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Leucocyte Numbers in Normal and Dwarf Beef Cattle Before and After Insulin Injections

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CONTENTS

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Leucocyte Numbers in Normal and Dwarf Beef Cattle Before and After Insulin Injections

C. W. Foley, J. F. Lasley and J. E. Comfort

Semi-lethal genes, such as those which allow the individual to develop to a certain state, or in some cases even to maturity, are of particular interest to the animal physiologist. Genes of this nature in the homozygous recessive state are responsible for dwarf calves of the snorter type.

Determination of the physiological cause of snorter dwarfism in beef cattle would be of great value to cattle producers and to research workers who are interested in problems related to the physiology of gene action. Such knowledge would be useful in devising labortaory methods of identifying carriers of the dwarf gene so they could be culled from our beef cattle population. In addition, it would be helpful in bringing about a better understanding of the physiology of normal individuals.

Results of initial experiments at the Missouri Agricultural Experiment Station (Foley *et al.,* 1957) indicated that, in general, dwarfs were more sensitive to insulin than normal animals. This was demonstrated by a more rapid and greater duration in the reduction of the blood glucose level in dwarfs after insulin injections than in normal animals. These results suggested that the pituitary and/or adrenals were not functioning normally in the dwarf and led to the further study of changes in kinds and numbers of leucocytes in the blood stream after insulin injections (Massey, 1957). The reason for this study was that changes in numbers and kinds of leucocytes in the blood stream after various forms of stress are a measure of the release, or of the action, of hormones from the anterior pituitary and the adrenal cortex (Gordon, 1955). These initial studies indicated that dwarfs were different from the normal individuals as indicated by a slower and smaller change in the neutrophils and lymphocytes after insulin injections.

After these promising initial results, a more detailed study was made of the response of dwarf, carrier and normal beef cattle to a high and a low dosage of insulin administered to each individual at intervals of 48 hours. Results of this study, involving glucose changes after insulin administration, have been reported elsewhere (Foley *et al.,* 1960). This report presents results of the same study related to leucocyte numbers before and after the administration of the two different dosages of insulin.

MATERIALS AND METHODS

Experimental Animals

Data were obtained from 36 different animals. This number included 12 mature pedigree-clean cows, 11 mature cows and one bull which were known carriers of the dwarf gene, and 12 dwarfs. Nine of the 12 dwarfs were considered mature. Two of the dwarfs were Aberdeen Angus; the other 10 were Herefords. The dwarfs were equally divided between the two sexes.

The pedigree-clean cows were from the University of Missouri's breeding herd at Weldon Springs. The carrier and dwarf animals were donated to the project by several breeders throughout the state.

At the time the data were collected from the pedigree-clean animals they were being maintained on native pasture. Approximately one-half of the data obtained from the dwarfs were obtained during the winter months when they were on low quality roughage. They were in poor condition at that time. The remainder of the data collected from the dwarfs were collected during the early summer when the dwarfs had access to green forage. Some of the data were obtained from the carrier animals when they were fed corn silage under dry lot conditions. The remainder of the data on carriers was obtained when they were on pasture.

The Insulin Tolerance Test

The insulin tolerance test was conducted in the following manner. Regular zinc insulin was injected into the jugular vein of the neck at the dosage level of 0.8 unit of insulin per kilogram of body weight. Prior to, and following the injection of insulin, blood was obtained from the animal's jugular vein with a type 16 gauge hypodermic needle. Approximately 10 cubic centimeters of venous blood was collected in a bleeding tube containing either potassium oxalate tablets or EDTA as anticoagulants. On the first day, seven blood samples were collected from each animal at definite time intervals before and after insulin injections. An initial blood sample was obtained prior to the administration of insulin, and this sample was considered as a control. The additional samples were collected at the following time intervals after insulin was administered: 30 minutes, 1, 2, 6, 10, and 12 hours. Forty-eight hours after the initial injection of insulin, the exact procedure was repeated, with one exception. This time, 0.3 unit of insulin per kilogram of body weight was administered to each animal instead of 0.8 unit.

Total and differential leucocyte counts were made on each blood sample. Dry slides were used for the differential counts. Duplicate blood smears were made of each sample of blood within 15 minutes after the sample was collected. At least 200 cells were counted per slide, under oil immersion, and the percentage of each kind of leucocyte was then calculated from the total number.

The method used for making the total white cell counts was the following. A sample of whole blood was diluted with an 0.8 percent solution of hydrochloric acid (9.6 cc. HCl to 500 cc. of distilled water) by means of a white cell pipette. The blood was drawn to the 0.5 mark on the stem of the pipette, and the diluting fluid to the 11.0 mark. This resulted in a dilution of one part of blood in 20 parts of diluting fluid. The blood and diluting fluid were mixed by shaking the pipette for two minutes. A small portion of the mixture was discarded by allowing a few drops to flow from the pipette.

The counting chamber was filled by allowing a small amount of the mixture to seep by capillary action under the cover glass of the counting chamber. The white blood cells were counted under the low power objective in the four corner groups of 16 squares of the hemacytometer

The formula for determining the total number of white blood cells per cubic millimeter of undiluted blood was:

$$\frac{\text{cells counted x 20 (dilution) x 10 (depth)}}{\text{number of square millimeters counted (4)}}$$

In cases when, for some reason, blood samples were missing, the missing data were calculated from data on samples taken in the same animal just before and just after the missing sample.

Data Calculations

The statistical calculations on the data were made on an electronic computor Type E-102, which is operated by the Department of Rural Sociology under the direction of Mr. C. L. Gregory. The statistical calculations consisted primarily of analyses of variance.

EXPERIMENTAL RESULTS

Total leucocyte counts as well as differential counts were made on each blood sample collected; thus results can be summarized according to the response of total leucocyte numbers to insulin injections, as well as the response of the neutrophils, lymphocytes, and eosinophils. Monocytes and basophils made up such a small percentage of the total number of leucocytes that they were not studied separately. Monocytes were included with the lymphocytes and the basophils with the eosinophils. Data for total leucocyte numbers following insulin injecions and for the different kinds of leucocytes will be presented separately .

Total Leucocyte Numbers in Dwarf, Carrier, and Pedigree-Clean Cattle Before and After Insulin Injections

Tables 1 gives total leucocyte numbers in beef cattle of the three genotypes for the dwarf gene, before and after insulin injections of 0.8 unit per kilogram of body weight. Similar data for the same animals after receiving an injection of 0.3 unit per kilogram of body weight are summarized in Table 2. Table 3 gives

TABLE 1-MEANS, STANDARD DEVIATIONS AND COEFFICIENTS OF VARIATION FOR TOTAL NUMBERS OF LEUCOCYTES PER mm^3 OF BLOOD IN DWARF, CARRIER AND PEDIGREE-CLEAN BEEF CATTLE BEFORE AND AFTER INJECTIONS OF 0.8 UNIT OF INSULIN PER KILOGRAM OF BODY WEIGHT

Time After Insulin	Dwarf		Carrier		Pedigree-Clean	
	Mean ± S.D.	C.V. %	Mean ± S.D.	C.V. %	Mean ± S.D.	C.V. %
Initial	9017 ± 3259	36	7659 ± 2104	27	7963 ± 1820	23
0.5 hour	7534 ± 2240	30	6809 ± 2856	42	7767 ± 1736	22
1.0 hour	7996 ± 1999	25	8000 ± 2288	29	8138 ± 1826	22
2.0 hours	9013 ± 3295	37	9371 ± 3593	38	9817 ± 1371	14
6.0 hours	11609 ± 4144	36	11763 ± 3102	26	11779 ± 2017	17
10.0 hours	11384 ± 4045	36	11213 ± 2396	21	12067 ± 3297	27
12.0 hours	11596 ± 3546	31	10434 ± 2439	23	11138 ± 2369	21

TABLE 2-MEANS, STANDARD DEVIATIONS AND COEFFICIENTS OF VARIATION FOR TOTAL NUMBERS OF LEUCOCYTES PER mm^3 OF BLOOD IN DWARF, CARRIER AND PEDIGREE-CLEAN BEEF CATTLE BEFORE AND AFTER INJECTIONS OF 0.3 UNIT OF INSULIN PER KILOGRAM OF BODY WEIGHT

Time After Insulin Injection	Dwarf		Carrier		Pedigree-Clean	
	Mean ± S.D.	C.V. %	Mean ± S.D.	C.V. %	Mean ± S.D.	C.V. %
Initial	9600 ± 3777	39	8438 ± 2085	25	7759 ± 1674	22
0.5 hour	7638 ± 3218	55	6334 ± 1585	25	7825 ± 2075	27
1.0 hour	7563 ± 3461	46	7588 ± 1463	19	9084 ± 2535	28
2.0 hours	7671 ± 2554	33	9521 ± 1592	17	10117 ± 2925	29
6.0 hours	8650 ± 4263	49	10459 ± 2086	20	9446 ± 2771	29
10.0 hours	9071 ± 4614	51	10138 ± 3074	30	9359 ± 2411	26
12.0 hours	8046 ± 5057	63	9663 ± 2227	23	9171 ± 3905	43

TABLE 3-ANALYSIS OF VARIANCE FOR TOTAL LEUCOCYTE DIFFERENCES BETWEEN GENOTYPES

Source	M.S.	D.F.	F Ratio
Genotype	2443	2	.98N.S.
Time intervals	47622	6	19.03***
Dosage	40932	1	16.36***
Genotype x time	3284	12	1.31N.S.
Genotype x dosage	4005	2	1.60N.S.
Time x dosage	9500	6	3.80***
Genotype x time x dosage	1409	12	.56N.S.
Within	2502	462	
Total		503	

***Probability of chance occurrence less than .005
N.S. Differences not significant.

the analysis of variance of the data from blood samples following the two dosage levels of insulin.

