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Breed Differences in the Number and Kinds of Leucocytes in Blood of Swine

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INTRODUCTION

Strain or breed differences in certain morphological or physiological characteristics are largely a reflection of genetic differences. Information regarding inherited differences of this nature is of great value because it should aid in the demonstration, directly or indirectly, of the physiological mechanisms involved in gene action.

Strain differences in the number of leucocytes have been observed in the mouse by Fekete (1941) and by Endicott and Cump (1947). Scarborough (1921) was one of the first to observe strain differences in leucocyte numbers in the rat which Reich and Dunning (1943) confirmed.

Chai (1957), in an experiment in which selection was practiced for large and for small body size in mice, discovered a pronounced difference in leucocyte numbers in the two strains. By comparing the variance of the F_1 and F_2 generations of the crosses between these two strains, it was found that approximately one-half of the total variance was of genetic origin.

Numerous studies have been reported concerning the leucocytes in the blood of swine, but no information is available concerning the comparative number in different breeds or strains maintained under similar environmental conditions. This study was designed to determine if breeds of swine differed in the kinds and numbers of leucocytes in the blood stream before and after a short period of mechanical stress.

MATERIALS AND METHODS

Thirty-six boars and 25 barrows from the Duroc, Hampshire, Poland China, and Landrace breeds were used in this investigation. Only the Polands and the

Landrace were from inbred lines. All pigs were fed the same ration *"ad lib."* from weaning to market weight, were penned in the same barn, and were handled in as nearly the same manner as possible during the feeding period. The experimental animals were between 150 and 200 pounds in weight when the blood studies were made.

Blood samples for study were obtained by catching the pigs by the snout with a pig catcher and then severing the tip of the tail. A sample of blood was collected in a glass tube containing potassium oxalate as an anticoagulant. A second blood sample was collected from each pig in the same manner in exactly four hours after the first except in a preliminary experiment in which blood samples were collected each hour from each pig for a period of six hours following the collection of the initial sample.

Blood samples from the entire group of experimental animals were collected on the same day with the exception of those from the eight pigs used in the preliminary study. These pigs were bled approximately ten days prior to the larger group.

Total leucocyte counts were made within 12 hours following collection with a 1:20 dilution of blood using 1 percent HCl as the diluent. Separate counts on each blood sample were made by two individual counters. The coefficient of correlation between the two different counts was 0.75 for all samples. An average of the two counts was used for the analysis of data.

The dry smears for the differential counts were stained and 100 cells were counted on each of the two slides made from each blood sample. An average of the two counts was used to calculate the percentages of different kinds of leucocytes. This was multiplied by the total numbers of leucocytes per mm^3 of blood to get the total numbers of leucocytes of each type in each blood sample. Since the eosinophils and basophils represented such a small proportion of the total cell number, they were not included in this study. Monocyte numbers which were also small were included with the lymphocytes.

The mechanical stress was that resulting from catching the pigs by the snout with a pig-catcher and then holding him for two to three minutes while the end of the tail was severed and the blood collected.

RESULTS

A preliminary study was made of the number and kinds of leucocytes in swine before and after mechanical stress in which boar and barrow littermate pairs from each of the four breeds were used. Results of this preliminary study are shown in Figure 1.

The number of lymphocytes per mm^3 of blood declined following the short period of mechanical stress. The initial average number before stress was 13,963 per mm^3 and the lowest average number was 10,075 per mm^3 of blood at the fifth hour following stress. The neutrophils decreased slightly in number one

