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Blood Sugar Level in Normal and Dwarf Beef Cattle Before and After Insulin Injections

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

"Snorter dwarfism in beef cattle is inherited as a simple Mendelian recessive trait. The increased frequency of "snorter" dwarfs in the United States in spite of the fact that they are not used for breeding purposes suggests that individuals heterozygous for the dwarf gene may be preferred in selection over those which are homozygous normal. If this is true, some physiological or morphological difference must exist between the homozygous normal and the heterozygous individuals. The effect of the individual gene in the heterozygous state must be rather slight, however, because its over-all phenotypic effect is not easily detected.

The economic importance of dwarfism in beef cattle led to numerous studies directed toward determining the physiological cause, or causes, of this genetic defect. The main objective was to find means of identifying heterozygous individuals without conducting an actual breeding test. Since various hormones in the body are known to be related to body size and development in other species, it was only natural that attention should be directed toward a study of the hormone content of the various endocrine glands of the dwarf. In general, it was found that dwarfs possessed normal amounts of hormones in the endocrine glands and especially in the anterior pituitary. The fact that the endocrine glands seemed to posses the normal amounts of hormones is no guarantee, however, that these substances function normally in the dwarfed individual. For this reason, attention was directed toward finding other means whereby flaws in the physiological make-up of the dwarf might be detected.

It is known that animals possess a number of homeostatic mechanisms in the body which keep many substances in the blood stream in proper balance. One of these involves the blood glucose level which must be maintained within a relatively narrow range. The ability to do this is dependent upon the interaction of several hormones produced by the endocrine glands. The injection of insulin, for instance, causes an almost immediate decrease in glucose in the blood stream. The hormones released from the anterior pituitary and the adrenal cortex are responsible for restoring the blood sugar level to normal once it is lowered by insulin. Thus, it seemed that a good means of determining if the pituitary and adrenal glands were functioning normally in the dwarf would be to lower the blood sugar with insulin and then compare the ability of dwarfs with normal individuals to restore the initial blood sugar level. If the dwarfs were deficient in this respect, it should be indicative of a pituitary and/or an adrenal insufficiency.

Preliminary studies conducted at the Missouri Agricultural Experiment Station which involved blood sugar determinations in beef cattle following the administration of insulin gave evidence that dwarfs restored their blood sugar level more slowly after insulin injections than normal appearing individuals. The experiments reported here had as their major objective the repetition of the preliminary studies, but on a larger scale and in more detail, to learn more about the physiology of dwarfism in beef cattle.

REVIEW OF LITERATURE

A review of the literature shows that many studies have been made of the blood glucose level in cattle, but there is considerable disagreement as to the normal level. Some blood sugar values given by various workers are summarized in Table 1. These range from 41 to 66 milligrams percent, so the normal blood sugar level must be somewhere within this range.

TABLE 1--THE BLOOD SUGAR CONTENT IN CATTLE

Author	Blood Sugar in Milligrams Percent	Method of Analysis
Hayden and Scholl (1924)	51.75	
Hayden and Fish (1928)	46.52	
Schlothauer (1928)	66.00	Folin-Wu
Hayden (1929)	41.15	
Anderson, Gayley and Pratt (1930)	51.20	
Hodgson, Riddell and Hughes (1932)	53.03	Micro Folin
Kennedy, Anderson, Bechdel and Shigley (1939)	52.12	Micro Folin
Jones (1943)	63.00	Folin-Wu

Some Factors Causing Variations in Blood Sugar Level in Cattle.

Probably one of the reasons that blood sugar levels vary so widely in different reports is that many factors cause variations in the blood sugar level. The literature has been reviewed to determine some of these factors.

Age. The age of the individual seems to be an important factor influencing the blood sugar level. Langstein and Newberg (1907) reported that they were able to find fructose in the fetal blood of ruminants, but most of the fructose disappeard from the blood stream at birth, possibly as a urinary constituent. Passmore and Schlossman (1938) reported a higher level of blood sugar in fetal sheep and goats than in their mothers.

Hodgson, Riddell and Hughes (1932) observed blood sugar levels of 100.4, 82.2 and 80.2 milligrams per 100 milliliters of blood in animals one to six days, one to four weeks and one to three months of age, respectively. As the age of the animals increased, the blood sugar level decreased until they were approximately two years of age. The results of Kennedy *et al.* (1939) agreed with these observations.

Lactation. No significant differences were detected by Scheurnert and Pelchrzin (1923), Moussu and Moussu (1926), Awdejewa, *et al.* (1927), Hayden and Fish (1928), Fish (1928), Hodgson, *et al.,* (1932) and Kennedy, *et al.,* (1939) in the blood sugar levels of lactating and non-lactating cows. However, other experimenters have obtained lower blood sugar levels in lactating animals than in those not lactating (Blackwood and Sterling, 1932; Widmark and Carlens, 1925; Schwarz and Mexler-Andelburg, 1928; Schlothauer, 1928; Hewitt, 1930 and Anderson, *et al.,* 1930).

Hewitt (1930) reported that with eight determinations, dry cows, on the average, gave a blood sugar content of 90.4 milligrams percent; comparable determinations of the blood of lactating cows gave 50.7 milligrams percent. Schlothauer (1928), Widmark and Carlens (1925), and Hodgson, *et al.* (1932) indicated that heavy producing cows have a slightly lower blood sugar level than poor producing cows.

Allcroft (1933) attributed considerable diurnal variation in the blood sugar level of lactating cows to the requirements of blood sugar for milk production. One group of investigators reported as much as 16 milligrams percent variation during the day in six lactating cows (Brown, *et al.,* 1936a).

Other Factors. Schuhecker (1925) and Awdejewa, *et al.* (1927) reported marked daily variations in the blood sugar of cattle. Kennedy, *et al.* (1939) reported marked diurnal variations in the blood sugar of calves and these variations were associated with the time of feeding. Studies conducted by Fish (1928) with five cows indicated that little fluctuation occurred in the blood sugar level during October, November and December. However, there was a uniform decrease during January and February and an increase in March. The blood sugar again decreased in April, increased in May and June and experienced a decline

again in July. This suggests a seasonal variation in the blood sugar level of cattle. Hodgson, *et al.* (1932) found that blood sugar determinations made during July and August gave considerably higher readings than those obtained during the winter months.

Hodgson, Riddell, and Hughes (1932) found no significant difference in the sugar content of the blood of mature animals of various breeds. An average of 44 determinations on each of four breeds gave the following results in milligrams percent of blood: Ayrshire 53.1, Guernsey 53.6, Holstein 52.8, and Jersey 52.5. Auger (1927), however, stated that breed might have some effect on blood sugar level and that certain breeds are more sensitive to insulin than others.

Hewitt (1930) reported blood sugar values as high as 362 milligrams percent for heifers during estrus. Observations on five heifers by Hodgson, Riddell and Hughes (1932) indicated a distinct rise in blood sugar during estrus. In some instances this amounted to as much as a 40 percent increase. In no instance, however, were readings of more than 90 milligrams percent obtained.

In a study involving five heifers, Hodgson, *et al.* (1932) found that during fasting over a period of nine days the blood sugar decreased to less than 50 percent of its initial value. This decrease was continuous and uniform until the seventh day, at which time a substantial increase was noticed. Robertson and Thin (1953) stated that cows which were starved at the peak of lactation showed a statistically significant fall in the blood sugar level on the first day of starvation with a subsequent rise to the prestarvation level on the second and the third days. There was a temporary rise in blood sugar level after feeding was resumed. Turk and Work (1933) were unable to change the glucose level of the blood of lactating cows by increasing the fat content of the ration.