As shown in Table 3, cattle of the three different genotypes did not differ significantly in the total numbers of leucocytes when all data were combined for the analysis. At the higher dosage level, the response was almost identical for all three genotypes. This is illustrated more clearly in Figure 1. When the smaller dosage of insulin was administered to the same animals 48 hours after the first and heavier dosage, there was still no significant difference among responses of cattle of the three genotypes. Figure 2 shows, however, that there was more variation between genotypes after the second insulin injection, with the dwarfs showing the smallest number of leucocytes per mm^3 of blood.

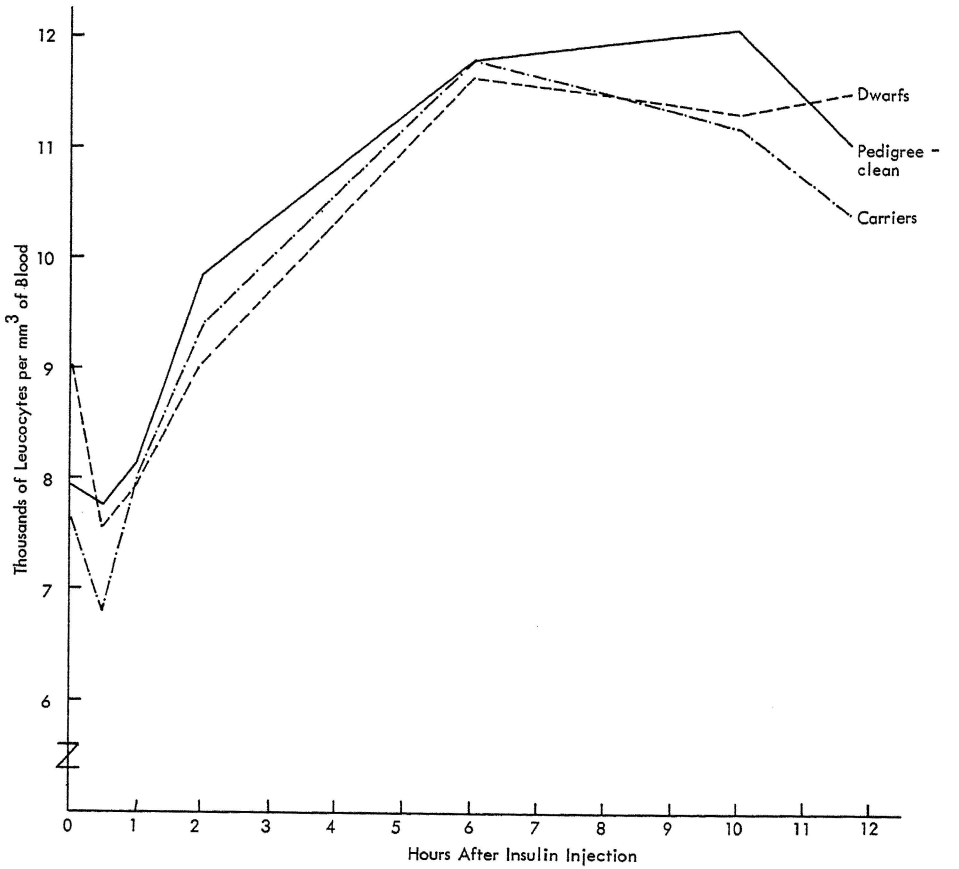

Figure 1. The total number of leucocytes per mm^3 of blood in dwarfs, carriers and pedigree-clean beef cattle before and after an intraveneous injection of .8 unit of insulin per kilogram body weight.

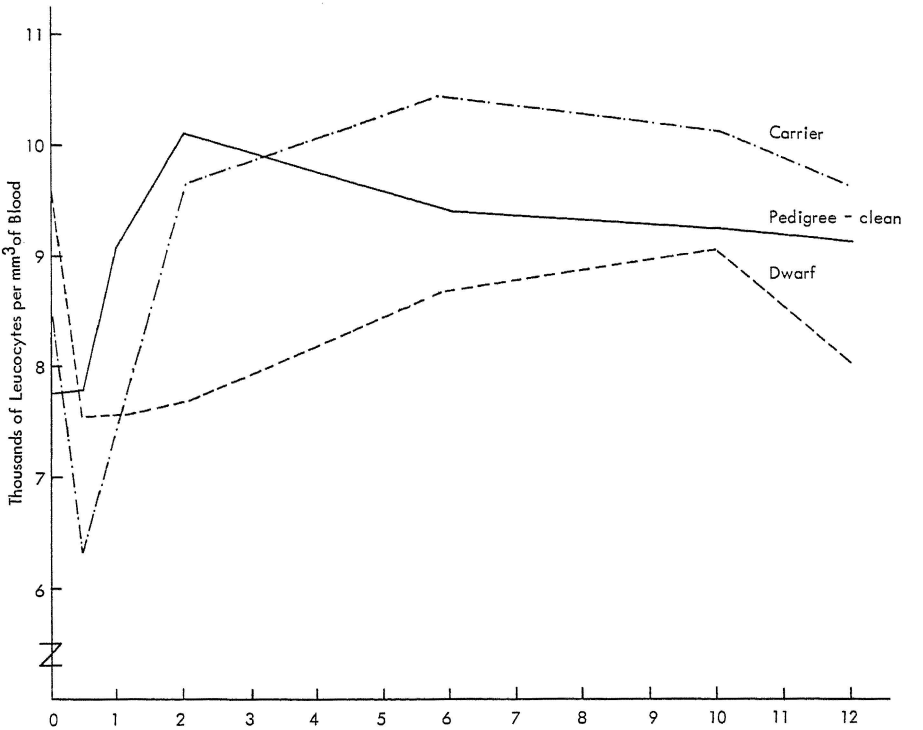

Figure 2. The total number of leucocytes per mm^3 of blood in dwarfs, carrier and pedigree-clean beef cattle before and after an intravenous injection of .3 unit of insulin per kilogram of body weight (48 hours after an injection of .8 unit of insulin).

Dwarfs varied more in total leucocyte numbers than did pedigree-clean and carrier animals, as shown by the size of the coefficients of variation in Tables 1 and 2. Carriers varied slightly more than pedigree-clean animals in leucocyte numbers after the large dosage of insulin but not after the second and smaller dosage of the hormone. Dwarfs showed a still further increase in variation in leucocyte numbers after the second hormone injection. This may have been due to the physiological mechanism responsible for maintaining leucocyte numbers within the normal range being inadequate in some dwarfs and more nearly adequate in others.

As a group, the animals showed a definite change in total leucocyte numbers following insulin injections. This change was highly significant ($P < .005$) as shown in Table 3. However, animals of the three different genotypes did not differ significantly in changes in leucocyte numbers, as indicated by the non-significant genotype x time interval interaction, also shown in Table 3. Both the dwarfs and carriers showed a definite drop in total leucocyte numbers within one-half hour after insulin was injected, regardless of whether they received the large or the small dosage of insulin. After receiving the high dosage of the hor-

mone, leucocyte numbers in the pedigree-clean animals decreased by only 2.5 percent and after the low dosage the leucocytes actually increased 1 percent. Leucocyte numbers decreased 16.4 percent in dwarfs and 11.1 percent in carriers after the large dosage of insulin, and 20.4 percent in dwarfs and 25 percent in carriers after the low dosage of insulin. Analysis of variance, however, showed the only significant drop in leucocyte numbers was in the carriers, one-half hour after they had received the lower dosage of insulin.

One hour after insulin injections, some increase had occurred in total leucocyte numbers in all genotypes, with the least rapid recovery being observed in the dwarfs. The peak in leucocyte numbers following the heavy dosage of insulin was reached between the sixth and tenth hours following insulin injections, with the total leucocyte numbers in all genotypes ranging from 11,609 to 12,067 or representing increases from 28.7 to 53.5 percent over the initial levels. No significant differences were noticed between the three genotypes in the levels of increase. Percentagewise, however, the increase over the initial level was smallest in the dwarfs.

After the second injection of a smaller dosage of insulin, which was administered 48 hours after the first, heavy dosage (Table 2), the number of leucocytes in the blood of dwarfs never returned to the initial level from the initial drop. The initial level was reached again and surpassed in the carriers two hours after insulin was injected. This was followed by a later peak at six hours. Pedigree-clean animals did not show a drop in leucocyte numbers following the second insulin injection and reached a peak of 10,117 cells per mm^3 of blood two hours after the hormone injection.

The reason for the initial drop in leucocyte numbers following insulin injections is not fully understood and probably warrants further study. The difference between carriers and pedigree-clean animals does not seem great enough to suggest it would be of value in identifying carriers of the dwarf gene. From the physiological standpoint, however, it is of considerable interest. Possibly the leucocytes of dwarfs and carriers were altered in some way after insulin injections so that they became more susceptible to hemolysis than the leucocytes of pedigree-clean individuals. Or, there may have been some sort of a direct action of insulin upon the leucocytes which caused them to hemolyze and disperse more readily. It is also possible that the decrease in leucocyte numbers could be due to an increase in blood volume or the cells could have been taken out of circulation by some physiological mechanism.