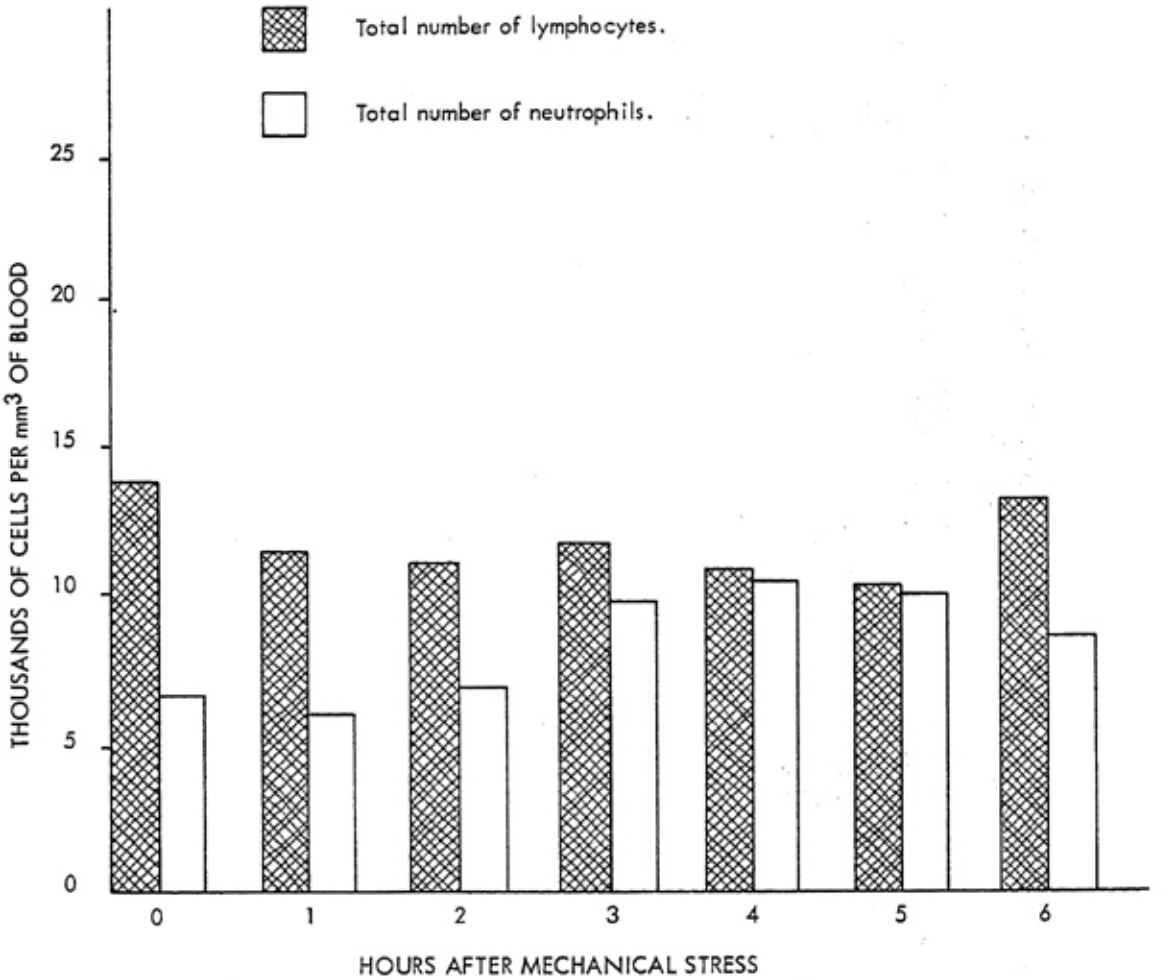

Figure 1. Showing total numbers of lymphocytes and neutrophils in pigs before and after mechanical stress. (n = 8)

hour after stress and then increased to a peak of 10,469 per mm^3 of blood at the end of four hours. This was an average increase of 3,719 cells per mm^3 of blood over the initial level.

The results of this preliminary study suggested that changes in numbers and kinds of leucocytes following the kind of mechanical stress used were near their maximum levels at the fourth hour. Therefore, in subsequent experiments with a larger group of animals, two blood samples from each pig, one obtained before stress and the other four hours later, were studied.

The total number of leucocytes per mm^3 of blood before and after mechanical stress in 61 boars and barrows of four breeds is shown in Table 1. Highly significant breed differences ($P < .01$) were obtained in total numbers of leucocytes both before and after stress. Pigs of the Landrace breed had the highest average total leucocyte number of around 23,000 per mm^3 whereas the Durocs were the lowest with an average of 16,000 per mm^3 of blood.

A comparison of the total number of lymphocytes per mm^3 of blood before and after mechanical stress in the same boars and barrows is shown in Table 2.