Schuhecker (1925), Allcroft (1933), Hitchcock and Phillipson (1946), Richter (1928), Bottomly, *et al.* (1939), and Reid (1950) found that neither condition nor plane of nutrition had much of an effect on the blood sugar level. Fasting for 24 hours was not effective in changing the blood sugar level in animals in good condition, but it had some effect in animals (sheep) in poor condition or those near the end of prognancy. Little change in the blood sugar level was noted by Reid (1951a) after sheep were fasted for 48 hours. Gowen and Tobey (1931) reported that fasting animals before an injection of insulin had little effect upon their response to the injection.

Effect of Insulin on the Blood Sugar Level in the Bovine.

The intravenous injection of insulin in animals, as a general rule, causes a rapid decrease in the blood sugar level. In some instances, animals go into a coma and may die unless glucose is administered intravenously. The lower level where symptoms of hypoglycemia occur is not constant for all species or for all individuals within a species. It appears that convulsions or other symptoms of hypoglycemia distress are induced more readily in monogastric animals than in ruminants.

In a study with six cows conducted by Auger (1927) hypoglycemic disturbances were observed only three times when the blood sugar level was reduced below 30 milligrams percent. One cow went into a semi-coma but an injection of glucose quickly restored this animal to normal. Reid (1951 b) found that injections of larger dosages of insulin did not cause a greater fall in the glucose level in the blood than small dosages. A single dose of insulin, whether it was one or 10 units per kilogram of body weight, dropped the blood sugar 17 to 25 milligrams percent within 30 minutes. The rate of fall of the sugar level was not affected by an increased dosage over one unit per kilogram of body weight. Individual differences were found in the depth to which blood sugar levels fell. Some animals showed a decline to 10 milligrams percent, while others showed a drop to as low as 4 to 7 milligrams percent. In one animal, the blood sugar level fell to 3 milligrams percent and hypoglycemia convulsions resulted. Increasing the dosage of insulin increased the period of hypoglycemia in most animals. Reid considered this duration of hypoglycemia as a measure of an animal's sensitivity to insulin.

Reid (1951 a) was of the opinion that the relative constancy of the blood sugar at a level of 5 to 10 milligrams percent over a long period of time suggests that a new level of equilibrium has been established between the rate of glucose output by the liver and the utilization by the extra hepatic tissue. The hormones of the pancreas, anterior pituitary, and the adrenal cortex, in turn, control the metabolism within the liver. The balance between these hormones governs the level at which the blood glucose is homeostatically held by the liver. An insulin injection upsets the established endocrine balance; therefore, resulting in a drop in the blood glucose.

Reid suggests that the constant minimum blood glucose level following the injection of insulin indicates a new balance between these hormones. This new balance is reached when the tissues become saturated with insulin. Reid suggested that one unit per kilogram of body weight was sufficient for this purpose. Increasing the dosage, within limits, merely permits a state of "saturation" to be maintained over a longer period of time. Brown, Petersen and Gartner (1936 a) also stated that after the tissues had become saturated with insulin, additional injections did not result in an additional effect on the blood sugar level of dairy cattle.

Measurement of Insulin Action.

Animals maintain their blood sugar level within a narrow range as a result of a balance between glycogenolysis, the splitting up of glycogen to glucose, and the removal of the sugar from the blood stream. These processes involve several hormones which act synergistically or antagonistically in carbohydrate metabolism (Engel, 1953). Following the administration of insulin, the blood glucose level is lowered because of an increased glucose uptake by the tissues (Wall,

et al., 1957) and not because of a decreasing glucose output by the liver. Thus, the site of insulin action appears to be the cell membrane. The return of the normal glucose level after the injection of insulin is brought about by a sudden increase in the release of glucose from the liver in response to the hypoglycemia (Dunn, *et al.,* 1959).

Scott and Dotti (1932) and Reid (1950) reported that the degree to which the blood sugar is lowered is not in direct proportion to the amount of insulin given. In other words, a large dose of insulin does not cause a greater depression of the blood sugar level than a small one. The effect of a large dose of insulin expresses itself in a greater duration of hypoglycemia. The quantitative determination of insulin action, therefore, must involve the measurement of both blood sugar depression and its duration. Normally, a decrease in blood sugar of 50 percent can be expected but the duration of hypoglycemia is of greater significance than its degree (Cantarow and Trumper, 1955).

Sensitivity to insulin has been expressed in several ways. Himsworth (1935) measured the sensitivity of man to insulin by measuring the area enclosed by the depression curve below the resting level of blood sugar for the first 15 minutes. Scott and Dotti (1932) measured insulin sensitivity in terms of the blood sugar depression at what they regarded as the optimum interval, 30 minutes, after insulin administration. Heinbecker, *et al.* (1939), in studies conducted with dogs, stated that insulin action in its entirety seemed best expressed by the area confined by the blood sugar curve at the initial post-absorptive level. No direct proportionality was found between insulin dosage and such areas. Mirand and Osborn (1955) used still a different method of determining the sensitivity of mice to insulin. They expressed sensitivity to insulin on the basis of the amount of insulin required to produce convulsions in various individuals.

MATERIALS AND METHODS

Experimental Animals.

In this study data were obtained from 36 animals. This included 12 dwarfs, 12 pedigree-clean cows, and 11 mature cows and one bull which were known carriers of the dwarf gene. Nine of the 12 dwarfs were considered mature. Two were Angus and 10, Herefords. The dwarf animals were equally divided in number between the sexes.

The pedigree-clean animals used in these experiments were from the University of Missouri's breeding herd at Weldon Springs. The carrier and dwarf animals were obtained from several breeders throughout the state.

At the time the data were collected from the pedigree-clean animals they were maintained on native pasture. Approximately one-half of the data from

the dwarf animals was obtained during the winter months when they were allowed only low quality roughage. These animals were in poor condition. The remainder of the data collected from the dwarf animals was collected during the early summer. At that time the animals were on green forage. The data obtained from the carrier animals were taken, for the most part, while the animals were receiving silage under dry lot conditions. The remainder of the carrier data was acquired from carriers on pasture.

The Insulin Sensitivity Test.

The insulin sensitivity test was conducted in the following manner. Regular zinc insulin was injected into the jugular vein of the neck at a 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 16-gage hypodermic needle. Approximately 10 cubic milliliters of venous blood was collected in a bleeding tube containing an anticoagulant. Either potassium oxalate tablets or EDTA was used as an anti-coagulant.

On the first day, seven blood samples were collected at definite time intervals before and after insulin injections. An initial blood sample was obtained prior to the administration of insulin. This sample was considered as a control. The additional samples were collected at the following time intervals; 30 minutes, 1, 2, 6, 10, and 12 hours after the insulin injections. Forty-eight hours after the initial injection of insulin, this exact procedure was repeated on the same animals, with one exception. This time 0.3 unit of insulin per kilogram of body weight was used instead of 0.8 unit.

The blood sugar responses of the animals at the different insulin dosages were compared and used as a measure of insulin sensitivity. Two methods of blood sugar analysis were used. These were the Nelson-Somogyi method and the modified Folin-Wu.

