Genetic control of immune responsiveness in the chicken

Promotor: dr. C.C. Oosterlee, hoogleraar in de veeteeltwetenschap

A.J. van der Zijpp

GENETIC CONTROL OF IMMUNE RESPONSIVENESS IN THE CHICKEN

Proefschrift
ter verkrijging van de graad van
doctor in de landbouwwetenschappen,
op gezag van de rector magnificus,
dr. C.C. Oosterlee, hoogleraar in de veeteeltwetenschap,
in het openbaar te verdedigen
op vrijdag 11 juni 1982 des namiddags te vier uur
in de aula van de Landbouwhogeschool te Wageningen.

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" .... grant me the strength to change what I can change, the courage to bear what I cannot change and the wisdom to know the difference"

Reinhold Niebuhr

NN08201, 900

STELLINGEN

1. Het kenmerk antilichaamproductie vertoont voldoende genetische variatie voor individuele selectie.
   Dit proefschrift.

2. Selectie op verhoogde activiteit van het immuun systeem zal gebaseerd moeten zijn op minstens drie kenmerken: de antilichaamproductie, de celgebonden immuniteit en de fagocytose.
   Biozzi, G., D. Mouton, O.A. Sant'Anna, H.C. Passos, M. Gennari, M.H. Reis, V.C.A. Ferreira, A.M. Heumann, Y. Bouthillier, O.M. Ibanez, C. Stiffel, and M. Siqueira (1979). Current Topics in Microbiology and Immunology 85: 31-98.
   Mouton, D., C. Stiffel, and G. Biozzi (1981). Immunologic defects in laboratory animals 1: 19-47.

3. Het onderzoek naar mogelijkheden voor selectie op ziekteresistentie moet primair gericht zijn op de waarde van weerstandskenmerken tegen ziekteverwekkende factoren.
   Biozzi, G., D. Mouton, O.A. Sant'Anna, H.C. Passos, M. Gennari, M.H. Reis, V.C.A. Ferreira, A.M. Heumann, Y. Bouthillier, O.M. Ibanez, C. Stiffel, and M. Siqueira (1979). Current Topics in Microbiology and Immunology 85: 31-98.
   Dit proefschrift.

4. Het voorstel van Almlid om aparte indexen voor specifieke en niet-specifieke weerstand op te stellen is niet in overeenstemming met de talloze wisselwerkingen, waarop het afweersysteem is gebouwd.
   Almlid, T. (1981). Livest. Prod. Sci. 8: 321-330.
   Unanue, E.R. (1981). Adv. Immunol. 31: 1-136.

5. Het is niet vanzelfsprekend de met vaccinatie beoogde bescherming weer te geven in de vorm van antilichaamtiters.
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6. De veronderstelling, dat selectie op productiekenmerken gepaard gaat met toenemende gevoeligheid voor infectieuze ziekten, is onjuist.

7. De indeling in primaire en secondaire productiekenmerken gaat voorbij aan het vitale belang van levensvatbaarheid.

8. Het onderzoek in de zoötechniek en de diergeneeskunde wordt te weinig gesteund door economische evaluatie van dierziekten.
   Ellis, P.R., and A.D. James (1979). Veterinary Record 105: 523-526.
   Renkema, J.A., en A.A. Dijkhuizen (1979). Tijdschr. Diergeneesk. 104: 977-985.

9. Het gebruik van commerciëel productiemateriaal voor proeven bevordert de doorstroming van kennis verkregen uit onderzoek naar de veehouderijpraktijk.
   Hann, C.M. and W. Hartmann (1981). World's Poult. Sci. J. 37: 138-141.

10. De nadelige gevolgen van contactovergevoeligheid bij onderzoekers kunnen worden opgevangen door vakgroepen c.q. afdelingen in te richten naar discipline.
    Harrington, C.J. (1981). Contact Dermatitis 7: 126.

11. Interne onderzoeksrapporten miskennen de waarde van internationale wetenschappelijke communicatie voor het Nederlandse zoötechnische onderzoek.

12. Niet een hoge correlatie tussen de eindwaarderingen gegeven voor meerkeuze toetsen en 'essay' type examenvragen, maar de doelstellingen van het te examineren vak moeten van doorslaggevende betekenis zijn bij beslissingen over het type examen.

13. Het regelmatig terugkerend advies aan vrouwelijke studenten in de studierichting Zoötechniek om zich te specialiseren in de pluimveeteelt, getuigt niet van kennis van de positie van de vrouw in deze bedrijfstak.

14. Het discriminerend onderscheid, dat bij publicatie van wetenschappelijke artikelen gemaakt wordt tussen vrouwelijke en mannelijke auteurs door vermelding van voornamen, respectievelijk initialen, dient geen enkel belang.

Proefschrift van A.J. van der Zijpp
Genetic control of immune responsiveness in the chicken

Wageningen, 11 juni 1982

VOORWOORD

Mijn belangstelling voor de immunologie werd gewekt door mijn werk, het produceren van bloedgroep reagentia, voor de Stichting Bloedgroepen Onderzoek. De resultaten van het resistentie-onderzoek bij muizen van dr. G. Biozzi en zijn medewerkers (Institut Curie, Parijs) zijn richting gevend geweest bij de keuze van het onderwerp van onderzoek. Mijn promotor, prof.dr. C.C. Oosterlee, dank ik voor de betoonde belangstelling voor dit onderwerp en voor het bieden van mogelijkheden dit onderzoek op te zetten.
Daadwerkelijke steun was niet alleen belichaamd in de persoon van zijn assistent, maar ook in hulp bij het bloed afnemen.

Het tot een succesvol einde brengen van een onderzoek middels publicatie is afhankelijk van alle medewerkenden. Wanneer één medewerker een steekje laat vallen kan dat het einde van een proef betekenen. De hier aangegeven rangorde is dus geen aanwijzing voor het belang van Uw aandeel. Alle bijdragen waren even belangrijk voor het welslagen van het onderzoek, waarvoor mijn hartelijke dank.

Naast de belangstelling van mijn promotor prof.dr. C.C. Oosterlee voor het onderzoek, dank ik ook de collega's dr.ir. G.A.A. Albers, dr. J.H. Boon, dr.ir. A. Kloosterman, ir. T. van der Lende en dr. H.S. Siegel voor hun inbreng bij het doornemen van proefopzetten en manuscripten.

Dankzij de soms verbluffende snelheid van mijn assistent, de heer M.G.B. Nieuwland, is het mogelijk geweest voor dit onderzoek bloed af te nemen van minstens 15.000 kuikens, 30.000 sera te testen en vele andere werkzaamheden uit te voeren. Tevens werd bijstand verleend door de heren P.C. Groebe, W. Hazeleger en R. van den Brink.

Voor de verzorging van de kippen waren verantwoordelijk de bedrijfsleider, de heer A. van Zeist, en de heren A. van den Dool, A. Rodenburg, J.M. van der Linden en J. Veldhuis. Tevens verleenden de heren A. van den Dool en A. Rodenburg assistentie bij vaccineren en immuniseren. Personeel van de proefaccommodatie "De Haar-varkens en kalveren" was behulpzaam bij de verzorging van kuikens in de zeugenstal.

Bij de statistische verwerking hebben een aantal leden van de vakgroep Veefokkerij zich verdiept in de eigenaardigheden van resistentieproeven.

Mw. G.J. Gijsbertse-Huiberts heeft het manuscript in verschillende vormen snel en nauwgezet getypt. De heer W. Heije heeft de grafieken getekend en mw. M. Felius verzorgde de illustratie voor het omslag. Daarna heeft de off-

setdrukkerij van de Landbouwhogeschool de vermenigvuldiging van dit proefschrift verzorgd.

Dr. B. Kouwenhoven van de Gezondheidsdienst voor Pluimvee was altijd bereid ons te adviseren bij entschema's en de verwerking van monsters op snelle en adequate wijze te doen uitvoeren.

Mijn werk is een combinatie van onderwijs en onderzoek. Veel doctoraalstudenten en stagiaires hebben zich bekwaamd in het immunologisch onderzoek, waarbij mijn inzicht in zowel het onderzoeksgebied als de menselijke verstandhouding aanmerkelijk is vergroot. Tot de doctoraalstudenten behoorden: ir. H.G.W. Schouten, mw.ir. T.C. de Jager, ir. E.S. Elzinga, ir. A. Hol, ir. J. Samplonius, ir. E.J. Entzinger, ir. C. Geerse, mw.ir. G. de Boer-Oosterhof, ir. K. Frankena, mw.ir. J. Boneschanscher, mw.ir. S.Ch. van Mourik, mw.ir. P. Luiting, mw.ir. E.C. Janson-Hogenhuis, mw.ir. B.A. Goedvriend-Overweg, mw.ir. F.R. Leenstra, ir. J.M. Rooyakkers, H. Oude Vrielink, H.J. Flipsen, G. Bogaard, W.A.J. Veldman, A.J. Schutte, M. Verbrugge en D.S. Koorn. Tijdens hun stageperiode hebben aan het onderzoek meegewerkt: ing. T. Zandstra, ing. L. Appel, J. Hoekstra, J. Bosma en G. Coes. Gedurende een kort dienstverband hebben ir. P.M.J. Duysings, mw.ir. A. Keus en mw.ir. C.M. Bakker (ondersteuning LEB-fonds) zich voor het onderzoek ingezet.

Tenslotte wil ik iedereen bedanken, die heeft bijgedragen aan dit onderzoek, maar niet met name is genoemd.

CONTENTS

INTRODUCTION

Disease is a major threat to poultry production. Medication, vaccination, hygienic measures and eradication have significantly contributed in combating disease. However the fact that our poultry is not or inadequately resistant to many disease agents and that remedies for new pathogens have to be found, has created a demand for genetic improvement of disease resistance.

Genetic improvement of disease resistance may be possible via two approaches: breeding for disease resistance to specific diseases or breeding for general disease resistance (Gavora and Spencer, 1978). Although improvement of specific resistance to disease can lead to major results, for example in resistance to Marek's disease, it is not practicable to apply deliberate exposure of breeding populations or challenge separate populations of sibs for each disease agent. Indirect selection for disease resistance is therefore required. Indirect selection may take two forms: selection for parameters valuable for resistance to specific diseases and selection for criteria of general disease resistance. In the first chapter the possibilities for these approaches are reviewed.

Early contributions to the development of general disease resistance came from Sang and Sobey (1954), working with rabbits, followed by the studies of Biozzi and coworkers (Institut Curie, Paris). In mice Biozzi et al. (1979) studied the antibody response to multideterminant antigens in five breeding experiments. Mouton et al. (1981) carried out selective breeding for high and low in vitro response to phytohaemagglutinin, a cell-mediated response, and Buschmann and Meyer (1981) selected for high and low phagocytic activity. Further studies of these selection lines have revealed relationships between immune parameters and the value of these selection criteria for resistance to various pathogens.

In this study agglutinin antibody production to sheep red blood cells (SRBC) has been chosen as a possible parameter of general disease resistance in the chicken. In chapter 2 and 3 the genetic control of this trait is discussed for primary and secondary responses. Evidence of genetic differences for cell-mediated responses, via stimulation with phytohaemagglutinin, and antibody production to SRBC is presented in chapter 4.

Antibody production to SRBC is affected by several environmental aspects. Amongst these are dose (chapter 4), previous stimulation with the antigen (chapter 3) and vaccinations before injection with SRBC (chapter 5). Genetic

origin by dose interactions are presented in chapter 4.

The studies presented here and other preliminary work have been the basis of a selection experiment started in 1980 with ISA Warren for high and low antibody production to SRBC. Besides production traits (see chapter 1 for a discussion of relations with immune parameters) also the haemagglutinin-inhibition titres to inactivated Newcastle disease vaccine are being recorded. These selection lines will be utilised for further study regarding genotype-environment interactions, in particular for their reactions to many pathogens and in stress situations (Siegel, 1980).

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CHAPTER 1

BREEDING FOR IMMUNE RESPONSIVENESS AND DISEASE RESISTANCE

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SUMMARY

Incorporation of disease resistance into breeding programmes would be enhanced by adequate methods. General disease resistance instead of resistance to specific diseases might be a useful concept. Knowledge of genetic resistance to specific diseases has not yet produced much evidence for general mechanisms except the involvement of the B major histocompatibility complex in viral oncogenesis. Since immune responsiveness is controlled by genes within and outside the B complex, the search for genetically determined immune criteria is needed to define general resistance to disease. Selection for immunoglobulin levels or the antibody response to sheep erythrocytes are the first investigations in this area.

Studies concerning the genetic relation between disease resistance and production traits are scarce. The negative genetic correlation between body weight, and antibody production and resistance to MD, may cause reconsideration of selection goals in broiler breeder programmes. Evaluation of selection methods for production traits can be expected awaiting knowledge of relationships with disease resistance, either specific or general.

1. INTRODUCTION

Intensive poultry production, because of economic risk, has created a demand for a status of continuous health of the flocks. Many efforts have been made by vaccination, hygienic measures, eradication and medication to reduce losses due to disease. Despite of increasing recognition of the importance of disease problems, availability of new vaccines and medicine, we still are in a situation, that the chicken cannot cope adequately with the pathogenic environment. Also the introduction of a vaccine to protect for a new disease agent, gives difficulties in reorganizing the present vaccination schemes.

A solution of this dilemma would be genetic improvement of disease resistance. However a lack of knowledge regarding resistance mechanisms for specific disease agents combined with a lack of interaction between basic immunologists and pathologists inhibit the development of a rationale to improve disease resistance (see Rose et al., 1981). Poultry breeders, and the pharmaceutical industry too, would benefit if criteria of disease resistance were clearly

defined. Then breeding programmes for disease resistance could be designed and the effects of genetic improvement of production traits on disease resistance could be investigated. Gavora and Spencer (1978) have defined specific and general disease resistance and discussed the feasibility of practical application. The purpose of this article is to discuss components of general disease resistance in relation to genetic improvement and their bearing on production and mortality.

2. THE AVIAN DEFENCE SYSTEM

The chicken employs various protective mechanisms to promote survival. These protective mechanisms can be classified as specific or non-specific. Non-specific reactions are the stress response and the non-immunological action of macrophages, complement, lysozyme, pH of body fluids, interferon. The immune system reacts specifically to invading organisms, in close co-operation with the non-immunological functions, and interacting with the stress response (Siegel, 1980). The avian immune system is characterised by the presence of central lymphoid organs, the bursa of Fabricius and the thymus and peripheral lymphoid tissue but by the absence of organised lymph nodes (Firth, 1977). The central organs regulate bursal-derived (B) and thymus-derived (T) lymphocytes with respectively antibody and cell-mediated responses. Antibodies, belonging to different classes IgM, IgG, IgA and possibly IgE have specialized functions. Cooperation between B and T lymphocytes is often necessary for antibody responses. Also close interaction between lymphocytes and macrophages is a condition for some responses. And T lymphocytes have specialized functions characterized as help, suppression, delayed hypersensitivity, and cytotoxicity (Glick, 1979; Chi et al., 1981).

During an immune response memory is established. Besides the direct response to a vaccine, memory formation is the major reason for vaccination.

The immune response is dependent upon the site of entry, the presentation of antigen, the response of antigen-sensitive cells and the regulation of the response (Tizard, 1979). Although most antigens cause a response affecting all available defence mechanisms, only some may be effective in eliminating the antigen. Therefore subgroups of viruses, bacteria, protozoa or fungi with similar aetiology may have characteristic major responses in common.

Immune responsiveness develops with age. Differentiation in development of specific and non-specific reactions occurs. Complement activity is low in

the embryo, but increases with age (Skeeles et al., 1980). Phagocytes are active in the cellular defence in the embryo allowing the immune system to develop (Seto, 1981). About the time of hatching the capacity for immune responsiveness emerges due to quantity and differentiation of immuno-competent cells. Seto (1981) also pointed out that thymus-derived cells may limit the onset of immunocompetence in neonatal chicks. Maternal antibodies can provide temporary protection, when the immune system is still immature.

3. GENETIC REGULATION OF HIGH AND LOW IMMUNE RESPONSIVENESS IN MICE

In mice Biozzi et al. (1979) and Mouton et al. (1981) have carried out six selective breedings: five for high and low antibody production to natural, multideterminant antigens and one for high and low in vitro response to phytohaemagglutinin, a cell-mediated response.

The quantitative antibody response was subject to polygenic control. The effect of the genes accumulated in the high and low lines was not restricted to the antigens used during the selection. This effect was essentially non-specific and it concerned many unrelated antigens and the synthesis of every immunoglobulin class. The differences in antibody production between high and low lines were a faster rate of multiplication and differentiation of B lymphocytes in the high line and a stronger catabolic activity of macrophages in the low line. Thus, an inverse relationship existed between antibody synthesis and bactericidal capacity. No differences in cell-mediated immunity between high and low lines have been detected.

The agglutinin antibody response to sheep erythrocytes was regulated by a group of about ten independent loci. Two linkages have been shown: one for the immunoglobulin allotype locus and one for the H-2 (Major Histocompatibility Complex) locus. The classic H-2 linked immune response genes in comparison with the response to sheep erythrocytes were different in two respects. At a threshold dose of sheep erythrocytes two loci instead of one controlled the response and low response was dominant.

The genetic control of cell-mediated immunity was also polygenic, but independent of the humoral response. Lines selected for high and low in vitro response to phytohaemagglutinin show similar responses to B cell mitogens.

In mice selection for general disease resistance thus should include at least the criteria antibody production, macrophage activity and cell-mediated response. Results from challenge experiments (Biozzi et al., 1979) indicate,

that extreme values for these criteria as a result of selection lead to increased susceptibility to some pathogens. In addition these lines prove to be useful for research regarding the nature of the defence mechanisms operating against specific pathogens.

4. GENETIC ASPECTS OF IMMUNE RESPONSIVENESS IN THE CHICKEN

Genetic differences in immune responsiveness of the chicken can be attributed to the major histocompatibility complex, immunoglobulin allotypes and to genes not associated with either MHC or allotypes.

4.1 The major histocompatibility complex

The B blood group locus codes for antigens common to erythrocytes and lymphocytes. The relationship with skin graft rejection was first described by Schierman and Nordskog (1961). The major histocompatibility complex (MHC) is characterized by a series of functions, all concerned with the immune response: graft-versus-host reaction (Lee and Nordskog, 1980), mixed leukocyte reaction (Miggiano et al., 1974), serum haemolytic complement level (Chanh et al., 1976), immune responses to synthetic polypeptides (Benedict et al., 1975; GÜnther et al., 1974; Pevzner et al., 1978) and other antigens (Karakoz et al., 1974; Pevzner et al., 1975), resistance to Marek's disease (Briles et al., 1977) and to lymphoid leukosis (Schierman et al., 1977). The B complex can now be described as a three-locus model (Pink et al., 1977). For completeness see the review of Longenecker and Mosmann (1981). Genes outside the MHC also control the immune response, as shown by Palladino et al. (1977). Two lines identical for the MHC by serological and functional tests were injected with four antigens. The differences between the lines were quantitative in nature and directed to non-MHC-associated responses. Morrow and Ablanalp (1981) reported quantitative differences between inbred lines for the in vitro mitogenic response to PHA and ConA. One of at least two major genes controlling ConA response may be linked to the MHC.

4.2 Other genetic variation in the immune response

Differences between inbred lines in humoral response to various antigens were observed by Balcarova et al. (1973 and 1973). Generally the levels of

response for various antigens parallelled each other with one deviation. The capability for formation of antibodies was inherited as a dominant trait. Palladino et al. (1977) showed quantitative differences in antibody responses, mostly in the 17S fraction, between MHC identical lines. And Rees and Nordskog (1980) screened 11 stocks for serum immunoglobulin levels and found highly significant differences, not related to the B complex. Breed and strain differences in the antibody response to SRBC were also detected by Van der Zijpp (1978).

Miggiano et al. (1976) demonstrated that a single dominant gene controlled the ability of blood leukocytes from two strains to respond to a T-cell mitogen. There was no linkage with the MHC, immunoglobulin allotype loci nor sex. The cell-mediated response to diphteria toxoid, studied in broiler breeder lines, reflected possible genetic variability (Klesius et al., 1977). Differences in delayed hypersensitivity were found by Palladino et al. (1977) in two lines identical for the MHC. Control by more than one gene of the in vitro response to PHA and ConA was observed by Morrow and Ablanalp (1981).

In 1972 Garnett and Roberts analysed percent gamma globulin levels in young chicks and estimated a heritability of .26. Large maternal effects at hatch were found. Data concerning IgG levels were analysed by Krieg et al. (1978). Heritabilities for IgG level at four and eight weeks of age were .89 and 1.18 respectively. The increase from four to eight weeks of age was also genetic in nature (h^2 = .75). Heritabilities for the haemagglutinin titres to SRBC in various stocks are summarized in Table 1. The level of these heritabilities for the primary response agrees with the estimates of realized heritability (.17 to .44) from a selection experiment for high and low antibody titres by Siegel and Gross (1980). Additive genetic variation for the persistency of the antibody response was also shown, but not for a lack of persistency. Non-additive gene action influenced the antibody response, too.

