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CONGRESS

Some aspects of breeding for resistance (*) with special emphasis on mice and pig experiments carried out at the Ludwig-Maximilian-University, Munich

H. KRÄUSSLICH

Institute of animal breeding, Ludwig-Maximilians-University of Munich
Veterinärstraße 13, 8000 Munich 22, Federal Republic of Germany

Summary

The following aspects of breeding for resistance are discussed :
— recording of diseases in farms under milk recording,
— measuring indicators for specific diseases,
— selection for pathogene unspecific resistance mechanisms,
— selection for agent specific resistance mechanisms.

The main results of experiments for selection on resistance to infection in mice and pigs carried out at the Ludwig-Maximilians-Universität München are presented. Until now no well defined selection criteria are available for breeding purposes. Available results support the intensification of research in this field.

1. Introduction

Breeding aims for domestic animals which are used for intensive animal production consider characters of :

— Production,
— Reproduction,
— Constitution.

During the past two decades considerable genetic improvement has been reached in production traits. But more and more problems are reported regarding reproduction and disease suceptibility. It seems that measurements of feeding, management, hygiene, chemotherapy and vaccination are not sufficient to secure productivity in an intensive production system with high performance per animal. Geneticists are fre-

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quently blamed for the arising problems. They are said to have neglected the constitution of the animals in selection. Geneticists justify the overemphasis on production traits in selection with economic demands and the lack of constitutional parameters which can be used as selection criteria. Since 1970 our team at the Veterinary Faculty of the Munich University (BUSCHMANN, MEYER, KRÄUSSLICH) has carried out experiments for selection on resistance to infection in mice and pigs. The most significant results of these experiments will be reported here and compared to those obtained in other species.

2. Incidence of diseases

There are two approaches to use disease incidence as selection parameters :

— Recording of diseases in progeny groups,

— Measuring indicators of diseases in breeding males or in progeny groups.

2.1. *Disease frequencies*

Disease recording programmes are on a large scale at present carried out only in dairy herds in Scandinavia. The success of direct selection on low disease frequencies, however, is limited primarily because diseases are all-or-non traits and adjustment on systematic environmental effects on the occurence of diseases is much more difficult than on production traits. Thus, heritability estimates are low and not very reliable.

2.2. *Indicators of diseases*

Most investigations in cattle are conducted on metabolic diseases, primarily ketosis and hypocalcaemia and on mastitis.

Ketosis : It is well documented that there are great individual and breed differences in bovine ketosis. Possible markers in bulls for predicting susceptibility to ketosis in their daughters are blood levels of metabolic components, relevant enzymes and hormones. Several components of the so-called « Metabolic Profile » have been shown to exhibit genetic variation (ROWLANDS, 1974). ALMLID (1980) exposed bulls which were kept in a performance test to an energy-deficient situation by starvation for 48 hours. Individual variation in the plasma levels of ketone bodies, glucose and thyroxine were found. Repeatabilities between tests conducted at the same animal ranged between 0.49 and 0.74.

Since the effects of starvation of a bull and ketosis are physiologically different processes the value of such traits in indirect selection has to be evaluated experimentally.

Hypacalcaemia : HENRICSON *et al.* (1975) found a significant correlation between serum-Mg-level of heifers and the frequency of paresis puerperalis in older half sisters. The heritability of the serum-Mg-level was estimated with $h^2 = 0.10$ in heifers and $h^2 = 0.19$ in first calvers. GRAF *et al.* (1977) found significant differences between halfsister groups in the difference between the serum-Ca-level 24 hours after parturition and the serum-Ca-level before calving.

Mastitis : As indicator of subclinical mastitis, primarily cell counts are used.

PHILIPPSON *et al.* (1978) gave a review on heritability estimates. Since low cell numbers might also be an indicator of insufficient immune responsiveness, it is doubtful whether cell numbers are suitable selection criteria.

PAAPE *et al.* (1977) found a significant variation between cows in the ability of milk to support phagocytosis by neutrophil polymorphonuclear (PMN) leukocytes, and that elucidation of these factors may help in reducing the incidence of bovine mastitis.