It is realized that the Folin-Wu method is not the most economical, the most convenient, the easiest, the most specific or the most reproducible; but this method is widely used in clinical laboratories. As in practically all of the methods now available for the estimation of glucose in blood, it is based on the reduction of alkaline oxidizing reagents by the blood filtrates. According to Kock and Hanke (1953), Cantarow and Trumper (1955) and Somogyi (1930) this reduction is not necessarily due to glucose alone, but may also be due to other substances such as creatinine, uric acid, thioneine, ergothioneine, and especially gluthathione. These substances are present chiefly in the erythrocytes.

When glucose or other reducing sugars are heated in alkaline solution with the cupric ion, the sugar and its various decomposition products reduce the copper, with the result that insoluble Cu_2O is formed. This is not a stoichiometric reaction, and depends on conditions of temperature, duration of heating, degree of alkalinity, etc. The ratio of glucose to Cu_2O formed may be varied over a

wide range. For any given set of conditions, however, the amount of Cu_2O will depend to a large extent on the amount of sugar present. After the heating period, the Cu_2O is estimated by allowing it to reduce phosphomolybdic acid with a proportionate formation molybdenum blue which is determined colorimetrically.

The following procedure was used in running the Folin-Wu determinations. Five milliliters of whole blood was added to 35 milliliters of distilled H_2O in an Erlenmeyer flask. To this, 5 milliliters of a 10 percent sodium tungstate solution was added. Five milliliters of 2/3N H_2SO_4 was added next while gradually shaking the flask. The contents of the flask were then filtered into a dry tube. Precaution was taken not to allow the blood to stand with the sodium tungstate alone for such a procedure would cause a rapid loss of the glucose in the blood sample.

One milliliter of the filtrate was transferred to a specially prepared blood sugar tube. This tube is constructed in a way to prevent oxidation of the cuprious compound by the air. To the 1 milliliter of filtrate, 1 milliliter of distilled H_2O was added. Two milliliters of copper alkaline tartrate solution was carefully added to each tube. The tubes were immersed in boiling water and heated for exactly six minutes and were then cooled by immersing them in cold running water for 10 minutes. Two milliliters of phosphomolybdic acid reagent was added next to each tube and mixed. The sample was diluted to 25 milliliters with distilled H_2O and mixed by inversion.

A representative portion of this mixture was measured on a Fisher clinical electrophotometer.

With the Nelson-Somogyi method the following procedure was used. One milliliter of blood was measured into a 25 milliliter test tube. Then 9½ milliliters of barium hydroxide was added while rotating the tube. Nine and one-half milliliters of zinc sulfate solution was added while mixing. This was shaken vigorously and filtered through Whatman No. 1 paper or equivalent.

One-half milliliter of barium zinc filtrate was measured into a tube and 1 milliliter of alkaline copper reagent was added. Each tube was mixed by tapping. A marble was placed on top and the tubes were heated in a vigorously boiling water bath for 20 minutes. The tubes were allowed to cool for one minute at room temperature. One milliliter of arsenomolybdate reagent was added to each tube and mixed. The mixture was diluted to the 10 milliliter mark with H_2O and mixed by inverting. The absorbance was then measured at 540 Mμ with a photocolorimeter. The Nelson-Somogyi determinations were made by the Missouri agricultural research clinical laboratory.

To better evaluate the depth and duration of the insulin-induced hypoglycemia, the area of blood sugar changes was calculated using the initial blood sugar reading as the base line. The area of change in the blood sugar value was determined for each individual by plotting the blood sugar values on graph

paper with time plotted on the horizontal axis and the milligram percent of blood sugar on the vertical axis. A straight line was drawn from the value of each sample to that of each succeeding one. This resulted in deviations above and below the base line, depending upon the time interval considered. The areas of deviations were then calculated for each reading by using the formulas for the area of triangles and rectangles.

Data Calculations.

Statistical calculations on the data obtained in this study 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 an analysis of variance and simple correlations.

RESULTS AND DISCUSSION

Comparison of Two Methods of Blood Sugar Analysis.

In this study both the Nelson-Somogyi method, which is claimed to measure glucose only, and the Folin-Wu method, which measures non-sugar reducing substances as well as glucose, were used to measure blood sugar. It has been found that the various methods of blood sugar estimation give widely different results for the distribution of sugar between the plasma and the red corpuscles. The average discrepancies between methods are not great for estimations on the plasma but are considerable for estimations on the corpuscles. The discrepencies between the blood sugar methods are due to the presence of a non-glucose reducing substance in the corpuscles which is present in tungstic filtrates and is either present in lesser amounts or absent in the iron and zinc filtrates (Herbert and Groen, 1929; Reid, 1950).

A highly significant difference was found between the two methods of blood sugar analysis used in this study (Table 5). The mean blood sugar values for both the Nelson-Somogyi and the Folin-Wu methods are shown in Table 2. This table gives the mean values of blood sugar in milligrams percent for both methods on all genotypes at the different time intervals studied, as well as at the different insulin dosage levels. The data presented in this table show rather clearly that blood sugar values determined by the Folin-Wu method averaged higher at all intervals before and after insulin than those determined on the same blood samples by the Nelson-Somogyi method.

Cantarow and Trumper (1949) stated that the Folin-Wu method gives results too high for high sugar levels and too low for low blood sugar values. For instance, near the 50-milligram level the results obtained are 15 to 18 percent less than the true value. It has been reported that at below normal levels of blood sugar in man the Folin-Wu method gives an average error of 20 milligrams percent (Somogyi, 1930). The differences in estimates of blood sugar values in this study when data from all cattle were grouped were much less than this, averaging 6.56 milligrams percent in favor of the Folin-Wu method.

When a comparison was made of blood sugar values for the same blood samples by the two methods for each genotype of cattle some interesting results were obtained. These data are summarized in Table 3 for the high dosage of insulin and in Table 4 for the low dosage.

TABLE 2--AVERAGE BLOOD SUGAR VALUES IN CATTLE OF THREE GENOTYPES AS DETERMINED BY TWO METHODS OF BLOOD SUGAR ANALYSIS (IN MILLIGRAMS PERCENT).

Units of Insulin per Kg. of Body Weight	0.8 unit		0.3 unit	
Method of blood sugar analysis	Folin-Wu	Nelson-Somogyi	Folin-Wu	Nelson-Somogyi
Before insulin	64.2	55.5	64.9	55.3
After insulin:				
0.5 hour	33.4	29.7	36.5	28.3
1.0 hour	33.5	29.6	30.0	27.4
2.0 hours	35.0	28.5	34.8	28.7
6.0 hours	38.9	34.9	59.3	47.7
10.0 hours	53.2	47.7	63.9	56.5
12.0 hours	61.7	55.1	65.8	58.4

TABLE 3--AVERAGE DIFFERENCES IN BLOOD SUGAR VALUES IN DWARFS, CARRIERS AND PEDIGREE-CLEAN ANIMALS AS DETERMINED BY TWO METHODS OF BLOOD SUGAR ANALYSIS (0.8 UNIT OF INSULIN PER KG. OF BODY WEIGHT).

