of full-feeding at 2 and 3 weeks post-inoculation (PI), but Figure 2 showed this difference to be due primarily to the response of line 6-3. Line and line by dietary treatment interaction effect, did not significantly influence tumor size at any of the periods at which tumors were scored in these two experiments.

TABLE 3

Analysis of variance testing effect of genotype, dietary treatment, and interaction upon tumor development, experiments 3, 4 and 5

Source of variation	df	Mean squares for: Mean length of latent period	Mean tumor score (week PI) 2	3	4	6	8	10	Mean TPI
Experiment	2	17.51	2.92	1.01	1.39	2.05	2.46	2.41	0.69
B genotype (G)	1	0.00	0.01	2.85*	33.82*	96.28*	112.67*	125.63*	24.36*
Dietary treatment (D)	1	16.67*	2.29*	1.98*	1.56	0.36	0.47	0.40	0.00
G X D	1	0.32	0.02	0.02	0.06	0.06	0.03	0.23	0.09
Residual	18	0.46	0.16	0.42	0.79	0.31	0.20	0.14	0.07

*$P \leq 0.05$

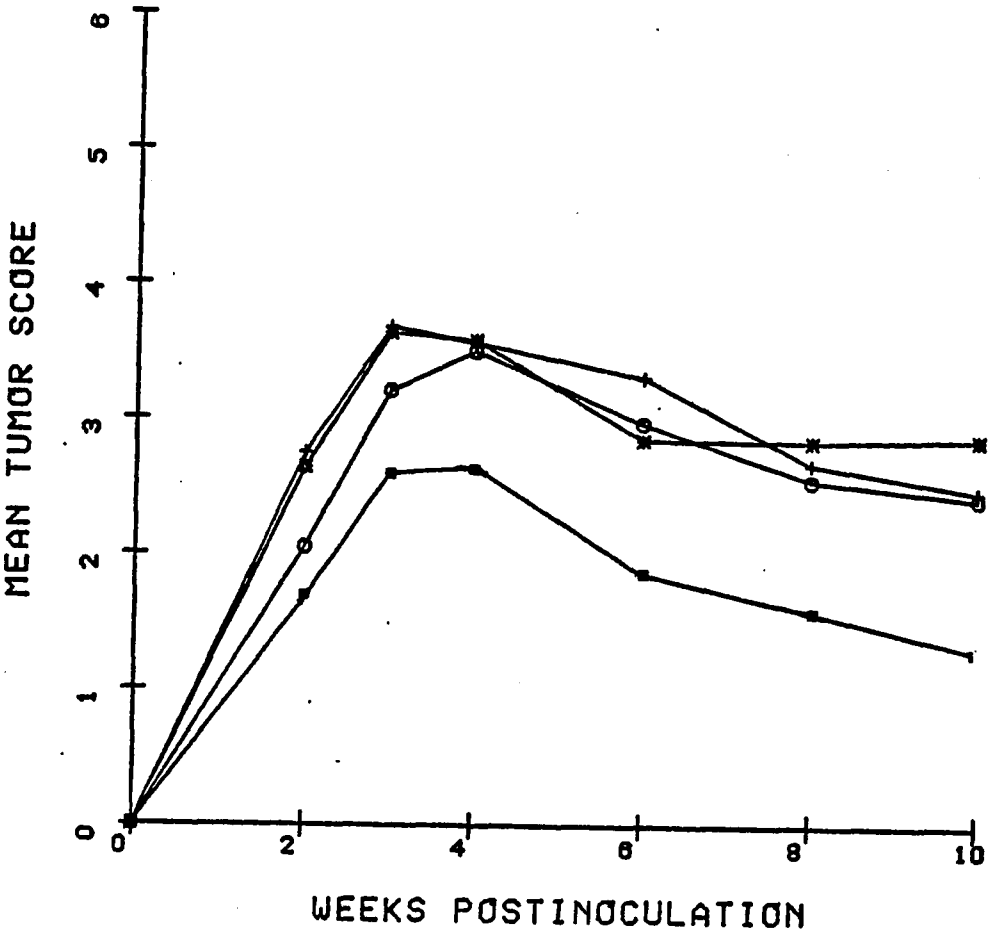

FIGURE 2. Mean tumor score of line 6-3 and line 105, respectively, at six different times PI for experiments 1 and 2. The mean tumor score of two replicates within line and dietary treatment was calculated for each experiment and the arithmetic mean of the experiment was plotted. The tumor score of chickens dying with tumor prior to 10 weeks entered into the determination of mean tumor score of a replicate in subsequent weeks. *, 6-3 Full-fed; ▪, 6-3 Restricted-fed; +, 105 Full-fed; O, 105 Restricted-fed.

Mean tumor score by B genotype and dietary treatment, averaged across experiments 3, 4 and 5, are graphed in Figure 3. Analysis of variance of these data are given in Table 3. B2B2 genotype had significantly smaller mean tumor score than B5B5 genotype at 3, 4, 6, 8 and 10, but not at 2 weeks PI. Restricted-fed chickens had significantly reduced mean tumor size at 2 and 3 weeks PI by 0.7 and 0.6 score, respectively, compared to full-fed chickens. Genotype by dietary treatment interaction effect for tumor size was minimal and not statistically significant.

Tumor Profile Index (TPI). A TPI was assigned to each chicken. Within a line, mean TPI for full-fed versus restricted-fed chickens was not consistent for experiments 1 and 2. Thus the analysis of the TPI data (Table 1) showed that the effects of line, dietary treatment and line by dietary treatment interaction were not significant.

In experiments 3, 4 and 5, the mean TPI for B2B2 hosts was 2.7 compared to 4.7 for B5B5 chickens. Differences in mean TPI between full-fed and restricted-fed chickens were small and not significant (Table 3). The effect of B genotype was significant but that of B genotype by dietary treatment interaction was not.

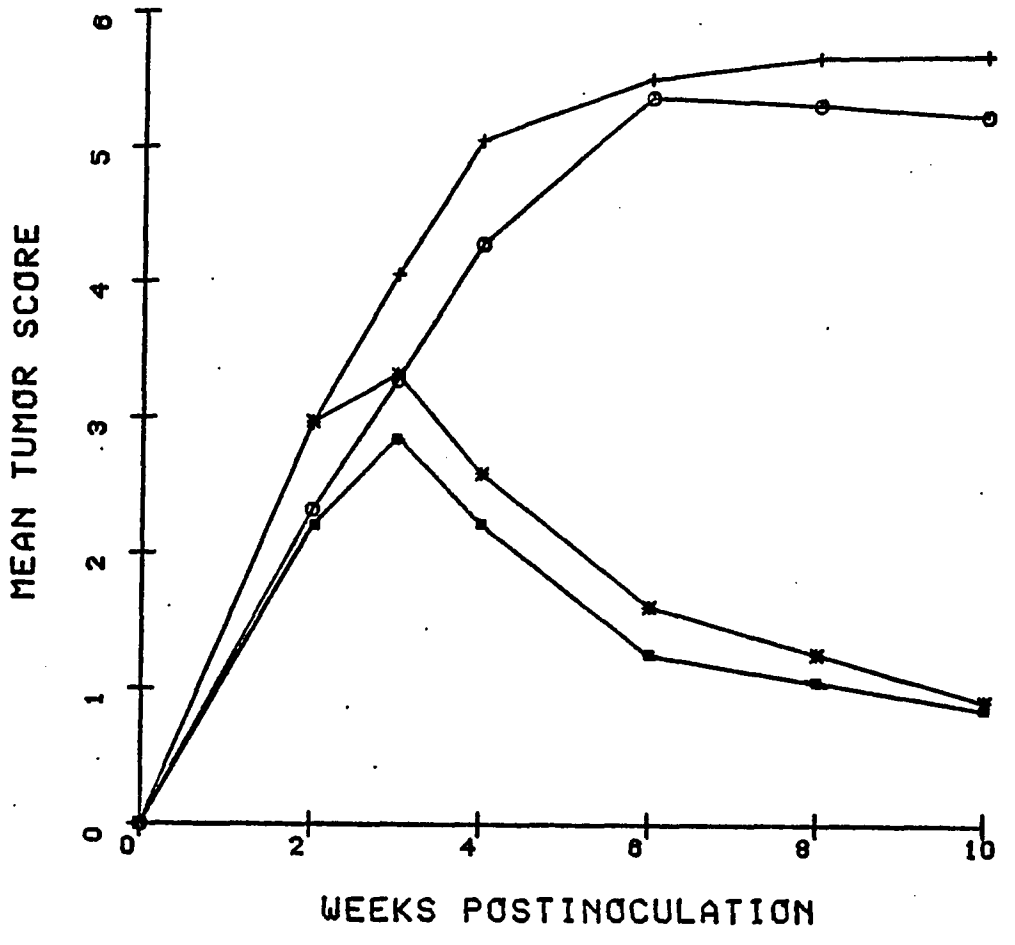

FIGURE 3. Mean tumor scores of B2B2 and B5B5 chickens, respectively, at six times PI for experiments 3, 4 and 5. The mean tumor score of two replicates within B genotype and dietary treatment was calculated for each experiment and the arithmetic mean of the experiment means plotted. The tumor score of chickens dying with tumor prior to 10 weeks entered into the determination of mean tumor score of a replicate in subsequent weeks. *, B2B2 Full-fed; ▪, B2B2 Restricted-fed; +, B5B5 Full-fed; O, B5B5 Restricted-fed.

Summary. Susceptibility to tumor formation was significantly affected by the line by dietary treatment interaction effect. That is, in line 6-3 a higher percentage of restricted-fed than full-fed chickens developed tumors while the opposite was true in line 105. Line did not significantly affect the number of days to first appearance of tumor, mean tumor score at 2, 3, 4, 6, 8 and 10 weeks PI, or TPI. Forty percent protein-calorie restriction significantly reduced mean tumor score at both 2 and 3 weeks PI by 0.8 score compared to full-fed chickens. The effect of protein-calorie restriction on later tumor development or on TPI was not significant despite the fact that tumor score in 6-3 restricted-fed chickens was consistently below that of 6-3 full-fed chickens throughout the experimental period (Figure 2).

Susceptibility to tumor formation was significantly reduced in restricted-fed compared to full-fed chickens from the F2 generation progeny of lines 6-1 and 15-1. Protein-calorie restriction significantly delayed the appearance of tumor by 1.7 days and significantly reduced mean tumor score at 2 and 3 weeks after RSV-1 inoculation by 0.7 and 0.6 score, respectively, compared to full-fed chickens. F2F2 hosts had significantly smaller tumors between 3 and 10 weeks PI and a smaller mean TPI than B5B5 hosts. Mean tumor score of restricted-fed chickens was consistently below that of full-fed chickens of both genotypes throughout the 10 week experimental period (Figure

3), however, not significantly so after 3 weeks PI. Differences in mean TPI between restricted-fed and full-fed chickens were generally in favor of restricted-fed chickens, but the differences were not significant.

Comparison of 25 and 50 Percent Protein-calorie Restriction on Tumor Development

Effect of level of protein-calorie restriction upon RSV-induced tumor development was studied in experiment 6. In previous experiments, chickens were restricted 40%, based upon earlier work of Dr. R. C. Ringrose with this level of feed restriction in chickens (personal communications). In experiment 6, the two restricted groups received 75 and 50%, respectively, of the feed consumed by full-fed chickens. These levels of feed restriction were chosen to determine the effect of 25 and 50% restriction relative to that of 40% restriction used in previous experiments on tumor development.