GÖTZE *et al.* (1977) showed that lysozyme concentration in the blood and in the milk are independent parameters. Lysozym concentrations in the milk are influenced by stage of lactation and bacterial contamination of the milk. However, nothing is known about genetic variation of lysozyme concentration in the milk up to now.

3. Resistance against infectious diseases

Our knowledge about the complex gene systems operating on several levels in regulating the immune responsiveness has increased considerably during the last decade. Immunoglobulin markers, primarily allotypes (allelic types) made it possible to establish the cellular origin of antibodies and the phylogenetic descendance of immunoglobulins. Genetic control of immune responsiveness is exercised on two independent levels, such as the antigen recognition (ability to respond) and the antibody synthesis (magnitude of response) (afferent limb and efferent limb of immune response). The ability to respond is generally controlled by one or a few gene loci, whereas the magnitude of response can be considered as a typical quantitative character. It is furthermore to be expected that the studies of the major histocompatability complex (MHC) in domestic animals will reveal relationships between well defined diseases and certain alleles of the MHC as already shown in poultry (BRILES *et al.*, 1977).

3.1. *Pathogene-unspecific factors*

Pathogene-unspecific factors are those operating on skin, on mucous membranes, in secretions and components of the blood. The unspecific resistance factors of the blood are of particular interest, since many show continuous variation, and some can be studied by relatively simple *in vitro* tests. Genetic differences in macrophage function, as measured by carbon clearance, were shown by BUSCHMANN *et al.* (1972) and PASSWELL *et al.* (1974). The carbon clearance rate measures the phagocytosis of the mononuclear phagocytic system. In figure 1 strain differences in the carbon clearance rate in inbred strains of mice and their reciprocal crosses are shown. Sample regression lines of log. carbon concentration on time of blood removal adjusted to one starting point on the ordinate are drawn.

Heritability estimates of the granulopectic index (Kr) in females varied between 0.36 and 0.92 in inbred strains and reached 0.11 in non-inbred NMR/mice. In a two way selection experiment with laboratory mice on carbon clearance rate the estimates of realized heritabilities were 0.30 in the high line and 0.25 in the low (KRÄUSSLICH *et al.*, 1975) as it is shown in figure 2.

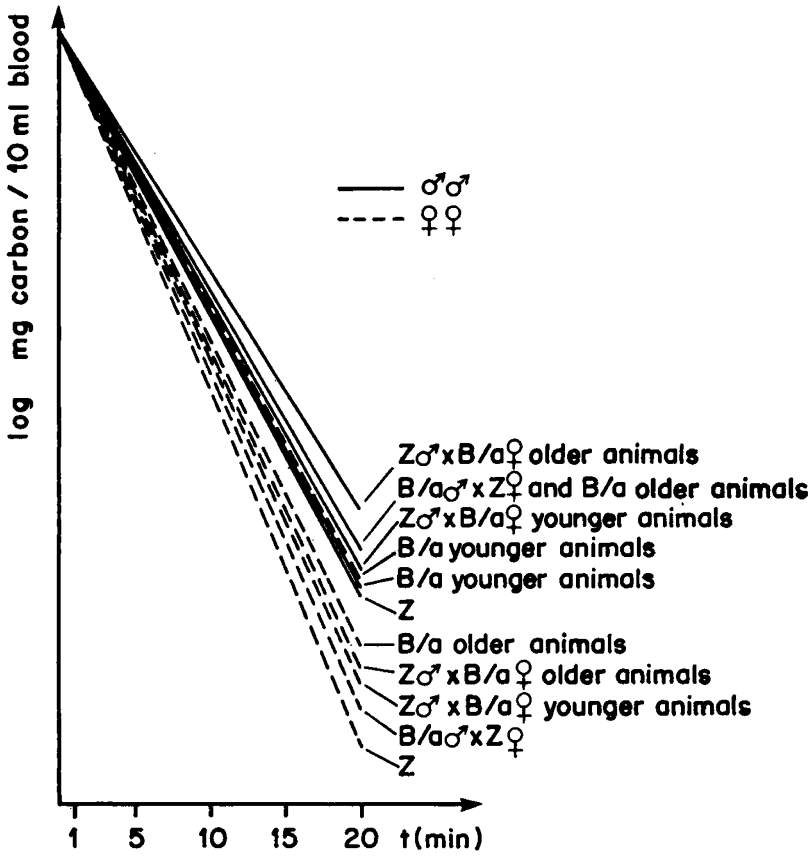

FIG. 1

Carbon clearance rate in inbred strains of mice and their reciprocal crosses. Shown are the sample regression lines of log carbon concentration on time of blood removal adjusted to one starting point on the ordinate (BUSCHMANN *et al.*, 1972).