The analysis of variance in Table 3 shows clearly that the dosage of insulin had an important influence on the change in leucocyte numbers following insulin injection. This is further demonstrated in Figures 3, 4, and 5. In all three genotypes, the larger dosage of 0.8 unit of insulin per kilogram of body weight caused a larger increase in the total leucocyte number than did the smaller dosage. This difference in response to the two different dosages of insulin might have been different if the hormone had been administered at longer time inter-

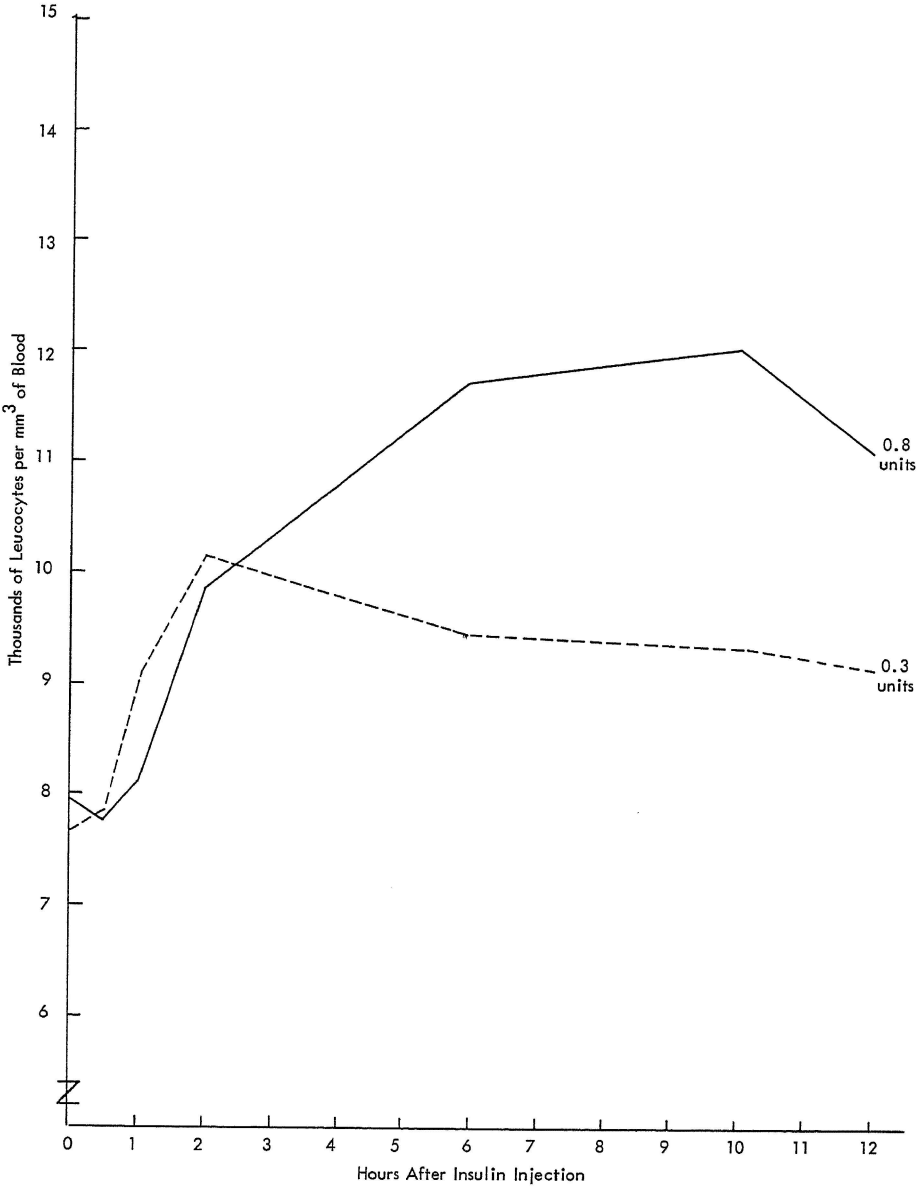

Figure 3. Total numbers of leucocytes per mm^3 of blood in pedigree-clean beef cattle before and after injections of 0.8 and 0.3 units of insulin 48 hours apart in the same animals.

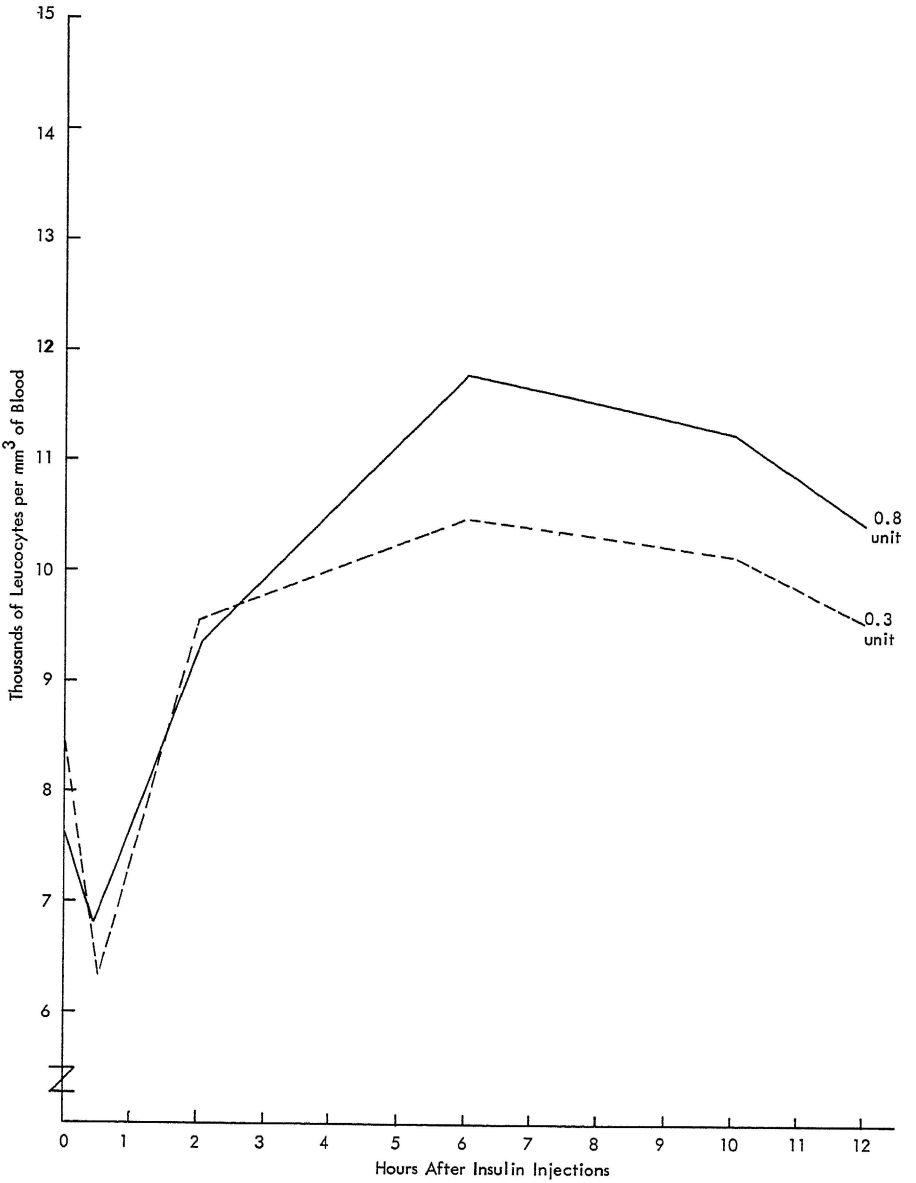

Figure 4. Total leucocyte numbers per mm^3 of blood in carrier beef animals before and after injections of 0.8 and 0.3 unit of insulin 48 hours apart in the same animals.

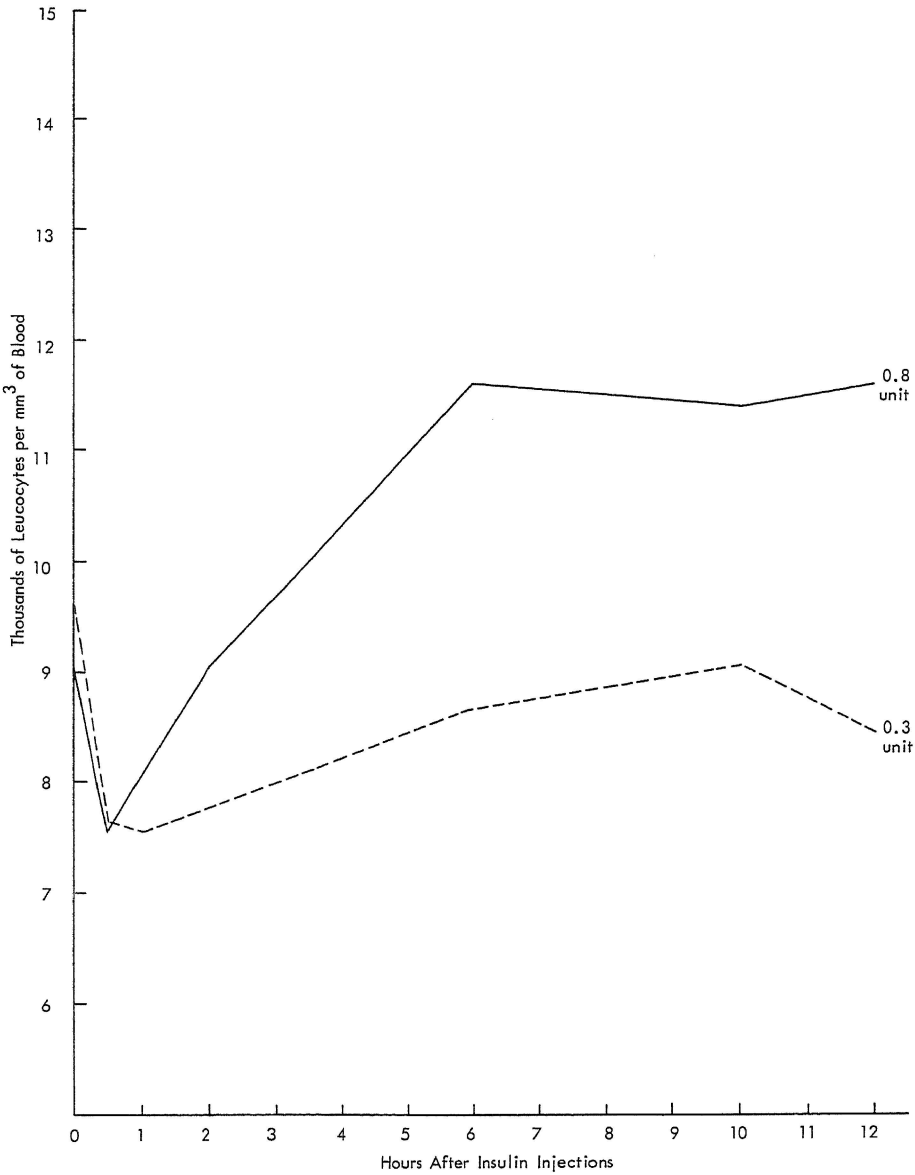

Figure 5. Total leucocyte numbers per mm^3 of blood in dwarf beef animals before and after injections of 0.8 and 0.3 unit of insulin 48 hours apart in the same animals.

vals. An experiment designed specifically to study the response of leucocyte numbers to a larger range of dosages with greater time intervals between injections in the same animals should be of considerable interest.