There were three hatches in this experiment, each designed identically. In each hatch chicks were assigned at random to one of twelve replicates of ten chickens each. Four replicates received the 50% protein-calorie restricted treatment, four the 25% protein-calorie restricted treatment and four were full-fed. Three replicates of each dietary treatment were inoculated with RSV-1, while the fourth served as a body weight control. Line 6K was used.

Body Weight Controls. Mean body weight of the uninoculated controls by dietary treatment, averaged across hatches, is plotted in Figure 4. After 12 weeks on the dietary treatments, the 25% protein-calorie restricted chickens of hatches 1, 2 and 3 weighed 76.3, 74.9 and 64.2%,

respectively, of that of full-fed chickens. Likewise, the 50% protein-calorie restricted chickens weighed 56.0, 53.1 and 45.9%, respectively, of that of full-fed chickens.

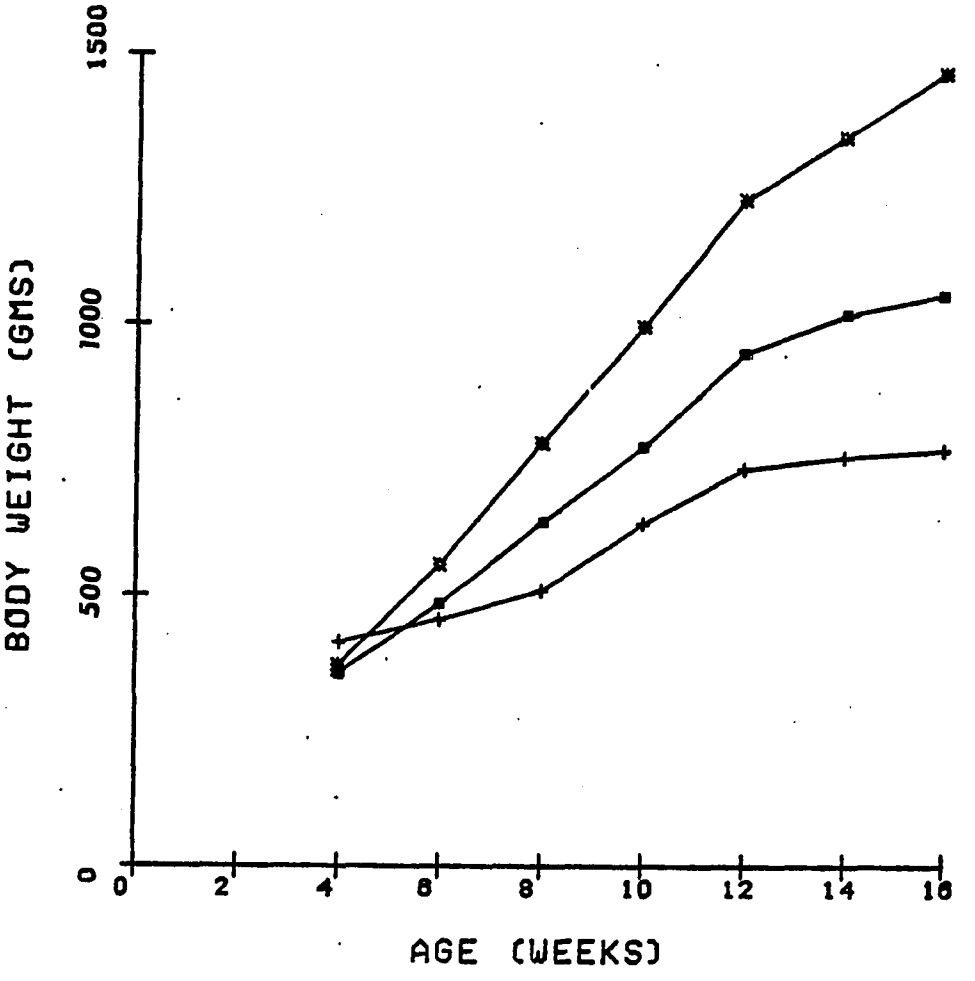

FIGURE 4. Mean body weight of uninoculated controls at various ages, experiment 6. Mean body weight was calculated for each replicate within a dietary treatment for each hatch and the arithmetic mean of the three hatches plotted. *, Full-fed; ▪, 25% Restricted-fed; +, 50% Restricted-fed.

Percentage of Chickens Developing Tumors. Percentages of chickens developing tumors by hatch and dietary treatment, averaged across replicates, are given in Table 4. By combining hatches susceptibility to RSV-induced tumor formation was lower in both 25 and 50% protein-calorie restricted chickens compared to full-fed chickens. An analysis of variance of these percentages, transformed to arcsins (Table 5), showed that dietary treatment effect did not significantly influence the percentage of chickens developing tumors.

TABLE 4

Mean percentage of line 6K chickens developing tumors by dietary treatment and hatch, experiment 6

Dietary Treatment	Hatch 1	2	3	Combined
Full-fed	55.2	73.3	72.6	66.9[a]
Restricted-fed (25%)	43.3	66.7	83.3	64.4[a]
Restricted-fed (50%)	16.7	73.3	77.8	55.9[a]
Overall mean	38.3	71.1	77.9	

Means having different superscripts are significantly different at $P \leq 0.05$. A mean seperation test (Duncan, 1955) was made.

TABLE 5

Analysis of variance testing effect of dietary treatment upon tumor development, experiment 6

Source of variation	df	Mean squares for: Mean % of chickens developing tumors[1]	Mean length of latent period	Mean tumor score (week PI) 2	3	4	6	8	10	Mean TPI
Hatch	2	1816.0	58.9	2.87	1.13	5.14	4.15	2.05	1.19	1.64
Dietary treatment	2	51.4	43.7*	3.97*	0.95*	0.44	0.01	0.39	0.04	0.16
Residual	22	145.5	3.6	0.22	0.27	0.39	0.49	0.71	0.73	0.38

[1]Percentages converted to arcsin before analysis

* $P \leq 0.05$

Latent Period. Time elapsing prior to first appearance of tumor was consistently longer in the 50% protein-calorie restricted than in the 25% protein-calorie restricted and full-fed chickens in all three hatches (Table 6). Full-fed chickens developed tumors 7.9 days after inoculation with RSV-1 while the 25 and 50% restricted-fed groups developed tumors at 8.3 and 11.9 days, respectively. The average delay with 50% protein-calorie restriction compared to 25% protein-calorie restriction and full feeding was 3.6 and 4.0 days, respectively. Dietary treatment significantly affected the length of time prior to first appearance of the tumor (Table 5). Duncan's multiple range test (Duncan, 1955) showed that 50% protein-calorie restriction significantly delayed tumor formation relative to full-feeding and to 25% restriction. Twenty five percent restriction was not significantly different than full-feeding.

TABLE 6

Mean number of days to first appearance of tumor by dietary treatment and hatch in line 6K, experiment 6

Dietary Treatment	Hatch 1	2	3	Combined
Full-fed	9.2	7.0	7.4	7.9[a]
Restricted-fed (25%)	10.0	7.8	7.0	8.3[a]
Restricted-fed (50%)	17.7	9.0	8.9	11.9[b]

Means having different superscripts are significantly different at $P \leq 0.05$. A mean separation test (Duncan, 1955) was made.

Tumor Score. Mean tumor score of line 6K by dietary treatment, averaged across hatches, is plotted in Figure 5. For each week that tumors were scored for size the mean tumor score of each of three replicates was subjected to analysis of variance (Table 5). Dietary treatment effect significantly influenced tumor size at 2 and 3 weeks PI. Fifty percent protein-calorie restricted-fed chickens had significantly smaller tumors compared to both 25% protein-calorie restricted and full-fed chickens at 2 and 3 weeks PI, as indicated by Duncan's multiple range test.

TPI. The mean TPI for the full-fed chickens was 2.8 compared to 2.6 for both restricted-fed treatment groups (Table 7). An analysis of variance of mean TPI's showed that dietary treatment effect did not significantly influence TPI.

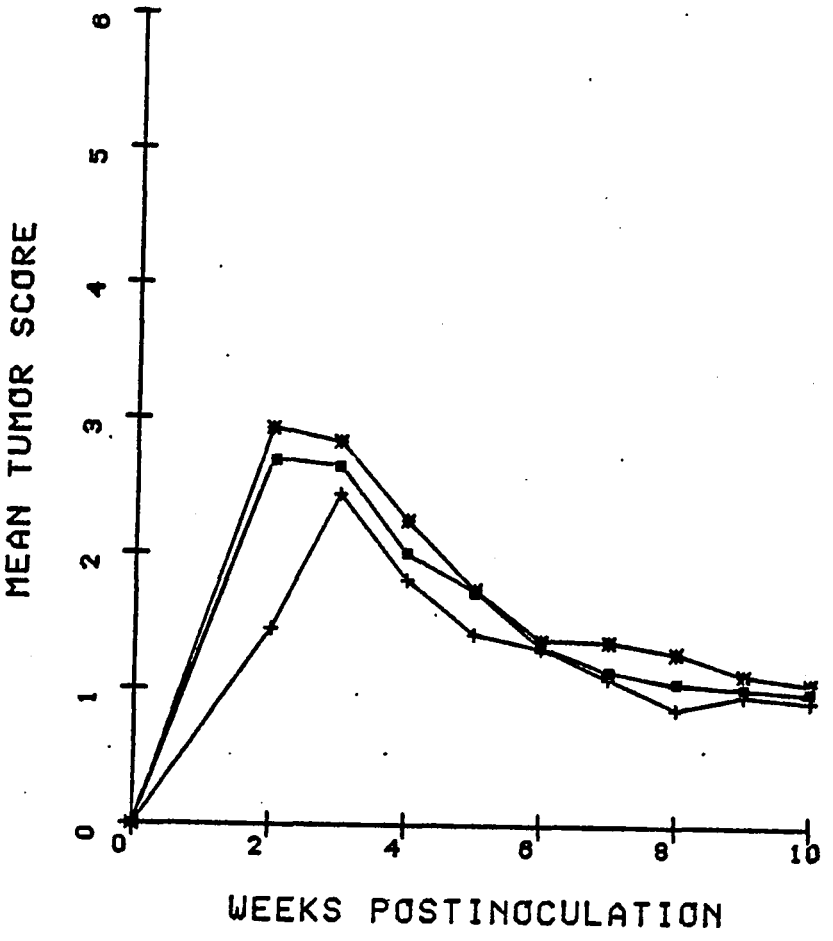

FIGURE 5. Mean tumor scores of line 6K by dietary treatment for experiment 6. The mean tumor score of three replicates was calculated for each hatch and the arithmetic mean of the hatch means was plotted. The tumor score of chickens dying with tumor prior to 10 weeks entered into the determination of mean tumor score of a replicate in subsequent weeks. *, Full-fed; ▪, 25% Restricted-fed; +, 50% Restricted-fed.

TABLE 7

Mean TPI by dietary treatment and hatch in line 6K, experiment 6

Dietary Treatment	Hatch 1	2	3	Combined
Full-fed	3.5	2.7	2.3	2.8[a]
Restricted-fed (25%)	3.0	2.6	2.2	2.6[a]
Restricted-fed (50%)	2.8	2.8	2.2	2.6[a]

Means having different superscripts are significantly different at $P \leq 0.05$. A mean separation test (Duncan, 1955) was made.