TABLE 1--THE TOTAL NUMBERS OF LEUCOCYTES PER mm^3 OF BLOOD BEFORE AND AFTER MECHANICAL STRESS IN BOARS AND BARROWS OF FOUR DIFFERENT BREEDS

Breed	No. of Pigs	Before Stress*			Four Hours After Stress*		
		Average	St. D.	C.V. %	Average	St. D.	C.V. %
Landrace	11	23, 028	4,373	19.0	23,387	3,988	17.1
Polands	16	20,494	4,974	24.3	22,850	5,160	22.6
Durocs	13	16,008	2,763	17.3	16,396	4,133	25.2
Hampshires	21	17,065	3,692	21.6	18,296	4,579	25.0
Total and Average	61	18,814	4,715	25.1	20,003	5,230	26.2

*Differences due to breeds very highly significant ($P < .01$)

TABLE 2--THE TOTAL NUMBERS OF LYMPHOCYTES PER mm^3 OF BLOOD BEFORE AND AFTER MECHANICAL STRESS IN BOARS AND BARROWS OF FOUR DIFFERENT BREEDS

Breed	No. of Pigs	Before Stress*			Four Hours After Stress**		
		Average	St. D.	C.V. %	Average	St. D.	C.V. %
Landrace	11	14,232	2,862	20.1	11,568	3,474	30.0
Polands	16	14,888	2,998	20.1	13,288	2,977	22.4
Durocs	13	12,554	2,180	17.4	10,523	2,562	24.4
Hampshires	21	14,288	2,516	17.6	11,819	2,736	23.2
Total and Average	61	14,066	2,715	19.3	11,883	2,972	25.0

* Probability of differences between breeds being due to chance less than .15.
** Probability of differences between breeds being due to chance less than .10.

In pigs from all four breeds mechanical stress decreased the number of lymphocytes per mm^3 of blood by an average of 2,183 cells (15 percent). Breed differences in changes in lymphocyte numbers from zero to four hours after stress were not significant, however, nor were there significant breed differences in total numbers of lymphocytes before or after the stress period (Table 4).

Data summarized in Table 3 show that the breeds differed significantly ($P < .01$) in numbers of neutrophils both before and after mechanical stress. As in the preliminary study, all four breeds showed increases in neutrophil numbers following mechanical stress. On the average these cells increased from 4,645 to 8,060 per mm^3 or an average increase of about 74 percent. The breeds did not differ significantly in the increases in neutrophil numbers due to stress (Table 4).

These results show rather clearly that the main breed differences in total leucocyte numbers were in the numbers of neutrophils rather than in numbers of lymphocytes. Furthermore, it was observed that numbers of neutrophils before and after stress were much more variable (C. V. of 59 and 68 percent) than were the numbers of lymphocytes (C. V. of 25 and 26 percent).

Data obtained from boar and barrow littermate pairs representing 15 litters of all four breeds were analyzed to determine if litters differed significantly in numbers and kinds of leucocytes (Table 5). None of the differences between litters within breeds were significant although all breed differences in this small group of pigs were again significant in all instances except in changes in numbers and kinds of leucocytes between zero and four hours after mechanical stress.

The coefficients of correlation between numbers of leucocytes before and after mechanical stress and rate of gain and backfat thickness are shown in Table 6. The data for average daily gain were obtained for the period from shortly after weaning to a weight of 200 pounds whereas backfat thickness was measured at 200 pounds of live weight in all animals. In general, all of the correlations on a within breed basis were low for both traits although a highly significant correlation ($P < .01$) was found between total neutrophils four hours after stress and rate of gain.

The only significant correlation ($P < .05$) concerning backfat thickness was that with changes in lymphocyte numbers per mm^3 of blood between zero and four hours following stress. None of the between-breed correlations were significant but nearly all were negative for both traits.

TABLE 3--THE TOTAL NUMBERS OF NEUTROPHILS PER mm^3 OF BLOOD BEFORE AND AFTER MECHANICAL STRESS IN BOARS AND BARROWS OF FOUR DIFFERENT BREEDS

Breed	No. of Pigs	Before Stress*			Four Hours After Stress**		
		Average	St. D.	C.V. %	Average	St. D.	C.V. %
Landrace	11	8,728	1,967	22.5	11,900	3,789	31.8
Polands	16	5,344	2,849	53.3	9,322	5,058	54.3
Durocs	13	3,442	2,215	64.4	5,873	4,458	75.9
Hampshires	21	2,719	2,092	77.0	6,441	3,895	60.5
Total and Average	61	4,645	3,148	67.8	8,060	4,746	58.9

* Probability of differences between breeds being due to chance less than .01.
** Probability of differences between breeds being due to chance less than .005.