Numbers of *Lymphocytes* Before and After Insulin Injections

The mean percentages of lymphocytes for cattle of the three different genotypes are shown in Tables 4 and 5. The blood of dwarfs contained a larger per-

TABLE 4-MEAN PERCENTAGE VALUES OF LYMPHOCYTES FOR PEDIGREE-CLEAN CATTLE, CARRIERS AND DWARFS FOLLOWING INJECTIONS OF 0.8 UNIT OF INSULIN

	% Lymphocytes in the blood					
	Clean		Carrier		Dwarf	
Time	Actual	% of Initial Sample	Actual	% of Initial Sample	Actual	% of Initial Sample
Initial	54.47	100.00	66.26	100.00	74.51	100.00
0.5 hour	51.31	94.00	64.39	97.18	67.06	90.00
1.0 hour	49.99	92.00	61.76	93.21	66.28	88.95
2.0 hours	42.15	77.38	58.37	88.09	64.02	85.92
6.0 hours	38.87	71.36	49.37	74.51	58.77	78.88
10.0 hours	37.49	68.83	46.89	70.77	63.91	85.77
12.0 hours	39.72	72.92	51.34	77.48	66.04	88.63

TABLE 5-MEAN PERCENTAGE VALUES OF LYMPHOCYTES FOR PEDIGREE-CLEAN CATTLE, CARRIERS AND DWARFS FOLLOWING INJECTIONS OF 0.3 UNIT OF INSULIN 48 HOURS AFTER AN INJECTION OF 0.8 UNIT PER KILLOGRAM BODY WEIGHT

	% Lymphocytes in the Blood					
	Clean		Carrier		Dwarf	
Time	Actual	% of Initial Sample	Actual	% of Initial Sample	Actual	% of Initial Sample
Initial	55.40	100.00	62.98	100.00	77.13	100.00
0.5 hour	59.78	107.91	59.43	94.36	72.17	93.57
1.0 hour	56.99	102.87	60.00	95.27	70.86	91.87
2.0 hours	41.17	74.30	57.93	91.98	70.01	90.77
6.0 hours	45.19	81.57	46.16	73.29	65.34	84.71
10.0 hours	47.90	86.46	49.16	78.06	75.29	97.61
12.0 hours	48.19	86.99	57.84	91.84	72.95	94.58

centage of lymphocytes than did the blood of pedigree-clean cattle; carriers were intermediate in this respect. In general, there was a reduction in the percentage of lymphocytes following insulin injections but there was a greater reduction in both pedigree-clean and carrier animals than in dwarfs.

The decrease in the percentage of lymphocytes after insulin was almost as great in carriers as in pedigree-clean animals, although a decrease seemed to take place more rapidly in animals of the latter genotype. When the second, smaller dosage of insulin was given (0.3 unit per kilogram body weight), pedigree-clean

animals actually showed a rise in the percentage of lymphocytes at one-half and one hour after the injection. A drop in lymphocytes to 74.3 percent of the initial sample had occurred at the end of two hours. The lowest percentage of lymphocytes occurred in the blood of carriers and dwarfs six hours after the hormone was administered.

The total or absolute number of lymphocytes per mm^3 of blood was calculated for each blood sample by multiplying the total leucocyte count by the percentage of lymphocytes determined from the differential count. Tables 6 and 7 give the lymphocyte totals; results of the analysis of variance are shown in Table 8.

TABLE 6.-MEANS, STANDARD DEVIATIONS AND COEFFICIENTS OF VARIATION FOR TOTAL NUMBERS OF LYMPHOCYTES PER mm^3 OF BLOOD IN DWARF; CARRIER AND PEDIGREE-CLEAN BEEF CATTLE BEFORE AND AFTER INJECTIONS OF 0.8 UNIT OF INSULIN PER KILOGRAM OF BODY WEIGHT

Time After Insulin Injection	Dwarf		Carrier		Pedigree-Clean	
	Mean ± S.D.	C.V. %	Mean ± S.D.	C.V. %	Mean ± S.D.	C.V. %
Initial	6704 ± 2471	37	5071 ± 1767	35	4209 ± 1072	25
0.5 hour	5025 ± 1658	32	4417 ± 2129	48	3900 ± 958	25
1.0 hour	5267 ± 1426	27	4934 ± 1862	38	4129 ± 1606	39
2.0 hours	6088 ± 2642	43	5025 ± 1706	34	4192 ± 1149	27
6.0 hours	6746 ± 2662	39	5625 ± 1428	25	4550 ± 1055	23
10.0 hours	7142 ± 2294	32	5229 ± 1402	27	4475 ± 1597	36
12.0 hours	7804 ± 2720	35	5304 ± 1657	31	4246 ± 919	22

TABLE 7-MEANS, STANDARD DEVIATIONS AND COEFFICIENTS OF VARIATION FOR TOTAL NUMBERS OF LYMPHOCYTES PER mm^3 OF BLOOD IN DWARF, CARRIER AND PEDIGREE-CLEAN BEEF CATTLE BEFORE AND AFTER INJECTIONS OF 0.3 UNIT OF INSULIN PER KILOGRAM OF BODY WEIGHT

Time After Insulin Injection	Dwarf		Carrier		Pedigree-Clean	
	Mean ± S.D.	C.V.%	Mean ± S.D.	C.V. %	Mean ± S.D.	C.V. %
Insulin	7238 ± 2145	30	5129 ± 1282	25	4196 ± 942	22
0.5 hour	5792 ± 2387	41	3650 ± 857	23	4609 ± 1728	37
1.0 hour	5338 ± 2196	41	4421 ± 556	13	4975 ± 890	18
2.0 hours	5367 ± 1602	30	5550 ± 1655	30	4113 ± 1219	30
6.0 hours	5542 ± 2522	46	4775 ± 1093	23	4238 ± 940	22
10.0 hours	6563 ± 2929	45	4638 ± 976	21	4459 ± 1190	27
12.0 hours	5759 ± 3662	64	4979 ± 1055	21	4492 ± 2546	57

Dwarfs had the largest number of lymphocytes in their blood stream and the pedigree-clean animals the least, with the carriers intermediate. The overall difference between genotypes in lymphocyte numbers was highly significant ($P<.01$) and there was a difference ($P<.05$) between lymphocyte numbers at the various time intervals following insulin injections. No significant difference

TABLE 8-ANALYSIS OF VARIANCE FOR TOTAL LYMPHOCYTE NUMBERS IN THE BLOOD OF DWARF, CARRIER AND PEDIGREE-CLEAN ANIMALS

Source	M.S.	D.F.	F. Ratio
Genotype	51197	2	35.801**
Time intervals	3209	6	2.243*
Dosage	2010	1	1.400N.S.
Genotype x time intervals	1897	12	1.320N.S.
Genotype x dosage	1816	2	1.269N.S.
Time x dosage	1499	6	1.040N.S.
Genotype x time x dosage	704	12	.492N.S.
Within	1403	462	
Total		503	

* Probability of chance occurrence less than .05.
** Probability of chance occurrence less than .01.
N.S.Differences not significant.

was found between the two dosages of insulin nor were any of the interactions significant.

Because none of the interactions were significant, the data concerning differences between genotypes were studied in more detail. Tukey's range test was used to determine the least significant difference required between genotypes ($P<.05$). It was found that a significant difference existed between all three genotypes in the total number of lymphocytes following the higher dosage of insulin. When the smaller dosage was given, dwarfs differed from the pedigree-clean and the carrier animals but there was no significant difference between the two latter genotypes.

The number of lymphocytes present in the initial blood sample of pedigree-clean individuals was 4,209 mm^3. One-half hour following the administration of 0.8 unit of insulin per kilogram of body weight, a slight decrease in the number of lymphocytes had occurred. Within one hour following the insulin injection a noticeable increase in lymphocytes over the level one-half hour after insulin was administered was observed. This increase continued for at least ten hours, surpassing the initial level at the end of six hours. At the lower dosage of insulin, the lymphocyte number in the initial blood sample comparable to the initial blood sample taken 4 hours earlier before the heavy dosage of insulin was given. After the lower dosage of insulin, however, a lymphocytosis occurred for about an hour, then a fluctuation in lymphocyte numbers was observed.

The response of the lymphocytes to insulin observed in the carriers was similar to that observed in the pedigree-clean animals. This appeared to be true after both dosages of insulin. One difference, however, was that the initial number of circulating lymphocytes was greater in the carrier than in pedigree-clean animals and remained higher throughout the test period of 60 hours.

The response of lymphocytes to insulin was somewhat different in dwarfs than in carrier and pedigree-clean animals. A lymphopenia occurred in dwarfs but the return in the number of lymphocytes to that found in the initial blood

samples occurred more slowly. At the lower dosage of insulin, the lymphopenia existed for the entire twelve-hour period considered.