Summary. Protein-calorie restriction did not significantly influence the percentage of chickens developing tumors. Large hatch differences may be due to variable genetic susceptibility of the chickens to RSV-1 or variable patterns of nutritional utilizations between hatches.

Fifty percent protein-calorie restriction significantly delayed the appearance of tumor and significantly reduced mean tumor score at 2 and 3 weeks PI compared to 25% protein-calorie restriction and full-feeding. The influence of 50% protein-calorie restriction on tumor development after 3 weeks PI was not statistically significant.

The difference between the effect of full-feeding and 25% restriction was not significant for any of the traits studied in this experiment using line 6K.

Effect of Age at RSV-inoculation On Tumor Development

Before studying the effect of lengthening the period of protein-calorie restriction prior to RSV-inoculation, the effect of age of the chicken at RSV-inoculation on tumor development had to be investigated. Since older chickens would be inoculated with RSV-1 in these future experiments, it became necessary to know whether or not the age of the chicken at RSV-inoculation would influence susceptibility to tumor formation, tumor size and TPI.

In experiment 7 approximately 30 line 6K chickens were hatched every 2 weeks over a 10 week period. When the oldest chickens were 14, and the youngest 4 weeks old, all chickens were inoculated with RSV-1. In this experiment, effect of hatch was confounded with effect of age at RSV-inoculation.

In experiment 8 there was one hatch of approximately 200 line 6K chickens. Thirty were inoculated with RSV-1 at 4 weeks of age. Every 2 weeks thereafter for 10 weeks 30 more chickens were inoculated. A different inoculum preparation of RSV-1 was used for each age group of chickens in this experiment. Different virus preparations were used for each lot of chickens since experience had shown that diluted virus could not be stored more than 24 hours without losing titer. Thus in this experiment virus preparation and age of chickens at RSV-inoculation were confounded.

Percentage of Chickens Developing Tumors. Susceptibility to RSV-induced tumor formation was higher for chickens inoculated at 4 weeks of age than for other age groups when experiments 7 and 8 were combined (Table 8).

In experiment 7 susceptibility to tumor formation declined as age at inoculation increased with the exception of chickens inoculated at 14 weeks of age. Since hatch effect, if present, was confounded with effect of age of the host at RSV-inoculation, it is possible that the first hatch (chickens inoculated at 14 weeks of age) was genetically more susceptible to RSV-1 than the later hatches.

In experiment 8, chickens inoculated at 12 and 14 weeks of age appeared to be the least susceptible to RSV-induced tumor formation. The variation among the four younger groups was relatively small and may have been due to differences in titer of the different RSV-1 preparations used.

TABLE 8

Percentage of line 6K chickens developing tumors by age at inoculation, experiments 7 and 8

Age at Inoculation (Weeks)	Experiment 7[1]	Experiment 8[2]	Combined
4	83.3	70.0	75.9
6	75.0	66.7	70.7
8	58.6	73.3	66.1
10	47.1	60.0	53.1
12	39.4	36.7	38.1
14	72.2	29.0	52.2
Overall	61.4	55.8	58.6

[1]Chickens hatched at 2 week intervals, all inoculated on the same calendar date.

[2]All chickens hatched at one time, groups of 30 inoculated at 2 week intervals.

Tumor Score. Mean tumor score by age at RSV-inoculation for each week that tumors were scored in experiment 7 are given in Table 9. A separate analysis of variance of tumor score was made for each of the six periods that tumors were scored for size. The chicken was the experimental unit. In each analysis the sources of variation were age of host at inoculation, sex and interaction of sex and age of host at inoculation. In each analysis the effect of age of the host at RSV-inoculation was significant. Sex and interaction effects were not significant. At 4 weeks PI through termination of the experiment, chickens inoculated at 4 weeks of age had significantly larger tumors than chickens inoculated at older ages. (Comparisons of mean tumor score should be made within a column, i.e. week PI).

Mean tumor score by age at RSV-1 inoculation for each week that tumors were scored in experiment 8 are given in Table 10. A separate analysis of variance of tumor score was made for each of the six periods that tumors were scored for size. The chicken was the experimental unit. In each analysis the sources of variation were age at inoculation, sex and interaction of sex and age at inoculation. The effect of age of the host at RSV-inoculation was significant in each analysis. Sex and interaction effects were not significant. At 6, 8 and 10 weeks PI, chickens inoculated at 4 weeks of age had significantly larger tumors than those inoculated at 8, 10, 12 and 14 weeks of age with one

exception--those inoculated at 14 weeks of age at 6 weeks PI. At 6, 8 and 10 weeks PI, chickens inoculated at 6 weeks of age had intermediate sized tumors at 6 and 8 weeks PI.

TPI. Mean TPI's by age at RSV-inoculation are also given in Tables 9 and 10 for experiments 7 and 8, respectively. In experiment 7 (Table 9) chickens inoculated at 4 weeks of age had significantly higher TPI's than chickens of all other age groups. In experiment 8 (Table 10) chickens inoculated at 4 and 6 weeks of age had significantly higher TPI's than the other groups.

TABLE 9

Least squares mean tumor score and TPI by age at inoculation, experiment 7

Age at Inoculation (Weeks)	Mean Tumor Score (Week PI) 2	3	4	6	8	10	Mean TPI
4	3.2^{a}	4.3^{a}	4.0^{a}	3.3^{a}	3.0^{a}	2.8^{a}	3.8^{a}
6	2.9^{ab}	3.5^{b}	2.1^{b}	1.2^{b}	0.9^{b}	0.6^{b}	2.4^{b}
8	3.0^{ab}	3.6^{ab}	2.5^{b}	1.3^{b}	1.2^{b}	1.2^{b}	2.5^{b}
10	2.7^{b}	3.2^{c}	2.3^{b}	1.0^{b}	0.8^{b}	0.7^{b}	2.4^{b}
12	2.0^{c}	2.4^{bc}	1.6^{b}	0.5^{b}	0.2^{b}	0.1^{b}	2.1^{b}
14	2.9^{ab}	3.2	1.7^{b}	1.0^{b}	1.0^{b}	1.0^{b}	2.4^{b}

Chickers hatched at 2 week intervals, are inoculated on the same calendar date. Means within a column having different superscripts are significantly different at $P \leq 0.05$. Bayesian k-ratio t (LSD) test (Waller and Duncan, 1969) was made for mean tumor scores within each week PI and for mean TPIs.

TABLE 10

Least squares mean tumor score and TPI by age at inoculation, experiment 8

Age at Inoculation (Weeks)	Mean Tumor Score (Week PI) 2	3	4	6	8	10	Mean TPI
4	3.2^{a}	4.1^{a}	3.7^{a}	3.1^{a}	3.1^{a}	3.0^{a}	3.8^{a}
6	3.0^{a}	3.7^{ab}	3.1^{ab}	2.4^{ab}	2.2^{ab}	2.2^{ab}	3.4^{a}
8	2.9^{a}	3.2^{bc}	2.5^{bc}	1.4^{bc}	1.5^{b}	1.3^{b}	3.0^{b}
10	2.3^{b}	2.7^{c}	1.8^{c}	1.2^{c}	1.1^{b}	1.1^{b}	2.3^{c}
12	2.3^{b}	3.0^{c}	3.0^{ab}	1.2^{c}	1.0^{b}	1.0^{b}	2.6^{bc}
14	2.1^{b}	3.0^{c}	3.0^{ab}	1.8^{abc}	1.2^{b}	1.1^{b}	2.8^{bc}

All chickens hatched at one time, groups of 30 inoculated at 2 week intervals. Means within a column having different superscripts are significantly different at $P \leq 0.05$. Bayesian k-ration t (LSD) test (Waller and Duncan, 1969) was made for mean tumor scores within each week PI and for mean TPI's.

Summary. Younger chickens (i.e., 4 weeks of age at RSV-inculation) appeared to be more susceptible to RSV-induced tumor formation than chickens varying in age up to 14 weeks of age at time of inoculation. The incidence of tumors in chickens inoculated after 6 weeks of age varied between the two experiments and is difficult to evaluate. The variation is due in part to the different procedures in experiments 7 and 8 and also to possible differences in the genetic susceptibility of the host to RSV-1 in the different hatches within experiment 7.

In both experiments chickens inoculated with RSV-1 at 4 weeks of age had larger tumors than chickens inoculated at older ages particularly after 3 weeks PI. Besides having larger tumors, chickens inoculated at 4 weeks of age were less able to regress RSV-induced tumors as indicated by large TPI's. There was no significant difference in mean TPI's of chickers inoculated at 10, 12 and 14 weeks of age in either experiment.

Effect of Length of 40 Percent Protein-calorie Restriction prior to RSV-inoculation On Tumor Development

In all previous experiments, chickens were placed on restricted diets 2 weeks prior to RSV-inoculation. The effect of lengthening the period of protein-calorie restriction prior to inoculation with RSV-1 was studied in experiments 9 and 10. Lengthening the period of restriction may enhance the depressing influence of feed restriction on tumor growth.

Line 6K chickens were placed on restricted diets at 4 and 6 weeks of age with RSV-1 inoculation at 8 weeks in experiment 9 and at 10 weeks of age in experiment 10. Thus, over the two experiments the length of feed restriction period prior to virus inoculation ranged from 2 to 6 weeks. The restricted chickens received 60% of the feed consumed by the full-fed chickens.

The chickens in each experiment were randomized to twelve replicates of approximately 10 chickens each with approximately equal numbers of each sex per replicate. Four replicates were restricted-fed beginning at 4 weeks, four restricted beginning at 6 weeks of age and four were fed *ad libitum* throughout the experiment. In experiment 9 three replicates of each dietary treatment were inoculated with RSV-1 at 8 weeks of age and the fourth replicate served as a body weight control. In experiment 10 all four replicates

of each dietary treatment were inoculated with RSV-1 at 10 weeks of age.

The feed for the restricted groups was not supplemented with minerals and vitamins in experiments 9 and 10.

Body Weight Controls. Mean body weight of uninoculated controls in experiment 9 by dietary treatment are plotted in Figure 6. Chickens restricted at 4 weeks of age weighed 61.7% of that of full-fed chickens after 14 weeks of restriction while chickens restricted at 6 weeks of age weighed 62.2% after 12 weeks of restriction.