TABLE 4--CHANGES IN TOTAL NUMBERS OF LEUCOCYTES, LYMPHOCYTES AND NEUTROPHILS IN BOARS AND BARROWS OF FOUR DIFFERENT BREEDS FOUR HOURS AFTER MECHANICAL STRESS AS COMPARED TO THE NUMBERS BEFORE STRESS

Breed	No. of Pigs	Total No. Leucocytes*		Total No. Lymphocytes*		Total No. Neutrophils*	
		Average	St. D.	Average	St. D.	Average	St. D.
Landrace	11	359	1,355	-2,663	1,205	3,173	1,087
Polands	16	2,356	700	-1,600	623	3,978	949
Durocs	13	388	946	-2,031	562	2,431	892
Hampshires	21	1,231	558	-2,426	600	3,721	556
Total and Average	61	1,189	425	-2,168	363	3,415	397

* Probability of breed differences being due to chance more than .25.

TABLE 5--INFLUENCE OF LITTER AND BREED ON THE NUMBER AND KINDS OF LEUCOCYTES IN THE BLOOD OF GROWING-FATTENING PIGS BEFORE AND AFTER A SHORT PERIOD OF MECHANICAL STRESS
(Fifteen boar-barrow littermate pairs)

	F-Ratio from Analysis of Variance***	
	Between Breeds	Between Litters Within Breeds
Initial number of leucocytes	6.64**	1.31
Initial number of lymphocytes	4.58*	1.09
Initial number of neutrophils	14.50**	0.73
Number of leucocytes 4 hours after stress	4.81**	1.30
Number of lymphocytes 4 hours after stress	10.88**	1.00
Number of neutrophils 4 hours after stress	4.51*	0.94
Changes in leucocyte numbers from 0 to 4 hours	0.99	1.05
Changes in lymphocyte numbers from 0 to 4 hours	0.85	0.83
Changes in neutrophil numbers from 0 to 4 hours	0.38	1.42

* Differences were highly significant ($P<.05$).
** Differences were very highly significant ($P<.01$).
*** Degrees of freedom between breeds were 3 and between litters within breeds 11.

TABLE 6--CORRELATIONS BETWEEN POST WEANING GAINS AND BACKFAT THICKNESS AT 200 POUNDS WITH NUMBERS OF LEUCOCYTES BEFORE AND AFTER MECHANICAL STRESS

	Coefficients of correlation			
	Rate of Gain		Backfat	
	Between Breeds	Within Breeds	Between Breeds	Within Breeds
Degrees of Freedom	3	57	3	57
Before Stress:				
Total leucocytes	-.62	.09	-.85	.26
Total lymphocytes	-.44	.25	-.45	.22
Total neutrophils	-.44	-.08	-.70	.22
Four Hours After Stress:				
Total leucocytes	-.60	.04	-.83	.09
Total lymphocytes	-.42	.19	-.52	-.09
Total neutrophils	-.52	-.34**	-.60	-.18
Changes From Zero to Four Hours After Stress:				
Total lymphocytes	-.54	.01	.37	-.27*
Total neutrophils	-.91	-.09	-.58	.06

* Probability of chance occurrence less than .05.
** Probability of chance occurrence less than .01.

DISCUSSION

A short period of mechanical stress resulting from catching pigs by the snout with a pig catcher caused a definite change in the numbers and kinds of leucocytes in the blood stream within four hours. The decline in numbers of lymphocytes per mm^3 of blood and the increase in neutrophils is similar to observations made in laboratory animals by other workers. Dalton and Selye (1939) reported a decrease in lymphocytes and eosinophils and an increase in numbers of neutrophils in the blood of animals subjected to severe exercise.

Daugherty and White (1944) showed that single injections of pituitary adrenotrophic hormone in mice, rabbits, and rats produced, within a few hours, a decrease in total lymphocyte numbers and an increase in the number of polymorphonuclear leucocytes. They concluded that the lymphopenia was a specific response to the pituitary adrenotrophic hormone because it did not occur in adrenalectomized animals treated with the hormone. On the other hand, the poly-morphonuclear response was not specific in that it occurred in adrenalectomized animals given a variety of agents. They concluded that the regulation of the numbers of blood lymphocytes was under pituitary control. Later work reviewed by Gordon (1955) shows that the endocrine system plays an important role in the regulation of the numbers of leucocytes in the blood stream. This

endocrine control is brought about by (1) the influence of the endocrines on the organs which produce the leucocytes, (2) their influence on the distribution of the leucocytes in the tissues, and (3) their influence on the eventual destruction or disposal of the leucocytes within the body.