Gross et al. (1980) utilized three pairs of lines, selected in opposite directions for antibody response to SRBC, persistency of the antibody response to SRBC and corticosterone response to social stress to test their ability to resist infectious agents. None of the selected lines was superior in all comparisons. Only the non-persistent line generally performed the worst. Because other immune functions, like macrophage activity and cell-mediated immunity were not known, a detailed explanation like Biozzi et al. (1979) presented for mice, was not available. Krieg et al. (1978) measured IgG levels of the serum of four lines, challenged with avian myeloblastosis virus.

TABLE 1. HERITABILITY ESTIMATES OF HAEMAGGLUTININ TITRES TO SRBC

Stock	Primary/secondary response	Day post immunization 5	7
WL[1)]	primary	nd[3)]	.26
WPR[2)]	primary	.39	.14
WPR[2)]	secondary	.28	.11
Warren[2)]	primary	.57	nd
Warren[2)]	secondary	.14	nd

1) Van der Zijpp and Leenstra, 1980.

2) Van der Zijpp (unpublished results)

3) Not done.

Differences between lines and between inoculated chicks and controls were significant. Chicks dying from the virus infection had lower IgG values, also compared with controls, than those of the same line which stayed clinically healthy. Thus the ability to mount an antibody response seems to be valuable for resistance.

4.3 Choice of criteria for selection

No single immunological parameter appears to be sufficient in characterizing general immune responsiveness. Although many defence traits may be expected to be genetically determined to some extent, little is known yet of genetic relationships amongst them. Even the role of the B complex in quantitative responses is not often researched. A choice of parameters for an index of immune responsiveness cannot be made until this information becomes available.

Polygenic control of humoral responses, measured either as immunoglobulin levels in serum or as the response to SRBC, has been shown. Immunoglobulin levels in serum represent the response to a wide spectrum of unknown antigenic stimuli in contrast to the response to the multi-determinant antigen SRBC. It will be interesting to see whether these different criteria for selection will

lead to different consequences for the defence system and disease resistance. Similar questions have to be answered regarding the age of immunization and the length of the period between immunization and measurement of the response.

High or low humoral immune responsiveness may also be gained by selection with pathogenic stimulation like Salmonella pullorum (Pevzner et al., 1981), Escherichia coli (Soller et al., 1981), and Newcastle disease virus (Peleg et al., 1976). Although the genetic mechanism is similar to that of the response to SRBC, it is of interest to know the value of these criteria for disease resistance to S. pullorum, E. coli and Newcastle disease virus, respectively, and the nature of responses to other antigens.

Selection for one type of response can be correlated to other immune differences, as Biozzi et al. (1979) have experienced. Before any conclusions are made about criteria for disease resistance, these correlated responses should be known. The same is true, when selection lines are used to detect the nature of resistance to specific pathogens.

5. GENETIC RESISTANCE TO SPECIFIC DISEASES

Criteria for general disease resistance may be derived from the resistance mechanisms operating for specific diseases or groups of diseases. Detailed information of these defence mechanisms appears to be scarce as indicated by the proceedings of the 16th British Poultry Science Symposium on Avian Immunology (Rose et al., 1981). The development of vaccination procedures and of genetic resistance to specific diseases is therefore delayed. Mortality due to specific disease agents proves to be useful (see Gavora and Spencer, 1978), but is not a likely criterion of selection for general disease resistance.

One area of possible genetic improvement of the immune system is age related susceptibility. Because of immunological immaturity the young chick may suffer from diseases like infectious bursal disease, avian encephalomyelitis, Marek's disease and Escherichia coli and Salmonella infections. Measurement of early immune competence can be complicated by the presence of maternal antibodies. Maternal antibodies provide protection in the first few weeks of life against diseases like Newcastle disease, adenovirus infections, infectious bursal disease, avian encephalomyelitis, Marek's disease. For other diseases immune competence at an early age may relate to cell-mediated or local immunity, or macrophage activity. Therefore general disease resistance also provides a context for age related immunity.

In the following sections the mechanisms of genetic resistance to Marek's disease, lymphoid leukosis and Newcastle disease only will be discussed. Genetic information on many diseases appears to be scarce.

5.1 Marek's disease

Mortality due to Marek's disease (MD) has a quantitative genetic basis (Friars et al., 1972; Yamada, 1974; Gavora et al., 1974; Maas et al., 1981). Von Krosigk et al. (1972) found the heritability of mortality to be somewhat higher when chicks were inoculated with infected blood (.20) instead of contact exposed (.10). The genetic correlation between mortality caused by injection and by contact exposure to MD virus was .38. Hartmann and Sanz (1971) calculated phenotypic correlations of .80 in purebreds and .66 in crossbreds. In another study Hartmann and Sanz (1974) indicated the presence of maternal effects by comparing heritabilities based on full and half sib groups. Maternal antibodies probably accounted for this maternal effect. The procedure of exposure to MD thus influences progress by selection based on mortality and may effect the type of resistance attained.

Immunity to MD seems to have antivirus and antitumour aspects. Payne et al. (1976) suggested that first resistance to the virus and its replication occurs. Reduced viral activity prevents damage to the immune system. Secondly, the immunological rejection of malignant, transformed lymphocytes takes place. The ability of strains of chickens (often inbred) to resist MD depends on their genetic background. But the nature of the inoculum leads to variable results. Gavora et al. (1974) studied the response to JM-V inoculation and to the BC-1 isolate of MD virus. Ranking of the strains showed that mortality caused by JM-V inoculation agreed with the known resistance to MD with one exception. The lack of correlation (.03) between MD incidence in BC-1 challenged females and JM-V inoculated males may indicate resistance to a lymphoid tumour transplant in the case of JM-V and to the virus in case of BC-1. When Gavora et al. (1977) compared the response of strains to JM-V and Olson's transmissible lymphoid tumour, they also observed good agreement between resistance to JM-V and the known resistance to MD. Fabricant et al. (1978) reported that transplant tumours occurred independently of genetic susceptibility to MD virus. But the incidence of MD virus-induced host tumours was dependent upon genetic susceptibility to MD. This may mean that resistance to the virus and its replication is of major importance, because damage to the immune system probably over-

rules resistance, if present, to malignant transformation.

A more practical alternative of selection for MD resistance may be the response post-vaccination with cell-associated or cellfree turkey (HVT) or attenuated MD herpesvirus. Spencer et al. (1974) found reduced variation in MD incidence after vaccination, but the ranking of strains resembled those among unvaccinated chicks. No strain by vaccine interactions were observed. Yamada (1974) and Crittenden et al. (1972) also reported no virus-strain interactions. Dambrine (personal communication) tested eight commercial layer stocks. No correlation in susceptibility was found between birds vaccinated with cellfree HVT and unvaccinated birds, after challenge. Possibly the presence of maternal antibodies in combination with cellfree HVT has prevented the exact expression of genetic susceptibility in these stocks. Cho (1977) showed possible genetic differences in HVT viraemia between two strains of chickens vaccinated with cell-associated HVT. The viraemia titres were dependent on dose and declined with age. Von den Hagen and Löliger (1980) measured precipitating MD viral antigen in feather follicles after vaccination with cellfree HVT or MD contact infection. The levels of precipitating MD virus antigen appeared to agree with the expected genetic susceptibility of the strains, although a direct correlation with MD mortality was not found. The infection level of the feather follicle epithelium after vaccination deserves more attention as a criterion of selection, especially since this trait may indicate resistance to viral replication. The interference of type of vaccine with maternal antibodies and the significance of the presence of viral antigen in the feather follicles for MD mortality (Payne et al., 1976) require further investigation.

Despite the quantitative nature of resistance to MD the alleles of the B bloodgroup locus also contribute to genetic constitution (Hanson et al., 1967). The frequent presence of certain B alloalleles, B^{21} for example, in populations selected for MD resistance (Briles et al., 1977) and the decline of the frequency of B^{21} in subsequent, vaccinated generations (Oosterlee, 1980) indicates their value for resistance. Now Ir genes, mapping within the immune response region of the B complex, have been found to control the resistance to MD (Pevzner et al., 1981). The exact nature of genetic resistance to MD coded by the MHC is not clear. Pazderka et al. (1975) suggested that proliferation of lymphocytes may be affected. Recently Bacon et al. (1981) found that some genes of the B haplotype may resist tumour formation to Rous sarcoma virus, MD and lymphoid leukosis. Thus differences in the B complex between lines may explain the independence between the fate of transplant tumours and genetic susceptibility to MD (Fabricant et al., 1978).

The genetic control of MD susceptibility as expressed in resistant and susceptible lines has not yet produced conclusive evidence of defence mechanisms responsible for resistance. The role of the B complex in relation to activity of lymphocytes, as given by Lee et al. (1981), should be clarified. Because MD resistance is quantitative in nature and other systems like T cell alloantigens (Fredericksen et al., 1977) also have an effect, more general phenomena like cell-mediated host responses and viraemia may be valuable parameters. Only the ability to reject tumour cells, a trait associated with the MHC, appears to be of value for resistance to other diseases like lymphoid leukosis.

5.2 Lymphoid leukosis

Lymphoid leukosis (LL) tumours are caused by viruses of the avian leukosis/sarcoma group. The Rous sarcoma viruses (RSV) can be divided into five subgroups depending on their association with LL virus. Thus RSV can be used to study genetic control of resistance to different subgroups of LL virus (Crittenden, 1975). Host resistance may be genetically determined at two levels: 1) cellular resistance to virus infection, which is controlled by single genes, but specific for the subgroup of the virus, and 2) resistance to tumour development in LL virus infected chickens.

Tumour formation after RSV injections, subgroup A and B in left and right wingweb respectively, was studied in three populations by Hartmann et al. (1978). Independent genetic regulation for infection resistance to subgroups A and B was found. The accuracy of testing individual genotypes for resistance to subgroups A and B was slightly reduced in comparison with progeny groups. The frequency distribution of tumours in progeny groups differentiated well between homozygous resistant, heterozygous and homozygous susceptible parents.

Schierman et al. (1977) reported that the ability to regress RSV-induced tumours is a dominant trait, controlled by genes within or closely linked to the MHC of the chicken. Collins et al. (1977) observed also an association between the fate of tumours and the B genotypes. In 1980 Collins et al. reported on RSV tumour regression (subgroups A, B and C) in a series of inbred lines. Large differences in regression between lines were found, despite sharing of B locus alloantigens of some lines. Other genes, either non-B or B linked (Ir) genes must therefore be involved (also Marks et al., 1979). Gebriel et al. (1979) found close linkage between the RSV genes, controlling tumour regression to subgroup A, and the immune response locus (Ir-GAT), which controls

antibody production to the amino acid polymer GAT.

Chickens selected to regress RSV-induced tumours were more resistant to MD and to challenge with JM-V tumour cells (Carte et al., 1972). But Calnek et al. (1975) showed, that there is not a common basis for resistance to RSV-induced tumours and to MD. Two strains, resistant to MD, and two strains, susceptible to MD, were tested. One of the susceptible strains was also unable to regress RSV-induced tumours. A generally low immune competence may account for this result. Hartmann et al. (1973) injected two groups of cockerels of three lines with either avian myeloblastosis virus (AMV) or a lymphosarcoma strain. There were large mortality differences between lines due to AMV inoculation, but not after lymphosarcoma injection. The level of exposure may have been too high in the last instance. The correlation of family mortality between the two types of infection was .30. Resistance to MD, LL and RSV-induced tumours was studied by Bacon et al. (1981) in a population segregating for B locus antigens B^2 and B^5. The haplotypes B^2B^2 and B^2B^5 showed greater resistance to tumours induced by all three viruses than the B^5B^5 haplotype. Thus some genes of the B haplotype may control a general ability to cause tumour regression. Pevzner et al. (1981) and Gebriel et al. (1979) have shown that the Ir region, characterized by the response to the synthetic polypeptide GAT, is probably responsible for resistance to MD and to RSV, respectively.

Cellular resistance to LL virus infection can be directly improved by selection after inoculation of the wingweb with RSV. Tumour regression appears to be controlled by genes of which some associate with the B locus including the Ir region. General resistance to tumours caused by different viruses seems to originate also in the MHC.

5.3 Newcastle disease

Cole and Hutt (1961) observed significant differences in mortality after wingweb inoculation with the Roakin strain of Newcastle disease virus (NDV) between lines and sire families. Heritabilities for mortality through nine days post challenge at two weeks of age with NDV varied from .07 to .17 in a study by Gordon et al. (1971). Different methods of challenge in consecutive generations resulted in consistent differences in mortality between resistant and susceptible selection lines. But the low level of additive genetic variance points to the need for better criteria for resistance to NDV.

Heller et al. (1981) observed no differences in haemagglutination-

inhibition (HI) titres between Bedouin fowl, commercial White Leghorns and their reciprocal crosses. The chicks were intramuscularly inoculated with either attenuated or inactivated NDV at four weeks of age. Peleg et al. (1976) followed the same inoculation procedure in White Rock chickens. Challenge, 12 days post-inoculation, indicated that inactivated NDV provided better protection. Protection improved with increase in HI titres. The heritabilities for HI titres due to attenuated virus and inactivated virus were .31 and .60 respectively. The genetic correlation was .49. Since the HI titre response to attenuated NDV may be the direct result of tissue susceptibility to virus multiplication (Peleg et al., 1976) the response to inactivated NDV should be more effective in improving resistance to NDV genetically. It remains to be seen whether HI titre is the only key to NCD resistance.

TABLE 2. SIRE AND DAM COMPONENTS OF VARIANCE AND PATERNAL HERITABILITIES COMPUTED FROM HI TITRES POST NDV VACCINATION

Stock	Inactivated			Attenuated			Author
	σ_s^2	σ_d^2	h_s^2	σ_s^2	σ_d^2	h_s^2	
White Rock	.48	.03	.60	.59	1.43	.31	Peleg et al., 1976
White Rock	.28	.36	.41		nd[2)]		Soller et al., 1981
White Plymouth Rock		nd		-.16	.73	[1)]	Van der Zijpp (unpublished results)
Warren	.09	.33	.14		nd		Van der Zijpp (unpublished results)

[1)] Not calculated because of negative variance component

[2)] Not done.

In Table 2 sire and dam components of variance and paternal heritabilities are summarized. Despite vaccination at an age when maternal antibody is no longer present in all studies, maternal effects were present except for the response to inactivated NDV in the study of Peleg et al. (1976). Vaccination with inactivated NDV appears most promising for improvement of HI titres by selection.

Soller et al. (1981) reported, that antibody responses to inactivated NDV and to heat killed Escherichia coli were not genetically related. Gross et al. (1980) selected for high and low antibody response to SRBC. The lines were vaccinated with NDV at one day of age and challenged at four weeks of age. None of the high responder line chickens died, but 84% of the low line expired. Also HI titre differences were noted, indicating that antibody production post-SRBC immunization may represent a component of general resistance. In our Warren (medium heavy, brown egg producer) base population we found variable genetic correlations (.53 to -.54) between agglutinin antibody titres to SRBC and HI titres to inactivated NDV (Van der Zijpp, unpublished results).

Selection for HI titres to inactivated NDV may be a possibility to improve resistance to Newcastle disease. So far correlated responses, with antibody titres to Escherichia coli and SRBC, have been variable.

5.4 Generality

Three diseases have been discussed because of the genetic component playing a part in resistance. Knowledge of the disease agent and of the response of the chicken is a condition to gain understanding in the possible presence of genetic resistance. Differences in heritability estimates of total mortality in comparison with specific mortality caused by MD and also mortality caused by NDV compared with HI titres to NDV illustrate the need for definition of the response. Because mortality is not a desired parameter to measure resistance for breeding programmes, other defence mechanisms are preferable. HI titres post NDV vaccination and viraemia titres post vaccination may become such selection criteria.

Although genetic differences have contributed to understanding of resistance mechanisms, especially for MD, this approach may not be the most useful considering general aspects. Genetic differences may well produce the most pronounced and therefore most specific mechanisms in disease resistance. But underlying more general immune processes also take place. The ability to resist tumour formation may serve as an example. Because we can never disentangle the specific and the general aspects of a response to disease agents, we will have to resort to the MHC and to multideterminant antigens (see Biozzi et al., 1979) to evaluate the general aspects of disease resistance.

6. RELATIONSHIP WITH PRODUCTION TRAITS

In addition to the search for criteria for selection for disease resistance correlations with production traits may pose further problems in breeding programmes. As Gavora and Spencer (1978) explained, estimates of genetic correlations in populations suffering from disease are less useful because no discrimination of effects due to disease and genetic potential is possible. These estimates are also very useful to establish the effect of selection for production traits on disease resistance.

In 1972 Han and Smyth reported that selection for rapid growth rate would result in increased MD susceptibility. Their observations were based on four pairs of lines selected for growth rate. Gavora et al. (1974) studied mortality due to injection with MD virus in one set of progeny and examined egg production in the other set of full sisters, adventitiously exposed to MD. Leghorn birds genetically more resistant to MD tended to have lower adult body weight (r_g = -.37 to -.43), matured earlier and produced smaller eggs. A similar exercise was carried out studying mortality due to MD in one set of sibs and egg production rate in another set of MD vaccinated sibs. A positive genetic correlation between resistance to MD and egg production rate of vaccinated birds was observed, indicating that selection for high egg production may at least maintain genetic resistance to MD in vaccinated birds.

Strains selected for high egg production less frequently harboured LL virus or group specific viral antigen in albumen of eggs in comparison with random-bred control strains (Spencer et al., 1979). These differences between control and selected strains of chickens were not related to genetic cellular resistance to virus infection. In a follow up, Gavora et al. (1980) showed that hens infected with LL virus performed less well in all important production traits. The difference in production traits between selected and control strains was partly (4 to 14% in egg production) caused by difference in frequency of LL virus shedding hens and therefore not only due to genetic gain.

Correlations between live weight and haemagglutinin antibody titres to SRBC indicated a negative genetic relationship (Van der Zijpp, in preparation). Siegel and Gross (1980) observed significantly heavier 4-week body weights in the low line selected for antibody response to SRBC than either the control or high line. The lines selected for persistency showed no differences in production traits. After infection with E. coli and Staphylococcus aureus Gross et al. (1980) found reduced growth and weight loss respectively in high and low

lines. These effects were more severe in the high line, where mortality was also higher. The capability of the high line to produce more antibodies may be partly responsible for these effects. Since resistance to MD was related to decreased body weight the involvement of antibody production in the response to MD may be the explanatory factor. Although Han and Smyth (1972) showed that higher titres of antibody did not provide protection to MD, antigen and antibody titres were positvely related.

So far information regarding correlations between disease resistance, immune parameters and economically important traits is scarce. The negative genetic relationships between body weight, and MD resistance and the humoral response may lead to reconsideration of selection goals in the broiler breeding industry.

7. CONCLUDING REMARKS

The discovery, that the bursa of Fabricius and the thymus are the bases of humoral and cell-mediated immunity in chickens (Glick et al., 1956; Warner et al., 1962), has stimulated research into lymphocyte functions and their interactions with other cells necessary to mount an immune response. This knowledge is still fragmentary in the chicken (Higgins, 1981) and does not yet provide a secure framework of immune components responsible for resistance to specific diseases nor for general disease resistance.

The B major histocompatibility complex in the chicken controls many functions related to immunity. Resistance to viral oncogenesis, caused by MD, RS and LL viruses, may be a common trait coded by the B complex. But differences in immune responsiveness are also non-MHC-associated. It is likely, that comparable to mice and men (Van Rood et al., 1978) resistance to some diseases is closely associated with the B complex and resistance to other diseases is coded by genes outside, or not only by, the B system. Therefore the development of genetically determined immune criteria, not involving mortality or severe production losses due to disease, is most necessary to define resistance to specific diseases and general disease resistance.

Evaluation of the host defence with a 7 parameter assay system to control therapy of human cancer (Hersh et al., 1981) and the development of an index of natural resistance, based on bactericidal effect of blood serum, phagocytic and lysozyme activity (Pavel et al., 1981) are examples of aggregates of parameters for control of, respectively, malignant diseases, or generally disease.

Almlid (1981) proposed the development of, respectively, a non-specific and a specific antimicrobial power index as marker traits for resistance to infectious diseases in dairy bulls.

Present research is still in the stage of investigating specific diseases and single defence characters. Increased effort in this area will produce correlations with general disease resistance and production traits. The negative genetic relationship between live weight and antibody formation and resistance to MD may already alter breeding plans in the broiler breeder industry. Despite the problems described by Gavora and Spencer (1978) to incorporate disease resistance in chicken breeding programmes, consideration of immune parameters can be foreseen in the near future.