Numbers of *Neutrophils* After Insulin Injections

Table 9 shows percentages of neutrophils before and after the injection of 0.8 unit of insulin in the blood of animals of the three different genotypes. The

TABLE 9-MEAN PERCENTAGE OF NEUTROPHILS FOR PEDIGREE-CLEAN CATTLE, CARRIERS AND DWARFS FOLLOWING INJECTIONS OF 0.8 UNIT OF INSULIN

	% neutrophils in the blood					
	Clean		Carrier		Dwarf	
Time After Insulin Injection	Actual	% of Inital Sample	Actual	% of Initial Sample	Actual	% of Initial Sample
Initial	28.24	100.00	25.83	100.00	20.33	100.00
0.5 hour	34.06	120.61	29.20	113.05	26.21	128.92
1.0 hour	36.88	130.59	31.63	122.45	28.60	140.67
2.0 hours	48.11	170.36	35.95	139.18	31.21	153.52
6.0 hours	52.52	185.98	45.70	176.92	37.93	186.57
10.0 hours	55.81	197.63	49.91	193.22	34.86	171.47
12.0 hours	54.22	192.00	44.72	173.13	31.45	154.70
Average	44.26	166.20	37.56	152.99	30.08	155.98

percentage of these cells increased in all genotypes, beginning with the first blood sample, taken 30 minutes after insulin injection, and continuing to increase for several hours after the hormone was administered. Greatest increase in percentage of neutrophils was reached in the pedigree-clean animals 10 hours after insulin was injected; this increase was 197.63 percent of the initial level. The carriers also reached a peak of 193.22 percent of the initial level at the tenth hour, whereas the dwarfs reached their peak of 186.57 at the sixth hour following administration of the hormone. The dwarfs, however, did not show as high or as sustained an increase as the pedigree-clean or the carrier animals.

Table 10 shows percentages of neutrophils for animals of the three different genotypes after the second injection (0.3 unit of insulin per kilogram of body weight within 48 hours of the first and larger injection). Animals of all genotypes again showed an increase in neutrophil numbers, beginning with the blood sample taken 30 minutes after the insulin was injected. The degree of increase and the sustained period of increase were not as great, as a general rule, as that observed after the administration of the first (large) dosage of the hormone. The peak increase for the pedigree-clean animals was again reached at 10 hours after insulin injection with an increase over the initial level of 187.82 percent. In the carriers, the peak was reached at six hours at 184.07 percent of the initial level, whereas in the dwarfs it was reached at six hours and was 170.19 percent of the initial reading.

TABLE 10-MEAN PERCENTAGE VALUES OF NEUTROPHILS FOR PEDIGREE-CLEAN CATTLE, CARRIERS AND DWARFS FOLLOWING INJECTIONS OF 0.3 UNIT OF INSULIN 48 HOURS AFTER AN INJECTION OF 0.8 UNIT PER KILOGRAM BODY WEIGHT

Time After Insulin Injection	Percent Neutrophils in the Blood					
	Pedigree-clean		Carriers		Dwarfs	
	Actual	% of Initial Sample	Actual	% of Initial Sample	Actual	% of Initial Sample
Initial	22.82	100.00	26.05	100.00	17.88	100.00
0.5	23.27	101.97	29.89	114.74	21.35	119.41
1.0 hour	26.58	116.48	30.49	117.04	22.47	125.67
2.0 hours	42.22	185.01	35.22	135.20	24.48	136.91
6.0 hours	42.44	185.98	47.95	184.07	30.43	170.19
10.0 hours	42.86	187.82	46.07	176.85	21.27	118.96
12.0 hours	39.72	174.06	45.55	174.86	24.42	136.58
Average	34.27	158.55	37.32	150.46	23.19	134.62

The total or absolute number of neutrophils was determined in each blood sample, as was the number of lymphocytes, by multiplying the total number of leucocytes per mm^3 of blood by the percentage of neutrophils as determined from the differential count. The mean numbers of neutrophils per mm^3 of blood, together with the standard deviations and coefficients of variation for the groups at each bleeding, are presented in Tables 11 and 12. In contrast to the lymphocytes which decreased in numbers following insulin injections, the neutrophils showed a rise in numbers in all of the groups studied. This is similar to changes in neutrophils observed by other workers after injections of hormones or after various other forms of stress. Dalton and Selye (1939) reported an increase in the number of neutrophils in animals after severe exercise. Daugherty and White (1944), working with mice, rats, and rabbits noticed a decrease in the total number of lymphocytes and an increase in the number of polymorphonuclear leucocytes following a single injection of pituitary adrenotrophic hormone.

TABLE 11-MEANS, STANDARD DEVIATIONS AND COEFFICIENTS OF VARIATION FOR TOTAL NUMBERS OF NEUTROPHILS PER mm^3 OF BLOOD IN DWARF, CARRIER AND PEDIGREE-CLEAN BEEF CATTLE BEFORE AND AFTER INJECTIONS OF 0.8 UNIT OF INSULIN PER KILOGRAM OF BODY WEIGHT

Time After Insulin Injection	Dwarf		Carrier		Pedigree-Clean	
	Mean ± S.D.	C.V.%	Mean ± S.D.	C.V.%	Mean ± S.D.	C.V.%
Initial	1879 ± 1044	56	1967 ± 1148	58	2234 ± 823	37
0.5 hour	2034 ± 906	45	1888 ± 968	51	2567 ± 750	29
1.0 hour	2967 ± 1966	66	2542 ± 1181	46	2959 ± 1187	40
2.0 hours	2804 ± 1270	45	3309 ± 1547	47	4704 ± 840	18
6.0 hours	4525 ± 2146	47	5542 ± 2221	40	6221 ± 1482	24
10.0 hours	4063 ± 1744	43	5625 ± 1679	30	6809 ± 2893	42
12.0 hours	3809 ± 1993	52	4713 ± 1902	40	6067 ± 1733	29

TABLE 12-MEANS, STANDARD DEVIATIONS AND COEFFICIENTS OF VARIATION FOR TOTAL NUMBERS OF NEUTROPHILS PER mm^3 OF BLOOD IN DWARF, CARRIER AND PEDIGREE-CLEAN BEEF CATTLE BEFORE AND AFTER INJECTIONS OF 0.3 UNIT OF INSULIN PER KILOGRAM OF BODY WEIGHT*

Time After Insulin Injection	Dwarf		Carrier		Pedigree-Clean	
	Mean ± S.D.	C.V. %	Mean ± S.D.	C.V.%	Mean ± S.D.	C.V. %
Initial	1896 ± 1413	75	2375 ± 1208	51	1654 ± 591	36
0.5 hour	1596 ± 803	50	2000 ± 924	46	1846 ± 1054	57
1.0 hour	1738 ± 1379	79	2492 ± 1239	50	2313 ± 955	41
2.0 hours	1967 ± 1241	63	3584 ± 1601	45	4321 ± 1580	37
6.0 hours	2825 ± 1984	70	5059 ± 1418	28	4184 ± 1705	41
10.0 hours	2363 ± 1782	75	4963 ± 2111	43	4004 ± 1174	29
12.0 hours	2163 ± 1703	79	4267 ± 1692	40	3559 ± 1383	39

*This injection of insulin given to the same animals 48 hours after the injection of 0.8 unit per kilogram of body weight.

The analysis of variance for numbers of neutrophils per mm^3 of blood before and after two different dosages of insulin is summarized in Table 13. This analysis showed a highly significant difference ($P<.01$) between genotypes, time intervals after insulin injections, and different dosages of insulin administered, as far as neutrophil numbers were concerned. In addition, a highly significant ($P<.01$) genotype x time interaction and a significant ($P<.05$) genotype x dosage interaction were observed. According to Snedecor (1956), significant interactions, such as observed in this portion of the study, mean that less attention can be paid to significant differences between the main effects such as genotype, time interval, and dosage levels.

TABLE 13-ANALYSIS OF VARIANCE FOR NEUTROPHIL DIFFERENCES BETWEEN GENOTYPES

Source	M.S.	D.F.	F. Ratio
Genotype	27500	2	29.98**
Time	39693	6	43.27**
Dosage	40822	1	44.50**
Genotype x time	2820	12	3.07**
Genotype x dosage	6259	2	6.82*
Time x dosage	3229	6	3.52N.S.
Genotype x time x dosage	386	12	.42N.S.
Within	917	462	

* Probability of chance occurrence less than .05.
** Probability of chance occurrence less than .01.
N.S. Differences not significant.

The average numbers of neutrophils per mm^3 of blood at different times after insulin injection and after two different dosages of insulin are presented in Figures 6 and 7. After a heavy dosage of insulin the increase in neutrophil numbers was more rapid and greater in the pedigree-clean animals and was slower and smaller in the dwarfs. Carrier animals were intermediate in this respect. At

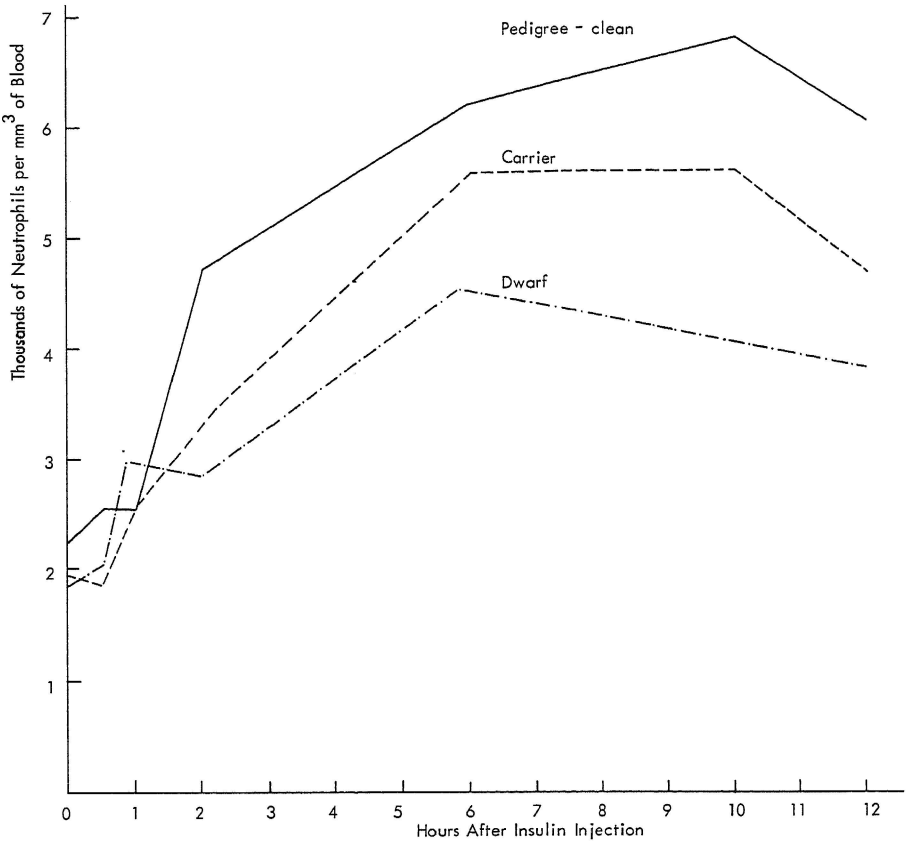

Figure 6. Number of neutrophils in dwarf, carriers and normal beef animals before and after injections of .8 unit of insulin per kilogram of body weight.