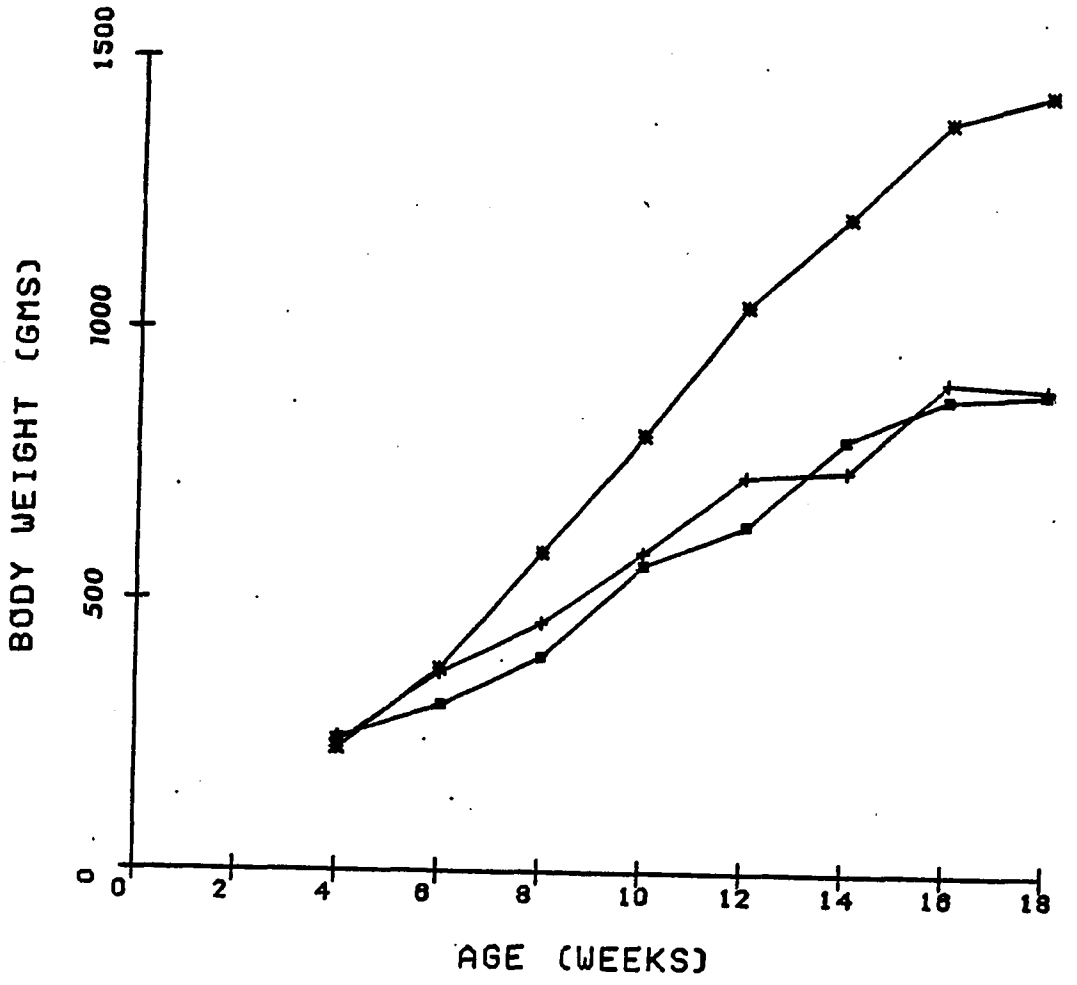

FIGURE 6. Mean body weight of uninoculated controls at various ages, experiment 9. Mean body weight was calculated and plotted for each replicate of body weight controls. *, Full-fed; +, Restricted-fed at 6 weeks of age; ▪, Restricted-fed at 4 weeks of age.

Percentage of Chickens Developing Tumors. Feed restriction starting at 4 and 6 weeks of age had no significant effect on the susceptibility of line 6K to RSV-induced tumor formation in either experiment (Tables 11 and 12). In experiment 9, 56.7% of the full-fed chickens developed tumors while 67.7 and 63.0% of the chickens restricted at 6 and 4 weeks of age developed tumors (with all chickens inoculated with RSV-1 at 8 weeks of age). In experiment 10, 65.0% of the full-fed chickens developed tumors while 82.8 and 70.0% of the chickens restricted at 6 and 4 weeks of age developed tumors, respectively (with all chickens inoculated with RSV-1 at 10 weeks of age).

Latent Period. In experiment 9 time elapsing prior to first appearance of tumor was significantly longer in both treatments of restricted chickens than in full-fed chickens (Tables 11 and 13). Full-fed chickens developed tumors at 7.8 days after inoculation with RSV-1 while chickens restricted at 6 and 4 weeks of age developed tumors at 9.9 and 9.0 days, respectively.

In experiment 10 restriction at 6 weeks of age delayed tumor appearance by 1.8 days but not significantly so (Tables 12 and 14).

TABLE 11

Analysis of variance testing effect of dietary treatment upon tumor development, experiment 9

Source of variation	df	Mean squares for: Mean % of chickens developing tumors[1]	Mean length of latent period	Mean tumor score (weeks PI) 2	4	6	8	10	Mean TPI
Treatment	2	31.2	3.25*	0.47*	1.28	1.48	1.73	1.53	0.87
Residual	6	36.8	0.31	0.02	0.80	0.51	0.63	0.70	0.20

[1]Percentages converted to arcsin before analysis

*$P \leq 0.05$

TABLE 12

Analysis of variance testing effect of dietary treatment upon tumor development, experiment 10

Source of variation	df	Mean squares for: Mean % of chickens developing tumors[1]	Mean length of latent period	Mean tumor score (week PI) 2	4	6	8	10	Mean TPI
Treatment	2	6.3	4.17	0.13	1.13*	2.21*	1.17*	0.67	0.36
Residual	9	146.2	3.07	0.15	0.22	0.28	0.27	0.38	0.13

[1]Percentages converted to arcsin before analysis

*$P \leq 0.05$

TABLE 13

Mean number of days tc first appearance of tumors by dietary treatment in line 6K, experiment 9

Dietary treatment	Latent period (days)
Full-fed	7.8 ± 0.4^{a}
Restricted at 6 weeks of age	9.9 ± 0.4^{b}
Restricted at 4 weeks of age	9.0 ± 0.0^{b}

All chickens were inoculated with BSV-1 at 8 weeks of age. For feed restricted chickens the duration of restriction was 12 and 14 weeks, respectively. A mean separation test (Duncan, 1955) was made. Means having different superscripts are significantly different at $P \leq 0.05$.

TABLE 14

Mean number of days to first appearance of tumor by dietary treatment in line 6K, experiment 10

Dietary treatment	Latent period (days)
Full-fed	7.6 ± 0.4^{a}
Restricted at 6 weeks of age	9.4 ± 0.7^{a}
Restricted at 4 weeks of age	7.6 ± 0.3^{a}

All chickens were inoculated with RSV-1 at 10 weeks of age. For feed restricted chickens the duration of restriction was 14 and 16 weeks, respectively. A mean separation test (Duncan, 1955) was made. Means having different superscripts are significantly different at $P \leq 0.05$.

Tumor Score. Mean tumor scores of line 6K by dietary treatment, averaged across replicates, is plotted in Figures 7 and 8 for experiments 9 and 10, respectively. A separate analysis of variance of tumor score was made for each week that tumors were scored for size. The mean tumor score of each replicate was subjected to analysis of variance (Tables 11 and 12).

In experiment 9 dietary treatment significantly affected tumor score at 2 weeks PI but not thereafter, but Figure 7 shows that the tumor score of chickens restricted at 4 weeks of age was consistently below that of full-fed chickens and chickens restricted at 6 weeks of age (2 weeks prior to virus inoculation) from 4 weeks on. A mean separation test (Duncan, 1955) showed that both restricted-fed treatment groups had significantly smaller tumors than full-fed chickens at 2 weeks PI.

In experiment 10 dietary treatment significantly affected tumor score from 4 weeks through 8 weeks after inoculation with RSV-1 at 10 weeks of age. A mean separation test (Duncan, 1955) showed that both restricted-fed treatment groups had significantly smaller tumors than full-fed chickens at 4, 5, 6, 7 and 8 weeks PI.

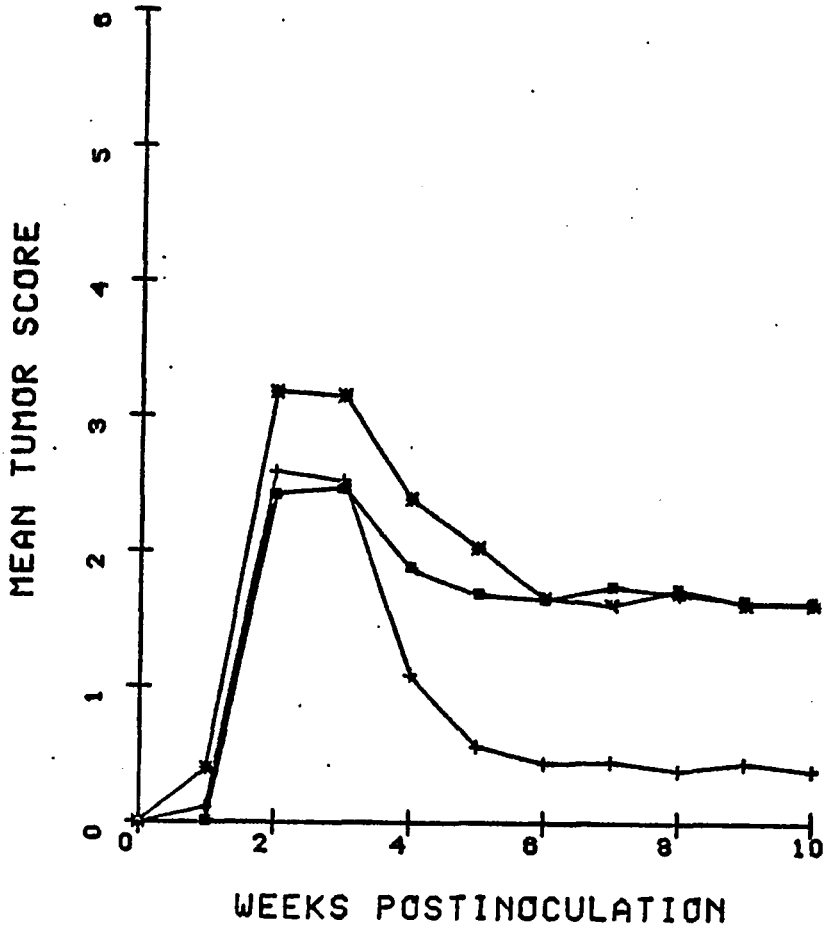

FIGURE 7. Mean tumor score of line 6K by dietary treatment for experiment 9. All chickens were inoculated with RSV-1 at 8 weeks of age. The mean tumor score of three replicates was calculated and the arithmetic mean of the replicate means was plotted. The tumor score of chickens dying with tumor prior to 10 weeks entered into the determination of mean tumor score of a replicate in subsequent weeks. *, Full-fed; ▪, Restricted-fed at 6 weeks of age; +, Restricted-fed at 4 weeks of age.