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CHAPTER 2

GENETIC ANALYSIS OF THE HUMORAL IMMUNE RESPONSE OF WHITE LEGHORN CHICKS

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ABSTRACT

Total agglutinin antibody titers, 2-mercapto ethanol (2-ME) sensitive and 2-ME resistant antibody titers were determined in 598 White Leghorn chicks after intramuscular injection with sheep red blood cells. Antibody titers were determined on day 0 and on days 3, 7, 10, 13 post injection. Mean total titer (5.2, $\log_2$ value) was highest on day 7. Females showed a significantly higher response to injection with sheep red blood cells than males. Also, significant hatch effects were noted.

Heritability estimates generally varied from 0 to 0.5 for all parameters. In the earlier stages of the immune response the sire estimate of heritability for total and 2-ME sensitive antibody titer was higher than the dam estimate. Additive genetic correlations between 2-ME sensitive (days 3 to 13) and resistant (days 7 to 13) antibody titers were negative, varying from - 0.30 to - 0.93. The response to selection for total antibody titer is, therefore, not easily predicted.

INTRODUCTION

Genetic variation in resistance to disease has long been noted (Hutt, 1958). Utilization of this genetic variation has not been applied frequently, due to easy access of vaccines and the random nature of infections. Considering specific diseases, often little is known about the contribution to resistance of the humoral and cellular responses and nonspecific mechanisms (Gavora and Spencer, 1978). Selection for components of the immune system, followed by challenge infections could lead to a better understanding of disease resistance (Biozzi et al., 1975).

Heritability studies for a range of immunological parameters and species have been carried out. In mice realized heritability values were found of about 0.2, using sheep red blood cells (SRBC), bovine serum albumin and Salmonellae as antigens and measuring the response as agglutinin titer (Biozzi et al., 1975; Passos et al., 1977; Siqueira et al., 1976). Garnett and Roberts (1972) calculated heritabilities of the percent gamma globulin level of chicks from hatch to 7 weeks of age. The sire estimate of heritability of

the pooled data was 0.26 and the dam estimate was 0.67. Large maternal effects at hatch were suggested.

In this study the feasibility of selection for agglutinin antibody titers was investigated. We present heritability data of agglutinin antibody titers in White Leghorn chicks of about six weeks of age after stimulation with SRBC. The 2-mercapto ethanol (2-ME) resistant and sensitive fractions (IgG and IgM type antibodies respectively) were determined, so separate heritabilities for these parameters in the course of the immune response were calculated. The increase and decrease of total agglutinating antibodies has also been taken into account.

MATERIALS AND METHODS

Chicks. The ninth generation of a strain of White Leghorns, kept individually in battery cages and selected for egg production traits at 62 weeks of age, provided the parent stock for this study (Van Albada and Timmermans, 1973). The effective population size was never less than 60 animals per generation.

Eleven sires were each mated with 4 dams. Full brother-sister matings were excluded. The parents, after an initial selection for minimum semen and egg production, were randomly chosen and were randomly assigned to matings. Females were inseminated once weekly. The number of offspring per sire varied from 38 to 70 (average 54) and per dam from 0 to 21 (average 14).

Two separate hatches of chicks were reared, with an age difference of 2 weeks. At hatch chicks were wingbanded. They were kept in groups of about 100 animals in a chickenhouse, partitioned by wiremesh walls into 6 floorpens. The chicks were not vaccinated. They received ad libitum water and a starter and grower feed. At 41 days of age the chicks were weighed and sexed. The number of chicks per hatch and per sex were: hatch 1, 132 cockerels, 146 pullets; hatch 2, 164 cockerels, 156 pullets; total, 296 cockerels, 302 pullets.

Immunization. At an age of 40 days the chicks were injected intramuscularly with 1 ml packed SRBC (about 26×10^9 cells). The SRBC were obtained in a heparin solution from six Texel sheep and washed three times in physiological saline (0.9% NaCl). The dose was injected in two equal portions, one into each thigh muscle.

Preparation of serum samples. The chicks were bled from the wing vein on the day of immunization and 3, 7, 10 and 13 days later. Before drawing blood the syringe was washed with a heparin solution to prevent clotting. After

centrifugation the plasma was harvested and stored at - 20 C.

Haemagglutinin assay. Total antibody titers were determined starting with solutions containing 50 µl plasma and 50 µl phosphate buffered saline solution (PBS). Plasma samples were titrated individually (microtiter system Dynatech). Packed SRBC were resuspended into PBS to make a 2% solution. Titers were expressed as the $\log_2$ of the reciprocal of the highest dilution giving complete agglutination. A half point was added if the next higher dilution showed partial agglutination. Two-ME resistant antibodies were determined by mixing equal volumes of serum and 0.2M 2-ME in PBS and incubating at 37 C for 30 min prior to serial dilution (Delhanty and Solomon, 1966). Agglutination tests were performed and the titer was recorded as 2-ME resistant antibody. The reduction of the total titer due to 2-ME treatment was called 2-ME sensitive antibody. The repeatibility of total titer determinations on the same day was 0.97 $\pm$ 0.01; with different time intervals the repeatibility decreased to 0.94 $\pm$ 0.02. During the assay period two batches of 2-ME were used. The repeatibility of titers of samples tested on the same day but treated with fresh or 4 week old 2-ME solution was 0.74 $\pm$ 0.09.

Statistical analysis. After checks for skewness and kurtosis the data were analysed with the Harvey (1977) LSML 76 program. The following parameters were analysed: total antibody titer, 2-ME resistant antibody titer, 2-ME sensitive antibody titer, the linear regression coefficient of total antibody titers on day 0, 3 and 7 (b_i = b increase), the linear regression coefficient of total antibody titers on day 7, 10 and 13 (b_d = b decrease).

The following model was used to describe the data:

$$Y_{ijklm} = \mu + a_i + b_{j:i} + c_k + d_l + e_{ijklm}$$

Y_{ijklm} represented the value of the mth animal, μ = population mean, a_i = effect of the ith sire (i = 1,11), $b_{j:i}$ = effect of the jth dam (j = 1,44) mated to the ith sire, c_k = effect of the kth sex (k = 1,2), d_l = effect of the lth hatch (l = 1,2), e_{ijklm} = remainder.

Effects of sire and dam were considered random; hatch and sex were assumed to be fixed. Previously the interaction between sex and hatch and the linear and quadratic regressions on weight had been checked for significant contributions to the model. Neither proved to be significant. The relevance of the natural antibodies to SRBC present on day zero for the titers at days 3, 7, 10 and 13 was tested by adding the linear regression on total antibody titer at day zero to the model.

Two estimates of heritability, a sire (h^2_s) and a dam (h^2_d) estimate, based on the sire and dam components of variance, were calculated. Heritability calculations were also carried out separately for each sex and hatch group, omitting the appropiate effect from the model.

Additive genetic correlations, based on paternal half sibs, were calculated between 2-ME sensitive and resistant antibody titers.

RESULTS

Means and standard deviations for total, 2-ME resistant and 2-ME sensitive antibody titers are presented in Figure 1.

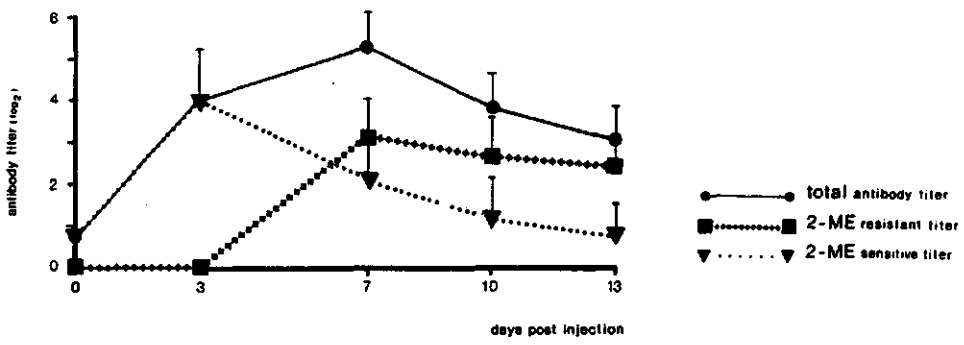

FIG. 1. THE HUMORAL IMMUNE RESPONSE OF WHITE LEGHORN CHICKS: MEAN ANTIBODY TITERS AND STANDARD DEVIATIONS.

Not until day 7 post injection 2-ME resistant antibodies were detected in considerable quantities. The total antibody titers on day 0 and 3 thus represent almost completely 2-ME sensitive antibodies. It is also evident that on day 0 natural antibodies were present in some chicks.

Except for the 2-ME resistant antibody titers on days 0 and 3 all other parameters showed no significant deviations from normality due to skewness or kurtosis. Since virtually all 2-ME resistant antibody titers on days 0 and 3 had a value 0, we have omitted this information from further calcuation.

The linear regression on covariable total antibody titer at day 0 was significant ($P < 0.005$) for total antibody titers on days 10 and 13, for 2-ME resistant antibody titer on day 10 and for 2-ME sensitive antibody titer on

TABLE 1. ANALYSES OF VARIANCE FOR TOTAL ANTIBODY TITERS FROM 0 TO 13 DAYS POST INJECTION (MEAN SQUARES)

Source of variation	df	Days post injection 0	3	7	10	13
Sires	10	3.58^{***}	6.08^{***}	3.75^{**}	3.55	2.84
Dams	31	0.52	1.78^{*}	1.25^{***}	1.77^{***}	1.34^{***}
Sex	1	1.40	8.13^{**}	6.83^{***}	9.81^{***}	6.52^{***}
Hatch	1	0.47	167.57^{***}	6.38^{***}	6.47^{***}	0.37
Remainder	554	0.44	1.10	0.61	0.59	0.54

k_1 = 13.86, constant associated with σ_d^2 of the dam MS

k_2 = 15.18, constant associated with σ_d^2 of the sire MS

k_3 = 54.04, constant associated with σ_s^2 of the sire MS

* $P < 0.05$
** $P < 0.01$
*** $P < 0.005$

TABLE 2. ANALYSES OF VARIANCE FOR 2-MERCAPTO ETHANOL RESISTANT ANTIBODY TITERS FROM 7 TO 13 DAYS POST INJECTION (MEAN SQUARES)

Source of variation	df	Days post injection 7	10	13
Sires	10	2.30^{*}	10.35^{***}	3.68^{***}
Dams	31	1.05^{*}	1.15^{***}	1.00^{***}
Sex	1	11.23^{***}	17.12^{***}	5.63^{***}
Hatch	1	36.93^{***}	2.28	15.58^{***}
Remainder	554	0.63	0.62	0.53

For constants and levels of significance see Table 1.

TABLE 3. ANALYSES OF VARIANCE FOR 2-MERCAPTO ETHANOL SENSITIVE ANTIBODY TITERS FROM 0 TO 13 DAYS POST INJECTION (MEAN SQUARES)

Source of variation	df	Days post injection 0	3	7	10	13
Sires	10	3.61***	5.97***	5.74*	6.98***	2.92
Dams	31	0.52	1.66*	2.47***	2.07***	1.48***
Sex	1	1.40	8.01**	0.54	1.01	0.03
Hatch	1	0.43	155.70***	12.61***	16.42***	20.74***
Remainder	54	0.44	1.04	0.81	0.77	0.66

For constants and levels of significance see Table 1.

TABLE 4. ANALYSES OF VARIANCE FOR REGRESSIONCOEFFICIENTS (INCREASE AND DECREASE) OF TOTAL ANTIBODY TITERS (MEAN SQUARES)

Source of variation	df	b_i	b_d
Sires	10	2.24	9.87***
Dams	31	3.23*	2.68
Sex	1	3.61	0.01
Hatch	1	1.17	10.23*
Remainder	564	1.98	1.86

For constants and levels of significance see Table 1.

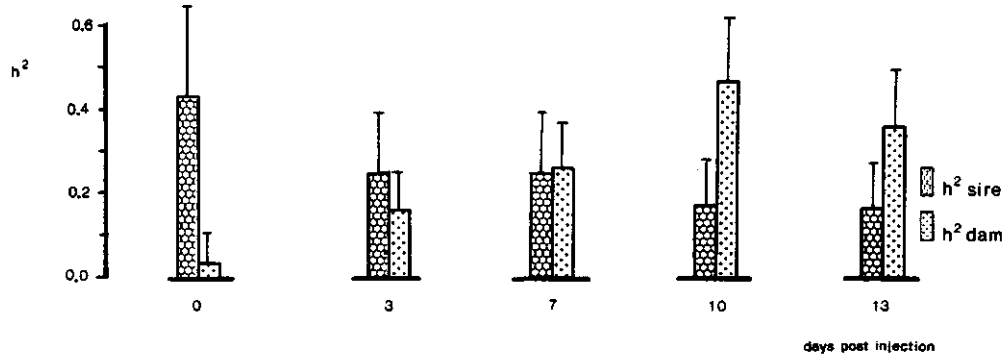

FIG. 2. HERITABILITY ESTIMATES AND STANDARD ERRORS FOR TOTAL ANTIBODY TITERS.

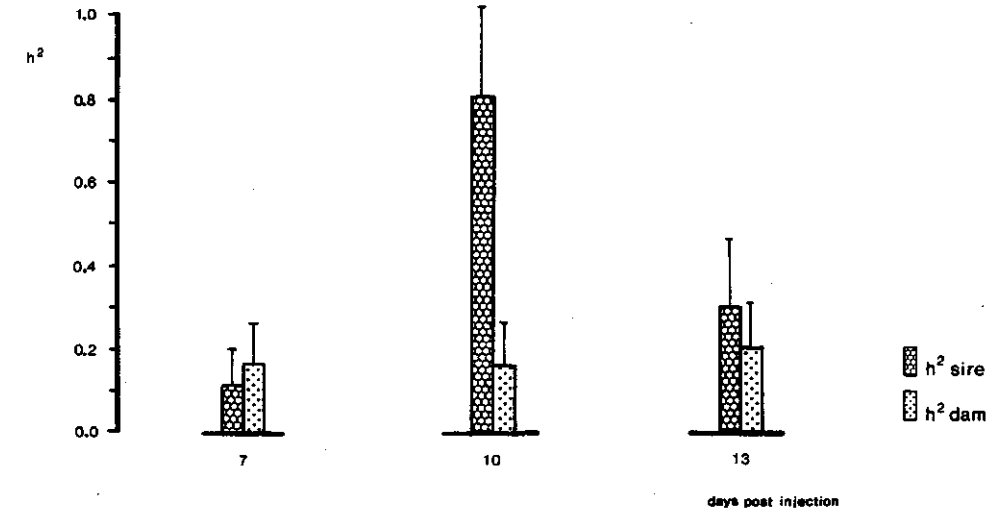

FIG. 3. HERITABILITY ESTIMATES AND STANDARD ERRORS FOR 2-ME RESISTANT ANTIBODY TITERS.

day 13.

The pullets generally showed a somewhat higher titer than the cockerels. From day 3 onwards these differences are highly significant for total antibody titers (Table 1), from day 7 for 2-ME resistant antibody titers (Table 2) and only on day 3 for 2-ME sensitive antibody titers (Table 3). Sex differences were not apparent for the regression coefficients b_i and b_d (Table 4).

The second hatch of chicks had significantly higher total antibody titers on days 3, 7 and 10 than hatch 1 (Table 1). Significant hatch effects were also found for 2-ME resistant and 2-ME sensitive antibody titers (Tables 2, 3). Only for the regression coefficient b_d was a significant effect shown (Table 4).

Several sire and dam components of variance were not significantly different ($P \geq 0.05$) from zero. These can be found in Tables 1, 2, 3 and 4. The heritability estimates h^2_s and h^2_d are presented in Figures 2, 3, 4 and 5 for total, 2-ME resistant, 2-ME sensitive antibody titers, and for the regression coefficients b_i and b_d, respectively. Heritability values for antibody titers and regression coefficients varied generally between 0 and 0.5 with an exceptionally high estimate on day 10 for the h^2_s of the 2-ME resistant antibody titer.

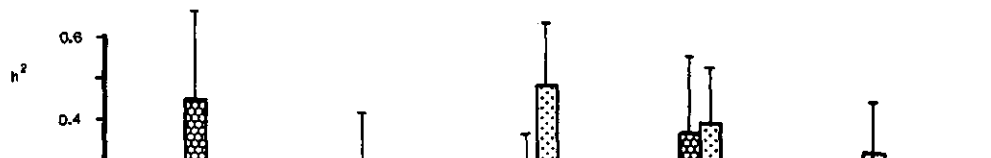

FIG. 4. HERITABILITY ESTIMATES AND STANDARD ERRORS FOR 2-ME SENSITIVE ANTIBODY TITERS.

For total antibody titers a trend with days post immunization is detectable: the h^2_s showed a decrease with time, the h^2_d an increase (Fig. 2). A similar trend for 2-ME sensitive antibody titers can be seen in Figure 4, where

TABLE 5. GENETIC CORRELATIONS BETWEEN 2-MERCAPTO ETHANOL SENSITIVE AND RESISTANT ANTIBODY TITERS AND STANDARD ERRORS

2-mercapto ethanol sensitive days post injection	2-mercapto ethanol resistant days post injection 7 r	SE	10 r	SE	13 r	SE
0	0.24	± 0.40	0.01	± 0.35	0.74	± 0.23
3	-0.56	± 0.32	-0.37	± 0.32	-0.75	± 0.25
7	-0.49	± 0.44	-0.81	± 0.20	-0.30	± 0.39
10	-0.80	± 0.31	-0.93	± 0.13	-0.50	± 0.32
13	-0.23	± 0.50	-0.64	± 0.31	0.05	± 0.47

TABLE 6. PHENOTYPIC CORRELATIONS BETWEEN 2-MERCAPTO ETHANOL SENSITIVE AND RESISTANT ANTIBODY TITERS

2-mercapto ethanol sensitive days post injection	2-mercapto ethanol resistant days post injection 7	10	13
0	0.05	0.11**	0.25**
3	0.31**	0.15**	0.09*
7	-0.59**	-0.40**	0.21**
10	-0.46**	-0.60**	0.31**
13	-0.40**	-0.39**	0.56**

* $P < 0.05$
** $P < 0.01$

h^2_s is considerably larger than h^2_d in the beginning of the immune response, but h^2_d is higher from day 7 post immunization and after.

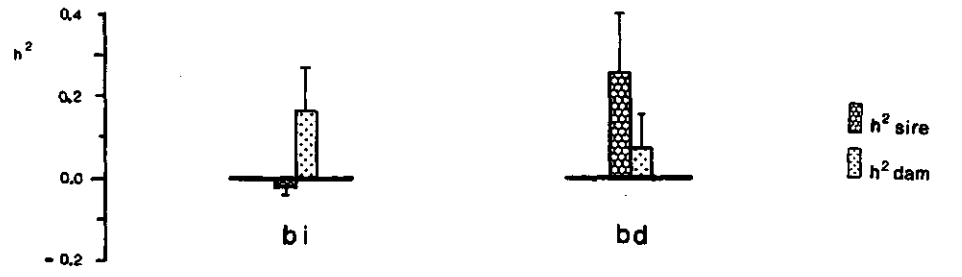

FIG. 5. HERITABILITY ESTIMATES AND STANDARD ERRORS FOR THE LINEAR REGRESSION COEFFICIENTS OF INCREASE AND DECREASE OF TOTAL ANTIBODY TITER.

Heritability calculations, separately carried out within each sex and hatch class, produced a variable, inconsistent picture.

Genetic correlations between 2-ME sensitive and 2-ME resistant antibody titers are presented in Table 5. Generally the relationship between the two parameters was negative, especially on days 3, 7 and 10. But the correlations between the 2-ME sensitive antibody titer on day 0 and the 2-ME resistant antibody titers were positive and were close to zero except for day 13. Phenotypic correlations were negative, with a range of - 0.39 to - 0.60 on days 7 to 13 for 2-ME sensitive and days 7 and 10 for 2-ME resistant antibody titers. All other phenotypic correlations were positive (Table 6).

DISCUSSION

The highest mean total antibody titer value was 5.2 on day 7. Subba Rao et al. (1977), in chicks immunized intravenously, found a mean titer of 5.8 (4.6 for 2-ME resistant titer) at 4 weeks of age and 8.0 (6.2 for 2-ME resistant titer) at 8 weeks. In a study with adult White Leghorn laying hens, Van der Zijpp (1978) found an average titer of 6.3 at days 6 and 10 after intramuscular immunization with 0.5 ml packed SRBC. In the same study the hens res-

ponded with an average titer of 10.0 to a dose of 3 ml packed SRBC. Therefore the 40 day old chicks in our experiment had probably not yet reached immunological maturity in their response to SRBC.

Sex effects, as shown by the higher titers of the pullets, have been found in humans, too. Grundbacher and Shreffler (1970) found higher immunoglobulin levels for young females, but males showed a more marked increase with age. In the Syrian hamster females produced significantly higher numbers of haemolytic plaque forming cells, but no significant effects were shown for agglutinin titers (Blazkovec et al., 1973).