Taux d'élimination du carbone dans les lignées consanguines de souris et leurs croisements. La figure montre les droites de régression du log de la concentration en C en fonction du moment de la prise de sang, ajustées sur une ordonnée à l'origine commune.

Some correlated selection responses were found. The absolute and relative liver and spleen weights differed significantly between the lines (fig. 3) indicating that the number of phagocyting cells was increased by the selection on carbon clearance rate. The *in vitro* phagocytic activity of the single cell however, remained unaltered.

After immunizing the mice with sheep erythrocytes definitely higher titres of specific mercapthoethanol-sensitive as well as mercaptoethanol-resistant hemolysins and hemagglutinins were detected in the positive selected line (BUSCHMANN *et al.*, 1976 ; fig. 4). On a singular cell basis, however, there were no differences in antibody production between lines. In the number of plaque forming cells/10^6 spleen cells the

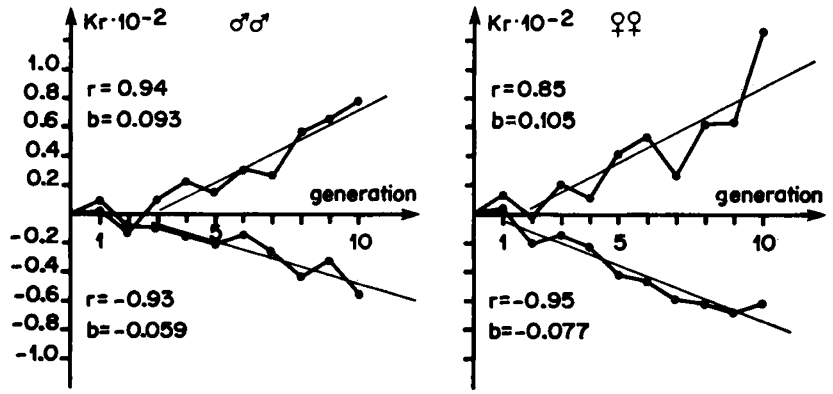

FIG. 2

Selection response per generation for the granulopeptic index (Kr) (KRÄUSSLICH *et al.*, 1975).

Réponse à la sélection pour l'indice granulopeptique (Kr).

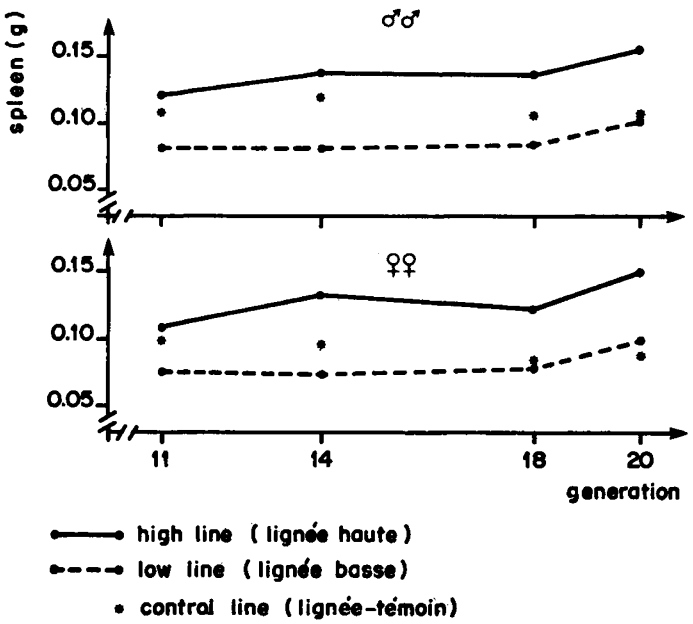

FIG. 3

Mean values of spleen weights in generation F 11 to F 20 (F 14, F 18 and F 20 without selection).