Breed differences in leucocyte numbers should be largely the result of genetic differences. Therefore, this suggests that there may be a genetic control of the pituitary and adrenal cortical hormones which seem to be responsible for regulating leucocyte numbers. A further study of the data, however, suggests that breed differences observed in leucocyte numbers in this study may not be due to the combined action or interaction of the pituitary and adrenal cortical hormones. The reason for this statement is that breed differences in lymphocyte numbers were not significant, whereas, there were significant differences in neutrophil numbers. As pointed out by Daugherty and White (1944), several factors other than the pituitary and adrenal hormones affect the number of neutrophils in the blood stream of laboratory animals. Thus, breed differences in neutrophil numbers may have been due to factors other than the pituitary and adrenal hormones.

The failure of breeds to react differently to mechanical stress, as reflected in changes in leucocyte numbers, indicates that the response was not under genetic control or that the mechanical stress applied was not severe enough to bring out breed differences that might have existed. A further study using methods of more severe mechanical stress or by using various hormones such as insulin, adrenalin, cortisone or ACTH would be of considerable interest and possibly might show breed differences in changes in leucocyte numbers after the special forms of stress.

Although breed differences in leucocyte numbers were highly significant in this study, no significant litter differences were observed. An explanation of this observation might be that genetic differences between breeds could be more of a non-additive genetic nature involving genes with dominant, overdominant or epistatic effects. Differences in litters could be due largely to additive genetic effects plus those due to certain environmental conditions common to littermates. Although confounded with environment common to littermates, the results suggest that leucocyte numbers are affected very little by additive gene action. On the otherhand, significant breed differences suggest an important non-additive genetic effect on leucocyte numbers.

Although correlations between numbers of leucocytes and rate of gain and backfat thickness were low, some were statistically significant. This shows that a physiological relationship does exist between some of these factors and that a more extensive and detailed study is warranted. Possibly a study of leucocyte numbers in pigs that make extremely fast gains as compared to those which make extremely slow gains would be very fruitful.

SUMMARY

Total and differential leucocyte counts were made on the blood of 61 purebred boars and barrows of the Duroc, Landrace, Poland, and Hampshire breeds before and after a short period of mechanical stress.

The breeds differed significantly in total leucocyte numbers before stress and four hours later. They also differed significantly in number of neutrophils but not in numbers of lymphocytes. Variation in numbers of neutrophils in individuals within the breeds was also much greater than that in numbers of lymphocytes. A further analysis of the data failed to reveal significant differences between litters in either lymphocyte or neutrophil numbers.

A preliminary study of eight boar-barrow littermate pairs from the four differenct breeds showed that the short period of mechanical stress caused a decrease of about 15 percent in lymphocyte numbers and an increase of about 74 percent in neutrophil numbers. In general, the extreme changes in these leucocyte numbers had occurred by four to five hours after the mechanical stress was applied.

Breed or litter differences in changes in lymphocyte or neutrophil numbers caused by the short period of mechanical stress were not statistically significant. Possibly the stress applied was not great enough to bring about extreme reactions as measured by changes in leucocyte numbers.

All coefficients of correlation between leucocyte numbers before and after the short period of mechanical stress and rate of gain from weaning to 200 pounds and backfat thickness at 200 pounds were low. In a few instances, however, they were statistically significant. This indicates a possible physiological relationship between the characters studied.

LITERATURE CITED

Chai, C. K., 1957. Leukopenia: an inherited character in mice. Science, 126, No. 3264, p. 125.

Dalton, A. J. and H. Seyle, 1939. "Blood picture during alarm reaction." Folia Haematology, 62:397-407.

Daugherty, T. F. and A. White, 1944. Influence of hormones on lymphoid tissue structure and function. The role of the pituitary adrenotrophic hormone in the regulation of the lymphocytes and other cellular elements in the blood. Endocrinology, 65: No. 1, pp. 1-14.

Endicott, K. M. and H. Cump, 1947. Hemograms and muelograms of healthy female mice of C-57 brown and CFW strains. Blood, Special Issue, No. 1, pp. 60-63.

Fekete, E., 1941. Histology in biology of the laboratory mouse. (G. D. Snell, Editor) Philadelphia: Blakiston Company, pp. 42-92.

Gordon, A. S., 1955. Some aspects of hormonal influences upon leukocytes. Annals of the N.Y. Academy of Sciences, 59: art. 5, pp. 907-927.

Reich, C. and W. F. Dunning, 1943. Studies on the morphology of the peripheral blood of rats. I. Normal rats. Cancer Research 3:248-257.

Scarborough, Robert A., 1931. The blood picture of normal laboratory animals. Yale Journal of Biol. and Med. 3:267-282.