The hatch effect may have been caused by climatic conditions. The first hatch of chicks was injected after three days with outside temperatures of 30 C. The second hatch was immunized when outside temperatures were not higher than 20 C. Subba Rao and Glick (1977) found that chicks, 4 weeks old, kept at an environmental temperature of 32.2 C before immunization, gave a significantly lower response than chicks held at 24 C or 7.2 C. Thus, our results may have been influenced by temperature. However, the chicks of the second hatch were also slightly, though not significantly, heavier at 41 days of age.

The heritability estimates generally agree with data of similar experiments (Biozzi et al., 1975; Garnett and Roberts, 1972; Passos et al., 1977). A revealing result is the heritability level of the natural agglutinins. These antibodies of 2-ME sensitive nature may represent antibodies to SRBC-like antigens. These natural antibodies are important only at lower levels of measurable response (days 10 and 13).

The high estimates of h^2_s for total and 2-ME sensitive titers in relation to h^2_d in the beginning of the immune response require further attention. The analysis within females and males revealed similar trends, but differences between sexes were inconsistent, so sex linkage cannot possibly be held responsible for this effect. An explanation for the low h^2_d estimate at day zero could be the transfer of fluctuating amounts of natural antibodies to SRBC from the hen to her chicks which thereby affects their immune response to SRBC-like antigens. Then the variation of antibody titers on day 0 within full-sib groups of chicks may increase and the dam estimate of heritability will be lowered.

The application of 2-ME produces a comparative estimate of antibody activity, in our case agglutination, and not of actual quantities of 7S and 19S antibodies produced (Osler, 1978). The assumption that destruction of the -SH bonds only affects the reactivity of IgM antibodies can also be disputed.

Despite these limitations we have considered more closely the relationship between 2-ME resistant and sensitive antibodies in the following paragraphs.

Sufficient additive genetic variation is present to select for total titers at either day three or seven (h^2_s = 0.26 for both) post immunization. Selection for total titer in this phase of the immune response regards both 2-ME resistant and 2-ME sensitive antibodies. However, the effectiveness of selection for total titer may be questionable because of the negative genetic correlations of about - 0.5. Biozzi et al. (1975) found increased levels for all immunoglobulin classes in immunized and nonimmunized mice of the line selected for high agglutinin antibody titers in comparison with the low line. They commented that the inheritance pattern is probably different for each class of immunoglobulin, although their selection results indicate a positive genetic relationship. Evidence for independent genetic regulation of the kinetics of the IgM response and level of IgG response to SRBC has been presented by Seman et al. (1978) in a study of inbred strains of mice. Our results indicate that chicks have a genetic constitution which is geared to either high 2-ME sensitive antibody titers and low 2-ME resistant titers or vice versa. This negative relationship is reflected to a lesser extent in the phenotypic correlations.

Antibodies are produced by B-cells, stimulated due to a series of cell interactions (McConnell, 1976) involving also macrophages and various T-cell populations. Further research comparing functions of cell populations of genetically extreme animals may produce a better understanding of these negative correlations.

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CHAPTER 3

GENETIC ANALYSIS OF PRIMARY AND SECONDARY IMMUNE RESPONSES IN THE CHICKEN

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ABSTRACT

Total agglutinin antibody titers, 2-mercaptoethanol (2-ME) sensitive and 2-ME resistant antibody titers were determined in 561 White Plymouth Rock chicks. At 38 days of age chicks were intramuscularly injected with .25 ml packed sheep red blood cells (SRBC) and at 66 days of age with .5 ml SRBC. Antibody titers were determined on day 0, 3, 5, 7 and 10 postinjection.

Mean total titer (5.2, $\log_2$ value) was highest on day 7 for the primary response and for the secondary response peak titer (7.0) was measured on day 5. The primary response was characterised by a very low level of 2-ME resistant antibodies. The secondary response consisted of both types of antibody.

The heritability estimates indicate, that selection for total antibody titer of the primary response can be based on the titer level of day 5 post-injection (h^2_s = .39). For the secondary response day 3 (h^2_s = .34) and 5 (h^2_s = .28) postinjection can be considered.

Phenotypic correlations between total antibody titers of first and second injection are not significant on day 5, 7 and 10 post second injection. The negative relationship between primary and secondary 2-ME sensitive antibody titers in contrast with a positive relationship between primary 2-ME sensitive antibody titers and secondary 2-ME resistant antibody titers explains this result.

Additive genetic correlations between total antibody titers of first and second injection tend to be quite negative on day 5, 7 and 10 post second injection. Selection for an improved prophylactic response therefore appears not to be easily achieved, either via selection of parameters of the primary immune response or via the secondary response.

INTRODUCTION

Quantitative genetic control of the primary antibody response in the chicken has been shown (Siegel and Gross, 1980; Van der Zijpp and Leenstra, 1980). In these studies sheep red blood cells (SRBC) were chosen as immunizing agent. SRBC are complex, multideterminant natural antigens provoking a T-B cell dependent antibody response. Most common pathogens also possess complex anti-

genic structures. Polygenic regulation of the response to these polydeterminant immunogens can be expected (Biozzi et al., 1979).

If the primary antibody response to SRBC is chosen as a selection criterion, correlated effects regarding the secondary response may be expected. Because vaccination is based on the development of immunological memory, the consequences of selection for the prophylactic response should be recognised. The term prophylactic response is used to designate the ability to mount a secondary immune response after vaccination or a primary immunization. Biozzi et al. (1979) indicate that both primary and secondary responses to SRBC in mice are submitted to the same genetic control at least partially. The genes regulating antibody production operate on the synthesis of both 19S and 7S antibodies. Sant'Anna et al. (1979) conclude that in mice immunized with Salmonella antigens, a smaller number of genes regulate the secondary response compared with the primary response. Another example of different genetic regulation of primary and secondary responses in mice in relation to H-2 linkage has been presented by Ando and Fachet (1977).

In this study the quantitative genetic regulation of primary and secondary antibody responses to SRBC is analysed in a flock of White Plymouth Rock (WPR) chicks. Because of their predictive significance special attention will be paid to the phenotypic and genetic correlations between primary and secondary antibody responses.

MATERIALS AND METHODS

Chicks. The experimental animals belonged to an experimental WPR strain formed by crossing and backcrossing. The parent stock have been selected for body weight at 56 days of age.

Twentyone sires were randomly mated with 93 dams with a minimum of 3 hens per sire. A minimum family size of four chicks per hen was accepted, so some selection for reproductive ability has occurred.

Six hundred chicks, hatched on the same day and wingbanded at hatch, were reared as one group in a floorpen. They received ad libitum feed until seven weeks of age, followed by a somewhat restricted feeding scheme according to live weight. Water was always provided ad libitum. At 35 and 63 days of age the chicks were weighed and sexed.

Vaccination. At one day of age chicks were intramuscularly vaccinated for Marek's disease and spray vaccinated for infectious bronchitis. At 8 and 52 days spray vaccinations for Newcastle disease and at 35 days of age vaccination via

the drinking water for infectious bursal disease (IBDV) followed. Always attenuated (live) virus vaccines were used.

Immunization. At an age of 38 days the chicks were injected intramuscularly with .25 ml packed SRBC mixed with .75 ml physiological saline (.9% NaCl). The SRBC were obtained in a heparin solution from six unrelated Texel sheep and washed three times in physiological saline. The dose was injected in two equal portions, one into each thigh.

At an age of 66 days the same procedure was carried out injecting a dose of .5 ml packed SRBC mixed with .5 ml physiological saline.

Preparation of plasma samples and the hemagglutinin assay. The chicks were bled from the wing vein on the day of injection and 3, 5, 7 and 10 days later. Treatment of plasma and the hemagglutinin assay are described by Van der Zijpp and Leenstra (1980). The total and 2-mercaptoethanol (2-ME) resistant antibody titers were expressed as the $\log_2$ of the reciprocal of the highest dilution in which agglutination occurred. The reduction of the total titer due to 2-ME treatment was called 2-ME sensitive antibody.

Statistical analysis. After checks for skewness and kurtosis the data were analysed with the Harvey LSML 76 (1977) program. The following parameters were analysed: total antibody titer, 2-ME resistant antibody titer and 2-ME sensitive antibody titer. Complete datasets of 561 chicks were available for computer processing: 276 cockerels and 285 pullets.

The following model was used to describe the data:

$$Y_{ijkl} = \mu + a_i + b_{j:i} + c_k + e_{ijkl}$$

Y_{ijkl} represented the value of the lth animal, μ = population mean, a_i = effect of the ith sire (i = 1, 21), $b_{j:i}$ = effect of the jth dam (j = 1, 93) mated to the ith sire, c_k = effect of the kth sex (k = 1, 2), e_{ijkl} = remainder. Effects of sire and dam were considered random; sex was assumed to be fixed.

Two estimates of heritability were calculated: a sire (h^2_s) and a dam (h^2_d) estimate, based on the sire and dam components of variance.

Phenotypic and additive genetic correlations based on paternal half sibs, were calculated between antibody titers of primary and secondary response.

RESULTS

Means for total, 2-ME resistant and 2-ME sensitive antibody titers are

presented in Figure 1. The primary immune response consisted almost completely of 2-ME sensitive antibodies, with a peak measured on day 7 postinjection. The secondary response had a peak level of total antibody on day 5. This peak measurement coincided with the peak level of 2-ME resistant antibodies. The 2-ME sensitive antibody level increased until day 3 post second injection and then declined very slowly. The peak total antibody titer of the secondary response measured 7.0 and of the primary response 5.2.

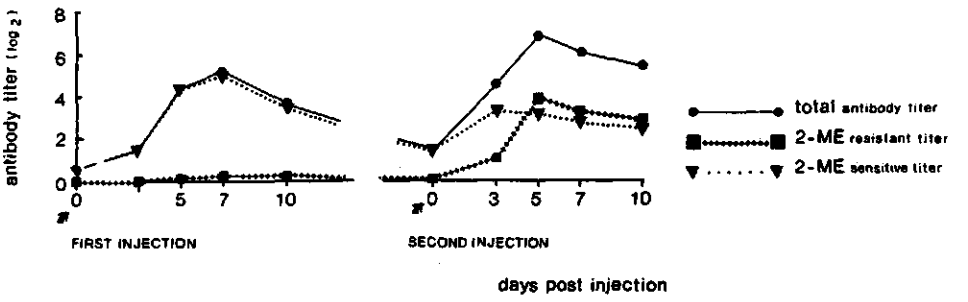

FIG. 1. THE HUMORAL IMMUNE RESPONSE OF WPR CHICKS: MEAN ANTIBODY TITERS OF PRIMARY AND SECONDARY RESPONSE.

Except for the 2-ME resistant antibody titers during the primary response and on the day of second injection all other antibody titers showed no significant deviations from normality due to skewness or kurtosis. Because these 2-ME resistant antibody titers nearly all had a value zero no further calculations were carried out.

Effect of sex. In Table 1 the analysis of variance is presented for total antibody titers of the primary response, but 2-ME sensitive antibody titers followed the same pattern. During the primary response cockerels showed a higher response of total and 2-ME sensitive antibodies. Sex effects were significant on day 3, 7 and 10 post first injection. In Tables 2, 3 and 4 analyses of variance for antibody titers post second injection are presented. Cockerels had higher total antibody titers than pullets on day 0 and 3 post second injection and higher 2-ME resistant antibody titers on days 0, 3, 5, 7 and 10 post second injection. Two-ME sensitive titers favoured the pullets on day 3, 5, 7 and 10 post second injection.

TABLE 1. ANALYSES OF VARIANCE FOR TOTAL ANTIBODY TITERS FROM 0 TO 10 DAYS POST FIRST INJECTION (MEAN SQUARES)[1]

Source of variation	df	Days post primary injection 0	3	5	7	10
Sires	20	.81*	3.19	17.80***	6.75	7.05**
Dams	72	.38***	1.98*	6.18***	4.12***	3.17***
Sex	1	.29	6.62*	12.54	9.56*	30.60***
Remainder	467	.23	1.47	3.52	2.31	1.65

[1] k_1 = 5.87, constant associated with σ^2_d of the dam MS; k_2 = 6.55, constant associated with σ^2_d of the sire MS; k_3 = 26.57, constant associated with σ^2_s of the sire MS.

* P < .05
** P < .01
*** P < .005

TABLE 2. ANALYSES OF VARIANCE FOR TOTAL ANTIBODY TITERS FROM 0 TO 10 DAYS POST SECOND INJECTION (MEAN SQUARES)[1]

Source of variation	df	Days post second injection 0	3	5	7	10
Sires	20	2.79***	4.60***	5.62***	3.35	2.97
Dams	72	.91***	1.74***	2.33***	2.17***	2.00***
Sex	1	6.39***	5.91*	.17	.37	.36
Remainder	467	.56	1.02	1.43	1.28	1.24

[1] For constants and levels of significance see Table 1.

Primary antibody response. Twice, on days 3 and 7, the random sire effect was not significantly different ($P \geq .05$) from 0 (Table 1). The heritability estimates h^2_s and h^2_d for total antibody titers are presented in Figure 2. The estimates varied between .11 to .39 for h^2_s and between .21 and .51 for h^2_d. The 2-ME sensitive antibody titers showed heritabilities of almost equal size.

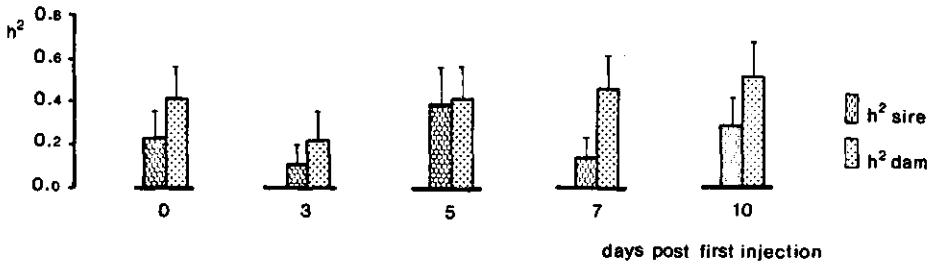

FIG. 2. HERITABILITY ESTIMATES AND STANDARD ERRORS FOR TOTAL ANTIBODY TITERS POST FIRST INJECTION.

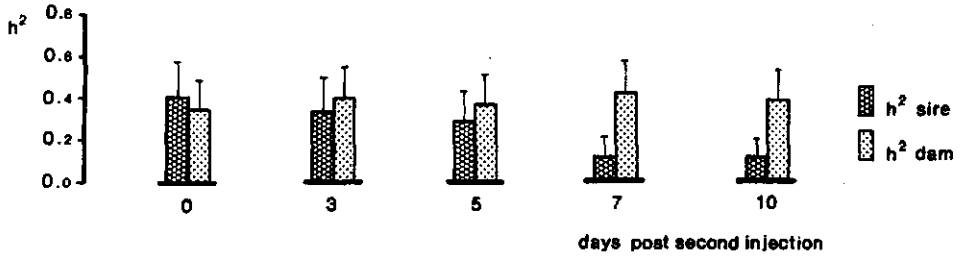

FIG. 3. HERITABILITY ESTIMATES AND STANDARD ERRORS FOR TOTAL ANTIBODY TITERS POST SECOND INJECTION.

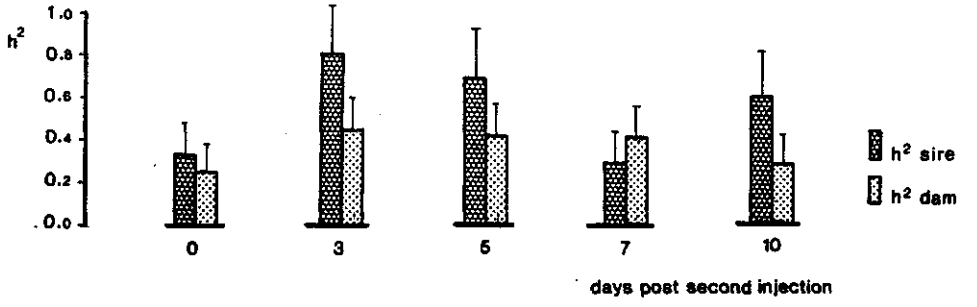

FIG. 4. HERITABILITY ESTIMATES AND STANDARD ERRORS FOR 2-ME SENSITIVE ANTIBODY TITERS POST SECOND INJECTION.

TABLE 3. ANALYSES OF VARIANCE FOR 2-ME SENSITIVE ANTIBODY TITERS FROM 0 TO 10 DAYS POST SECOND INJECTION (MEAN SQUARES)[1]

Source of variation	df	Days post second injection 0	3	5	7	10
Sires	20	1.80***	11.49***	10.90***	4.44***	6.29***
Dams	72	.63*	2.30***	2.45***	1.85***	1.46***
Sex	1	3.26**	.65	11.39***	2.86	.08
Remainder	467	.45	1.18	1.34	1.07	.96

[1] For constants and levels of significance see Table 1.

TABLE 4. ANALYSES OF VARIANCE FOR 2-ME RESISTANT ANTIBODY TITERS FROM 3 TO 10 DAYS POST SECOND INJECTION (MEAN SQUARES)[1]

Source of variation	df	Days post second injection 3	5	7	10
Sires	20	11.32***	9.74***	2.31***	3.85***
Dams	72	1.36***	1.68***	.93**	1.34***
Sex	1	10.13***	8.48***	1.17	.07
Remainder	467	.76	.91	.62	.71

[1] For constants and levels of significance see Table 1.

Secondary antibody response. In Tables 2, 3 and 4 generally significant sire and dam effects are shown, except for sire effects of total antibody titers on day 7 and 10 post second injection. Heritability estimates are presented in Figures 3, 4 and 5. For total antibody titers h^2_s varied from .10 to .34 and h^2_d varied from .36 to .41. Two-ME sensitive titers had higher heritabilities: h^2_s varied from .29 to .80 and h^2_d from .27 to .44. Very high heritabilities are shown for the 2-ME resistant antibody titers: h^2_s varied from .28 to 1.20

and h^2_d from .29 to .47. The titers on day 0 of the second injection have been excluded in the above consideration, because these titers were the result of the first injection.

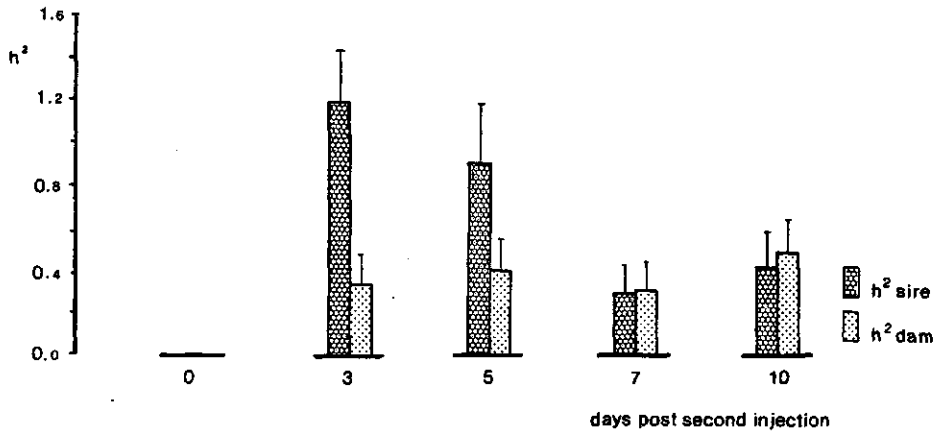

FIG. 5. HERITABILITY ESTIMATES AND STANDARD ERRORS FOR 2-ME RESISTANT ANTIBODY TITERS POST SECOND INJECTION.

Relationship between primary and secondary response. Genetic and phenotypic correlations between total antibody titers of first and second injection are presented in Table 5 and 6, respectively. Phenotypic correlations were positive for days 0 and 3 post second injection, because the level of antibodies on day 0 post second injection was the result of the primary immunization. On day 5, 7 and 10 post second injection the relation was not significant. The genetic correlations followed a pattern of a positive correlation, becoming negative on days 5 and 7 post second injection.

The correlations between 2-ME sensitive antibody titers of first and second injection were generally larger and more often had a negative sign (Tables 7, 8). The genetic correlations already were negative on day 3 post second injection. Phenotypic correlations were positive on day 0 (result of primary immunization), but always negative on days 3, 5, 7 and 10 post second injection.

The relationship between 2-ME sensitive titers of first injection and 2-ME resistant titers of second injection was positive for genetic and phenotypic correlations (Tables 9, 10).