the dosage of 0.3 unit of insulin per kilogram of body weight, administered 48 hours after the heavier dosage of 0.8 unit of insulin, the increase in neutrophil numbers was again greater in pedigree-clean individuals up to two hours after insulin was injected but declined to a level below that of carriers six hours after the hormone was administered. Dwarfs again showed a much smaller rise in neutrophil numbers than was noticed after the larger dosage of insulin (Figure 6).

The analysis of variance showed that the heavier dosage of insulin (0.8 unit) caused a greater rise of neutrophil numbers than the smaller dosage. As shown in Figures 6 and 7 the heavier dosage of insulin also seemed to bring about a larger difference between pedigree-clean and carrier individuals than the lighter dosage. This suggests that further experiments should be conducted in which large dosages of insulin are administered to animals of the two genotypes for the purpose of identification of carriers.

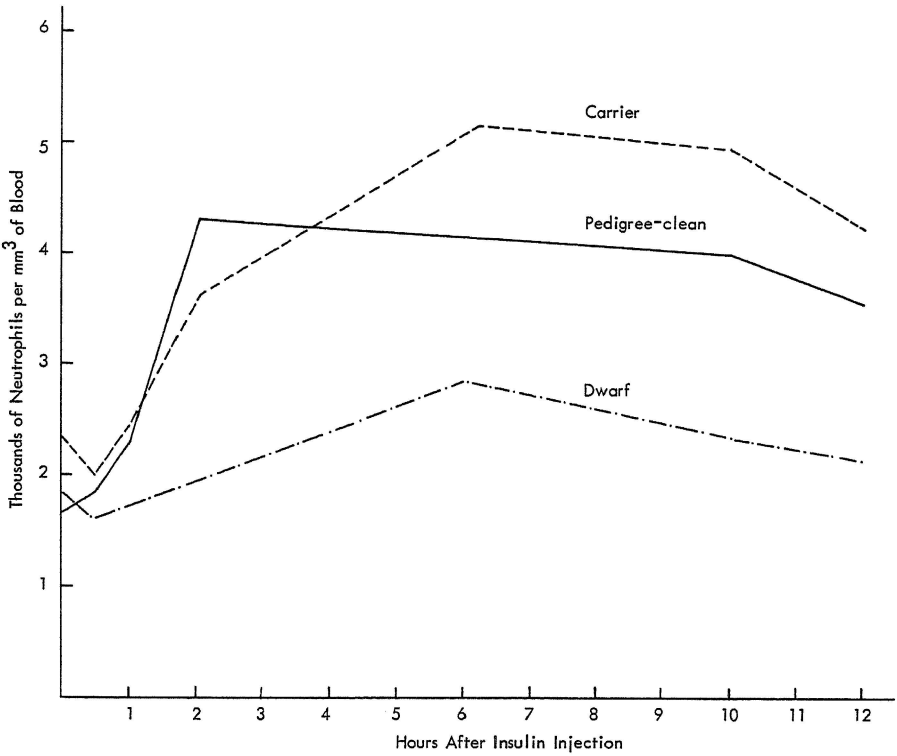

Figure 7. Number of neutrophils in dwarf, carrier and normal beef animals before and after intravenous injections of .3 units of insulin per kilogram of body weight.

The significant interaction between genotypes and time intervals after insulin injection ($P<.01$) shows that the three genotypes differed in number of neutrophils per mm^3 of blood at different times after the administration of the hormone. This was previously observed by Massey (1957). In Figures 6 and 7, the pedigree-clean animals show a more rapid increase in neutrophil numbers at two hours following insulin injections than do the carriers or the dwarfs.

The significant interaction between genotypes and insulin dosage ($P<.05$) shows that the genotypes differed in their response to the high and the low dosage of insulin. These results also suggest that a heavier dosage of insulin, or possibly some other hormone or stressing agent that had a more severe effect, might give a greater difference between the genotypes in neutrophil numbers. The fact that the dwarfs were less able to respond to the insulin than the carriers and the pedigree-clean animals indicates that their homeostatic mechanism, responsible for maintaining neutrophil numbers at a certain level, is abnormal. This same conclusion was reached with the same animals in a study of glucose content of the blood following injections of these two different dosages of insulin, reported by Foley *et al.* (1960).

Numbers of Eosinophils Before and After Insulin Injections

Table 14 shows percentages of eosinophils determined from the different counts in blood samples of dwarfs, carriers, and pedigree-clean animals after an injection of 0.8 unit of insulin per kilogram of body weight. Eosinophils in all

TABLE 14-MEAN PERCENTAGE VALUES OF EOSINOPHILS FOR PEDIGREE-CLEAN CATTLE, CARRIERS AND DWARFS FOLLOWING INJECTIONS OF 0.8 UNIT OF INSULIN PER KILOGRAM OF BODY WEIGHT

Time After Insulin Injection	Percent of Eosinophils in the Blood					
	Pedigree-Clean		Carriers		Dwarfs	
	Actual	% of Initial Sample	Actual	% of Initial Sample	Actual	% of Initial Sample
Initial	17.27	100.00	7.89	100.00	5.14	100.00
0.5 hour	14.61	84.60	6.89	87.33	6.70	130.35
1.0 hour	13.11	75.91	6.59	83.52	5.11	99.42
2.0 hours	9.72	56.28	5.66	71.74	4.75	92.41
6.0 hours	8.59	49.74	4.92	62.36	3.29	64.01
10.0 hours	6.68	38.68	3.19	40.43	1.62	31.52
12.0 hours	6.04	34.97	3.92	49.68	2.49	48.44
Average	8.23	56.70	5.58	65.84	4.16	77.69

three genotypes showed a gradual decline in percentages during the period of observation after insulin was administered. In pedigree-clean and carrier animals this decline began in the blood sample collected 30 minutes after insulin was administered, but in the dwarfs the percentage increased at 30 minutes and then showed a small decline at one hour. In general, the decline in eosinophil numbers occurred more rapidly in the pedigree-clean animals than in either the carriers or the dwarfs. The overall response was greatest in animals free of the dwarf gene, least in the dwarfs, and intermediate in the carriers.

The percentages of eosinophils in animals of the three different genotypes, after a second injection of 0.3 unit of insulin per kilogram of body weight given 48 hours after the first dosage, are shown in Table 15. In general, the decline in

TABLE 15-MEAN PERCENTAGE VALUES OF EOSINOPHILS FOR PEDIGREE-CLEAN CATTLE, CARRIER AND DWARFS FOLLOWING INJECTIONS OF 0.3 UNIT OF INSULIN PER KILOGRAM OF BODY WEIGHT FOLLOWING WITHIN 48 HOURS OF AN INJECTION OF 0.8 UNIT PER KILOGRAM OF BODY WEIGHT

Time After Insulin Injection	Pedigree-clean		Carriers		Dwarfs	
	Actual	% of Initial Sample	Actual	% of Initial Sample	Actual	% of Initial Sample
Initial	21.77	100.00	10.95	100.00	4.96	100.00
0.5 hour	16.92	77.72	10.67	97.44	6.46	130.24
1.0 hour	16.42	75.42	9.49	86.67	6.65	134.07
2.0 hours	16.59	76.21	6.83	62.37	5.48	110.48
6.0 hours	12.35	56.72	5.86	53.52	4.21	84.86
10.0 hours	9.95	45.71	4.75	43.38	3.42	68.95
12.0 hours	12.05	55.35	4.92	44.93	2.60	52.42
Average	15.15	64.52	7.64	66.39	4.83	96.84

eosinophil percentages was not as great, especially in the dwarfs, as it was after injection of the first (and larger) dosage of the hormone. All blood samples in the pedigree-clean and carrier animals showed a decline in eosinophil percentages after the insulin was injected but this decline occurred sooner in those which were pedigree-clean. Dwarfs again showed an increase in eosinophil numbers following the insulin injection. This increase lasted for at least two hours, which was longer than it lasted after the first injection of the hormone.