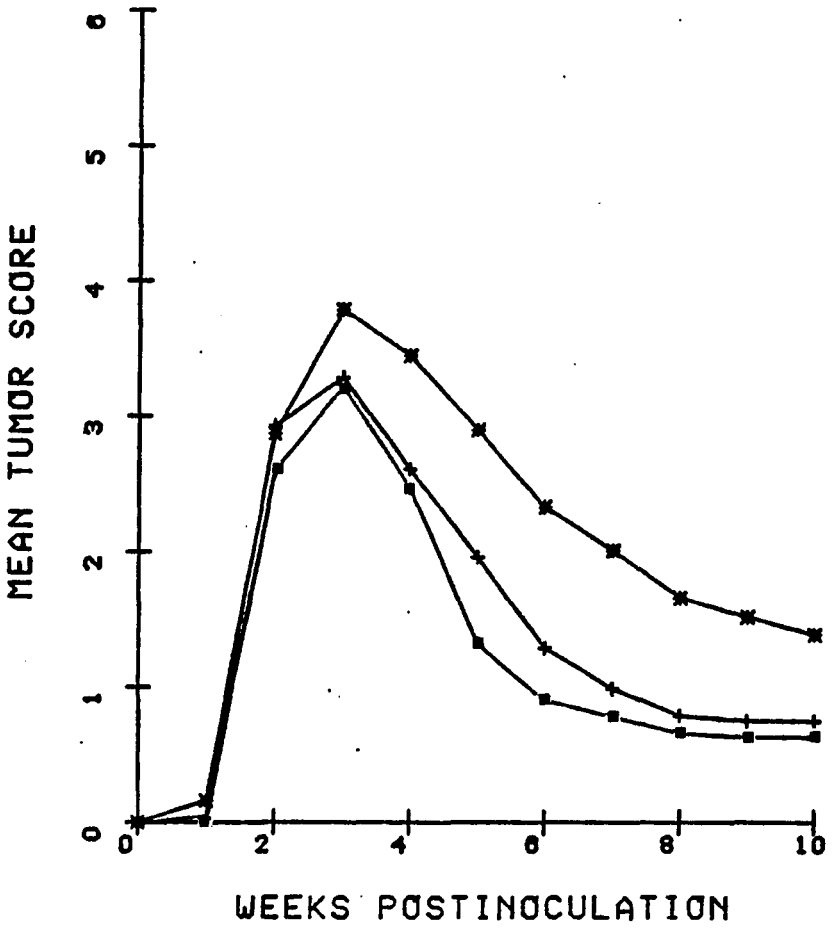

FIGURE 8. Mean tumor score of line 6K by dietary treatment for experiment 10. All chickens were inoculated with RSV-1 at 10 weeks of age. The mean tumor score of four replicates was calculated and the arithmetic mean of the replicate means plotted. The tumor score of chickens dying with tumor prior to 10 weeks entered into the determination of mean tumor score of a replicate in subsequent weeks. *, Full-fed; ▪, Restricted-fed at 2 weeks of age; +, Restricted-fed at 4 weeks of age.

TPI. In experiment 9 the mean TPI for chickens restricted at 4 weeks of age was 1.9 compared to 2.8 for full-fed chickens and chickens restricted at 6 weeks of age (Table 15). Mean TPI for chickens restricted at 4 weeks of age was significantly lower than that for the other two dietary treatments.

In experiment 10 the mean TPI for chicken restricted at 4 and 6 weeks of age was 2.5 and 2.6 compared to 3.0 for full-fed chickens (Table 16). The differences among these mean TPI's were not statistically significant.

TABLE 15

Mean TPI by dietary treatment in line 6K, experiment 9

Dietary treatment	Mean TPI
Full-fed	2.8 ± 0.2[a]
Restricted at 6 weeks of age	2.8 ± 0.4[a]
Restricted at 4 weeks of age	1.9 ± 0.3[b]

All chickens were inoculated with RSV-1 at 8 weeks of age. For feed restricted chickens the duration of restriction was 12 and 14 weeks, respectively. A mean separation test (Duncan, 1955) was made. Means having different superscripts are significantly different at $P \leq 0.05$.

TABLE 16

Mean TPI by dietary treatment in line 6B, experiment 10

Dietary Treatment	Mean TPI
Full-fed	3.0 ± 0.3^{a}
Restricted at 6 weeks of age	2.6 ± 0.2^{a}
Restricted at 4 weeks of age	2.5 ± 0.3^{a}

All chickens were inoculated with RSV-1 at 10 weeks of age. For restricted chickens the duration of feed restriction was 14 and 16 weeks, respectively. A mean separation test (Duncan, 1955) was made. Means having different superscripts are significantly different at $P \leq 0.05$.

Summary. Forty percent restriction 2, 4 and 6 weeks prior to virus inoculation appeared to have no effect on the susceptibility to tumor formation in line 6K in experiments 9 and 10. Similarly in experiment 6 50% protein-calorie restriction 2 weeks prior to RSV-inoculation had no effect on susceptibility to tumor formation in line 6K.

In experiment 9 the two restricted groups (restricted 2 and 4 weeks prior to RSV-inoculation) developed tumors later and had significantly smaller tumors at 2 weeks PI than the full-fed groups. In experiment 10 both restricted groups (restricted at 4 and 6 weeks prior to virus inoculation at 10 weeks of age) had significantly smaller tumors at 4 through 8 weeks PI than the full-fed group. Lengthening the period of restriction combined with later RSV-inoculation apparently delayed the effect of dietary treatment on tumor size (Figures 7 and 8). However, in experiment 10 the differences in mean TPI's among the three dietary treatment groups were not significant (Table 16). Thus even though tumors were smaller in the restricted groups the outcome of the tumor using TPI as the criterion, was not affected, but presumably tumor burden was smaller in restricted groups.

The Delayed Wattle Reaction Test as an in vivo Measure of Cell-mediated Immune Response

Before studying the effect of protein-calorie restriction upon immunological capacities, the feasibility of using the delayed wattle reaction (DWR) test was investigated. The DWR test is an *in vivo* test in which for a given chicken the difference between the thickness of the wattle challenged with diphtheria toxoid (DT) and that of a control wattle injected with phosphate buffered saline (with 1% normal chick serum) is used as a measure of cell-mediated immune responsiveness. This was experiment 11.

DWR Test in (6-1 X 15-1) F2 Chickens. In this part of the experiment, F2 generation progeny of a cross of lines 6-1 and 15-1 were used. B2B2, B2B5 and B5B5 chickens of both sexes were utilized in the study. Thirty-one chickens were immunized at 13 weeks of age with DT and challenged with DT three times at 2 week intervals for one test. Fifteen non-immunized chickens served as controls. Five of the 15 control chickens were tested for a response to DT at each challenge period. The control chickens did not respond in any of the tests.

Wattles were measured at 24, 36 and 48 hours after each challenge with DT to determine the time of maximum response. The repeatability of a single measurement of the thickness of a given wattle at a given time, as determined by intraclass correlation, was 0.99, thus, a wattle was

measured only once at a given time in subsequent challenges. In all three challenges the response at 24 hours after challenge was significantly lower than the responses at 36 and 48 hours which were not significantly different from each other. Thus, in subsequent DWR challenges, for convenience, wattles were measured at 48 hours after challenge with DT.

The 48-hour response resulting from each of the three challenges for each immunized chicken was subjected to an analysis of variance and an estimate of repeatability of response obtained. Repeatability was estimated to be 0.48. Since this is only moderate repeatability each chicken in subsequent DWR tests was challenged three times.

Mean response of males and females by B genotype for each challenge are given in Table 17 together with the number of chickens tested. Ignoring B genotype the mean response for females was 1.06 mm compared to 0.57 mm for males. The mean response, averaged across all three challenges for each chicken was subjected to analysis of variance (Table 18). The effect of sex was significant. Effect of B genotype and the interaction of B genotype and sex effect were not significant.

TABLE 17

Mean response (millimeters) to DT of (6-1 X 15-1)F2 progeny by sex and B genotype for each of three challenges, experiment 11

	Male			Female		
Challenge	B2B2	B2B5	B5B5	B2B2	B2B5	B5B5
1	0.37	0.60	0.48	1.09	0.79	1.25
2	0.53	0.78	0.53	1.10	1.11	1.15
3	0.65	0.68	0.63	0.90	1.09	1.20
Combined	0.52	0.68	0.54	1.03	1.00	1.20
Number	6	4	4	7	7	4

TABLE 18

Least squares analysis of DWR test in (6-1 X 15-1) F2 generation progeny

Source of variation	df	Mean square
B Genotype	2	2.6
Sex	1	199.0*
B genotype X Sex	2	5.1
Residual	26	8.1

* $P \leq 0.05$

DWR Test in (6-1 X 15-1) F3 Chickens. A total of 68 male and female F3 generation progeny of crosses of lines 6-1 and 15-1 were tested in two hatches. Twelve chickens were in the first hatch, 52 in the second. Only B2B2 and B5B5 chickens were included in the study. Chickens were immunized at 13 weeks of age in both hatches. The criterion of response for each chicken was the response at 48 hours averaged across three challenges.

Mean response of males and females by B genotype are given in Table 19 together with the number of chickens tested. The mean response for females was 0.47 mm compared to 0.20 mm for males. The mean response for each chicken was subjected to analysis of variance (Table 20). Sex, B genotype, and the sex by B genotype interaction effects were not significant.

DWR Test in Lines 6-3, 7-2 and 105 Chickens. Forty-six adult chickens were tested. Nine females from each line and nine 6-3 and ten 7-2 males were tested. No 105 males were tested. The criterion of response for each chicken was the mean response at 48 hours after each challenge over the three challenges.

The mean responses of males and females by line are given in Table 21 together with the number of chickens tested. In line 6-3 and 7-2 females responded to a greater extent than males. In both sexes, the mean response for lines 7-2 was greater than that for line 6-3. The mean response for each chicken was subjected to analysis of

variance (Table 22). Line, sex and line by sex interaction effects were significant.

TABLE 19

Mean response (millimeters) to DT of (6-1 X 15-1) F3 progeny by sex, B genotype and hatch, experiment 11

Hatch	Male		Female	
	B2B2	B5B5	B2B2	B5B5
1	0.18	0.15	0.14	0.53
2	0.14	0.25	0.24	0.75
Combined	0.15	0.23	0.22	0.70
Number	13	18	18	19

TABLE 20

Least squares analysis of variance of DWR test in (6-1 X 15-1)F3 generation progeny, experiment 11

Source of variation	df	Mean square
Hatch	1	663.6
B Genotype	1	1750.4
Sex	1	3668.4
B genotype X Sex	1	1740.7
Residual	63	3163.5

None of the mean squares tested was significant, $P \leq 0.05$.

TABLE 21

Mean response (millimeters) to DT of lines 6-3, 7-2 and 105 by line and sex, experiment 11

Line	Male	Female
6-3	0.25 (9)	1.19 (9)
7-2	0.51 (10)	2.63 (9)
105	NT	0.43 (9)

Number in parenthesis represents number of chickens tested.

NT = not tested

TABLE 22

Least squares analysis of variance of DWR test in lines 6-3, 7-2 and 105, experiment 11

Scurce of variation	df	Mean square
Line	2	989.2*
Sex	1	2179.9*
Line X Sex	1	3C2.5*
Residual	41	49.0

* $P \leq 0.05$

Summary. In the DWR test females from the F2 generation progeny of a cross of lines 6-1 and 15-1 responded to DT challenge to a greater extent than did males from the same cross. The effect of B genotype upon the immune response was not significant.

The DWR response to DT in F3 generation progeny of a cross of lines 6-1 and 15-1 was smaller than that in the F2 generation progeny possibly due to different preparations of DT or to segregation of gene(s) involved in the response. There was no sex difference in response and B genotype did not significantly affect the DWR test in the F3 generation progeny.

The mean response to DT of line 7-2 chickens was greater than that of line 6-3 in both males and females. Within lines 7-2 and 6-3, the response to DT of females was greater than that of males as determined by the DWR test.

In this experiment (6-1 X 15-1)F2 and F3 generation progeny were low responders to DT using the DWR test. In evaluating cell-mediated immunocompetence in future protein-calorie restriction experiments, it is necessary to use stocks which are known responders. Since (6-1 X 15-1)F5 generation progeny were going to be used, the DWR test would be inappropriate.

Effect of Genotype and 40 Percent Protein-calorie Restriction on Specific Immunological Tests In Uninoculated Chickens

Forty percent protein-calorie restriction delayed the appearance of RSV-induced tumors (experiments 3, 4 and 5) and reduced tumor score during the first 3 weeks PI (experiments 1 through 5). The objectives of experiment 12 were to compare the immunological capabilities of full-fed versus protein-calorie restricted-fed chickens and of B2B2 versus B5B5 chickens. The immunological tests were a PHA assay as a criterion of cell-mediated immunity and antibody titer as a measure of humoral immunity. The restricted chickens received 60% of the feed consumed by the full-fed chickens beginning at 4 weeks of age.