TABLE 5. GENETIC CORRELATIONS (r_g) BETWEEN TOTAL ANTIBODY TITERS OF FIRST AND SECOND INJECTION AND STANDARD ERRORS

Days post first injection	Days post second injection: 0 r	SE	3 r	SE	5 r	SE	7 r	SE	10 r	SE
0	.31	± .30	-.06	± .34	.01	± .35	.02	± .44	.17	± .45
3	.11	± .39	.41	± .37	-.56	± .39	-.84	± .46	-.22	± .54
5	.05	± .30	.58	± .23	-.68	± .23	-.74	± .32	-.30	± .40
7	.71	± .23	.22	± .37	-.41	± .37	-.28	± .48	.07	± .51
10	.78	± .14	.42	± .28	-.66	± .25	-.33	± .39	.38	± .39

TABLE 6. PHENOTYPIC CORRELATIONS (r_p) BETWEEN TOTAL ANTIBODY TITERS OF FIRST AND SECOND INJECTION

Days post first injection	Days post second injection: 0	3	5	7	10
0	.16**	.02	-.04	-.01	-.03
3	.19**	.13**	-.05	-.01	.05
5	.34**	.23**	.03	.04	.06
7	.46**	.23**	.04	.08*	.13**
10	.57**	.29**	.09	.14**	.25**

* $P < .05$
** $P < .01$

TABLE 7. GENETIC CORRELATIONS (r_g) BETWEEN 2-ME SENSITIVE ANTIBODY TITERS OF FIRST AND SECOND INJECTION AND STANDARD ERRORS

Days post first injection	Days post second injection: 0 r SE	3 r SE	5 r SE	7 r SE	10 r SE
0	.31 ± .31	.14 ± .30	- .64 ± .24	- .58 ± .29	- .63 ± .25
3	.23 ± .39	-.45 ± .35	- .09 ± .38	- .78 ± .38	- .64 ± .34
5	.03 ± .31	-.41 ± .25	- .57 ± .23	- .76 ± .23	- .55 ± .24
7	.90 ± .24	-.47 ± .36	-1.00 ± .33	-1.09 ± .37	-1.11 ± .32
10	.80 ± .16	-.29 ± .29	- .59 ± .25	- .26 ± .34	- .42 ± .28

TABLE 8. PHENOTYPIC CORRELATIONS (r_p) BETWEEN 2-ME SENSITIVE ANTIBODY TITERS OF FIRST AND SECOND INJECTION

Days post first injection	Days post second injection: 0	3	5	7	10
0	.17**	-.03	-.10*	-.06	-.07
3	.11**	-.13**	-.14**	-.17**	-.13**
5	.27**	-.12**	-.18**	-.16**	-.16**
7	.37**	-.05	-.21**	-.13**	-.12**
10	.48**	-.03	-.12**	-.07	-.01

* $P < .05$
** $P < .01$

TABLE 9. GENETIC CORRELATIONS (r_g) BETWEEN 2-ME SENSITIVE TITERS OF FIRST INJECTION AND 2-ME RESISTANT TITERS OF SECOND INJECTION AND STANDARD ERRORS

2-ME sensitive titer Days post first injection	2-ME resistant titer Days post second injection 3 r	SE	5 r	SE	7 r	SE	10 r	SE
0	-.17	± .29	.66	± .21	.81	± .23	.99	± .13
3	.65	± .27	-.27	± .35	.32	± .39	.77	± .28
5	.70	± .15	.15	± .27	.37	± .28	.60	± .21
7	.51	± .31	.56	± .30	1.02	± .24	1.41	± .30
10	.49	± .23	.13	± .29	.03	± .35	.70	± .20

TABLE 10. PHENOTYPIC CORRELATIONS (r_p) BETWEEN 2-ME SENSITIVE TITERS OF FIRST INJECTION AND 2-ME RESISTANT TITERS OF SECOND INJECTION

2-ME sensitive titer Days post first injection	2-ME resistant titer Days post second injection 3	5	7	10
0	.06	.08*	.06	.04
3	.28**	.10*	.21**	.21**
5	.38**	.25**	.27**	.26**
7	.30**	.32**	.34**	.33**
10	.34**	.29**	.32**	.34**

* $P < .05$
** $P < .01$

DISCUSSION

The primary response was characterized by a normal pattern of response for total antibody titers (White and Nielsen, 1975; Van der Zijpp and Leenstra, 1980). The level of 2-ME resistant antibodies was quite low however. In a comparison of three dose levels in three commercial stocks Van der Zijpp (in preparation) found low 2-ME resistant titers at antigen levels of .1 and .01 ml SRBC in the Warren stock; levels in the WPR were intermediate. The low response of 2-ME resistant antibodies may be attributable not only to the low dose of antigen, but also to the particular WPR strain used in this study. Depression of primary total hemagglutinin titers was observed by Giambrone et al. (1977) after simultaneous injection of SRBC and infection with IBDV. Serum levels of IgM and IgG were not altered. The IBDV vaccination per se, in our experiment applied three days before first SRBC injection, may be responsible for the low level of 2-ME resistant antibodies, too. Also the vaccination program as a whole can adversely affect the production of 2-ME resistant antibodies (Van der Zijpp et al., 1982).

A secondary response is characterized by a higher peak titer, an earlier peak postinjection and a larger IgG production in comparison with a primary response. In our study these three conditions are fulfilled, but manipulation with dose of SRBC (.25 ml at first injection versus .5 ml at the second injection) can also contribute to this typical picture of a secondary response (Van der Zijpp, unpublished results). The importance of the immunization procedure on the phenotypic effect of genes regulating immunoresponsiveness has been stressed by Biozzi et al. (1979).

Selection for an improved primary antibody response can be based on the total antibody titer level at day 5 postinjection, considering the size of the heritability in this study, and the results of Siegel and Gross (1980). Selection for this parameter has consequences for the rate of antibody production postinjection, but also for the composition of the response (Biozzi et al., 1979) and for the persistence (Siegel and Gross, 1980).

Heritabilities of the secondary total antibody titers were of similar size, on day 3 and 5 postinjection, and can therefore be considered as a criterion of selection for the prophylactic response. There were no clear indications, that the genetic influence on the secondary response is less strong. Sant'Anna et al. (1979) and Lie (1979) reported that the primary response is genetically influenced to a greater extent than the secondary

response. Their conclusions may have been dependent on the sampling time post-injection, considering the variation in size of heritability on different days postinjection in our study.

The choice of a parameter for selection, from the primary or secondary response, depends also on the relationship between antibody titers of first and second injection. The positive phenotypic correlations of total antibody titers on day 0 and 3 were probably influenced by the antibody level due to first injection. On day 5, 7 and 10 post second injection these correlations were not significant; their value was determined by the negative relationship between 2-ME sensitive antibody titers of first and second injection and the positive relationship between 2-ME sensitive antibody titers of the first injection and 2-ME resistant antibody titers of the second injection. This inverse relationship between 2-ME sensitive antibody titers is visualised in Figure 6. Three groups have been formed according to 2-ME sensitive titer level on day 0 of the second injection. The high level group did not show any increase in 2-ME sensitive antibodies, but a strong increase in 2-ME resistant antibodies (Fig. 7) and the low level groups showed opposite tendencies in 2-ME sensitive titer level. A similar situation occurs when chicks have been injected with a high dose of SRBC at first; the secondary response existed of an increase in 2-ME resistant antibodies and a decrease, even, of 2-ME sensitive antibodies. A low dose at primary injection leads to increases in both types of antibody at the secondary response (Van der Zijpp, unpublished results).

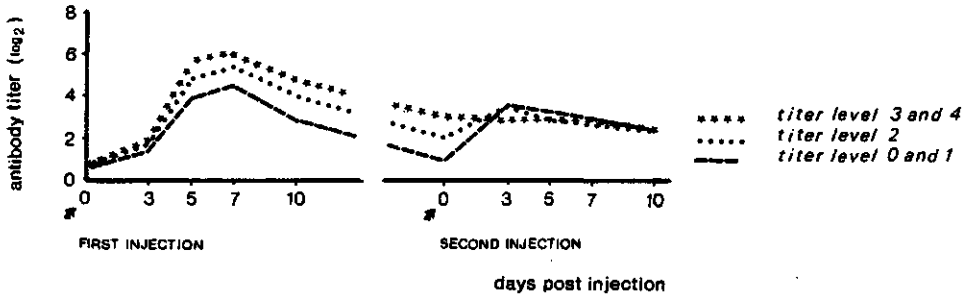

FIG. 6. TWO-ME SENSITIVE ANTIBODY TITER LEVELS IN RELATION TO THEIR DISTRIBUTION ON THE DAY OF SECOND INJECTION.

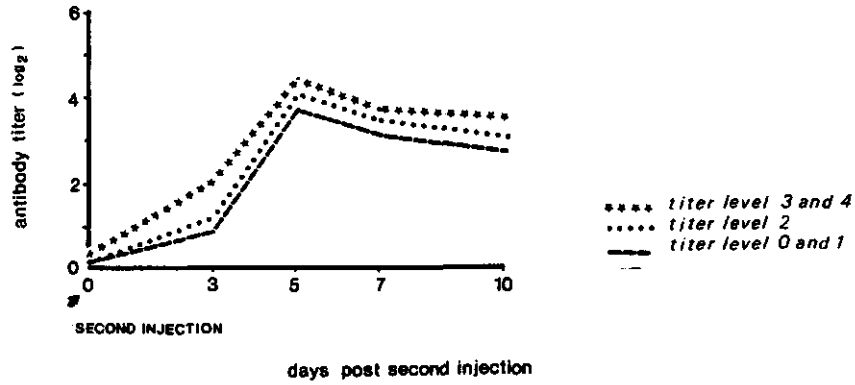

FIG. 7. TWO-ME RESISTANT ANTIBODY TITER LEVELS IN RELATION TO THE DISTRIBUTION OF 2-ME SENSITIVE ANTIBODY TITERS ON THE DAY OF SECOND INJECTION.

The immune status of the chick at the time of second injection, whether determined by genetic ability, vaccination program, or primary dose, influences the secondary response. Grantham and Fitch (1975) found in mice that high primary antibody levels inhibit antibody production during the secondary response. Especially IgG antibodies can suppress the secondary antibody response, in particular IgM production. In our study few 2-ME resistant antibodies were present during the primary response. But especially in the high responder group, they may have exerted a negative influence upon the production of secondary 2-ME sensitive antibodies.

The additive genetic correlations tended to have the same sign as the phenotypic correlations, but were larger in size. Different characteristics of cell populations, genetically determined, can account for these relationships. Trizio and Cudkowicz (1978) concluded that low dose priming in mice can activate T helper cells, but not B cells, leading to increased IgG: IgM ratios upon challenge. Peripheral T cells probably interact most effectively with IgM precursors in a primary response; upon challenge these T-cells stimulate the IgG precursors. In our study genetic differences in T helper cell populations may have been present, leading to genetic variation in the primary 2-ME sensitive antibody response and to a negative relationship between 2-ME sensitive antibody titers of primary and secondary response and a positive relation-

ship between 2-ME sensitive antibody titers of primary response and 2-ME resistant antibody titers of the secondary response.

In mice Biozzi et al. (1979) found marked differences in macrophage activity of the Low and High antibody responder line. In the Low line antigen is rapidly broken down; in the High line antigen is slowly destructed, stimulating lymphocytes to produce antibodies. This difference in macrophage activity leads to differences in the primary response, the High responder line producing 7S and 19S antibodies and the Low responder line 19S antibodies only. At a secondary response the High responders produce 7S antibodies only, the Low line produces 7S and 19S antibodies. In our experiment the genetic variation of the primary response could be caused by genetic differences in macrophage activity, followed by a secondary response with similar tendencies towards a higher level of 2-ME resistant antibodies in the high primary producers and a combination of a 2-ME sensitive and resistant antibody response in the low primary responders.

Our data showed that sufficient genetic variation is present for selection on primary and/or secondary antibody production. However this particular WPR strain appears to be characterized as a low responder, in combination with a low primary dose of SRBC antigen and the vaccination program. This may be the cause of negative genetic correlations between total antibody titers. Further research with different breeds and dose regimen is warranted before genetic improvement of the prophylactic response can be applied.

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CHAPTER 4

THE EFFECT OF GENETIC ORIGIN, SOURCE OF ANTIGEN AND DOSE OF ANTIGEN ON THE IMMUNE RESPONSE OF COCKERELS

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Submitted for publication

ABSTRACT

The total agglutinin antibody titers and the 2-mercaptoethanol resistant antibody titers were determined in cockerels of three genetic origins, i.e. White Plymouth Rock (WPR), White Leghorn (WL) and a medium heavy breed cross (Warren). At 34 days of age the chicks were injected intramuscularly with 1.0 ml of a 100 per cent, a 10 per cent or a 1 per cent suspension of packed sheep red blood cells (SRBC) in phosphate buffered saline. Antibody titers were determined on day 0, 3, 7 and 14 postinjection. The response of phytohemagglutinin (PHA) was measured at 49 days of age after intradermal injections of the antigen into the wingweb of the birds that had received the 100 per cent suspension of SRBC.

Differences between genetic groups resulted in significant differences for total antibody titers. The ranking of groups of different origin varied with day postinjection. The highest antigen dose yielded the highest antibody titers and there were no significant genetic origin-dose interactions. The WPR cockerels showed the greatest swelling in the PHA test, followed by the Warren and WL in that order, with differences being significant ($P < .05$). There was no correlation between antibody titers to SRBC measured on day 3, 7 and 14 postinjection and PHA response, neither overall nor within groups of different genetic origin. We tentatively conclude that selection for general immune responsiveness may mean combining parameters of antibody and cell-mediated immune responses.

The WPR cockerels were significantly ($P < .005$) heavier than the Warren cockerels which in turn were significantly heavier ($P < .005$) than the WL. Statistical analyses suggest that the antibody response may be independent of body weight differences within genetically similar groups.

INTRODUCTION

Genetic differences in antibody response have been reported for experimental random bred populations (Siegel and Gross, 1980; Van der Zijpp and Leenstra, 1980; Pevzner et al., 1981), between experimental strains (Gross and Colmano, 1971), inbred lines (Balcarova et al., 1973) and commercial strains

(Heller et al., 1981). In these studies one level of antigen was investigated which was determined by commercial practice, where vaccines were concerned, or by undefined experimental evidence. To separate two stocks of chickens at different levels of antigen application the lowest concentrations of sheep red cell antigen proved to be most useful (Gross, 1979). However no dose - strain interaction was reported.

Klesius et al. (1977) detected differences in cell-mediated immune response to diphteria toxoid in two commercial lines.

This study examined the effect of dose of sheep red blood cells (SRBC) on antibody response in three commercial stocks of different genetic origin with particular attention to dose - genetic origin interaction. Genetic differences between stocks were evaluated for the phytohemagglutinin (PHA) skin response, a cell-mediated immune reaction (Goto et al., 1978).

MATERIALS AND METHODS

Experimental chicks. One hundred one-day-old cockerels from each of three genetic origins, i.e., a commercial cross of White Plymouth Rocks (WPR), a commercial cross of White Leghorns (WL) and a commercial breed cross (ISA Warren, a medium heavy, brown egg layer) were reared together in one room, but the chicks of each genetic group were separated from the others by chicken wire. We realize that such rearing confounds genetic origin and location - within-the-room-effects. But we judged that this disadvantage was less than the effects of possible competitive interactions among chicks of quite different size and body weight when reared together. A starter and grower ration (23% crude protein, gross energy content 17 $kJ.g^{-1}$) and water were available ad libitum. Environmental temperature was gradually lowered from about 35 C to about 25 C during the first three weeks after hatching. In this period the light regime gradually changed from 20 hr light and 4 hr dark per day (20 L : 4 D) to 20 L : 12 D. Body weights were recorded at 1, 37 and 50 days of age, but only the body weights at 50 days of age are reported here. The vaccination program included Marek's disease, infectious bronchitis, infectious bursal disease and Newcastle disease. The Marek vaccine was applied intramuscularly; all other vaccines were administered by eyedrop.

SRBC immunization and plasma collection. At 34 days of age chicks were injected intramuscularly with 1.0 ml of a 100% (H), 10% (M) or 1% (L) suspension (v./v.) of packed SRBC in phosphate buffered saline (PBS) respectively in

all genetic groups. We did not use PBS-injected controls because in our experience chicks injected with PBS do not show an increase in antibody titers (Van der Zijpp, 1978; unpublished results). Per experimental group 28 to 33 chicks were immunized. The SRBC were obtained in a heparin solution from 12 Texel sheep and washed three times in PBS (phosphate buffered saline). The chicks were bled from the wing vein on the day of injection and 3, 7 and 14 days later. Before drawing blood the syringe was washed with a heparin solution to prevent clotting. After centrifugation the plasma was harvested and stored at -20°C.

Hemagglutinin assay. Details of the hemagglutinin test are given by Van der Zijpp and Leenstra (1980). Titers were expressed as the log 2 of the reciprocal of the highest dilution giving complete agglutination for total and 2-mercapto-ethanol (2-ME) resistant antibody titers.

PHA skin response. Bacto PHA-P (Difco Laboratories, Detroit, Mich.) was injected intradermally in chicks that had received the 100 per cent suspension of SRBC. The cockerels, 49 days old, were injected in the wingweb of the non-wingbanded wing with .125 ml PBS containing 1250 µ g PHA. In the control wing .125 ml PBS was injected. The thickness of the wingweb was measured by a micrometer with 100 g pressure at 0 and 24 hr. after injection. The wingweb swelling (WS) was calculated as the difference between the thickness of the wing after injection (24 hr.) and prior to injection (0 hr.). The wingweb index (WI) was calculated as the difference between WS of the PHA injected and the control wing.

Design and statistical analysis. The experiment was designed to analyse antibody titers with the following model:

$$Y_{ijk} = \mu + a_i + b_j + ab_{ij} + \beta_1 x_{ijk} + e_{ijk}$$

Y_{ijk} represented the value of the kth animal, μ = population mean, a_i = effect of ith dose (i = 1,3), b_j = effect of jth genetic group (j = 1,3), ab_{ij} = interaction between dose and genetic group, $\beta_1 x_{ijk}$ = linear regression of body weight at 37 days, e_{ijk} = remainder. The regression approach was always used. The interaction effect and the covariable body weight were not significant and therefore excluded from the model for final results regarding total antibody titers. The linear regression on titer at day 0 has been included in a model with genetic group and dose effects. Also an analysis of variance for total antibody titers was carried out separately for each genetic group. The model included effect of dose and covariable body weight at 37 days of age.

After it became obvious that the genetic groups differed considerably

in body weight, we decided to account for this effect by statistical analysis. The coherence between effect of genetic origin and the covariable body weight was shown by first fitting β_1, adjusted for μ and ignoring effects a_i and b_j and then fitting a_i and b_j, adjusted for each other, μ and β_1. Secondly effects a_i and b_j were fitted, adjusted for each other, μ and ignoring β_1, followed by fitting β_1, adjusted for μ, a_i and b_j. This procedure of describing reductions (R) in sums of squares has been given by Searle (1971).

The WS and WI data were processed in a similar way, but the dose effect was eliminated from the model and covariable body weight at 50 days of age was substituted. The relationship between total antibody titers and WI is expressed as the Pearson product-moment correlation.

Body weights at 50 days of age were analysed for effects of dose, genetic origin and their interaction.

The five per cent level of probability or lower ($P < .01$ and $P < .005$) were accepted as indicating a significant effect for the variable tested.

RESULTS

Body weight. The body weights at 50 days of age for the three groups of chicks of different genetic origin and for the three concentrations of SRBC are reported in Table 1.