The total number of eosinophils was also calculated, as were the numbers of lymphocytes and neutrophils, by multiplying the total number of leucocytes per mm^3 of blood by the percentage of eosinophils determined from the differential count. Tables 16 and 17 show mean numbers of eosinophils in animals of the three genotypes at each period before and after two different dosages of

TABLE 16-MEANS, STANDARD DEVIATIONS AND COEFFICIENTS OF VARIATION FOR TOTAL NUMBERS OF EOSINOPHILS PER mm^3 OF BLOOD IN DWARF, CARRIER AND PEDIGREE-CLEAN BEEF CATTLE BEFORE AND AFTER INJECTIONS OF 0.8 UNIT OF INSULIN PER KILOGRAM OF BODY WEIGHT

Time After Insulin Injection	Dwarf		Carrier		Pedigree-clean	
	Mean ± S.D.	C.V.%	Mean ± S.D.	C.V.%	Mean ± S.D.	C.V.%
Initial	438 ± 218	50	609 ± 450	74	1284 ± 621	48
0.5 hour	475 ± 318	67	496 ± 296	60	1146 ± 639	56
1.0 hour	413 ± 303	73	529 ± 405	77	1046 ± 660	63
2.0 hours	442 ± 414	94	550 ± 391	72	929 ± 539	58
6.0 hours	354 ± 267	75	600 ± 411	69	1004 ± 450	45
10.0 hours	246 ± 291	118	359 ± 294	82	796 ± 477	60
12.0 hours	250 ± 160	64	409 ± 301	74	629 ± 275	44

TABLE 17-MEANS STANDARD DEVIATIONS AND COEFFICIENTS OF VARIATION FOR TOTAL NUMBERS OF EOSINOPHILS PER mm^3 OF BLOOD IN DWARF; CARRIER AND PEDIGREE-CLEAN BEEF CATTLE BEFORE AND AFTER INJECTIONS OF 0.3 UNIT OF INSULIN PER KILOGRAM OF BODY WEIGHT*

Time After Insulin Injection	Dwarf		Carrier		Pedigree-clean	
	Mean ± S.D.	C.V.%	Mean ± S.D.	C.V.%	Mean ± S.D.	C.V.%
Initial	454 ± 450	99	925 ± 655	71	1721 ± 602	35
0.5 hour	459 ± 328	71	675 ± 459	68	1242 ± 601	48
1.0 hour	459 ± 314	68	716 ± 500	70	1588 ±1312	83
2.0 hours	379 ± 264	69	688 ± 473	69	1663 ± 863	52
6.0 hours	304 ± 284	93	671 ± 423	63	1134 ± 547	48
10.0 hours	375 ± 342	91	529 ± 361	68	975 ± 723	74
12.0 hours	188 ± 267	142	421 ± 391	93	1117 ± 759	68

*This injection of insulin given to the same animals 48 hours after the injection of 0.8 unit per kilogram of body weight.

insulin were administered. The analysis of variance of differences in eosinophils is presented in Table 18.

As shown by the coefficient of variation, the number of eosinophils in blood of the genotypes was much more variable than the numbers of neutrophils and lymphocytes. This was true both before and after injection of each dosage of insulin. The total number of eosinophils per mm^3 of blood fell within the normal range in dwarfs and carriers but was higher than usual in the pedigree-clean animals. The reason for this is not evident, although it is known that infestations with parasites or the presence of foreign proteins in the body will cause an increase in this type of cell (Vaugh, 1953).

Research work with many species of animals has shown that many different kinds of stress will cause a rapid reduction in the number of circulating eosinophils. The reliability of this reaction resulted in Thorn *et al.* (1948) devising a test for adrenal insufficiency based on this response. The reduction in the number of eosinophils is less in those individuals in which the adrenals are less active. The reduction in eosinophil numbers is brought about by the release of ACTH from the pituitary gland, which, in turn, stimulates the adrenal cortex to release the corticoids which cause the reduction in eosinophil numbers. Injections of insulin, as in this experiment, should test for any insufficiency in the pituitary reaction as well as that of the adrenals.

The analysis of variance of the data, summarized in Table 18, shows a highly significant difference ($P<.01$) between eosinophil numbers in the different genotypes, between time intervals after insulin injections, and among the different dosages. A significant ($P<.05$) genotype x dosage interaction was also

TABLE 18-ANALYSIS OF VARIANCE FOR EOSINOPHIL DIFFERENCES BETWEEN GENOTYPES

Source	M.S.	D.F.	F Ratio
Genotypes	11300	2	110.47**
Time	676	6	6.60**
Dosage	1534	1	14.99**
Genotype x time	97	12	.95N.S.
Genotype x dosage	29	2	6.15*
Time x dosage	53	6	.51N.S.
Genotype x time x dosage	57	12	.55N.S.
Within	102	462	
Total		503	

* Probability of chance occurrence less than .05.
** Probability of chance occurrence less than .01.
N.S. Differences not significant.

noted, which means that there was a variation in the different genotypes in numbers of eosinophils when the two dosages of insulin were administered. The data showed that the number of eosinophils per mm^3 of blood had increased in the carriers and pedigree-clean animals when the second injection of insulin was given 48 hours after the first. This was not true in the dwarf, however. In the 84 blood samples from dwarfs after the first injection of insulin, the average number of eosinophils per mm^3 of blood was 374. This was exactly the same

number that was found before and after the second injection of insulin was given. On the other hand, in the carriers the number of eosinophils increased from an average of 507 per mm^3 after the first injection to 661 per mm^3 of blood at the second injection for an increase of 23.29 percent. This was a significant rise ($P<.025$). In the pedigree-clean animals the average number of eosinophils per mm^3 of blood in 84 blood samples immediately following the injection of 0.8 unit of insulin per kilogram of body weight was 976, compared with 1,348 per mm^3 in the same animals when they received a second injection of insulin (0.3 unit per kilogram of body weight) 48 hours after the first injection. This was a rise of 38.11 percent and was very highly significant ($P<.005$).

The reason for the increase in eosinophil numbers in the carriers and pedigree-clean animals is not known, but it must have been associated in some manner with either the insulin injected or with the drawing of blood and the stress accompanying it during the first series of bleedings. The rise in eosinophil numbers in the second series of bleedings was greatest in the pedigree-clean animals, intermediate in the carriers, and non-existant in the dwarfs. Results of this portion of the study are of sufficient interest to warrant further experiments along this line.

Data in Figures 8 and 9 show a decline in eosinophil numbers in animals of the three genotypes after the insulin injections. This decline was rather irregular and erratic, but was greater in the pedigree-clean individuals than in either the dwarfs or carriers, as shown by a larger regression coefficient for eosinophil numbers on hours after insulin injections. Actually, little difference was noted in the rate of decline after either dosage of insulin, which would indicate that even the lower dosage of insulin was causing the optimum effect. The decline in eosinophil numbers apparently did not reach its extremely low level in either of the genotypes until 10 to 12 hours after the insulin injections. This is considerably slower than the decline observed in other species of animals following injections of the ACTH hormone. The insulin probably caused a decrease in eosinophil numbers by an indirect action on the adrenals through the pituitary gland to cause a release of the corticoids; this could have been responsible for the slow action.

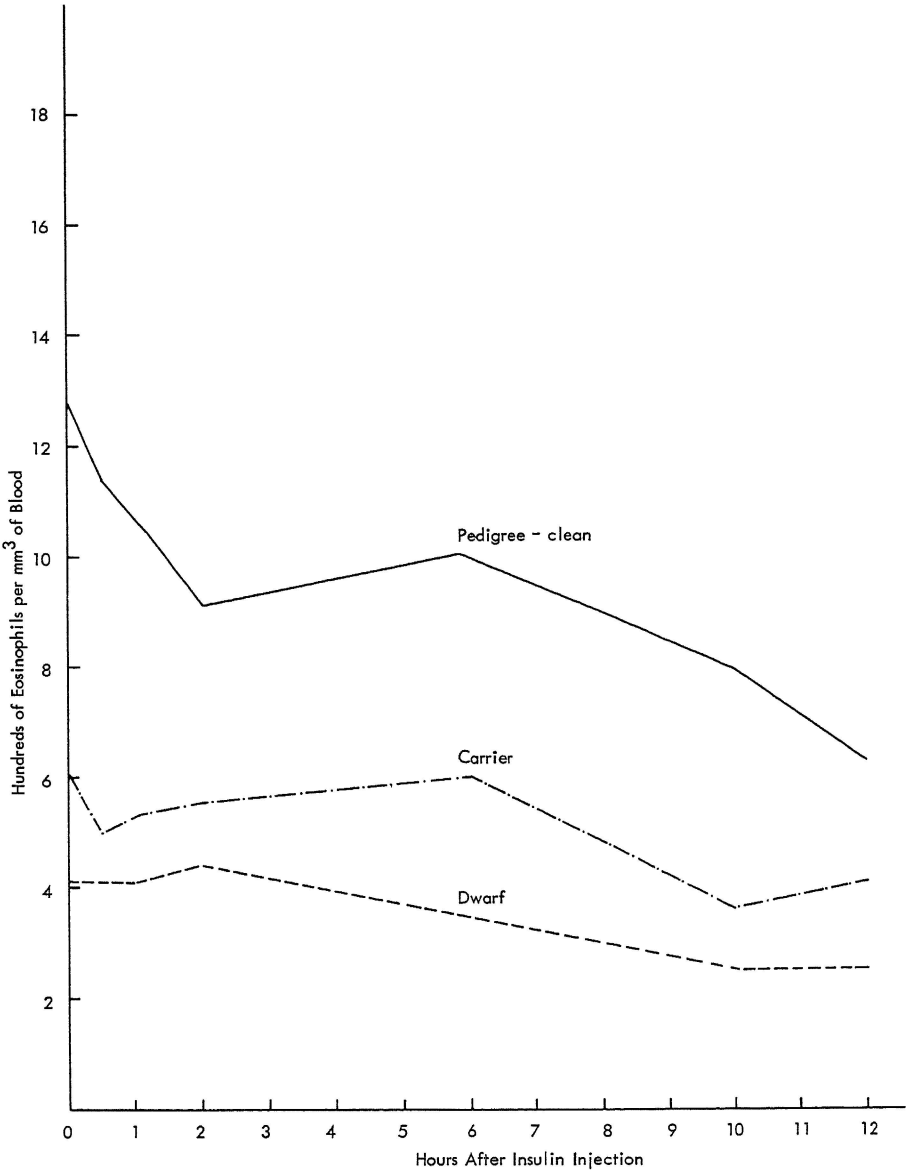

Figure 8. Hundreds of eosinophils per mm^3 of blood in beef cattle before and after insulin injections of .8 units per kilogram of body weight.