	Differences by Genotypes*		
	Dwarf	Carrier	Pedigree-Clean
Before insulin	-0.8	7.8	19.3
After insulin:			
0.5 hour	-4.5	3.5	17.8
1.0 hour	-7.5	0.8	19.8
2.0 hours	-3.6	5.6	17.6
6.0 hours	-2.5	3.2	11.1
10.0 hours	-1.9	4.2	14.4
12.0 hours	-5.4	5.7	19.4
Average	-3.74	4.40	17.06

* In mg. percent as determined by subtracting values determined by the Nelson-Somogyi method from those determined by the Folin-Wu method.

TABLE 4--AVERAGE DIFFERENCES IN BLOOD SUGAR VALUES IN DWARFS, CARRIERS AND PEDIGREE-CLEAN ANIMALS AS DETERMINED BY TWO METHODS OF BLOOD SUGAR ANALYSIS (0.3 UNIT OF INSULIN PER KG. OF BODY WEIGHT).

	Differences by Genotypes*		
	Dwarf	Carrier	Pedigree-Clean
Before insulin	2.9	8.6	17.4
After insulin:			
0.5 hour	-2.3	5.5	21.4
1.0 hour	-1.0	-0.5	9.3
2.0 hours	1.5	3.6	13.5
6.0 hours	8.5	7.9	18.2
10.0 hours	-1.3	5.6	18.0
12.0 hours	-3.6	9.0	17.0
Average	+0.7	5.67	16.40

* In mg. percent as determined by subtracting values determined by the Nelson-Somogyi method from those determined by the Folin-Wu method.

Results were very similar at both dosage levels. In the pedigree-clean cows the Folin-Wu method of blood sugar determination gave an average of 16 to 17 milligrams percent higher values than the Nelson-Somogyi method. This difference was only 4 to 6 milligrams percent in favor of the Folin-Wu method in the carrier cows whereas in the dwarfs the values were about the same or were slightly less as determined by the Folin-Wu method of glucose determination.

That the cattle of the three genotypes for the dwarf gene gave different values when the two different methods were compared is shown by a highly significant method of blood sugar analysis x genotype interaction in Table 5.

TABLE 5--MEAN SQUARES AND SIGNIFICANT DIFFERENCES BETWEEN DOSAGE LEVELS, METHODS OF BLOOD SUGAR ANALYSIS, TIME INTERVALS, GENOTYPES, AND THE INTERACTIONS INVOLVED.

Source	DF	MS	F Ratio
Between dosage level (.8 and .3)	1	3811	19.73**
Between methods (Folin-Wu and Nelson-Somogyi)	1	11363	58.82**
Between time intervals (1 to 7)	6	26790	138.68**
Between genotypes (clean, carrier and dwarf)	2	38105	197.26**
Level x time	6	1718	8.89**
Level x method	1	200	1.03NS
Time x method	6	120	1.62NS
Level x genotype	2	2583	13.37**
Time x genotype	12	629	3.26**
Method x genotype	2	7162	37.07**
Level x time x method	6	72	.37NS
Level x time x genotype	12	326	1.69NS
Method x time x genotype	12	57	.29NS
Level x method x genotype	2	2163	11.20**
Level x method x genotype x time	12	307	1.59NS
Within	924	193	
Total	1007		

* Probability of chance occurrence less than .05.

** Probability of chance occurrence less than .01.

NS Differences not significant.

Results from this portion of the study suggest that something may be present in the blood of the pedigree-clean animals that is lacking in the dwarfs and is present to a lesser extent in the known carriers of the dwarf gene. It is conceivable that this deficient substance, or substances, may be creatinine, uric acid, thioneine, ergothioneine and glutathione. According to Kock and Hanke (1953), Somogyi (1930), Cantarow and Trumper (1955) and Reid (1950) these substances account for the increased values of the Folin-Wu method (tungstic filtrate) over the values obtained with the Nelson-Somogyi method (zinc filtrate). These substances are present chiefly in the erythrocytes. Practically speaking, glutathione is the main substance responsible for this difference (Kock and Hanke, 1953).

The absence of glutathione in the dwarf animal could be closely correlated with its abnormalities since glutathione plays an extremely important role in many enzyme systems. According to Koeppe, Boyer, and Stulberg (1956) gluta-

thione is involved with the enzyme glyceraldehyde-3-phosphate dehydrogenase, which functions in carbohydrate metabolism.

Response of Beef Cattle to a High and a Low Dosage of Insulin.

Data presented in Figures 1 and 2 show rather clearly that beef cattle react differently to a high dosage of insulin than to a low dosage. In these figures the

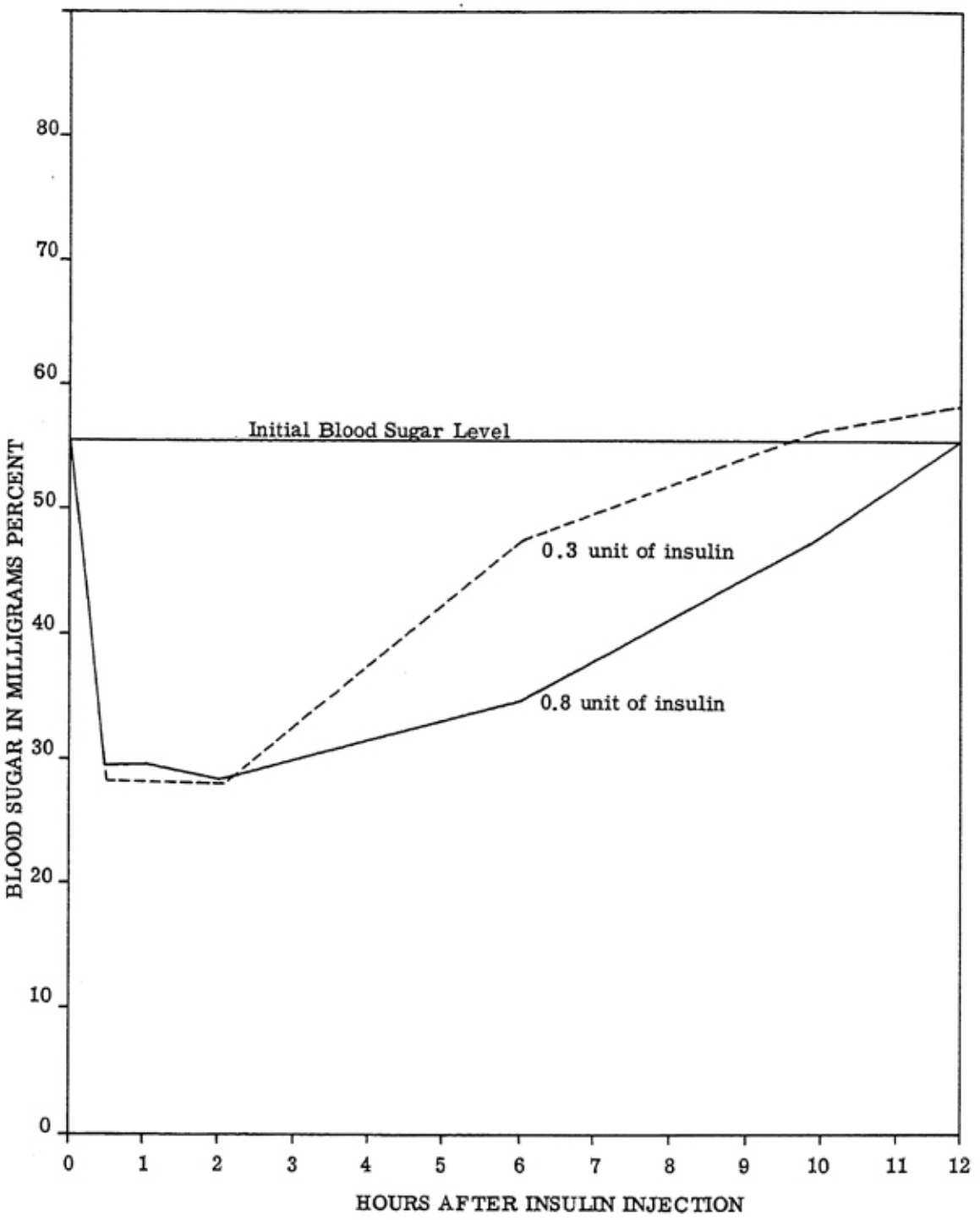

Figure 1. Influence of a heavy dosage and a light dosage of insulin on the blood sugar level in beef cattle (Nelson-Somogyi Method).