TABLE 1. AVERAGE BODY WEIGHTS (G) OF COCKERELS OF DIFFERENT GENETIC ORIGIN INJECTED WITH THREE DIFFERENT CONCENTRATIONS OF SRBC

Genetic origin	Concentrations of SRBC 1%	10%	100%
Warren	785.7	799.6	781.9
WPR	1795.5	1812.8	1816.3
WL	667.9	674.4	658.8

The differences in body weight among genetic stocks were highly significant ($P < .005$), but there were not significant differences due to different con-

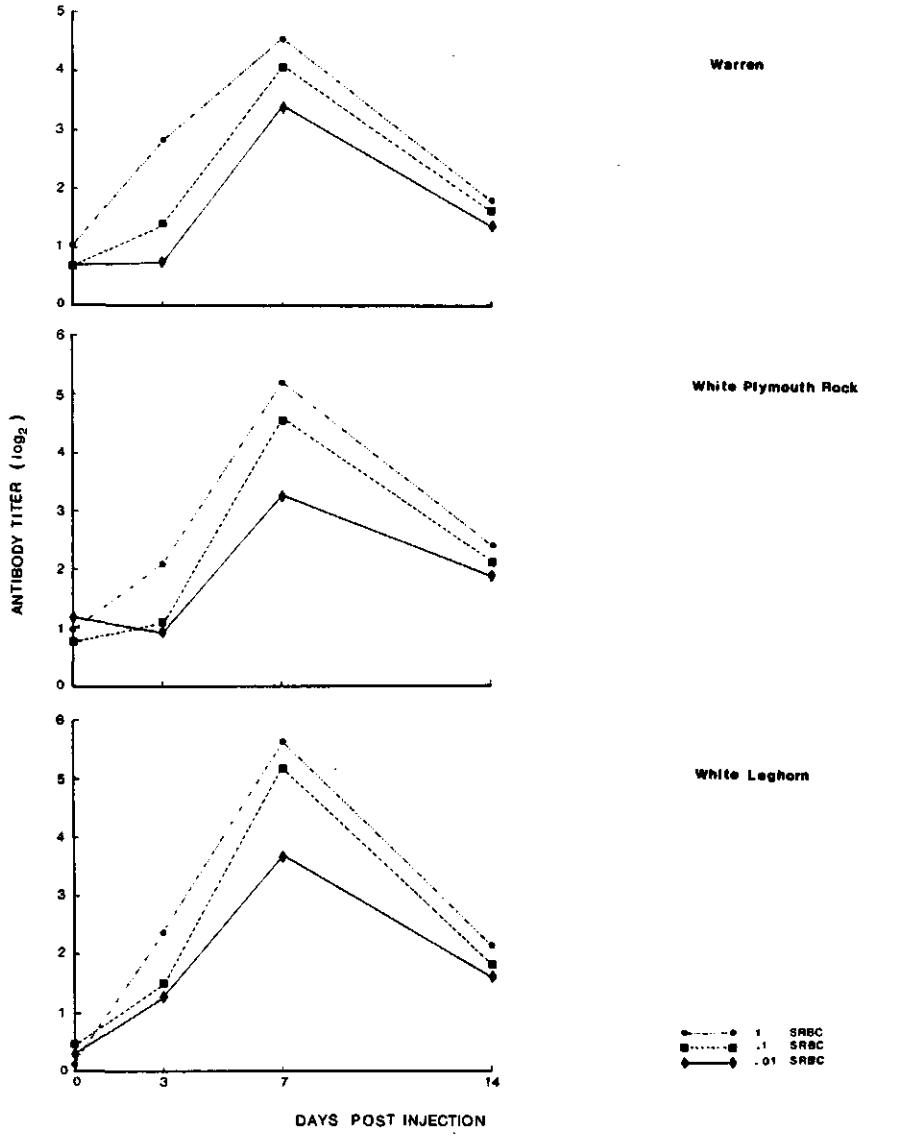

FIG. 1. THE TOTAL ANTIBODY RESPONSE AT THREE DOSAGE LEVELS OF WL, WARREN AND WPR STOCKS.

centrations of SRBC. No significant interaction between source of cockerels and dose of SRBC injected was found.

SRBC hemagglutinin titers. Total and 2-ME resistant antibody titers are presented for each genetic group in Figures 1 and 2. The 2-ME resistant antibodies appeared at day 3 postinjection in the M and H dose groups of the WPR stock; on day 7 and 14 they were present in all dose groups and appear to be dose dependent (Fig. 2).

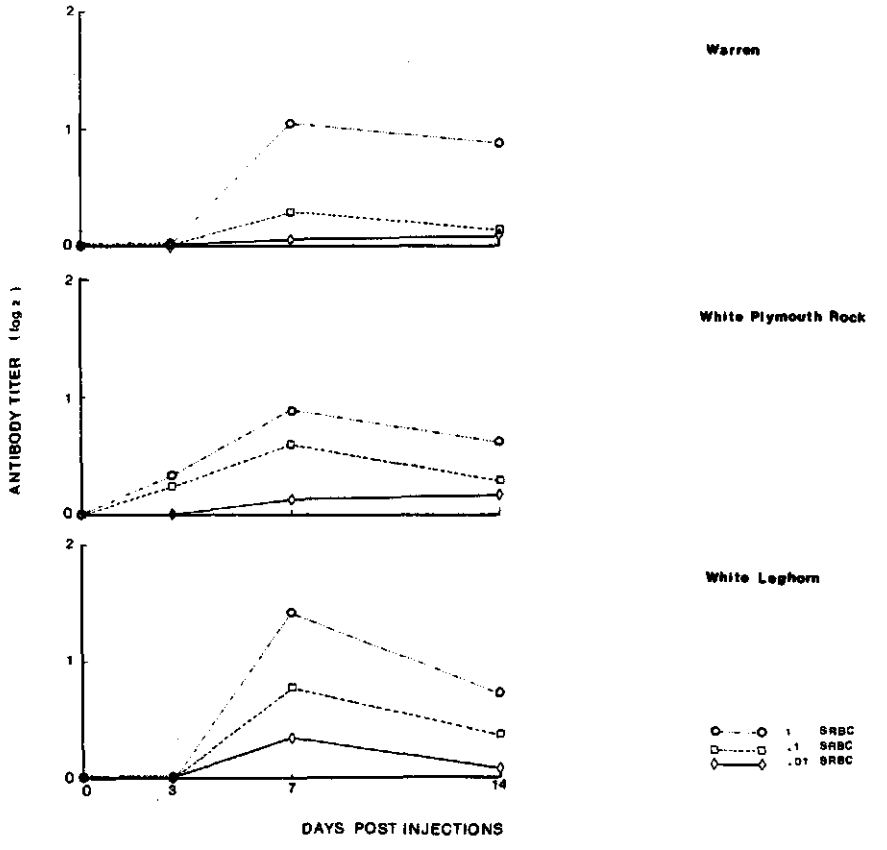

FIG. 2. THE 2-ME RESISTANT ANTIBODY RESPONSE AT THREE DOSAGE LEVELS OF WL, WARREN AND WPR STOCKS.

TABLE 2. ADJUSTED MEAN TOTAL ANTIBODY TITERS OF THREE GENETIC STOCKS, INJECTED WITH THREE DIFFERENT CONCENTRATIONS OF SRBC

Day post-injection	Genetic origin			Concentration of SRBC		
	Warren	WPR	WL	100%	10%	1%
0	.79	.96	.29			
3	1.54	1.29	1.74	2.25	1.32	1.01
7	4.01	4.34	4.83	5.10	4.65	3.47
14	1.57	2.13	1.85	2.12	1.86	1.61

TABLE 3. ANALYSES OF VARIANCE FOR TOTAL ANTIBODY TITERS BY SEQUENTIAL FITTING (MEAN SQUARES)

Source of variation	Reduction	Days postinjection 0	3	7	14
Genetic origin	$R(a_i/\mu,b_j,\beta_1)$	5.05^{***}	.77	13.21^{***}	2.68
	$R(a_i/\mu,b_j)$	11.62^{***}	4.03^{**}	13.81^{***}	6.35^{***}
Dose	$R(b_j/\mu,a_i,\beta_1)$		34.47^{***}	57.45^{***}	5.26^{***}
	$R(b_j/\mu,a_i)$		34.53^{***}	57.33	5.18^{***}
Regression of body weight	$R(\beta_1/\mu)$	14.05^{***}	7.54^{***}	1.61	7.61^{***}
	$R(\beta_1/\mu,a_i,b_j)$	.92	.05	.24	.51
Remainder		.54	.80	1.73	.94

* $P < .05$
** $P < .01$
*** $P < .005$

Significant differences ($P < .005$) between genetic stocks were found on day 0, 3, 7 and 14 postinjection for total antibody titers. All stocks showed a maximum total titer on day 7 postinjection (Fig. 1). The shape of the antibody response curve is unique for each genetic group, because the ranking of the stocks varied with day postinjection (Table 2) and differences in total antibody titer between different test days were always significant ($P < .005$; except $P < .05$ for the increase in titer from day 3 to 7). Genetic origin by day-postinjection interactions can therefore be deduced. The regression of total antibody titer at day of injection is significant only ($P < .05$) for the total titer at day 7 postinjection.

For total antibody titers in Table 3 the relationship between live weight and strain effect is illustrated by sequential fitting. The mean square of dose is expressed virtually as a constant, but the mean square of genetic origin depended on the fitting procedure except on day 7 postinjection. When the analysis of variance was carried out within each genetic group the covariable body weight at 37 days was never significant. An analysis restricted to the Warren and WL stocks once produced a significant ($P < .05$) contribution of the regression of body weight ($R\ (b_1/\mu)$): for total antibody titer at day 7.

The dose of SRBC affected the total antibody titers significantly ($P < .005$) on day 3, 7 and 14 postinjection. In comparison with the effect of genetic stock the dosage effect on day 3 and 7 was very large; on day 14 their contribution was of more equal size (Tables 2, 3).

Dose - genetic origin interactions were never significant.

PHA skin response. Before injection of PHA the thickness of the wingweb was not significantly different between left and right wing. In Table 4 the average wingweb thickness before injection is therefore presented. Effects of genetic origin on wingweb thickness before injection were highly significant ($P < .005$).

The WS results are also presented in Table 4. The slight swelling in the control wing was not significant except in the Warren stock ($P < .05$). The correlation (-.47 to -.64) between the WS and the thickness of the wingweb before injection was significant ($P < .05$) in the control wing, but not in the PHA injected wing.

The effects of genetic origin were not significant for the WS in the control wing, but were highly significant ($P < .005$) for the WS of the PHA injected wing and also for the WI.

For the WI the relationship between body weight at 50 days and effect of

genetic stock is given in Table 5. Within genetic groups the regression of WI on body weight at 50 days was never significant.

TABLE 4. WINGWEB SWELLING DUE TO PHA INJECTION IN THREE GENETIC STOCKS: AVERAGES AND STANDARD DEVIATIONS

Genetic origin	N birds	Wingweb thickness before injection	Wingweb swelling in mm	
			Control wing	PHA injected wing
Warren	24	1.46	.16 ± .34	3.16 ± .59
WPR	27	2.23	.08 ± .63	3.52 ± .85
WL	22	1.48	.09 ± .38	2.44 ± .81

TABLE 5. ANALYSIS OF VARIANCE FOR THE SWELLING INDEX BY SEQUENTIAL FITTING (MEAN SQUARES)

Source of variation	Reduction	Mean squares
Genetic origin	$R(a_i/\mu)$	6.38^{***}
	$R(a_i/\mu,\beta_1)$	1.56
Regression on body weight	$R(\beta_1/\mu)$	10.38^{***}
	$R(\beta_1/\mu,a_i)$	.73
Remainder		.76

* $P < .05$
** $P < .01$
*** $P < .005$

Relationship between total SRBC antibody titers and PHA skin response. No significant correlations between WI and total antibody titers on day 3, 7 and 14 postinjection were found within groups of different genetic origin, nor in the overall material. These correlations were significant ($P < .05$) only in the WPR stock ($r = -.41$) and the WL stock ($r = .46$) on the day of injection. The

general lack of relationship is also shown by comparing the ranking of genetic groups for WI and total antibody titer (Tables 2 and 4).

DISCUSSION

In this study the range of doses (.01 to 1 ml packed SRBC) was chosen so that an immune response might be expected. In a previous study it was shown that a very low dose (.0005 ml) does not invoke a response and a very high dose (6 ml) does produce the maximal attainable response (Van der Zijpp, 1978). At all stages of the immune reaction the antibody titers reflected the antigen dose level. Thus manipulation with concentration of SRBC can easily be applied to obtain a required antibody level.

Differences between genetic stocks were detected at all dose levels tested in our study, but there was no dose - genetic origin interaction. This is in contrast with the results of Gross (1979), whose data indicate the presence of dose - strain interactions, but intravenous administration of SRBC may have caused the difference with our results.

Separation of stocks was possible at all stages of the immune reaction to SRBC, but the ranking of the stocks was different for each test day in this experiment. Balcarova et al. (1973) indicated strain differences depending on titer measurements on day 5 and 7 postinjection. Van der Zijpp (1979) concluded that the ranking order of nine strains was fairly consistent from day 10 post-injection onwards, but Siegel and Gross (1980) demonstrated genetic variation in persistence of titers. Therefore the objective of separation of genetic stocks has to be defined before a choice of test days postinjection can be made.

Differences between genetic groups were already existent on the day of immunization. The higher level of natural antibodies in the WPR may have been influenced by their genetic capacity for antibody production and by their feed intake, because of SRBC-like antigens present on food particles. Neither explanation is likely, because the response to SRBC did not show secondary characteristics nor did the antibody response of the WPR excell.

In this material differences among cockerels of different genetic origins could partially be explained by body weight. For total antibody titers the WPR strain was responsible for this relationship between effect of genetic stock and body weight, because of their extreme weights in comparison with the Warren and WL. Generally correction for body weight is not significant within strains (Van der Zijpp and Leenstra, 1980) indicating independence of the antibody re-

sponse and body weight.

Cell-mediated immune responses can be detected by PHA skin responses (Goto et al., 1978) in the wattles. The wingweb test in contrast with wattle testing can be applied at all ages, but Klesius et al. (1977) observed also differences in duration of the cell-mediated immune response to diphteria toxoid. Duration and age dependence of the PHA test in the wingweb deserve further study.

The negative relation between thickness of wingweb and WS in the control wing may have been due to a more even distribution of PBS solution in the thicker wings. Goto et al. (1978) observed a swelling due to PBS injection, but the wattles returned to normal 6 hr. after injection. The same process of distribution may have occurred in the PHA injected wingweb, but here the accumulation of cells attracted by PHA probably counteracted the distribution process.

Differences between cockerels of different genetic origins could be detected for the cell-mediated response as measured by WS in the PHA injected wing and WI. However the effect of genetic origin was confounded with body weight.

Selection for antibody production in mice (Biozzi et al., 1979) does not affect the cell mediated immune response. Although we also found a lack of correlation between WI and antibody titer, only an analysis of genetic correlations within breeds or strains can support Biozzi's conclusions. Moreover our data arose from tests on the same chicks and experimentation with the same tests on different chicks of equal genetic background is needed to avoid interaction between immune responses and to substantiate this result.

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CHAPTER 5

THE IMMUNE RESPONSE OF THE CHICK FOLLOWING VIRAL VACCINATIONS AND IMMUNIZATION WITH SHEEP RED BLOOD CELLS

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ABSTRACT

The effects of viral vaccinations and immunization with sheep red blood cells (SRBC) on the humoral response of pullets were investigated. Pullets were vaccinated with Marek's disease virus, Newcastle disease virus (NDV), infectious bronchitis virus (IBV), and infectious bursal disease virus at appropriate ages used in commercial practice. At seven weeks, the pullets were intramuscularly immunized with SRBC. NDV and IBV antibodies were detected by hemagglutination-inhibition tests. Hemagglutination (HA) titers were established against SRBC.

IBV antibody titers were not affected by vaccination or by immunization with SRBC. NDV antibody titers were significantly increased by vaccination and by immunization with SRBC. The SRBC agglutinin response was also positively affected by vaccination. The HA titer increase consisted of a rise in 2-mercapto-ethanol (2-ME)-sensitive antibodies and a fall in 2-ME-resistant antibodies.

INTRODUCTION

When experiments are carried out to assess immune reaction, the possibility of infections interfering with the immune response to the antigen to be investigated must be considered. Specific-pathogen-free animals, pathogen-free housing, or vaccination programs could therefore be included in the experimental design. In long-term genetic experiments selecting for parameters of the immune response, vaccinations will often be used (19) to ensure viability of breeding stock when pathogen-free housing is not available for the large numbers of animals required.

In the present study, the effects of a vaccination program used in commercial practice on the immune response of the chicken against sheep red blood cells (SRBC) and vice-versa were investigated. The term vaccination will be used for the total vaccination program, and immunization will refer to injection with SRBC.

MATERIALS AND METHODS

Experimental animals. Hybrid pullets (Warren SSL) were used. The chicks were wingbanded, and at eight days of age they were debeaked. From hatch, the chicks were housed in slatted floorpens in four rooms at two locations. Unvaccinated chicks were at one location and vaccinated were at the other. The rooms were fumigated and disinfected before the experiment started. Sanitary precautions, temperature, light, and nutrition regimen were according to commercial practice.

Design. Both groups were divided into two groups each, which were either immunized with SRBC or left unimmunized. The groups were designated as follows: group I, 55 unvaccinated, unimmunized chicks (controls); group II, 55 unvaccinated, immunized chicks; group III, 230 vaccinated, unimmunized chicks; group IV, 230 vaccinated, immunized chicks.

Vaccination program. On the day of hatch, chicks were vaccinated intramuscularly (IM) with a minimum of 1×10^3 plaque-forming units per bird of Marek's disease vaccine (virus strain CVI/988/Rispens-Delvax), followed by eye-drop vaccination with 5×10^3 EID_{50} per bird of infectious bronchitis virus (IBV) (vaccine H120-Delvax). Newcastle disease virus (NDV) vaccinations were administered by eye-drop with 10^7 EID_{50} Hitchner B1 vaccine (Delvax) per bird at seven days of age, followed by 10^7 EID_{50} La Sota vaccine (Delvax) per bird at 5 weeks of age. A minimum dose of 50 EID_{50} of infectious bursal disease virus (IBDV) vaccine (strain LZD228-Delvax) per bird was given via the drinking water at 3 weeks of age.

Hemagglutination-inhibition (HI) test. The HI tests on NDV and IBV antibodies were expressed in $\log_2$ HI titers (1, 4). Twenty randomly chosen day-old chicks were sacrificed to determine the level of maternal antibody to NDV and IBV. At four and nine weeks of age, 10 plasma samples of groups I and II each, 25 samples of group III, and 120 samples of group IV were tested. The same chicks were sampled each time.

Agar-gel precipitation test (AGP). The AGP test (7) to IBDV was carried out at nine weeks of age. Twenty-four plasma samples of each group were tested.

Immunization and plasma preparation. At seven weeks of age, the chicks of groups II and IV were injected IM with 1 ml of packed SRBC (about 26×10^9 cells). The SRBC were obtained in a heparin solution from six Texel sheep and washed three times in physiological saline (0.9% NaCl). The dose was injected in two equal portions, one into each thigh.

The chicks were bled from the wing vein using heparin-rinsed syringes. The blood samples were stored for one night at 4 C, and then the plasma was harvested and stored at - 20 C.

Hemagglutination (HA) assay. Total and 2-mercaptoethanol (2-ME)-resistant hemagglutinin antibody titers (5, 22) were determined. The reduction of the total titer due to 2-ME treatment was called 2-ME-sensitive antibody.

Hemagglutinin assays were carried out for all groups at seven and nine weeks of age. Plasma samples were taken at eight weeks of age from all chicks of groups II and IV. Plasma from all plaque-test animals was tested also.

Direct hemolytic plaque test. This assay was carried out to obtain information on the cellular response against SRBC to support the serological data (HA). On the day of immunization with SRBC and 4, 7, 11, and 15 days later, a direct hemolytic plaque test (3) was carried out. Body, spleen, and bursa weights were also recorded. The distribution of chicks among groups is presented in Table 1.

TABLE 1. DIRECT HEMOLYTIC PLAQUE TEST: DISTRIBUTION OF CHICKS (N) AMONG GROUPS

	Group			
Day	I	II	III	IV
0	5	-	5	-
4	-	5	-	16
7	5	5	-	16
11	5	5	-	16
15	2	8	2	8

Groups I and III served as controls for groups II and IV, respectively, on day zero. Furthermore, group I served as a control for group III.

Statistical analysis. Spleen and bursa weights were corrected by applying the formula organ weight:body weight x 1,000 (9). The number of plaque-forming cells resulting from the direct plaque assay was transformed by: $\log_e$ (no. of plaques + 1). All parameters were checked for skewness and kurtosis. The

significance of differences between means was calculated by Student's t-test. The analysis of variance was based on a one-way classification (vaccination or immunization effect) including, if significant, covariables body weight and titer at day of immunization.

RESULTS

HI titers to NDV. HI titers to NDV (Fig. 1) were significantly higher in vaccinated chicks than in unvaccinated chicks at four ($P < 0.05$) and nine ($P < 0.001$) weeks of age. At nine weeks, SRBC immunization had not affected the HI titers of unvaccinated chicks, but it significantly increased the HI titers of the vaccinated animals ($P < 0.01$).