Poids moyen de la rate de la génération F 11 à la génération F 20 (F 14, F 18 et F 20 sans sélection).

lines did not differ significantly. This supports the assumption that the increase or decrease of the spleen weight was the main selection response. Resistance against *Listeria* infection was significantly higher in the high line. No difference was seen in resistance against *Salmonella typhimurium*. An interesting side effect was the difference between lines in spontaneous and benzopyrene induced mammary tumors (fig. 5 and 6). This finding favours the hypothesis that the spleen is the major site for the production of enhancing or blocking factors, and that its removal may inhibit the growth of tumors.

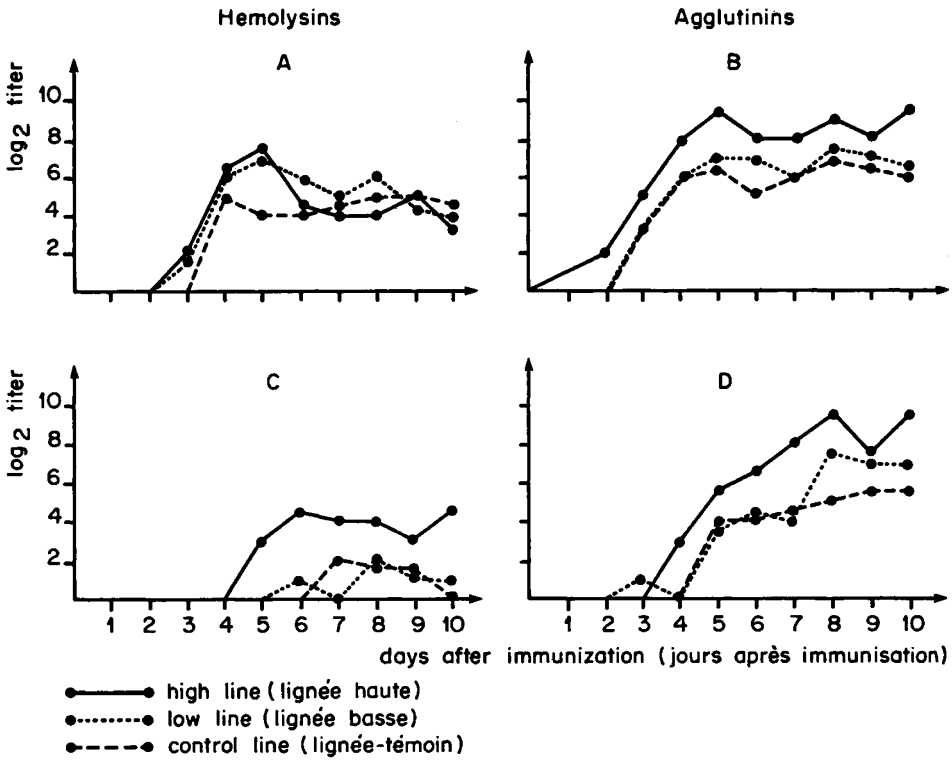

FIG. 4

Titer of mercaptoethanol-sensitive (A, B) and of mercaptoethanol-resistant (C, D) hemolysins and hemagglutinins after immunisation with sheep erythrocytes. Differences between lines selected for high and low phagocytic activity (BUSCHMANN *et al.*, 1976).

Titre en hémolysines et hémagglutinines sensibles (A, B) et résistantes (C, D) au mercaptoéthanol après immunisation avec des globules rouges de mouton. Différences entre des lignes sélectionnées pour haute et basse activité phagocytaire.

Differences in longevity and reproduction were found in generation 16 between lines (table 1).

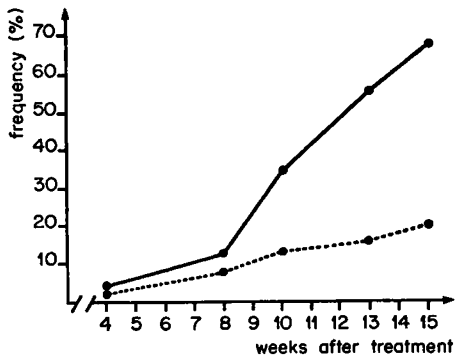

FIG. 5

Line differences in spontaneous mammary tumor incidence ; n = 30 females bred per line.