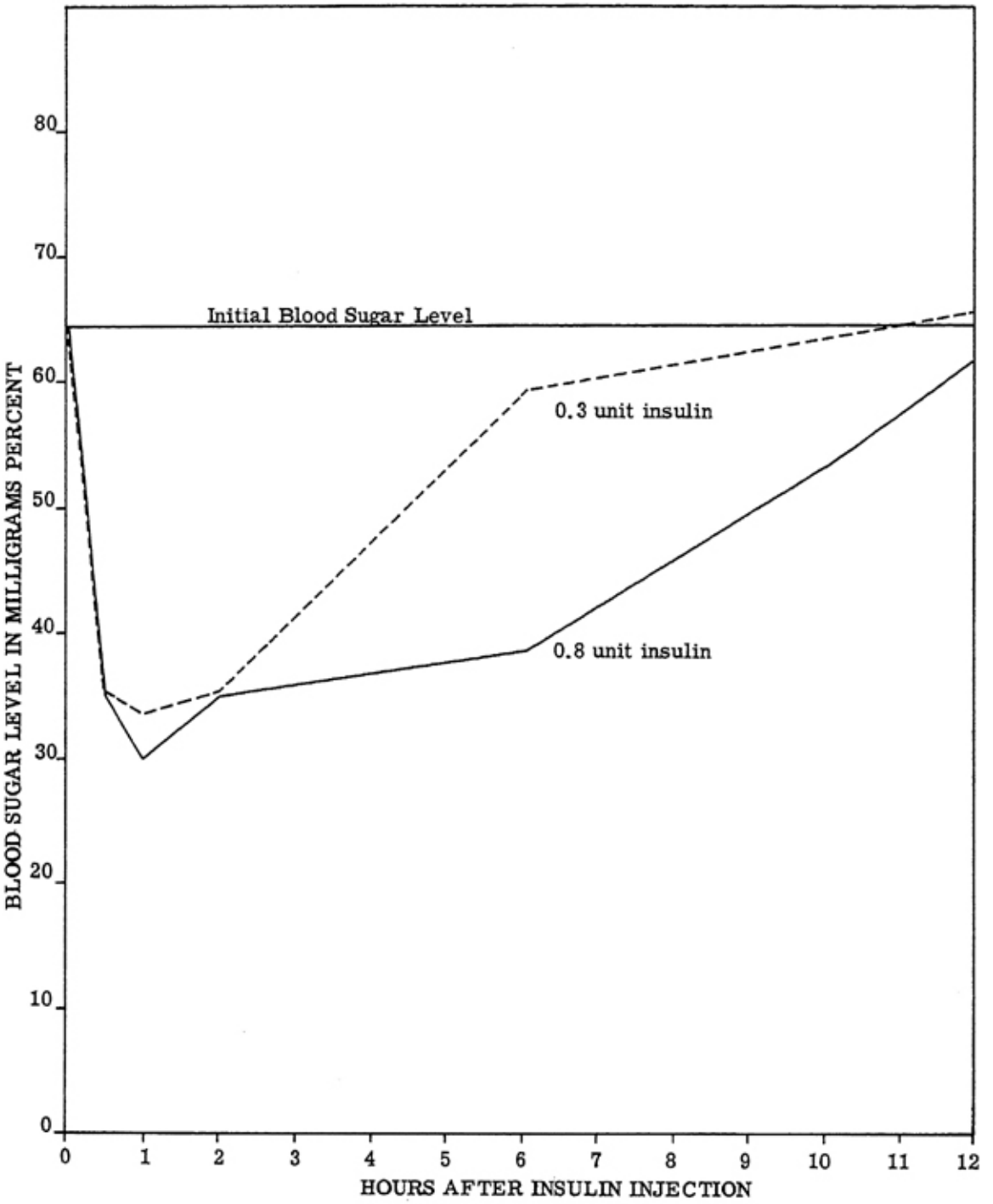

Figure 2. Influence of a heavy and a light dosage of insulin on the blood sugar level in beef cattle (Folin-Wu Method).

data are grouped for all 36 cattle, regardless of genotype; the statistical analysis in Table 5 shows a highly significant difference ($P<.01$) in blood sugar levels between the two dosages. Results were also very similar regardless of the method used to determine the sugar content of the blood. On the average, injection of 0.8 unit of insulin per kilogram of body weight did not cause a greater or a more rapid decrease in the blood sugar level. The main difference between the effects of the two dosages of the hormone were that the blood sugar level was depressed a longer period of time when the larger dosage was given.

These results are in excellent agreement with those of workers who have experimented with other species of animals. Reid (1950) working with sheep found that an increase of insulin above one unit per kilogram of body weight did not cause an increased depression of the glucose level but expressed itself in a greater duration of hypoglycemia. Scott and Dotti (1932) and Heinbecker, *et al.* (1939) made similar observations.

In comparing the effects of the genotype for the dwarf characteristic on blood changes following insulin administration, it was found that an extremely significant difference existed between the various genotypes (Table 5). Tables 6 and 7 give mean values for the various blood samples at the different time intervals following insulin injections. The observed values for the blood sugar determinations appeared to be within the accepted range. Regardless of the method of blood sugar analysis used, the initial values were greatest in the pedigree-clean animals, intermediate in carriers, and lowest in dwarfs. The values found for all animals at the initial bleeding with the Folin-Wu method ranged from 60.6 to 98.7 milligrams percent for the pedigree-clean animals with a mean value of 80.17 milligrams per cent. For the carriers the mean value obtained was 61.72, with a range of 50.8 to 80 milligrams percent. The dwarf animals ranged from 21.5 to 85.0 milligrams percent, with a mean of 50.75.

When the Nelson-Somogyi method was considered it was observed that the pedigree-clean animals had an initial blood sugar value which ranged from 50.9 to 80.8 percent, with a mean value of 60.89. The mean obtained for the carriers was 53.97, and the range was from 44.7 to 70.6 milligrams percent. For the dwarf animals a mean of 51.63 with a range in value of 38.8 to 64.8 milligrams percent was obtained. The overall average for all animals with both methods was 59 milligrams percent.