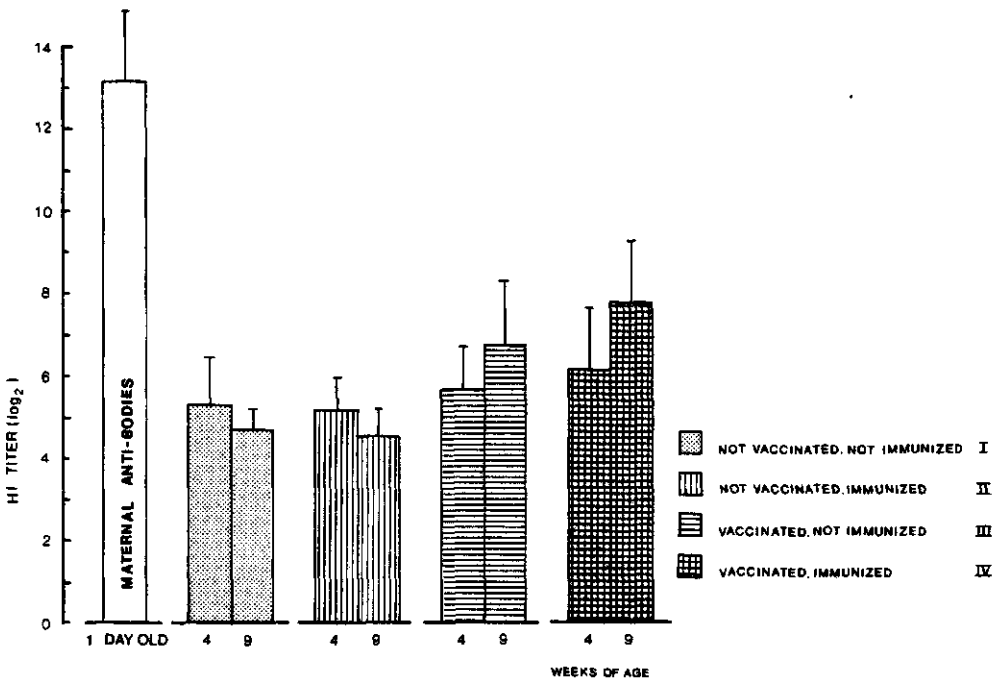

FIG. 1. THE EFFECT OF VACCINATION AND IMMUNIZATION ON HI TITERS TO NDV: MEANS AND STANDARD DEVIATIONS. IMMUNIZATION TOOK PLACE AT 49 DAYS OF AGE; TITERS DETERMINED AT 4 AND 9 WEEKS OF AGE.

HI titers to IBV. When the chicks were four weeks of age, HI titers to IBV were not influenced by vaccination. At nine weeks of age, vaccination had a significant effect ($P < 0.05$) on only the SRBC-immunized group. No effects due to SRBC immunization could be established.

AGP test. The AGP test for groups I and II was negative. All animals in groups III and IV except one were positive.

HA titers to SRBC. The total HA titers to SRBC (Fig. 2) on day of immunization, which were almost completely 2-ME-sensitive, were significantly enhanced ($P < 0.001$) by vaccination.

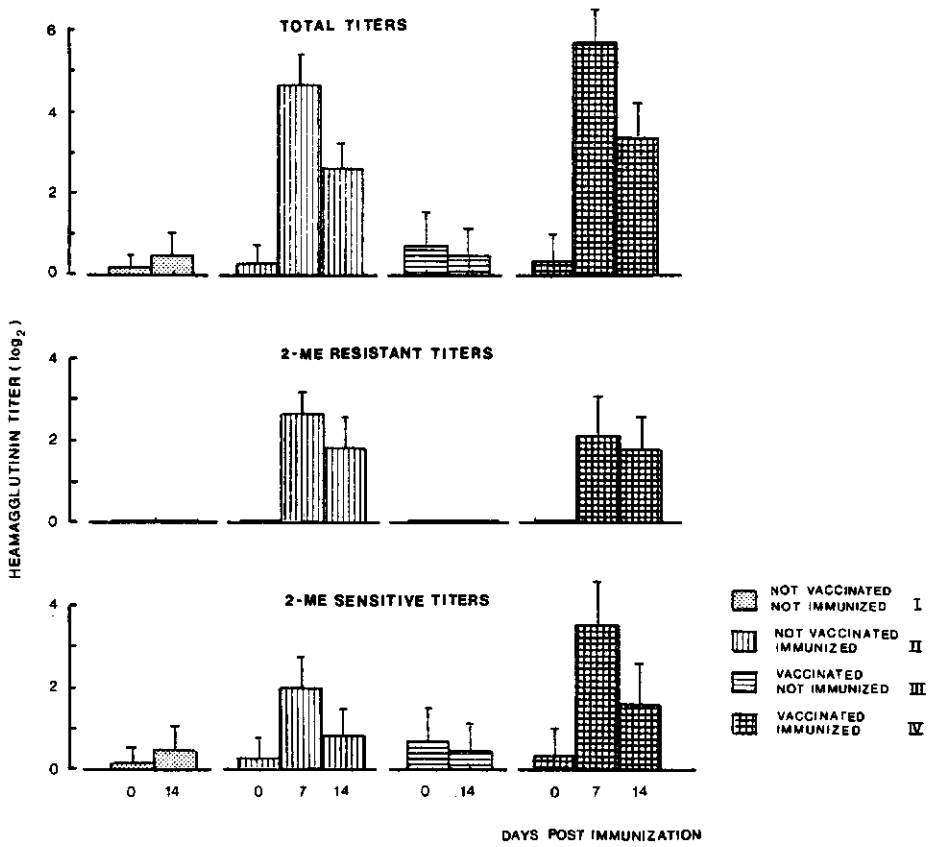

FIG. 2. THE EFFECT OF VACCINATION AND IMMUNIZATION ON HA TITERS TO SRBC: MEANS AND STANDARD DEVIATIONS.

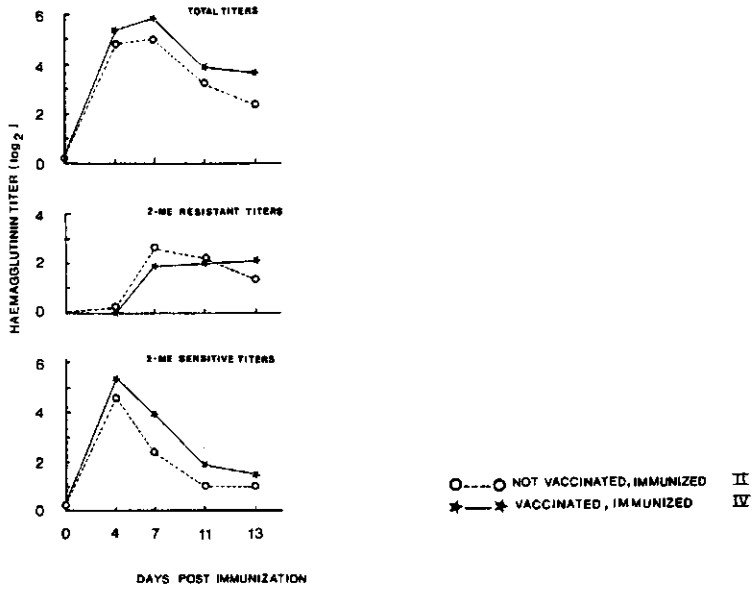

FIG. 3. THE EFFECT OF VACCINATION ON HA TITERS TO SRBC OF CHICKS USED FOR THE DIRECT HEMOLYTIC PLAQUE-TEST.

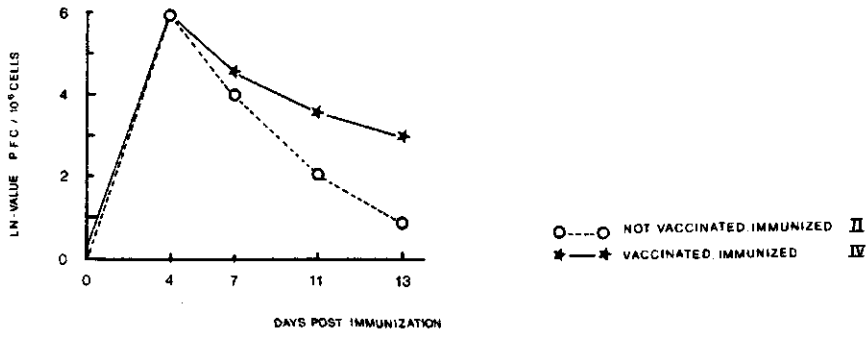

FIG. 4. THE EFFECT OF VACCINATION ON THE NUMBER OF DIRECT PLAQUEFORMING CELLS (PFC).

On day 14 post immunization this effect was absent (group I versus group III). The effect of vaccination on immunized groups was highly significant ($P < 0.001$) for all antibody titers on days 7 and 14, except for the 2-ME-resistant antibody titer on day 14. Vaccination influenced the relationship between 2-ME-resistant and -sensitive antibodies, apparently favoring the expression of 2-ME-sensitive antibodies.

The above findings were substantiated by the more detailed data from the limited number of plaque-test animals (Fig. 3). Significant increases due to vaccination were found for total HA titers on day 7 ($P < 0.01$) and day 15 ($P < 0.05$) for 2-ME-resistant titers on day 4 ($P < 0.01$) and day 15 ($P < 0.05$), and for 2-ME-sensitive titers on day 7 ($P < 0.01$).

Direct hemolytic plaque test. Fig. 4 illustrates the plaque test results of groups II and IV; group I produced negative results. Vaccinated chicks had significantly higher values on days 11 and 15 ($P < 0.05$ and $P < 0.01$, respectively).

Spleen and bursa weights. No significant effects due to vaccination or immunization could be detected for spleen:body weight ratios. Bursa:body weight ratios (Fig. 5) were not affected by immunization (group I versus II). The vaccinated immunized group (IV) showed a highly significant decrease compared with the unvaccinated immunized group (II) on days 4 ($P < 0.01$), 7 ($P < 0.001$), 11 ($P < 0.01$), and 15 ($P < 0.05$).

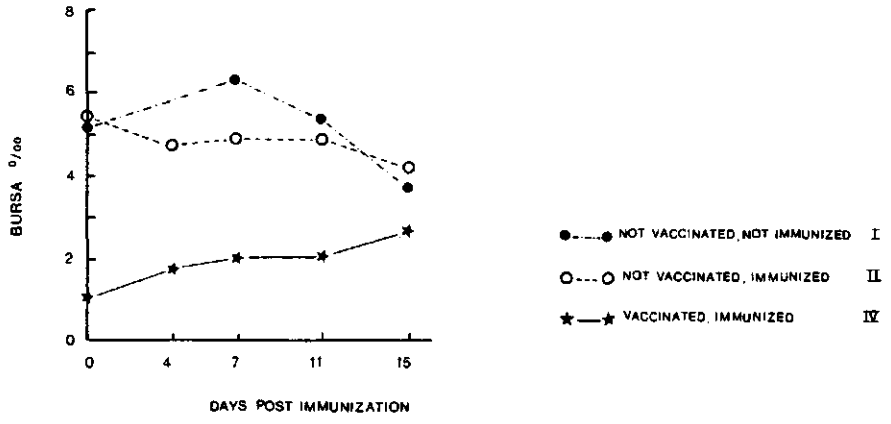

FIG. 5. THE EFFECT OF VACCINATION AND IMMUNIZATION ON THE BURSA:BODY WEIGHT RATIO.

DISCUSSION

SRBC HA titer values were in agreement with those obtained with White Leghorns (19, 22). The HI titer to IBV did not increase after vaccination with the strongly embryo-adapted H120 vaccine virus. This is in accordance with observations following vaccination of 3-week-old SPF birds via the ocular route with a similar vaccine (11). The significant but small rises of HI titers to NDV after vaccination may be explained by the high level of maternal HI titers to NDV. The AGP test indicated that the unvaccinated chicks had not been infected with IBDV.

The antibody response to SRBC in our study was significantly increased in vaccinated birds. Similar results of viral vaccination have been reported earlier. Chicken-egg-grown influenza virus vaccine or chicken-egg-grown purified influenza virus produced powerful isoagglutinins and isohemolysins in humans (20). Inactivated IBV vaccine, injected subcutaneously, stimulated agglutinin production against horse red blood cells and SRBC (18). The enhanced formation by vaccination of different antibody specificities, including those against SRBC, may be due to the injection of chick embryo tissue or the virus envelope which has acquired chicken cell specificities. All vaccines, except Marek's disease, used in the present study were grown on chicken embryos and applied by eye drop or via the drinking water, but the possible priming function has resulted neither in largely increased levels of natural antibodies to SRBC nor in a typical secondary response.

The effects of viral infections on immune functions are very variable (23). Enhancement of antibody formation to several nonviral antigens in virus-infected mice has been reported (12, 13, 16). The enhancement depended on the nature of antigen injected, the interval between and the sequence of infection and immunization, and the immunizing dose. When serum immunoglobulin levels are elevated, often immunoglobulin M (IgM) levels are increased. The immunological relationship between virus vaccinations and SRBC immunization in our experiment may be comparable to the previously mentioned systems in mice, despite distinct characteristics of the vaccine viruses used.

SRBC and most viruses belong to the group of T-dependent antigens (2, 17). The influence of the preceding immune responses may be due to nonspecific helper and suppressor T-cell activity (6). The balance of cooperation and suppression depends on the interval between responses, as was found for two noncross-reacting antigens. Cooperation occurs after short periods, depression after

longer periods (6). In the present study, the last vaccination took place at five weeks of age, two weeks before immunization with SRBC. Because vaccination was carried out with live, attenuated virus vaccine, the response may be delayed owing to virus replication necessary to obtain an immunizing dose level. Therefore, cooperative effects may have occurred at the time of immunization with SRBC.

The increase of NDV antibodies in immunized chicks at nine weeks of age can be explained by the same hypothesis, assuming that continuation of formation of antibody to NDV has been positively influenced by the positive helper:suppressor T-cell ratio as a result of SRBC challenge. Nonspecific polyclonal hyperimmunoglobulinemia may also contribute to increased HI titers to NDV (14).

Vaccination caused a remarkable decrease in the bursa:body weight ratio. IBDV vaccines have been found to be responsible for damage to the bursa to various degrees (21, 24). Vaccines leading to severe damage can also negatively affect the response to following vaccinations like NDV (8, 15). A significantly reduced primary response to SRBC due to infection with IBDV and a simultaneous injection with SRBC at 28 days of age has been shown (10). The infected chicks had normal levels of serum IgM and IgG and lower levels of IgA. But in the present study, the response to SRBC immunization was elevated in the vaccinated group.

Interference of concurrent and successive immune responses appears to have determined our results. The assessment of immune reactions per se, therefore, should be accompanied by a careful description of environmental aspects like vaccination procedures, housing, and disease probability.

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GENERAL DISCUSSION

Resistance to infectious diseases in chickens is a highly desirable characteristic to maintain modern poultry production systems. In addition to traditional procedures like medication, vaccination and eradication to combat disease, genetic improvement of resistance to infectious disease may become important because the forementioned procedures are not always reliable and available and genetic resistance to disease can have a complimentary role in improving disease resistance (see for Marek's disease Spencer et al., 1972). The breeding goal for poultry can be described in terms of economically important production traits with reproduction and disease resistance as so-called secondary traits. However to define selection criteria for disease resistance is difficult. The large variation in disease agents threatening our poultry, their mutagenic ability and the complexity in response, specific and non-specific, to pathogens almost inhibit a rational approach to define selection criteria for disease resistance. A first possibility could be selection for resistance to specific diseases. For a variety of reasons this approach may be less desirable:

1. In poultry genetic resistance to a large number of pathogens is necessary. This means that a testing programme has to be set up, either deliberately exposing the breeding population itself or separate populations of sibs to keep the breeding population relatively disease free.
2. Information regarding resistance mechanisms is scarce for most diseases. Sometimes mortality caused by a specific disease can be measured (for Marek's disease and lymphoid leukosis, for example); sometimes disease symptoms may be used. This leads to recording of all-or-none traits, and possibly, low repeatibility, both resulting in low heritabilities.
3. Age and sex can have a significant effect on the incidence of some diseases. Lengthening of the generation interval and adaptation of the testing programme are the consequences.
4. Selection for specific disease resistance may lead to significant results (see Chapter 1), but does not necessarily imply positive effects on resistance to other disease agents.
5. Continuous medication may augment the survival of some mutants. Selection programmes for resistance to specific diseases cannot be succesful, when mutation of the disease agent takes place.

Because of the above considerations and the development of immunological

knowledge a second possibility, improvement of general disease resistance, is gaining attention. Gavora and Spencer (1978) have defined general disease resistance as "the ability to resist any alteration of the body by external causes (microorganisms and/or stress) which interrupts or disturbs proper performance". An optimal combination of defence mechanisms should be the basis of general disease resistance, enabling the animal to resist pathogens and stress. Selection criteria for general disease resistance, as a breeding goal, need to be developed. Almlid (1981) considered these traits as markers, which give information about disease resistance. These marker traits are useful on the condition, that they show: 1) manifestation at an early age, 2) high repeatibility of the test method, 3) high heritability, 4) high genetic correlation with resistance to specific diseases or groups of diseases. The research of Biozzi et al. (1979) to select for the antibody response to natural polydeterminant immunogens is a major effort to produce selection criteria for general disease resistance. Their findings indicate, that at least three major components of the defence system: macrophages, T and B lymphocytes, should be included to define selection criteria for general disease resistance in mice. Moreover by choosing polydeterminant antigens the response is essentially non-specific. In our own research work we have taken a similar approach by measuring the antibody response to sheep red blood cells (SRBC) in the chicken. Ultimately we want to evaluate this trait in terms of 1) value for resistance to specific diseases, 2) correlations, phenotypic and genetic, with other defence parameters, 3) relationship with economically important production traits. On the basis of our knowledge of the mouse and of the complexity of the defence system of the chicken we may expect that in the chicken a resistance index for general disease resistance (Pavel et al., 1981) has to be developed. The incorporation of disease resistance criteria in an index of overall performance (production, reproduction, disease resistance) will depend upon genetic and economic evaluation of all desired components in the resistance index.

In the studies presented here the antibody response to SRBC has been genetically evaluated under variable circumstances. Direct comparisons between experiments are not possible because of differences in stock, dose of SRBC, age at injection, vaccination schedule, and interval between injection and titer determination. However, the experiment discussed in chapter 4 yielded evidence for significant differences in total antibody response on all days post injection (0, 3, 7 and 14) between cockerels of three different genetic

origins. These significant differences were present at all dose levels of SRBC antigen.

Repeatibility represents the upper limit of the heritability. For total agglutinin titre to SRBC Van der Zijpp and Leenstra (1980) reported values above .94. The same level was found by Van der Zijpp and Zandstra (unpublished results) for total antibody titre and 2-ME resistant antibody titre. Evidence for lower repeatibility (.74) of 2-ME resistant antibody titre, caused by 2-ME solution of varying age, was presented by Van der Zijpp and Leenstra (1980). Generally the repeatibility of antibody titres was high, indicating a high degree of standardisation of blood sampling and laboratory testing.

The heritability estimates for total antibody titres of White Leghorn (chapter 2), White Plymouth Rock (chapter 3) and ISA Warren (chapter 1) stock varied from sometimes non-significant to often intermediate (.20 to .30) levels for the primary response. These estimates offer a perspective for individual selection as has already been shown by Siegel and Gross (1980). In our own selection experiment the agglutinin titres for the high line were 6.23 and 5.78 for pullets and cockerels, respectively, and for the low line 5.37 and 5.01 in the first selection generation. The control line showed antibody titres at day 5 post injection of 5.97 and 5.62. The heritability estimates of the secondary response were quite low in comparison with their primary equivalents. Presumably this second response is affected even more by environmental conditions. The negative genetic correlations between antibody responses of primary and secondary injection should be regarded with caution, because almost no equivalent research on this subject has been carried out. If these negative correlations prove to be general, then the impact of genetic improvement of primary responses on vaccination may become a primary concern.

Many environmental factors have been responsible for the differences in antibody titres to SRBC, presented in chapters 1 through 5. Here follows a discussion of some major non-genetic influences.

Dose of SRBC. The dose of SRBC did vary between .01 and 1 ml packed SRBC in the experiments described in chapters 1 to 5. In chapter 4 a linear effect of dose on antibody response was found. In Figure 1 the effect of dose of SRBC on total and 2-ME resistant antibody titre is presented (Van der Zijpp and Zandstra, unpublished results). The Warren pullets were injected when 11 weeks old with .01, .1 and 1 ml packed SRBC, respectively. For the highest dose the peak total antibody titre is reached earlier than for the medium and low dose.

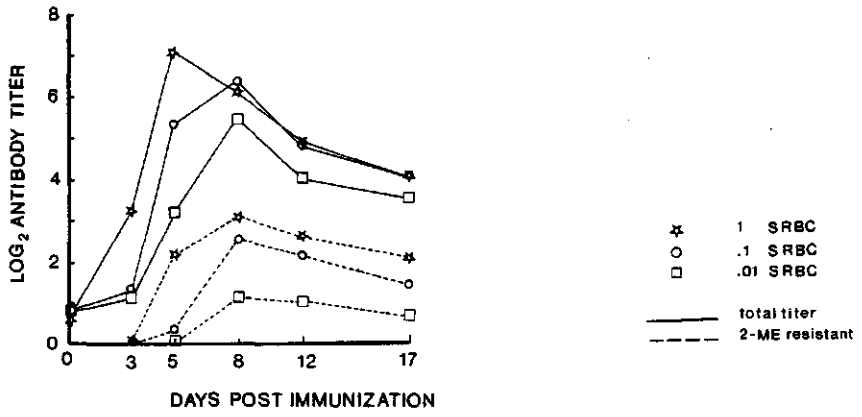

FIG. 1. THE EFFECT OF DOSE OF SRBC ON THE TOTAL AND 2-ME RESISTANT ANTIBODY TITRES.