Différences entre lignées pour la fréquence des tumeurs mammaires spontanées, n = 30 femelles reproduisant par lignée.

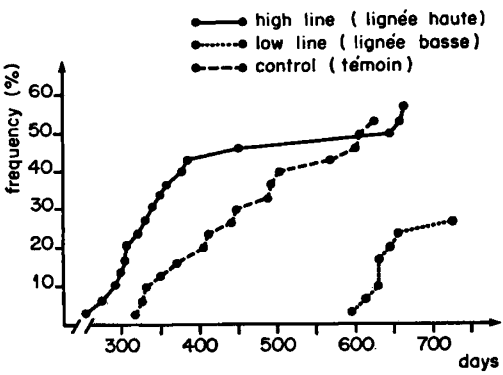

FIG. 6

Line differences in 3,4 benzopyrene induced tumors ; n = 200 per line, 50 p. 100 females and 50 p. 100 males.

Différences entre lignées dans la fréquence des tumeurs induites par 3,4 benzopyrène ; n = 200 par lignée, 50 p. 100 de femelles, 50 p. 100 de mâles.

The results of the selection experiment on carbon clearance rate show that unidirectional selection for a complex immunological trait might disturb the balance of the various resistance mechanisms which have been established by natural selection. Unfavourable correlated selection responses are to be expected.

Variation of the phagocytic activity was found in different pig breeds by BUSCHMANN *et al.* (1974). In pigs phagocytic activity of the polymorphonuclear (PMN) leukocytes was measured by the 14 C - glucose oxidation rate in the whole blood.

TABLE 1

Differences in longevity and reproduction between lines selected for carbon clearance rate (generation 16).

Différences de longévité et de reproduction entre des lignées sélectionnées pour le taux d'élimination du carbone (génération 16).

	Selected lines - *Lignées*		
	High *Haute*	Control *Témoin*	Low *Basse*
Character ..... *Caractère*			
N	30	30	30
Lifetime (days) ..... *Durée de vie*	399.17	457,80	585,71
Reproductive time (days) .... *Durée de reproduction*	215.96	266,30	201,73
Litters (number) ..... *Portées*	7.58	8,97	8,10
Offspring (number) ..... *Descendants*	80.13	86,27	78,67

Significant differences due to breed, age and immunization history were observed. Ranking the breeds from high to low according to their average P/R ratio (ratio of $^{14}CO_2$ for glucose-C-^{14} C by phagocytizing (P) compared to resting (R) blood cells) and according to their immune response to sheep erythrocytes gave a significant correlation between the 2 arrangements.

The following rankings were obtained :

a) P/R ratio ;

Belgian Landrace > *Duroc* > *Deutsches Edelschwein* > *Large White* > *Deutsche Landrasse* > *Hamphire* > *Piétrain* > *Angler Sattelschwein* > *Welsh*

b) Antibody response to sheep erythrocytes (number of plaque-forming cells in the spleen) :

Duroc > *Deutsches Edelschwein* > *Large White* > *Hamphshire* > *Deutsche Landrasse.*

It may be concluded that a significant correlation exists between the variation in the phagocyte function and the immune response to particular antigens like sheep erythrocytes in pigs. Other important antigens are those which can be analysed in blood serum. Recently, LIE (1980) demonstrated genetic variation in the serum activity of the enzyme lysozyme in cattle. In poultry genetic variation has been found in the activity of interferon in serum (HONG and SEVOINEN, 1971).

The cellular, T lymphocyte depending immune response is also polygenic regulated. STIFFEL *et al.* (1977) selected mice on the basis of lymphocyte stimulation by phytohemagglutinin as a mitogen. A heritability of 0.28 ± 0.08 was estimated. The humoral response was identical in both lines.