Since the results in Table 5 indicated a highly significant difference between genotypes, an attempt was made to determine if this difference was due to a large difference between two genotypes or to significant differences in all genotypes involved. To do this, the means of each genotype were tested against each other within methods and insulin dosage level. The mean values used were calculated for all 12 animals of each genotype and for all samples within a given dosage level. This gave a total of 84 blood samples for each mean. The values are shown in Table 8. To test the difference in these means the Q test was used. Tables 9 to 12 show these values and results.

TABLE 6--MEAN VALUES OF BLOOD SUGAR ESTIMATIONS IN MG. PER CENT AS DETERMINED BY THE FOLIN-WU AND NELSON-SOMOGYI METHODS OF ANALYSIS (.8 UNIT OF INSULIN PER KG. OF BODY WEIGHT).

Method	Genotype	Initial	Time intervals in hours after insulin injection					
			0.5	1	2	6	10	12
Nelson-Somogyi	Clean	60.89	33.96	30.17	27.10	42.22	61.27	67.40
	Carrier	53.97	32.00	31.50	29.87	33.90	42.45	49.33
	Dwarf	51.63	23.19	27.26	28.44	28.67	39.24	48.61
	Average	55.50	29.72	29.64	28.47	34.93	47.65	55.11
Folin-Wu	Clean	80.17	51.80	49.98	44.74	53.35	75.62	86.76
	Carrier	61.72	35.53	30.73	35.44	37.05	46.62	55.02
	Dwarf	50.84	18.72	19.76	24.83	26.18	37.36	43.17
	Average	64.24	35.35	33.49	35.00	38.86	53.20	61.65

TABLE 7--MEAN VALUES OF BLOOD SUGAR ESTIMATIONS IN MG. PER CENT AS DETERMINED BY THE FOLIN-WU AND NELSON-SOMOGYI METHODS OF ANALYSIS (.3 UNIT OF INSULIN PER KG. OF BODY WEIGHT).

Method	Genotype	Initial	Time intervals in hours after insulin injection*					
			0.5	1	2	6	10	12
Nelson-Somogyi	Clean	59.03	30.69	31.13	29.40	53.45	60.05	66.29
	Carrier	59.04	29.54	30.35	34.49	58.15	65.21	63.25
	Dwarf	47.84	24.75	20.82	22.10	31.60	44.15	45.55
	Average	55.30	28.33	27.43	28.66	47.73	56.47	58.36
Folin-Wu	Clean	76.45	52.10	40.47	42.86	71.65	78.00	83.32
	Carrier	67.60	35.00	29.82	38.06	66.00	70.80	72.20
	Dwarf	50.75	22.41	19.80	23.56	40.10	42.82	41.99
	Average	64.93	36.50	30.03	34.83	59.25	63.87	68.54

* This is the time interval after the injection of 0.3 unit of insulin per kilogram of body weight. This injection followed within 48 hours after an injection of 0.8 unit of insulin per kilogram of body weight in the same animals from which data were obtained for Table 6.

TABLE 8--AVERAGE VALUES OF BLOOD SUGAR ESTIMATIONS BY TWO DIFFERENT METHODS FOR ALL SAMPLES AT TWO INSULIN DOSAGE LEVELS.

Method of estimation	Units of insulin per kg. body weight	
	.8	.3
Nelson-Somogyi		
Pedigree-clean	46.74 mg. %	47.15 mg. %
Carrier	39.00 mg. %	48.57 mg. %
Dwarf	35.29 mg. %	33.83 mg. %
Folin-Wu		
Pedigree-clean	63.20 mg. %	63.55 mg. %
Carrier	43.16 mg. %	54.20 mg. %
Dwarf	31.55 mg. %	34.49 mg. %

TABLE 9--ANALYSIS OF VARIANCE FOR MEAN BLOOD SUGAR VALUES BETWEEN GENOTYPES WITHIN DOSAGE LEVEL AND METHOD (NELSON-SOMOGYI METHOD)

Source	.8 unit of insulin per kg. body wt.		
	D/F	MS	F Ratio
Between genotypes	2	2556	99.59**
Within	249	25	
Total	251		

DIFFERENCES IN TOTAL MEAN VALUES BETWEEN GENOTYPES WITHIN DOSAGE LEVEL AND METHOD

	Dwarf (35.29)	Carrier (39.00)
Clean (46.74)	14.44**	7.14*
Carrier (39.00)	3.6**	

* Probability of a chance occurrence less than .05.

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

TABLE 10--ANALYSIS OF VARIANCE FOR MEAN BLOOD SUGAR VALUES BETWEEN GENOTYPES WITHIN DOSAGE LEVEL AND METHOD (NELSON-SOMOGYI METHOD)

	.3 unit of insulin per kg. of body wt.		
Source	D/F	MS	F Ratio
Between genotypes	2	5555	17.23**
Within	249	322	
Total	251		

DIFFERENCES IN TOTAL MEAN VALUES BETWEEN GENOTYPES WITHIN DOSAGE LEVEL AND METHOD

	Dwarf (33.83)	Carrier (48.57)
Clean (47.15)	13.32**	1.42
Carrier (48.58)	14.75**	

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

TABLE 11--ANALYSIS OF VARIANCE FOR MEAN BLOOD SUGAR VALUES BETWEEN GENOTYPES WITHIN DOSAGE LEVEL AND METHOD (FOLIN-WU METHOD)

	.8 unit of insulin per kg. of body wt.		
Source	D/F	MS	F Ratio
Between Genotypes	2	21532	58.26**
Within	249	369	
Total	251		

DIFFERENCES IN TOTAL VALUES BETWEEN GENOTYPES WITHIN DOSAGE LEVEL AND METHOD

	Dwarf (31.55)	Carrier (43.16)
Clean (63.20)	31.65**	20.04**
Carrier (43.16)	11.61**	

** Probability of chance occurrence less than .05.

TABLE 12--ANALYSIS OF VARIANCE FOR MEAN BLOOD SUGAR VALUES BETWEEN GENOTYPES WITHIN DOSAGE LEVEL AND METHOD (FOLIN-WU METHOD)

Source	.3 unit of insulin per kg. of body wt.		
	D/F	MS	F Ratio
Between Genotypes	2	18491	34.45**
Within	249	538	
Total	251		

DIFFERENCES IN TOTAL VALUES BETWEEN GENOTYPES WITHIN DOSAGE LEVEL AND METHOD

	Dwarf (34.49)	Carrier (54.22)
Clean (63.55)	29.06**	9.33*
Carrier (54.20)	19.71**	

* Probability of a chance occurrence less than .05.

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

When 0.8 unit of insulin per kilogram of body weight was administered and the blood sugar determinations were made by the Nelson-Somogyi method of analysis, a highly significant difference was observed between the means of all three genotypes. However, the results obtained following the injection of insulin at 0.3 unit per kilogram of body weight were somewhat different. At this dosage level there was a highly significant difference between the dwarfs and the other two genotypes concerned, but none between the carrier and the pedigree-clean animals.

The dwarfed animals appeared to respond different than the other two genotypes at both dosages. This was also true for the blood sugar determinations obtained with the Folin-Wu method. At a dosage level of 0.8 unit of insulin per kilogram of body weight a highly significant difference between the mean values for all genotypes was found. A decrease in the insulin dosage to 0.3 unit per kilogram of body weight gave results somewhat comparable to those observed with the other method of blood sugar analysis. Here again the dwarfs differed significantly from the other two genotypes. A difference significant at the 5 percent level was observed between the mean values for pedigree-clean and known carriers.