There were two hatches in this experiment, each designed identically. In both hatches, chicks within each B genotype, were assigned at random to one of four replicates of approximately 10 chickens each with approximately equal numbers of each sex per replicate. Two replicates were then assigned to each dietary treatment (full-fed or 40% restricted-fed) within each genotype. Chicks were not RSV-inoculated.

PHA Assay. At 4, 5, 6, 7 and 8 weeks of age one chicken from each replicate was sacrificed for the PHA assay. A stimulatory index (SI) for each of two replicates within B genotype and dietary treatment for each of two

hatches was calculated. Mean SI by B genotype and dietary treatment for each week are given in Table 23. A separate analysis of variance of SI's was made for each week that a PHA assay was done using the SI for the chicken from a replicate as the experimental unit (Table 24). At 6 weeks of age the interaction of B genotype and dietary treatment was significant. Thus, 6-week-old B2B2 full-fed chickens had higher SI's than corresponding B2B2 restricted-fed chickens (Table 23). On the other hand, 6-week-old B5B5 restricted chickens had higher SI's than the B5B5 full-fed chickens.

TABLE 23

Mean stimulatory indices in the PHA assay by B genotype and dietary treatment and by age of the chicken, experiment 12

Age (Weeks)	B2B2		B5B5	
	Full-fed	Restricted	Full-fed	Restricted
4	13.7	6.5	7.0	4.2
5	4.1	4.5	6.4	4.4
6	25.1	1.8	2.1	8.0
7	1.1	1.0	1.2	1.3
8	1.6	2.6	1.4	1.4

Each mean is the arithmetic mean of four chickens.

TABLE 24

Analysis of variance testing effect of B genctype, dietary treatment, and interaction upon stimulatory indices in the PHA assay at 4, 5, 6, 7 and 8 weeks of age, experiment 12

Source of variation	df	Mean Squares				
		4	5	6	7	8
Hatch	1	52.2	5.0	830.3	1.5	1.1
B genotype (G)	1	77.0	3.2	280.2*	0.1	1.1
Dietary treatment (D)	1	95.6	3.0	313.8	0.0	1.0
G X D	1	18.2	7.3	829.2*	0.1	1.0
Residual	11	86.5	11.9	163.2	0.6	1.7

* $P \leq 0.05$

Antibody Titer. At 6 and 8 weeks of age serum from each of five chickens per replicate was tested for antibody production to sheep erythrocytes. Mean antibody titer for each replicate was calculated as the geometric mean of the antibody titers of the five chickens of that replicate. Antibody titers at 6 and 8 weeks of age by B genotype and dietary treatment across hatches are given in Table 25. In the B2B2 genotype mean antibody titer of full-fed chickens was nearly double that of feed restricted chickens at both 6 and 8 weeks of age. In the B5B5 genotype restricted chickens had the higher titers. The geometric mean antibody titer of each replicate was subjected to analysis of variance at 6 and 8 weeks of age (Table 26). Antibody titers were significantly higher in B5B5 chickens than in B2B2 chickens at 6 weeks of age but not at eight. Dietary treatment did not significantly affect antibody titers at either 6 or 8 weeks of age.

TABLE 25

Mean antibody titers by B genotype and dietary treatment, experiment 12

	6 weeks		8 weeks	
Genotype	Full-fed	Restricted	Full-fed	Restricted
B2B2	53.0	28.1	263.6	154.0
B5B5	72.8	78.2	205.3	310.8

The geometric mean antibody titer was calculated for a replicate which consisted of five chickens. Each mean in the table is the arithmetic mean of the four geometric means, two from each hatch. Each mean in the table represents the antibody titers of 20 chickens.

TABLE 26

Analysis of variance testing effect of B genotype, dietary treatment, and interaction upon antibody titer at 6 and 8 weeks of age, experiment 12

Source of variation	df	Mean squares	
		6 weeks	8 weeks
Hatch	1	0.054	0.476
B genotype (G)	1	0.426*	0.055
Dietary treatment (D)	1	0.035	0.000
G X D	1	0.069	0.106
Residual	11	0.039	0.039

* $P \leq 0.05$

Summary. Protein-calorie restriction had a limited influence on immunological capabilities as indicated by the PHA assay. Protein-calorie restriction may have enhanced the cell-mediated response in 6-week-old B5B5 chickens and depressed the response in corresponding B2B2 chickens. A secondary response was observed after the second immunization at 7 weeks of age as indicated by the higher antibody titers at 8 compared to 6 weeks of age. No effect of dietary treatment on antibody titer was observed.

B genotype influenced the results of the PHA assay and antibody titers at 6 weeks of age. B5B5 chickens had higher antibody titers that B2B2 chickens whereas the B2B2 chickens had a higher SI in the PHA assay than B5B5 chickens. Perhaps the humoral system in B5B5 chickens is more mature than that in B2B2 chickens at 6 weeks of age. In the PHA assay, lymphocytes from B2B2 chickens were stimulated to a greater extent than those from B5B5 chickens which may indicate a difference in cell-mediated immunological capabilities.

Effect of Genotype and 40 Percent Protein-calorie Restriction on Tumor Size

In all experiments tumors were subjectively scored for size. Tumor scores 4, 5 and 6 are dependent upon the size of the wing-web of the host. The effect of 40% protein-calorie restriction on tumor area was investigated using the formula for an ellipse (Schierman et al., 1977). B2B2 and B5B5 chickens from the F5 generation of a cross of lines 6-1 and 15-1 were utilized.

Chickens of each B genotype were randomized to four replicates of approximately 10 chickens each with approximately equal numbers of each sex per replicate. Two replicates were then randomly assigned to each dietary treatment (full-fed or restricted) within each genotype. In addition, two replicates of B2B5 chickens, one for each dietary treatment, were utilized as uninoculated body weight controls.

Body Weight Controls. Uninoculated B2B5 chickens monitored the effect of dietary treatment on body weight in experiment 13 (Figure 9). After 12 weeks on the dietary treatments, restricted chickens weighed 60.9% of that of full-fed chickens.

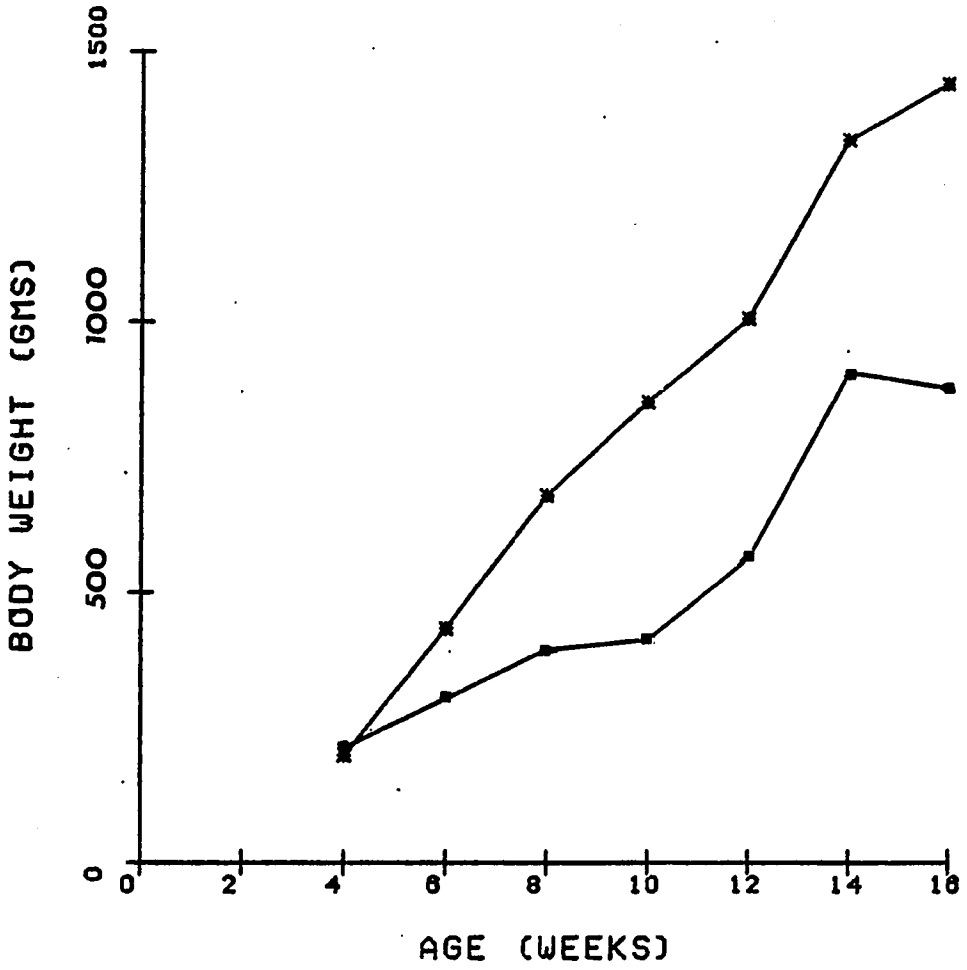

FIGURE 9. Mean body weight of uninoculated controls at various ages, experiment 13. Mean body weight of each replicate within a dietary treatment was calculated and plotted. *, Full-fed; ▪, Restricted-fed.

Tumor Score. Tumors were scored weekly. Mean tumor scores by B genotype and dietary treatment are plotted in Figure 10. A separate analysis of variance of tumor scores was made for each week tumors were scored for size. The mean tumor score of each of two replicates was subjected to analysis of variance (Table 27). Restricted-fed chickens had significantly smaller mean tumor score at 1 week PI but not thereafter, but B2B2 full-fed chickens had lower mean tumor scores after 2 weeks PI than B2B2 restricted-fed chickens. Moreover, B5B5 full-fed chickens had higher mean tumor score than restricted-fed chickens but the genotype by treatment interaction effect was not significant (Table 27 and Figure 10).

Tumor Area. Mean tumor areas by B genotype and dietary treatment are plotted in Figure 11. It is clear from the graph that mean tumor area of B5B5 full-fed chickens was much larger than that of B5B5 restricted-fed chickens. For B2B2 chickens no such influence of feeding regimen is apparent. A separate analysis of variance of tumor areas was made for each week tumors were measured. The mean tumor area of each of two replicates was subjected to analysis of variance (Table 28). Like tumor score, tumor area was significantly reduced by restricted feeding at 1 week PI. Genotype significantly influenced mean tumor area from the fourth through the tenth week PI. Treatment and genotype by treatment interaction effect significantly influenced mean tumor area for the fifth through the tenth week PI. The

genotype by treatment interaction effect on tumor area is clearly evident in Figure 11 since mean tumor area of B5B5 restricted-fed chickens was substantially smaller than that of B5B5 full-fed chickens. Mean tumor area of B2B2 restricted-fed chickens, on the other hand, was generally slightly larger than that of F2F2 full-fed chickens.

Summary. Protein-calorie restriction significantly reduced tumor size at 1 week PI using either tumor score or tumor area as the criterion of tumor size. Beginning at 5 weeks PI F genotype by dietary treatment interaction effect significantly influenced tumor area through the tenth week PI. The effect of restricted feeding is much greater in F5B5 than in F2F2 chickens when tumor area is the criterion than when tumor score is the criterion of response.