3.2. *Agent specific resistance mechanisms*

The « classical » selection experiments of BIOZZI *et al.* (1979) in mice have demonstrated that the humoral immune response against complex antigens is a quantitative character which is regulated by at least 10 loci, and that there is a genetic correlation between the responsiveness to different antigens, including antigens of pathogens. But, there was a similar ability for T cell mediated immunity in the high and low lines. This shows that selection can modify antibody response without changing the cell mediated immunity and reverse.

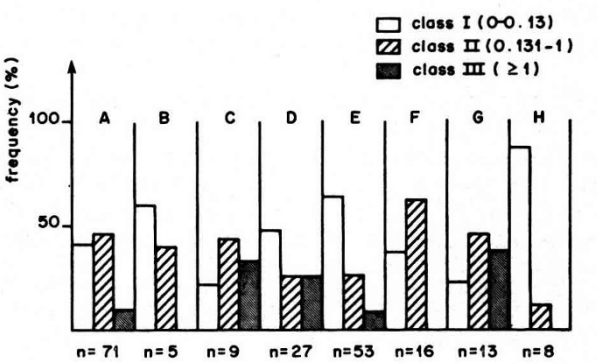

FIG. 7

Distributions of K-values of DNP-immunized pigs belonging to 8 different breeds (A-H) (BUSCHMANN, 1975).

Distributions de la réponse immunitaire au DNP (valeur de K) chez des porcs appartenant à 8 races différentes (A-H).

Genetic variation in humoral immune response has also been demonstrated in bulls by LIE (1979). The antibody titers against human serum albumin (HSA) and the total serum immunoglobulin levels were evaluated. Heritability estimates ranged between 0.14 and 0.56. The primary response (peak at 15 days after first injection) seems to be under stronger genetic influence than the secondary response (peak at 8 days after second injection). LIE assumes that the primary response reflects the genetic control of antigen recognition (ability to respond) and the secondary response reflects the magnitude of response (a quantitativ character). The answer to whether the primary or the secondary response should be the best criterion for the general immune response can only be given by correlation to disease date. An important condition for the practical application of the tests is the standardisation of the environment which could be achieved in performance testing stations for AI bulls.

In pigs, genetic variation in humoral immune response was also found. RADZIKOWSKY *et al.* (1974) demonstrated breed differences in the immune response to sheep erythrocytes measured by the number of plaque forming cells (PFC) of the spleen. High levels of cross-reacting antibodies, however, were found in the sera of non-immunised pigs. To avoid these crossreactions, immune response of different pig breeds to DNP hapten (2.4 Dinitrophenylsulfonic acid-Bovine Serum Albumine) was investigated (BUSCHMANN, 1975). In the serum of 49 p. 100 of the pigs no antibody activity was determined (Class 1), 38 p. 100 had weak antibody titres (Class II), and 13 p. 100 high antibody titres (Class III) in the serum. Figure 7 demonstrates considerable breed differences.

ALMLID *et al.* (1980) found in a two way selection experiment with goats a difference between selected lines in humoral response to diphtheria toxoide and human albumin.

SIEGEL & GROSS (1980) selected two lines of chickens on the basis of antibody response against sheep red blood cells. They found a genetically determined variation in antibody titres after an antigenic stimulation. Van der ZIJPP and LEENSTRA (1980) found in a *White Leghorn* population heritability estimates for humoral antibody response to sheep red blood cells between 0 and 0.5.

At the Ludwig-Maximilians Universität München a one way selection experiment with pigs, which had been immunized with dinitrophenylated bovine serum albumin (DNP-BSA) is in progress : There are only very weak titres of « naturally » occuring anti-DNP antibodies in pigs. A dose-response curve could be verified for DNP in pigs so that the specificity of the antibody reaction is established. Preliminary results are shown on fig. 8. The main conclusions from this selection experiment are up to now :