Apparently a high dose encouraged early formation of 2-ME resistant antibodies as shown by the levels on day 5 in Figure 1. In the study, presented in chapter 4, this effect, if present, could not be shown because no titre measurements were carried out between 3 and 7 days post injection. Van der Zijpp (1978) found that a very low dose (.0005 ml) did not provoke a response and that at very high doses (3 and 6 ml) the limits of antibody production capacity have been reached. Gross (1979) concluded that separation of stocks for antibody titres is easier at the lowest doses of SRBC, injected intravenously. This may be an indication of genotype-dose interaction. The range of doses of SRBC used in these studies is such, that a linear response might be expected and that genotype-dose interactions were absent, at least for the three stocks tested (chapter 4).

It will be necessary to check the effects of genetic improvement at a particular dose level for responsiveness at other doses. Biozzi et al. (1979) have shown, that high responder mice mount an antibody response at lower doses of SRBC than the low responder mice. This may be an advantage to combat infectious agents.

Age. For the primary response the age of first injection varied between 34 and 40 days of life in the present studies. Due to many confounding factors a direct comparison of the immune response to SRBC at different ages is not

possible. Seto (1973) reported a rapid increase in capacity to produce antibodies to mouse erythrocytes in WL chicks from 3-4 days of age up to 3 weeks. The peak titres were measured on day 5 post injection, after intravenous immunization. McCorckle and Glick (1980) have shown that maximum haemagglutinin values of SRBC occurred between 3 and 6 months of age and declined thereafter. Van der Zijpp (1978) recorded a slightly positive, but significant effect of an increase in age on the antibody titres to SRBC. Medium heavy cockerels were immunized in a weekly sequence with 3 ml SRBC from 6 to 17 weeks of age. In Figure 2 total and 2-ME resistant antibody titres are presented for ISA Warren pullets from 1 to 17 weeks of age (Van der Zijpp and Duysings, in preparation).

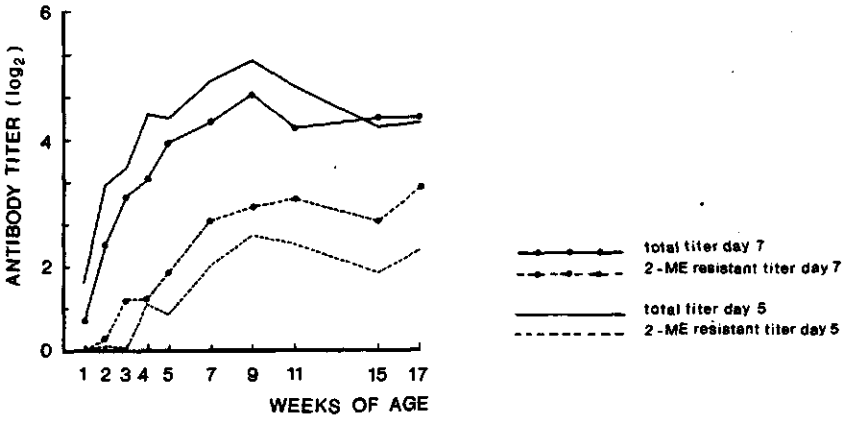

FIG. 2. THE EFFECT OF AGE ON THE TOTAL AND 2-ME RESISTANT ANTIBODY TITRES, DETERMINED 5 AND 7 DAYS POST INJECTION.

The antibody titres were determined on day 5 and 7 post intramuscular injection with SRBC. Maximum titres were reached at 9 weeks of age. The decline after 9 weeks may have been caused by an increasing ratio of body weight and dose of SRBC.

Chicks, 5 to 6 weeks old, have not yet reached their maximum capacity to produce agglutinin antibodies to SRBC. However, an early general immune response is a highly desirable trait to cope with juvenile diseases. Our selection experiment for antibody titre to SRBC, injected at 38 days of age, may reveal the consequences for immune competence at other ages.

Primary and secondary response. Sofar we have analysed secondary responses (chapters 1 and 3) in WPR and Warren stock. The dose regimen (.25 and .50 ml packed SRBC at primary and secondary immunization, respectively) was the same, but in the Warren the second injection was delayed till over 13 weeks of age compared to over 9 weeks in the WPR stock. Secondary responses, theoretically, can be characterised by a higher peak titre, an earlier peak post injection and increased IgG production in comparison with a primary response. In Figure 3 antibody titres of a secondary response of Warren pullets at 15 weeks of age are presented. The primary response of the same pullets is presented in Figure 1. The effect of dose of SRBC (.01, .1 and 1 ml SRBC) at secondary response was dependent on the primary dose level. Two-ME resistant antibodies, dependent also on dose of SRBC, were the main type of antibody produced in the secondary response. Another typical feature of the secondary response, higher peak, can be clearly manipulated by dose regimen. This result may be of value for vaccination procedures, where memory formation is a major aim to enable the animal to mount a quick and substantial response after challenge. If vaccination is applied to produce a high level of antibodies, then a high dose at primary immunization is required.

Generally, information retained from secondary responses should be accompanied by information regarding dose regimen at primary and secondary responses and probably, age.

Vaccination procedure. Except in FAPP (filtered air positive pressure) housing, rearing chicks is never possible without contact with disease agents. Protection can be provided by vaccinations; at the same time the immune system faces a repeated challenge. In chapter 5 the effects of a vaccination programme on the immune response to SRBC are discussed. In a follow-up with essentially the same vaccination programme, but in- or excluding the second NCD vaccination, we recorded no differences in response to SRBC (Van der Zijpp, unpublished results). The specific combination and sequence of vaccinations and possibly other uncontrollable antigens from the environment may thus affect the immune response to SRBC.

Interval between injection and titer determination. The most complete picture of the antibody response post injection is shown in chapter 3 and in Figures 1 and 3. Peak titres post primary injection appeared at 7 or 8 days post injection, but at high dose levels this peak moved to 5 days post injection. When 2-ME resistant antibody titres are produced at a primary response, their peak response appeared around 8 days post first injection. Peak total

titres for the secondary response were measured on day 5 post second injection.

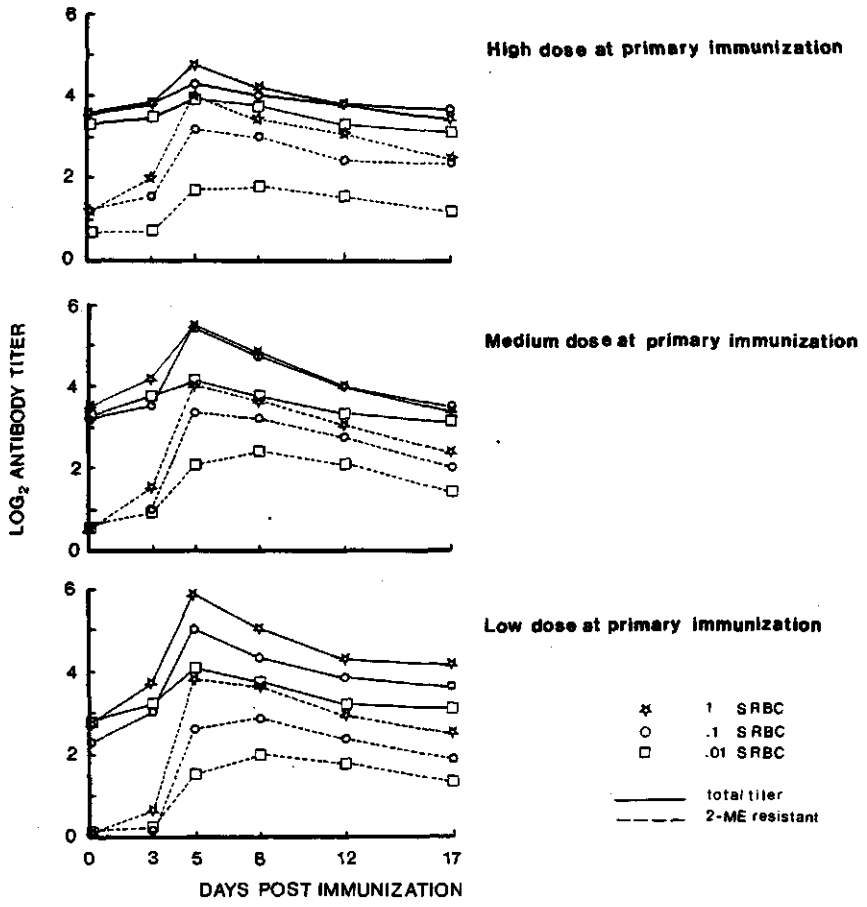

FIG. 3. THE EFFECT OF DOSE OF SRBC ON THE TOTAL AND 2-ME RESISTANT ANTIBODY TITRES POST SECOND INJECTION IN RELATION TO PRIMARY DOSE OF SRBC.

A lower dose at second injection did delay the secondary peak of 2-ME resistant antibodies (Fig. 3). Divergent selection for total antibody titres at 5 days post injection has been succesfully carried out by Siegel and Gross (1980). The high line chickens were less persistent, but they had significantly ($P \leq .05$) higher titres at 21 days post injection than the low line chickens. There was

no information on the interval between injection and peak antibody response.

Apart from the above mentioned external factors, others like sex (Van der Zijpp and Leenstra, 1980; Siegel and Gross, 1980), maternal antibodies (Van der Zijpp, 1978), environmental temperature (Subba Rao and Glick, 1977; Henken et al., in preparation), nutrition (Cheville, 1979), social interactions (Siegel and Latimer, 1975), and route of administration (Gross, 1979; Van der Zijpp and Geerse, unpublished results) can have an effect on the antibody response to SRBC. The selection criterion, the antibody response to SRBC, should therefore be explicitly defined with regard to the above terms.

Genetic improvement of disease resistance is an enormous challenge. Our approach by measuring the antibody response to SRBC may be right with regard to manifestation at an early age, high repeatibility and sufficiently high heritabilities for mass selection. However, the antibody response to SRBC is defined in the context of a series of external effects, which can or cannot be standardised. The value of this marker trait for the general capacity to produce antibodies will have to be proven. The behaviour of genetically different lines with regard to these external effects and to other antigens and pathogens will be decisive for the acceptance of the antibody response to SRBC as a valuable trait for general disease resistance.

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SUMMARY

Disease can be combated by medication, vaccination, hygienic measures, eradication and genetic resistance. Genetic resistance to infectious diseases is advantageous because of its permanent character in contrast with the aforementioned procedures. In the chicken genetic resistance to specific diseases like Marek's disease and lymphoid leukosis is well known. Despite this knowledge improvement of genetic resistance to specific diseases is not included extensively in breeding programmes yet. The need for infection of populations, the lack of knowledge of correlations with resistance to other diseases and production traits and the rare understanding of defence mechanisms are major drawbacks for application.

A different approach to genetic improvement of disease resistance is the composition of a series of defence traits, defining general resistance to disease. The major histocompatibility complex, the B-locus in the chicken, may be such a valuable marker especially for resistance to viral oncogenesis. The capacity to produce antibodies to a multideterminant antigen, like sheep red blood cells (SRBC) is another possible marker trait. Selective breeding for this trait in mice has shown, that the effects are non-specific. In this thesis genetic and environmental aspects of the agglutinin antibody response to SRBC in poultry are discussed.

The agglutinin antibody response to SRBC was polygenically determined. Heritability estimates for total antibody titre and 2-mercapto-ethanol (2-ME) resistant and sensitive antibody titres varied from non-significant to, usually, levels around .2 to .3 for the primary response in White Leghorn (WL), White Plymouth Rock (WPR) and ISA Warren populations. The heritabilities of a secondary response, measured in WPR and ISA Warren populations, were somewhat lower. The size of these heritability estimates offers a good perspective for mass selection as already shown in the first selection generation for high and low antibody production in our ISA Warren population. Moreover the repeatibility of this trait is very high, above .9.

Phenotypic correlations between primary and secondary total antibody titres were not significant in a WPR population. This result was explained by a positive relationship between primary 2-ME sensitive antibody titres and secondary 2-ME resistant antibody titres and a negative relationship between primary and secondary 2-ME sensitive antibody titres. Additive genetic correlations between total antibody titres of primary and secondary response were

quite negative. If these negative genetic correlations will be confirmed by other research workers, then the choice of the primary antibody response to SRBC as a selection criterion for general disease resistance becomes a major concern.

Differences between three stocks: WL, WPR and ISA Warren were found for antibody response to three doses of SRBC and for the cell-mediated response, the swelling of the wingweb post phytohaemagglutinin (PHA) injection. Genetic origin by dose of SRBC interactions were absent. However the response curve was different for each stock, indicating genetic origin by testday post injection interactions. Phenotypic correlations between total antibody titres to SRBC and PHA wingweb swelling were absent, overall and within stocks.

The antibody response to SRBC is influenced by many non-genetic factors. Important non-genetic effects were dose of SRBC, age of the chick, primary versus secondary response, vaccination programme, interval between SRBC injection and titre determination. Desirable traits of the defence system in combating infectious diseases are immune competence at an early age, responsiveness to low doses of infectious agents, a quick response post infection and development of memory. The selection criterion, therefore, has to be clearly defined and the effects of selection upon these desired characteristics will have to be evaluated.

Considering the heritabilities, the repeatibility and the manifestation at an early age, the haemagglutinin antibody response to SRBC offers a perspective for selection. The value of this trait for general disease resistance will have to be proven in the near future, when our selection lines for high and low antibody responsiveness have been established.

SAMENVATTING

Ziekten kunnen worden bestreden door toediening van medicijnen, vaccinatie, hygienische maatregelen, uitroeiïng en genetische resistentie. Genetische resistentie tegen infectieuze ziekten is blijvend aanwezig in tegenstelling tot de effecten van eerder genoemde bestrijdingsmaatregelen. Bij pluimvee is genetische resistentie tegen bepaalde ziekten, zoals ziekte van Marek en lymfoïede leukose welbekend. Ondanks de bekendheid met mogelijkheden tot genetische verbetering van resistentie tegen sommige infectieuze ziekten vindt geen toepassing in de pluimveefokkerij op grote schaal plaats. Oorzaken zijn de noodzaak om fokkerijstammen te besmetten, het gebrek aan kennis over verbanden met resistentie tegen andere ziekten en productie-eigenschappen en het beperkte begrip van weerstandsmechanismen tegen specifieke ziekten.

Een andere benadering van genetische verbetering van resistentie tegen ziekten is het samenstellen van een groep weerstandskenmerken, die tesamen de algemene resistentie tegen ziekten vormen. Het "Major Histocompatibility Complex", het B-systeem bij de kip, zou hiervan deel uit kunnen maken vooral vanwege haar rol bij weerstand tegen door virussen geïnduceerde tumoren. Een ander kenmerk, dat voor selectie voor de algemene weerstand tegen ziekten, kan worden gebruikt, is het vermogen antistoffen te produceren tegen een antigeen met vele determinanten, zoals schapen rode bloed cellen (SRBC). Selectie voor dit kenmerk in hoge en lage richting heeft bij muizen aangetoond, dat de effecten niet specifiek zijn. In dit proefschrift worden de genetische en milieuaspecten van de productie van agglutinerende antistoffen na injectie met SRBC bij pluimvee besproken.

Kuikens, verschillend in leeftijd van 34 tot 40 dagen werden geïmmuniseerd met SRBC in de dijbeenspieren. De toegediende dosis variëerde van .01 tot 1 ml "packed" SRBC. Indien een tweede dosis werd toegediend, was dit .5 ml na een primaire dosis van .25 ml SRBC. De kuikens waren per proef van verschillende herkomst, namelijk Wit Leghorn (WL), White Plymouth Rock (WPR) en ISA Warren. Ook verschilde het vaccinatieschema per proef. Antilichaamtiters in plasma werden minimaal vier keer bepaald gedurende een periode van maximaal twee weken na immunisatie. Naast de totale agglutinatie titers werden tevens de 2-mercapto ethanol (2-ME) resistente (IgG type antilichamen) en de 2-ME gevoelige (IgM type antistoffen) agglutinatie titers bepaald.

De productie van agglutinerende antistoffen na intramusculaire injectie met SRBC wordt bepaald door vele genen. Schattingen van de erfelijkheidsgraad

voor totale antilichaam titer, 2-ME resistente en gevoelige antilichaam titers variëerden van waarden niet significant ($P < .05$) verschillend van 0, tot, gewoonlijk, waarden tussen .2 en .3 voor de primaire response in WL, WPR en ISA Warren populaties. De erfelijkheidsgraden voor de secundaire response, geschat bij de WPR en de ISA Warren populaties, waren iets lager. De hoogte van deze erfelijkheidsgraad schattingen biedt goede mogelijkheden voor individuele selectie, zoals reeds is gebleken in onze eerste selectie generatie voor hoge en lage antilichaamproductie in een ISA Warren populatie. Bovendien is de herhaalbaarheid van dit kenmerk hoog, meer dan .9.

Phenotypische correlaties tussen primaire en secundaire totale antilichaam titers waren niet significant ($P \geq .05$) voor de WPR populatie. Een verklaring hiervoor was het positieve verband tussen primaire 2-ME gevoelige antilichaam titers en secundaire 2-ME resistente antilichaamtiters en het negatieve verband tussen primaire en secundaire 2-ME gevoelige antilichaam titers. De additief genetische correlaties tussen totale antilichaam titers voor de primaire en secundaire response waren negatief. Als deze negatieve genetische correlaties worden bevestigd door andere onderzoekers, dan zal de keuze van de primaire antilichaamproductie tegen SRBC als één van de selectiecriteria voor algemene ziekte resistentie moeten worden heroverwogen.

De antilichaam response tegen drie doses SRBC (.01, .1 en 1 ml "packed" cellen) was verschillend bij drie populaties: WL, WPR en ISA Warren. Eveneens werden verschillen gemeten voor de celgebonden immuniteit, gemeten als de zwelling na injectie met phytohaemagglutinine (PHA) in de vleugelhuid. Interacties tussen dosis SRBC en genetische herkomst waren afwezig. De response curve na injectie met SRBC was verschillend voor elke populatie. Interactie tussen genetische herkomst (populatie) en interval tussen injectie en titerbepaling is dus te verwachten. Phenotypische correlaties tussen totale antilichaam titers tegen SRBC en de PHA zwelling waren niet significant ($P \geq .05$), zowel binnen populaties als in het totale materiaal.

De antilichaamproductie tegen SRBC wordt beïnvloed door vele niet genetische factoren. Belangrijke niet genetische effecten waren de dosis SRBC, de leeftijd van het kuiken, primaire versus secundaire response, vaccinatieprogramma, interval tussen injectie met SRBC en titerbepaling. Gewenste kenmerken van het afweersysteem voor de bestrijding van infectieuze ziekten zijn immuuncompetentie op jonge leeftijd, reactievermogen op lage doses van infectieuze agentia, een snelle response na infectie en de aanleg van immunologisch geheugen. Het selectie criterium zal dus duidelijk gedefinieerd moe-

ten worden en de effecten van selectie op deze gewenste eigenschappen moeten worden onderzocht.

Gezien de hoogte van de erfelijkheidsgraden, de herhaalbaarheid en het voorkomen op jonge leeftijd biedt het kenmerk: productie van agglutinerende antistoffen tegen SRBC mogelijkheden voor selectie. De waarde van dit kenmerk voor algemene resistentie tegen infectieuze ziekten moet nog aangetoond worden in de nabije toekomst, wanneer onze selectielijnen voor hoge en lage antilichaamproductie zijn gevormd.

CURRICULUM VITAE

A.J. van der Zijpp werd geboren op 29 juli 1945 te Rottum (Fr.). In 1962 werd het einddiploma HBS-B behaald aan het Openbaar Lyceum te Heerenveen, in 1963 gevolgd door het einddiploma van de Princeton Township High School te Princeton, Illinois, Verenigde Staten. In september 1963 werd de studie aan de Landbouwhogeschool aangevangen. Deze studie met als hoofdvak Veeteelt en de keuzevakken Veevoeding, de Leer van het Grasland en de Teelt van Voedergewassen en het Natuurbehoud en Natuurbeheer werd afgesloten in september 1971. Van september 1971 tot oktober 1974 was de auteur verbonden als wetenschappelijk medewerker aan het Department of Agriculture, Reading University, Reading, Engeland. Vervolgens begon haar werkkring als wetenschappelijk medewerker bij de vakgroep Veefokkerij, maar zij was gedetacheerd bij de Stichting Bloedgroepen Onderzoek gedurende bijna twee jaar. Hier werd haar belangstelling gewekt voor het vakgebied immunologie. Sinds oktober 1975 is zij verbonden aan de vakgroep Veehouderij. In 1976 is een aanvang gemaakt met het onderzoeksproject: genetische en milieu aspecten van de immuunrespons bij kippen, in het kader van het onderzoeksthema Resistentie.