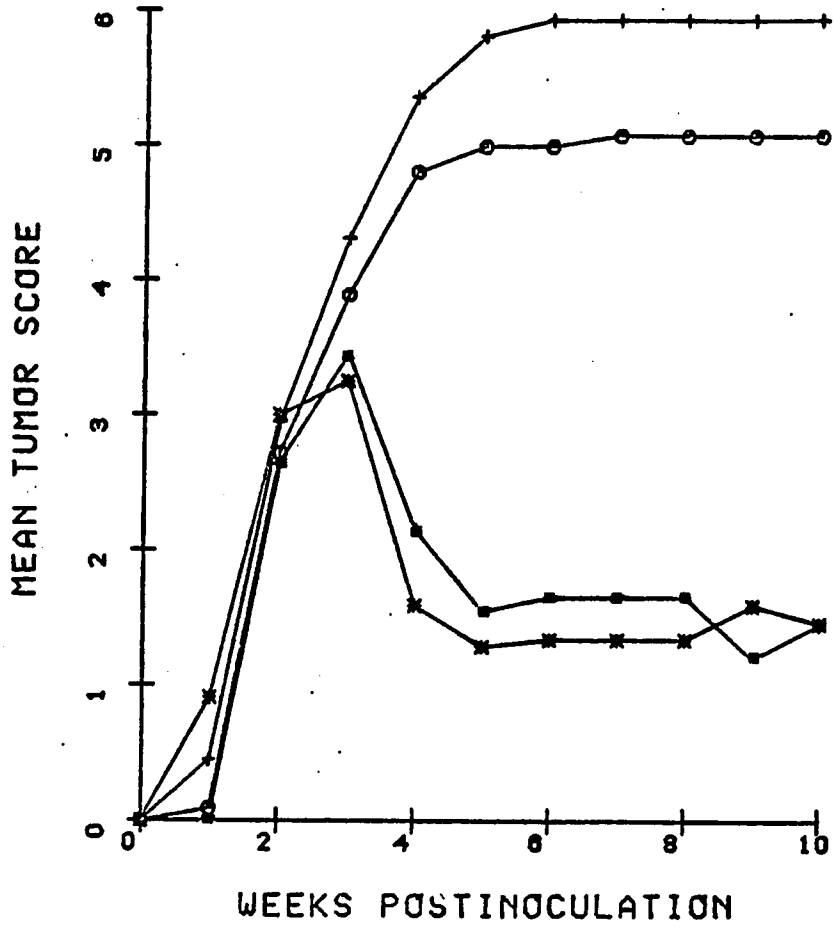

FIGURE 10. Mean tumor score of B2B2 and B5B5 chickens, respectively, each week PI for experiment 13. The mean tumor score of two replicates within a B genotype and dietary treatment was calculated and the arithmetic mean of the replicates was plotted. The tumor score of chickens dying with tumor prior to 10 weeks PI entered into the determination of mean tumor score of a replicate in subsequent weeks. *, B2B2 Full-fed; ▪, B2B2 Restricted-fed; +, B5B5 Full-fed; O, B5B5 Restricted-fed.

TABLE 27

Analysis of variance testing effect of genotype, dietary treatment, and interaction upon tumor score, experiment 13

Source of variation	df	Mean squares for mean tumor score (weeks PI)									
		1	2	3	4	5	6	7	8	9	10
Genotype (G)	1	0.07	0.00	4.89	20.6*	31.6*	31.4*	32.1*	32.1*	33.7*	32.7*
Treatment (T)	1	0.81*	0.17	0.11	0.0	0.2	0.2	0.2	0.2	0.8	0.4
G X T	1	0.15	0.01	0.85	0.6	0.6	0.8	0.7	0.7	0.1	0.4
Residual	4	0.03	0.04	0.23	0.5	0.7	0.7	0.8	0.8	0.8	0.5

*$P \leq 0.05$

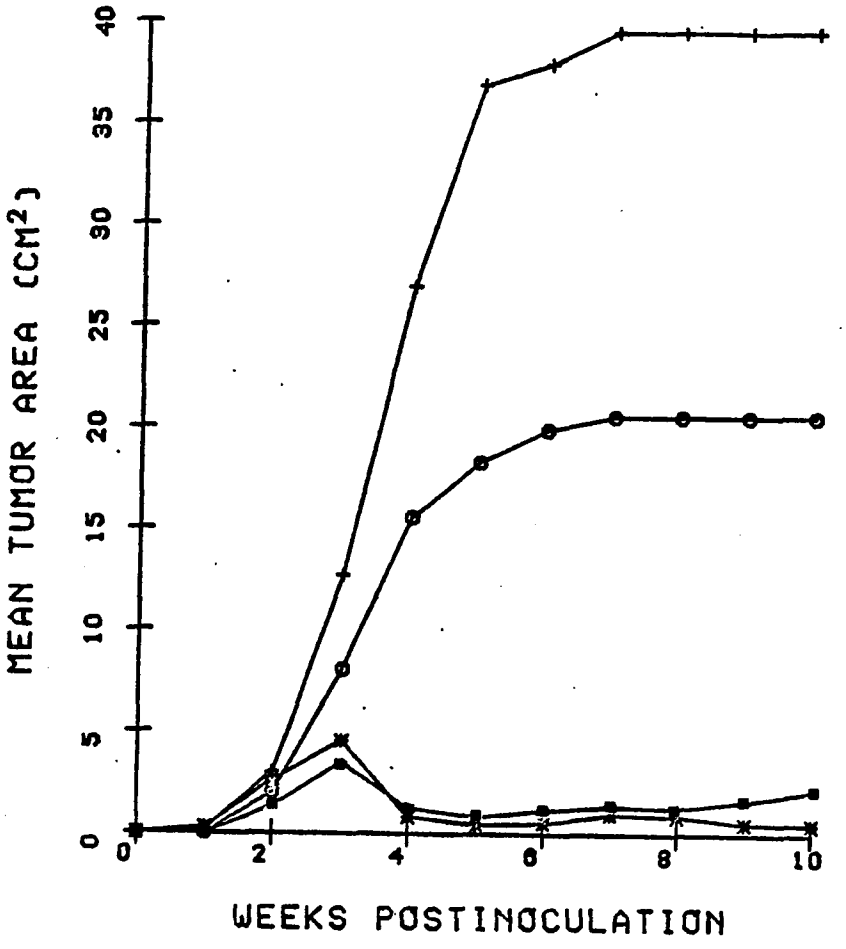

FIGURE 11. Mean tumor area of B2B2 and B5B5 chickens, respectively, each week PI for experiment 13. The mean tumor area of two replicates within a B genotype and dietary treatment was calculated and the arithmetic mean of the replicates plotted. The tumor area of a chicken dying with tumor prior to 10 weeks PI entered into the determination of mean tumor area of a replicate in subsequent weeks. *, B2B2 Full-fed; ▪, B2B2 Restricted-fed; +, B5B5 Full-fed; O, B5B5 Restricted-fed.

TABLE 28

Analysis of variance testing effect of genotype, dietary treatment, and interaction upon tumor area, experiment 13

Source of variation	df	Mean squares for mean tumor area (weeks PI)									
		1	2	3	4	5	6	7	8	9	10
Genotype (G)	1	0.01	0.55	81.98	821.1*	1458.3*	1581.2*	1675.9*	1684.9*	1684.0*	1659.5*
Treatment (T)	1	10.99*	2.65	16.33	60.3	167.2*	153.0*	173.8*	173.7*	160.7*	151.2*
G X T	1	0.01	0.04	6.32	70.2	181.0*	175.9*	188.5*	188.6*	202.7*	213.6*
Residual	4	0.01	0.95	12.85	26.1	8.6	4.7	3.2	3.2	3.6	4.5

*$P \leq 0.05$

V. DISCUSSION

Nutritional restriction reduced susceptibility to RSV-induced tumors, delayed the appearance of the tumor and suppressed tumor growth at least during the initial weeks PI. In addition, this research confirmed the findings of Collins et al. (1977) and Schierman et al. (1977) that B genotype decisively and significantly influenced the outcome of RSV-induced tumors. Forty percent protein-calorie restriction reduced tumor area of B5B5 restricted-fed chickens compared to B5B5 full-fed chickens but tumor area of B2B2 chickens was not affected by 40% protein-calorie restriction. The strong genetic control of tumor regression by B genotype may be related to different levels of immunocompetence of the immune systems in B2B2 and B5B5 chickens.

Effect of Genotype and of 40 Percent Protein-calorie Restriction and their Interaction on Tumor Development

Protein-calorie restriction influenced susceptibility to the formation of RSV-induced tumors differently in line 6-3 than in line 105. Relatively fewer tumors developed in line 105 than in line 6-3 as a result of dietary restriction indicating a genotype by environment interaction effect on

susceptibility to tumor formation. The wide variation in percentage of chickens developing tumors between experiments may be due in part to different RSV-1 inocula and also to variable genetic background of the hosts, particularly in noninbred line 105, between the two experiments. Fernandes et al. (1976b) showed that two strains of short-lived, autoimmunity-susceptible mice [DBA/2f and (NZB X NZW)F1] responded differently to dietary restriction. DBA/2f mice showed prolongation of life with protein restriction whereas (NZB X NZW)F1 mice exhibited prolonged life with caloric restriction.

Protein-calorie restriction significantly influenced tumor development to 4 weeks PI in lines 6-3 and 105 and in F2 generation progeny of lines 6-1 and 15-1. After 4 weeks PI, restriction did not significantly affect tumor score as a criterion of response, or TPI.

B genotype significantly influenced tumor development after 3 weeks PI. Mean tumor score and mean TPI were significantly smaller in B2B2 than in B5B5 chickens from the F2 and F5 generation of (6-1 X 15-1) from 3 through 10 weeks PI. Collins et al. (1977) observed that B genotype had a profound influence on the fate of RSV-induced tumors among the F2 generation progeny of a cross of lines 6-1 and 15-1. In B2B2, B2B5 and B5B5 segregants mean TPI was 2.9, 3.8 and 4.9, respectively.

On the other hand, a line effect was not detected. Mean tumor score and mean TPI were similar in line 6-3 and line 105. Cotter et al. (1973a) showed that line six had a significantly higher incidence of regression of RSV-induced tumors than did line 105. Using different lines, Gyles and Brown (1971) showed that genetic line significantly influenced tumor size.

Effect of the Severity and Timing of Protein-calorie Restriction on Tumor Development

Fifty percent protein-calorie restriction, similar to 40% protein-calorie restriction, delayed the appearance of tumor and reduced tumor score at 2 and 3 weeks PI compared to both 25% protein-calorie restriction and full-feeding. Apparently 25% protein-calorie restriction was not adequately limiting to affect tumor development. Ross et al. (1970) showed that level of calorie intake and proportion of protein in the diet modified the incidence of spontaneous chromophobe adenomas of the anterior pituitary gland of the male rat.

Restricting chickens for periods of either 4 or 6 weeks prior to inoculation with RSV-1, suppressed tumor score later than for chickens restricted only 2 weeks prior to inoculation. It is not known if this later effect on tumor score is actually due to lengthening the restriction period prior to inoculation or to a restriction of a specific mineral and/or vitamin, since feed in the experiments on

which this observation was based was not supplemented with minerals and vitamins. According to Jose and Good (1973b) resistance can be either increased or depressed depending upon the severity and the timing of the nutritional deprivation. Although the TPI of chickens restricted 4 and 6 weeks prior to inoculation was not significantly different than that of full-fed chickens, mean tumor score of restricted-fed chickens remained smaller than for full-fed chickens throughout the experiment.