Highly significant differences were also observed between the different dosage levels studied as well as for the various time intervals (Table 5). When

insulin was administered to the pedigree-clean cows at the rate of 0.8 unit per kilogram of body weight, decreases in the blood sugar of about 35 percent with the Folin-Wu method and 45 percent with the Nelson-Somogyi method were noticed. This is in agreement with other reports in the literature.

This decrease in blood sugar appears to be somewhat less sharp than is usually reported for normal non-ruminants. Previous investigators have indicated that an insulin injection of one unit per kilogram of body weight is sufficient to fully saturate the involved tissue (Reid, 1950). Increasing the dosage levels, within limits, would only maintain the saturated condition over a longer period of time. The result would be a greater duration of hypoglycemia. A dosage level of 0.8 unit should not completely saturate the tissue, and thus should enable the animal to begin the recovery from the hypoglycemia more rapidly.

Within six hours after the initial insulin injections the pedigree-clean individuals showed definite signs of returning their blood sugar to the initial level. They were back to the initial level at the tenth hour. This was true regardless of the method of glucose determination. When a second injection of 0.3 unit of insulin was administered to the pedigree-clean cattle, after a period of 48 hours had elapsed following the initial injection, comparable results were obtained. This is shown as an average for both methods of sugar determination in Figures 3 and 4.

When the same procedure was applied to known carriers of the dwarf gene, the initial drop in blood sugar was about 42 percent. The carriers appeared somewhat more sluggish in their response to insulin than the pedigree-clean animals. At the end of 12 hours they had not, as a group, been able to return their blood sugar to the initial level. Following the administration of 0.3 unit of insulin per kilogram of body weight to known carriers it was found that the initial drop in the blood sugar level was comparable or perhaps only slightly greater than it had been at the larger dosage of the hormone. Carrier animals appeared to have the ability to handle the smaller insulin dosage as well as the pedigree-clean animals.

Data from the dwarfs gave dissimilar results. Regardless of the amount of insulin given, the dwarfs experienced a much greater initial drop in blood sugar level. Either method of blood sugar analysis gave comparable results (Figures 3 and 4). Irrespective of insulin dosage, the dwarfs were unable to return their blood sugar to its initial level within the 12-hour period considered. Considering the pedigree-clean animals as normal, the dwarfs apparently did not have the ability to respond in a normal manner to insulin-induced hypoglycemia. Carriers appeared to be intermediate in this respect. This indicates that the mechanism concerned with maintaining the blood sugar within a narrow range is inadequate in the dwarfs.

These results lead one to believe that the genes responsible for snorter dwarfism in cattle are in some manner concerned with the anterior pituitary, since, according to Russell (1955), hypophysectomized animals are extremely

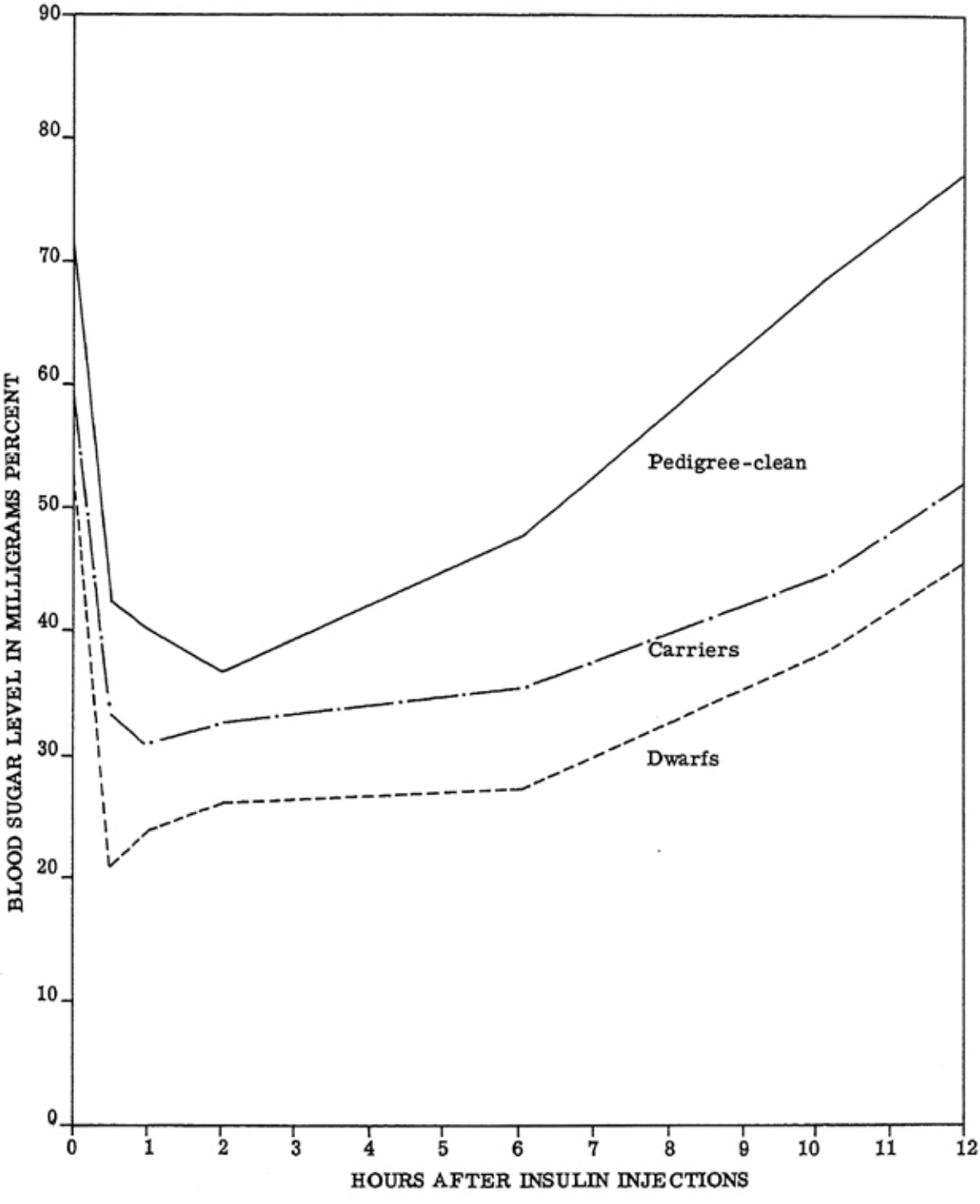

Figure 3. Response of dwarfs, known carriers and pedigree-clean beef cattle to a heavy dosage of insulin (0.8 unit per kilogram of body weight). Averages of the Folin-Wu and Nelson-Somogyi methods of blood sugar determination.