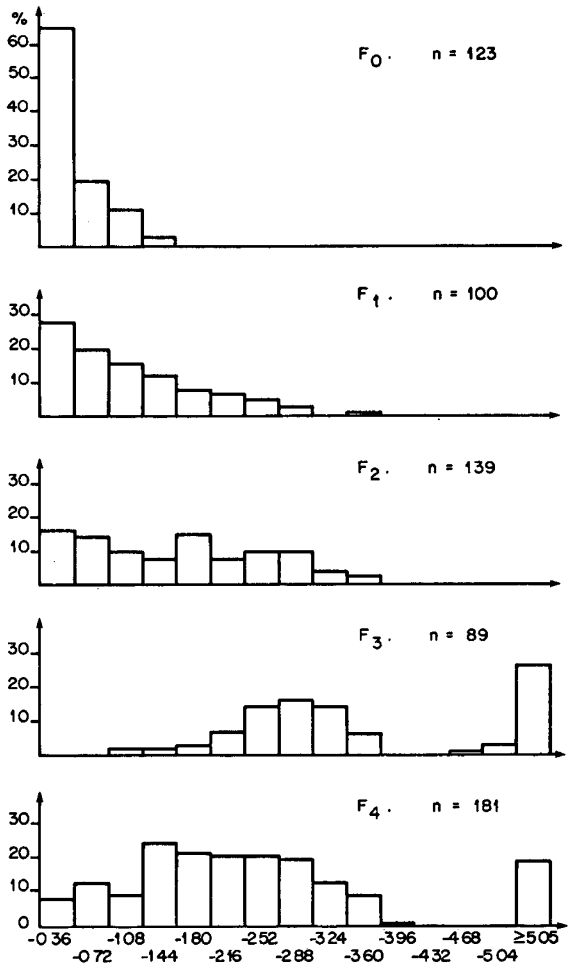

FIG. 8

Frequency distribution of pigs selected for high antibody-forming capacity to DNP-hapten (K. 10^2).

Distribution de fréquence de porcs sélectionnés pour une haute capacité à former des anticorps au DNP-hpatène (K. 10^2).

The character selected for is polygenic. Selection increased antibody responsiveness to DNP and to some related antigens per example T_4 phages. Parameters of the cellular immune response (phagocytosis of polymorphonuclear cells, stimulation of lymphocytes by various mitogenes, number of E and EAC rosette-forming cells in the blood) were not changed. This agrees with the findings of BIOZZI *et al.* (1979) in mice. It seems that selection can change antibody response without changing the cell mediated immunity and reverse in mice and pigs.

In the above mentioned selection experiment with pigs at the Ludwig-Maximilians-Universität München lymphocyte associated antigens are determined in the selec-

tion line and in the control line. Preliminary results support the hypothesis that immune response of pigs might be under the control of genes associated with the major histocompatilibity locus.

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Résumé

Quelques aspects de l'amélioration génétique de la résitance aux maladies à la lumière des expériences menées sur la souris et le porc à l'Université Louis-Maximilien de Münich

Les aspects suivants de l'amélioration génétique de la résistance sont discutés :
— enregistrement des maladies dans les élevages pratiquant le contrôle laitier,
— mesure d'indicateurs de maladies spécifiques,
— sélection pour des mécanismes de résistance non spécifiques,
— sélection pour des mécanismes de résistance spécifiques à certains agents pathogènes.

Les principaux résultats des expériences de sélection pour la résistance à l'infection chez la souris et le porc à l'Université Louis-Maximilien de Munich sont présentés. Jusqu'à présent aucun critère de sélection bien défini n'est disponible à des fins d'amélioration génétique. Les résultats obtenus montrent la nécessité d'intensifier les recherches dans ce domaine.

Zusammenfassung

Folgende Aspekte der Zucht auf Krankheitsresistenz werden diskutiert :
— Erfassung der Krankheitsfrequenz in Milchkontrollbetrieben,
— Messen von Indikatoren für spezifische Krankheiten,
— Selektion auf unspezifische Resistenzmechanismen,
— Selektion auf antigenspezifische Resistenzmechanismen.

Die wichtigsten Ergebnisse von Experimenten an der Ludwig-Maximilians-Universität München in denen Mäuse und Schweine auf Resistenz gegen Infektionen selektiert wurden, werden dargestellt. Derzeit gibt es noch keine definierten Selektionskriterien, die eine gerichtete Resistenzzucht ermöglichen. Es erscheint jedoch notwendig, die Forschung auf diesem Gebiet zu intensivieren.

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