In no experiments did restriction begin prior to 4 weeks of age. Restricting prior to 4 weeks of age likely would have a depressing effect on immunocompetence, since chicks develop immunologically at this time.

Effect of 40 Percent Protein-calorie Restriction On Tumor Size

Tumor area of protein-calorie restricted B5B5 hosts was significantly smaller than that of B5B5 full-fed hosts. Although B5B5 restricted hosts had smaller tumors than corresponding full-fed hosts, apparently the difference in tumor burden was too small and/or our criterion of measurement of tumor size too crude, to detect a real difference in TPI at the end of the experimental period. In B2B2 hosts the area of tumor and the fate of the tumor as measured by TPI were not different in the restricted compared to full-fed chickens.

The relatively smaller tumors in protein-calorie restricted than in the full-fed B5B5 hosts may have resulted from a limited supply of nutrients to the cancer cell. Transformation of a normal cell into a tumor cell clearly involves a profound switch in biological mechanism from a precisely regulated phenomenon characteristic of a normal resting cell to one involving persistently increased synthesis of nucleic acids, proteins and other substances specifically needed for continued cell growth and division (Braun, 1969). According to Tannenbaum (1944) carcinogenic agents produce the initial fundamental changes, in which the carcinogens transform normal cells into cancer cells, regardless of the diet, low or high calorie (Tannenbaum, 1944). Forty percent restriction inhibited the early growth and development of the tumor, suggesting that a limited supply of nutrients may not affect cellular transformation but may retard early tumor growth by limiting essential nutrients required for rapid cell proliferation.

Effect of 40 Percent Protein-calorie Restriction on Immunological Functions

Reduced susceptibility to tumor formation, and the retardation of tumor growth of protein-calorie restricted hosts during the first 3 weeks PI, may have reflected the effect of nutrient restriction on the immune response to the tumor. Several investigators showed in mice (Jose *et al.*, 1973a, 1973b; Cooper *et al.*, 1971, 1974; Bell and Hazell,

1975; Fernandes et al., 1976a, 1976c), rats (Jose et al., 1971b) and guinea pigs (Kramer and Good, 1977, 1978) that moderate dietary protein restriction depressed antibody production but permitted maintainence of, or even an increase in, certain kinds of cell-mediated responses. These included response to mitogens, defenses against certain viruses, rejection of skin allografts, and development of killer cells against allogeneic or syngeneic tumor cells.

Forty percent protein-calorie restriction had a limited effect on immunocompetence in this study based upon the PHA assay and antibody titer as measures of cell-mediated and humoral immunity, respectively. Spleen cells from 6-week-old E5E5 protein-calorie restricted chickens exhibited an enhanced cell-mediated response compared to corresponding full-fed chickens of the same genotype. On the other hand, in E2E2 chickens protein-calorie restriction depressed the cell-mediated response. Cooper et al. (1974) using two unrelated strains of mice (C3H/Bi and Sec/ReJ) demonstrated enhancement of the proliferative response of spleen cells from mice receiving 8% versus 27% protein in the diet to stimulation by PHA. On the other hand, Erickson et al. (1979a, 1979b) found that protein concentration did not affect PHA-stimulated T-cell transformation in mice fed purified diets containing 6, 10 or 30% casein. After 6 weeks of age protein-calorie restriction and E genotype did not influence cell-mediated immunocompetence in chickens as

measured by the PHA assay.

Forty percent protein-calorie restriction did not affect hemagglutinin antibody production to sheep erythrocytes at 2 and 4 weeks after feed restriction began. Erickson et al. (1979b) showed that level of dietary protein, level of caloric intake and the duration of nutritional manipulation influenced B cell transformation stimulated by lipopolysaccharide in mice. Kenney et al. (1968) found that protein-restricted rats had depressed hemagglutinin antibody titers to sheep erythrocytes. In that experiment, however, adult rats fed the low protein diet had lost 22-24% of initial weight when tested for antibody production. A possible explanation for the failure of protein-calorie restriction to influence antibody production to sheep erythrocytes may be that the restriction was too mild since chickens continued to gain weight throughout the experiment.

Effect of B Genotype on Immunological Functions

The B complex exerts control over numerous immunologic functions including regression of RSV-induced tumors (reviewed by Abplanalp, 1979). Because of the effect of B genotype on tumor regression, Collins et al. (1977) suggested that in B5B5 chickens immunological mechanisms failed to respond to the tumor, responded inadequately, or the response was negated, but they did not assay for either antibody or cell-mediated immune responses.

The immunocompetence of B2B2 versus B5B5 chickens were compared using the level of PHA-stimulated blastogenesis and antibody titers to sheep erythrocytes. The cell-mediated immune response of uninoculated 6-week-old B2B2 chickens may have been enhanced relative to B5B5 chickens using the PHA assay. But B5B5 chickens appeared to have enhanced antibody production. Even though this immunological difference was detected in uninoculated 6-week-old B2B2 and B5B5 chickens it may have been associated with the different ability of these genotypes to regress RSV-induced tumors.

Genetic Differences in Delayed Wattle Reaction Test

Lines 6-3 and 7-2 differed significantly in their response to DT using the delayed wattle reaction test even though both lines are considered to have identical allogantigen genotypes within the B complex (Pazderka et al., 1975; Gilmour et al., 1977). Significant differences between these lines also exist in degree of graft-versus-host reaction (Pazderka et al., 1975), delayed hypersensitivity (Gilmour et al., 1977), antibody production (Palladino et al., 1977) and in their ability to regress RSV-induced tumors (Marks et al., 1979).

The delayed wattle reaction test was used to determine the effect of B genotype on the cell-mediated immune response to DT in vivo. B2B2 and B5B5 chickens from the F2 and F3 generation of the cross of lines 6-1 and 15-1 failed to respond to DT in the delayed wattle reaction test. The

gene(s) coding for response to DT may be a non-E gene(s).

Effect of Age on Size and Regression of RSV-induced Tumors

Mean tumor score from 4 weeks through 10 weeks after inoculation with RSV-1, and mean TPI, were smaller in chickens inoculated at 8, 10, 12 and 14 weeks of age than in chickens inoculated at 4 weeks of age. Cotter et al. (1973b) showed that tumor regression failed to occur in line six chicks inoculated with RSV-1 at 1 and 14 days of age, but in chickens inoculated at 28 days of age the incidence of regression was 50%.

Regression of murine sarcoma virus (Moloney) (MSV)-induced tumors was dependent on genetic strain of mouse (Fefer et al., 1967). Of tumors induced in susceptible newborn BALB/c, C57BL/6 and (BALB/c X C57Bl/6)F1 mice, 3, 47 and 24%, respectively, of the primary tumors spontaneously regressed. All tumors induced in adult mice from these genetic strains completely regressed. Regression of MSV-induced tumors in BALB/c mice was found to be dependent on the age of the host (Fefer et al., 1969) and the immunologic competence of the host (Fefer et al., 1968). RSV-induced tumor regression in chickens is also host age dependent and may be related to the immunocompetence of the host.

Questions Raised by this Research

1. Using tumor area as the criterion, did 40% protein-calorie restriction reduce tumor size in lines 6-3 and 105? In experiments 1 and 2, tumor score only was used to evaluate the effect of protein-calorie restriction on tumor size. In experiment 13, 40% protein-calorie restriction significantly influenced tumor area in B2B2 and B5B5 chickens. Since some tumor scores depend on the size of the host's wing-web and wing-web size is influenced by body size which in turn is reduced as a result of feed restriction, tumor area may be a better measure of the effect of protein-calorie restriction on tumor size.

2. What effect would protein-calorie restriction have on tumor development in line 7-2 using RSV(RAV-49) to induce tumors? Throughout this research RSV(RAV-1) was used to produce sarcomas in susceptible chickens. Line 7-2 is genetically resistant to RSV(RAV-1) but segregates for susceptibility to RSV(RAV-49). Protein-calorie restriction may have a different effect on a different line of chickens using a different subgroup of RSV.

3. Would the effect of protein-calorie restriction be different if the dilution of RSV were changed? The effects of protein-calorie restriction on tumor growth and development might be greater if a higher dilution of virus was used. With virus of higher dilution, protein-calorie restriction might retard tumor growth to a greater extent and for a longer period of time. During this time

immunological mechanisms might be better able to regress the tumor.

4. Was the incidence or extent of metastasis influenced by protein-calorie restriction? In this research this aspect of tumor development was not studied. Protein-calorie restriction may have an effect on the incidence, time of appearance and extent of metastasic tumors.

5. Would the age of E5B5 chickens at RSV-inoculation influence tumor size and TPI? In the research involving age at inoculation line 6K with a high incidence of tumor regression was used. Collins et al. (1977) reported that 93% of B5B5 chickens inoculated at 6 weeks of age died with tumor. Perhaps if E5E5 chickens were older at the time of inoculation they would be more immunocompetent and have a lower incidence of RSV-induced tumor progresssion.

6. How is the DWB response controlled genetically? By crossing lines 6-3 and 7-2 and making reciprocal backcrosses it would be possible to determine if the immune response is controlled by one or many genes and whether it is sex-linked or dominant.

7. In what ways do B2B2 and B5B5 chickens differ in immunological capabilities? There is a vast difference in the ability of B2B2 and B5B5 chickens to regress tumors. Can this difference be shown to be due to differences in immunological competence? The immunological differences may be quite specific. For example, B5B5 chickens may be unable

to elicit an immune response against the tumor cells due to their failure to recognize tumor antigens as foreign.

8. What are the specific nutrients responsible for the effects observed in feed restriction experiments? A quantitative measurement, such as serum albumin level, lean body mass or percentage body fat, would indicate if the restricted chickens were nutrient deficient.

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APPENDIX

I. Mineral and Vitamin Composition of Starter Feed used in Experiments 1, 2, 3, 4, 5, 12 and 13

Ingredient	Composition of feed for: Full-fed	40% Restricted
Vitamin A (units/lb)	6260.0	10433.0
Vitamin D (units/lb)	835.0	1391.7
Vitamin E* (mg/lb)	2.5	4.2
Vitamin K* (mg/lb)	0.8	1.3
Riboflavin (mg/lb)	2.6	4.3
Pantothenic acid (mg/lb)	6.8	11.3
Niacin (mg/lb)	21.1	35.2
Choline (mg/lb)	630.0	1050.0
Calcium (%)	1.54	2.57
Total phosphorus (%)	0.51	0.85

*In addition to what was naturally in the feed.

II. Mineral and Vitamin Composition of Grower Feed used in Experiment 6

Ingredient	Composition of feed for: Full-fed	Restricted 25%	Restricted 50%
Vitamin A (units/lb)	5100.0	6800.0	10200.0
Vitamin D (units/lb)	835.0	1133.3	1670.0
Vitamin E* (mg/lb)	2.5	3.3	5.0
Vitamin K* (mg/lb)	0.8	1.1	1.6
Riboflavin (mg/lb)	3.3	4.4	6.6
Pantothenic acid (mg/lb)	5.5	7.3	11.0
Niacin (mg/lb)	21.2	28.3	42.4
Choline (mg/lb)	583.0	777.3	1166.0
Calcium (%)	1.52	2.03	3.04
Total phosphorus (%)	0.42	0.60	0.90

*In addition to what was naturally in the feed.