Regression Coefficients:
Dwarfs - 18.55
Carriers - 14.00
Normals - 40.03

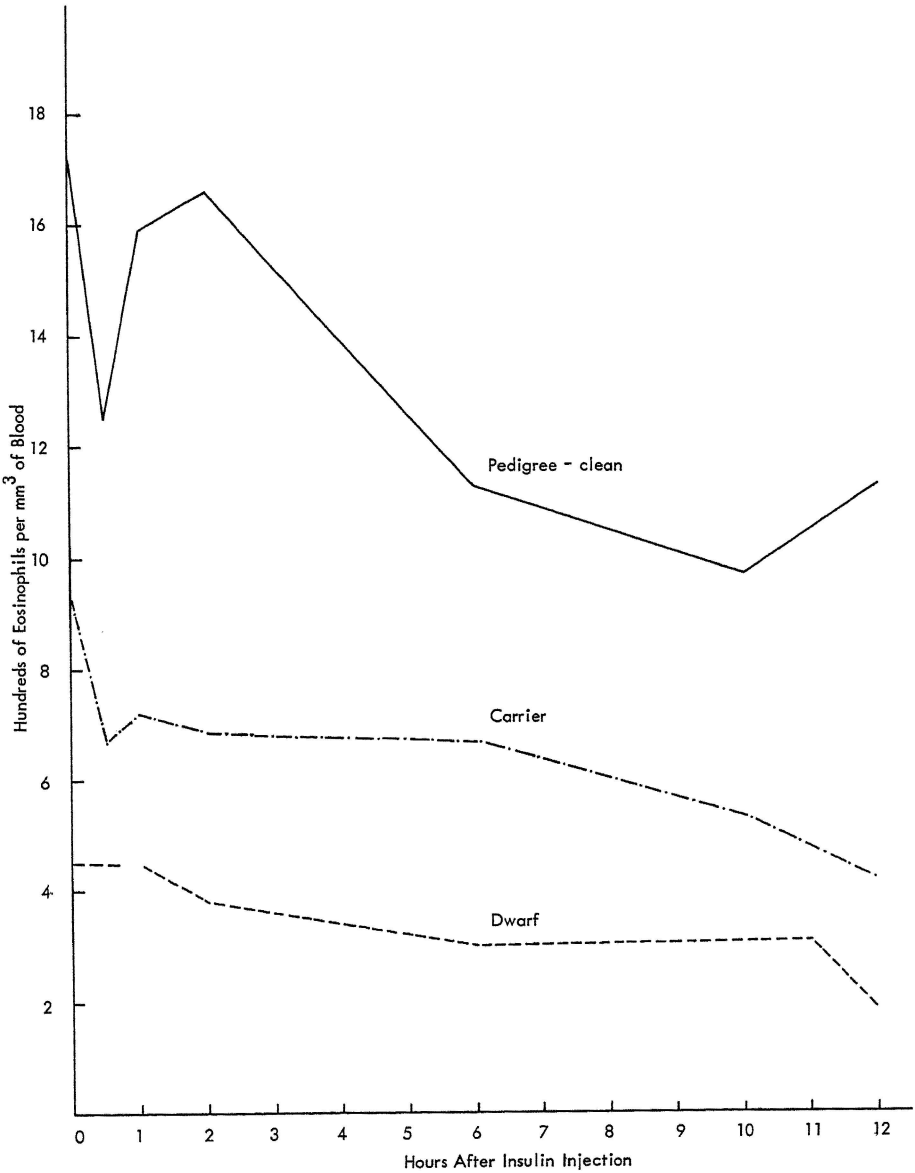

Figure 9. Hundreds of eosinophils per mm^3 of blood in beef cattle before and after insulin injections of .3 unit per kilogram of body weight.

Regression coeff.
Dwarfs = -17.45
Carriers = -27.98
Normals = -48.69

DISCUSSION

Animals possess a number of homeostatic mechanisms in the body which keep many substances in the blood stream in proper balance. The ability to maintain normal levels of various substances is dependent upon the interaction of several hormones produced by the endocrine glands.

Previous experiments at the Missouri Agricultural Experiment Station had tested the ability of dwarf cattle to return the blood sugar level to normal after it had been lowered by insulin injections. These preliminary experiments led to a more detailed study of sensitivity to insulin of cattle of the various genotypes for dwarfism as measured by changes in glucose levels in the blood. This study by Foley *et al.* (1960) was reported elsewhere and showed that the dwarfs were more sensitive to insulin than were pedigree-clean animals. This suggested that the pituitary-adrenal mechanism was not functioning normally in the dwarf and possibly was below par in carriers.

Changes in the numbers of various kinds of leucocytes in the blood stream following the insulin injections were studied at the same time as the insulin sensitivity (as measured by glucose changes). The reason for this study was that changes in different kinds of leucocytes after various kinds of stress are known to be related to pituitary and adrenal activity (Gordon, 1955). This should be another way of checking to see if dwarfs are deficient in the activity of the pituitary and adrenals.

No significant difference was noted in the number of leucocytes per mm^3 of blood in dwarfs, carrier, and pedigree-clean cattle. A highly significant increase ($P < .005$) did occur in leucocyte numbers, with a peak at six to ten hours in cattle of all genotypes when they received the high dosage of insulin. The rise in leucocyte numbers was greater when a high dosage of insulin was given than when a smaller dosage was given 48 hours later.

These results are similar to those reported for pedigree-clean cattle by Massey (1957) and Downs and Benson (1959), but are not in full agreement with the results of these workers concerning dwarf individuals. They found little or no increase in total leucocytes in dwarfs, whereas, in the present study, the increase in dwarfs was similar to that of pedigree-clean animals. In Massey's work, however, it was later discovered that he was counting cells, or fragments of cells, in addition to standard leucocytes, and these were largely responsible for the greater increase in cell numbers in the pedigree-clean animals, compared with dwarfs following insulin injections. Downs and Benson were able to confirm the results they obtained with cattle in dwarf and normal mice.

The total or absolute number of lymphocytes was determined from the total leucocyte and differential counts. In agreement with the results of other workers, lymphocyte numbers decreased after insulin injections. There was no significant difference among cattle of the three genotypes in this respect, however. A highly significant difference was observed in lymphocyte numbers among the three genotypes, especially after the injection of the heavy dosage of insulin. Cornelius

et al. (1956) also found that dwarfs possessed a larger percentage of lymphocytes than normal beef cattle.

A study of changes in neutrophil numbers following the injection of a high and a low dosage of insulin showed more positive results than the study of lymphocytes. A significant genotype difference was noted in neutrophil numbers; dwarfs possessed the lowest number, pedigree-clean animals the highest, and carriers were intermediate. Neutrophils increased much more, proportionately, than the lymphocytes decreased. Furthermore, the high dosage of insulin caused a much greater increase in neutrophils than the low dosage ($P<.01$). A highly significant ($P<.01$) interaction of genotype x time interval after insulin injection was also observed, with the dwarfs showing a delayed response in neutrophil numbers and a smaller increase, compared to the cattle of the other two genotypes. A significant genotype x dosage interaction ($P<.05$) was also observed, with the dwarfs again showing less response to the insulin as measured by neutrophil increases.

This failure of the dwarfs to show as great an increase in neutrophil numbers as pedigree-clean animals was also observed by Downs and Benson (1959) and Massey (1957. Deyoe *et al.* (1959), however, observed an opposite effect, with carrier cattle showing more of an increase in neutrophils than pedigree-clean animals. Their observations were made over a much shorter period of time, however, and therefore could not be compared with results of the other workers.

The deficiency in neutrophil response to insulin injections could indicate a deficiency of the hormones related to the pituitary and adrenals. This may not be true, however, since Dougherty and White (1944) reported that even though the lymphopenia following ACTH injections was a specific response of the adrenals, the increase in neutrophils was not specific because it could be induced in adrenalectomized animals given a variety of agents other than adrenotrophic hormones. That neutrophil numbers are under genetic control, at least in swine, has been indicated by the report of Milicevic *et al.* (1960).

A study of the eosinophils showed a significant genotype difference, before and after insulin injections. They were higher in pedigree-clean animals and lower in dwarfs with carriers intermediate. This may not have been strictly a genotype difference since the pedigree-clean animals were obtained from a different source than those of the other two genotypes and could have had a greater infestation of parasites than animals of the other genotypes. It was interesting, however, that pedigree-clean animals possessed a significantly higher number of eosinophils 48 hours after the first injection of insulin. The carrier cows also showed an increase, although it was less than that observed in pedigree-clean animals. Dwarfs showed no increase. Although the reason and mechanism responsible for eosinophil increases are unknown, this suggests that the mechanism responsible for the reaction may be lacking in the dwarf.

Dwarfs, and to a lesser extent carrier animals, did not show as much of an eosinopenia following insulin as pedigree-clean animals. Downs and Benson (1959) made similar observations. This suggests an adrenal insufficiency, but could be due to failure of the normal reaction that takes place via the pituitary.

In general, the results of this study confirm those reported by Foley *et al.* (1960) regarding insulin sensitivity studies as measured by blood glucose changes. Both studies suggest that the homeostatic mechanism responsible for maintenance of the normal glucose level and that of various leucocytes in the blood stream are deficient in the dwarf and to a lesser extent in the carrier animals. We are inclined to agree with Downs and Benson (1959) that the hypothalamic-hypophyseal-adrenocortical axis in dwarfs and carrier animals has been altered and this is responsible for the results obtained in this study.

CONCLUSIONS

Results obtained in this study indicated an abnormal response, especially in dwarfs and to a lesser extent in carriers, in the homeostatic mechanisms involved in regulating leucocyte numbers after insulin stress. This indicates that the animals' adrenal-pituitary axis was not functioning normally. Results may have been one of the side effects of the dwarf gene rather than a major or direct one.

Differences between carriers and pedigree-clean animals in some instances were significant, but were not of sufficient magnitude to be useful as a means of identifying carriers of the dwarf gene.

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