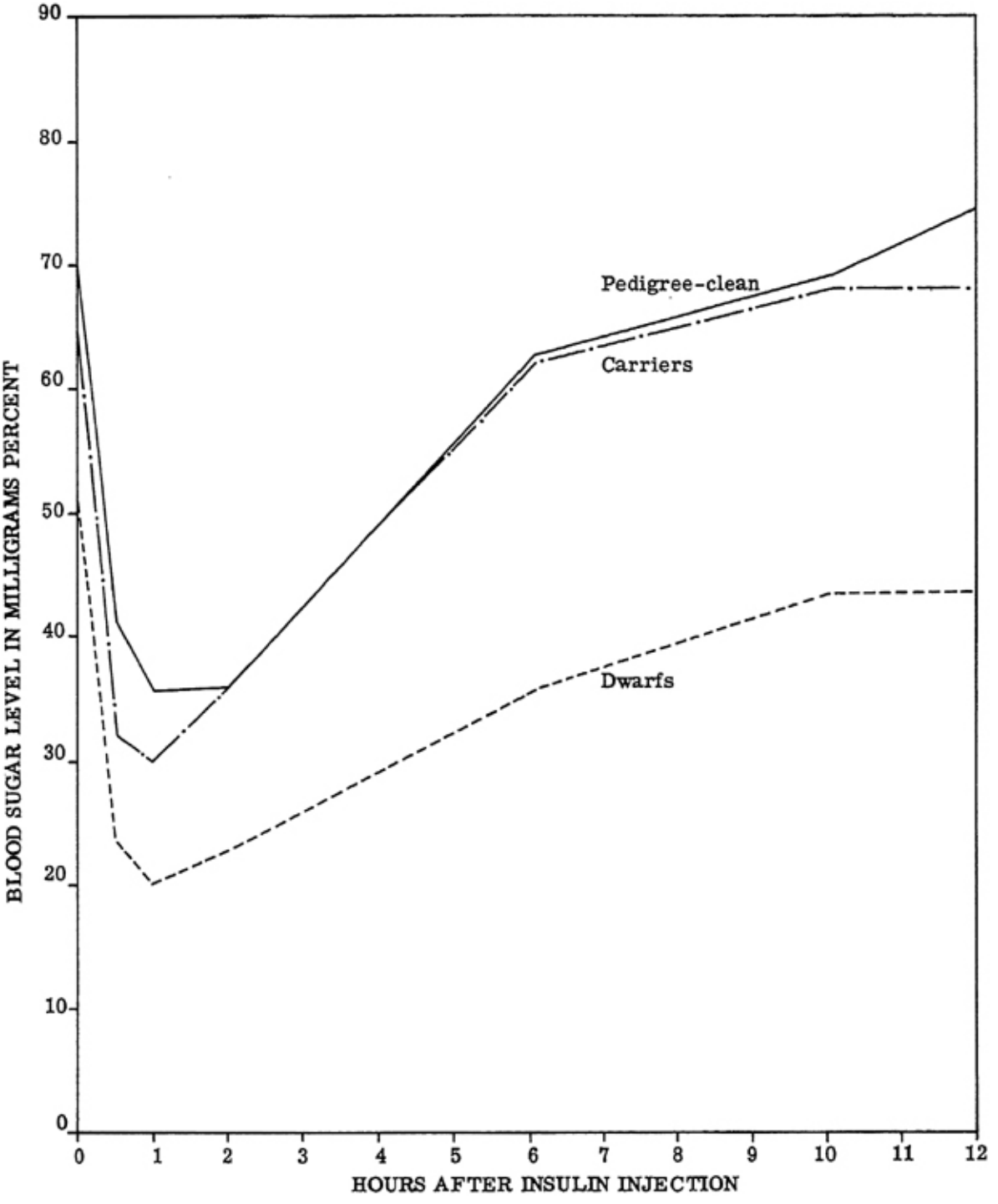

Figure 4. Response of dwarfs, known carriers and pedigree-clean beef cattle to a light dosage of insulin (0.3 unit per kilogram of body weight) 48 hours after a heavy dosage of 0.8 unit of insulin per kilogram of body weight. Averages of the Folin-Wu and Nelson-Somogyi methods of sugar determination.

hypersensitive to the hypoglycemic effects of insulin. This observation was first made in 1924 by Houssay and since confirmed in a variety of animal species and in clinical cases of hypopituitarism. A considerable part of the increased sensitivity may be an indirect result of adrenal cortical abnormalities. With humans, in the case of adrenocortical hypofunction following intravenous injections of a standard dose of insulin (0.1 or 0.05 units per kilogram of body weight) in the fasting state, the blood sugar falls rapidly, attaining minimum levels of about 30 to 40 milligrams percent in 20 to 30 minutes and returning to the fasting level in 1½ to two hours. In patients with Addison's disease, the blood sugar usually falls in 20 milligrams percent or less and remains at hypoglycemic levels for prolonged periods, sometimes showing no indication of an attempt to rise from the minimum levels attained.

A decreased growth hormone secretion is difficult to study since the symptoms of a deficiency are often in association with a deficiency of ACTH, TSH and gonadotrophic hormones (Cantarow and Trumper, 1955). This shows that one must remember that normal growth and development is dependent upon a large number of factors both intrinsic and extrinsic, and that a hyposecretion of any one hormone might influence other hormones which also play a role in normal growth. Since numerous reports have indicated that at least a large percentage of the dwarf animals are capable of reproduction it is assumed that the production of the gonadotrophic hormones approaches normal and need not be of concern in this discussion.

Crenshaw, *et al.* (1957) found that the condition of dwarfism was not due entirely to a lack of TSH. Their results did indicate that this type of dwarfism may be concerned with the anterior pituitary.

Table 5 shows that a significant interaction existed for blood sugar between insulin dosage level and time interval between blood collections. This indicates, as would be expected, that the effect of insulin injections varies with dosage level and time after injection. With the larger insulin dosage the blood sugar level would be expected to remain lower for a longer period of time. A significant interaction was also demonstrated between dosage and genotype. This implies, as already shown, the different genotypes respond differently to the two different dosage levels.

The interaction between genotype and time interval after insulin injection was also highly significant (Table 5) which means that at the different time intervals the reaction was different for each genotype. It was also found that a highly significant interaction existed between the method of sugar analysis, the dosage level of insulin, and the genotype of the individual concerned. These interactions perhaps take away from the importance of the significance of differences found for the separate sources of variation studied. When the different genotypes were concerned in the interaction, a significant difference usually occurred. This could be accounted for by the large F ratio found between geno-

types. The interactions between dosage level and method of sugar analysis; time intervals and methods; dosage level, time interval, and method; dosage, time interval, and genotypes; and method, time interval, and genotype were not found to be significant.

Area of Glucose Change After Insulin Injections.

Table 13 gives mean values for areas of blood sugar changes after insulin injections for both methods of sugar determinations. The larger the values for the areas the greater the degree of insulin sensitivity indicated.

TABLE 13--CALCULATED AREAS OF DEPRESSION OF BLOOD GLUCOSE BY INSULIN INJECTIONS IN BEEF CATTLE

Genotype	.8 unit insulin		.3 unit insulin	
	F.W.	N.S.	F.W.	N.S.
Dwarf	262	218	180	174
Carriers	253	209	111	87
Pedigree-clean	240	187	131	130

The statistical analysis of the data, including values determined by both the Folin-Wu and Nelson-Somogyi methods, is summarized in Table 14. A highly significant difference due to genotypes was found between the means of the glucose areas. Highly significant differences ($P<.01$) were also observed for blood sugar areas at the various time intervals following insulin administration, dosage levels of insulin, interaction between genotype and time interval after insulin, between time intervals, interaction between dosage level and genotype, and be-

TABLE 14--ANALYSIS OF VARIANCE FOR BLOOD SUGAR AREAS ANALYZED BY BOTH METHODS OF BLOOD SUGAR METHODS. (BOTH METHODS COMBINED)

Source	DF	MS	F Ratio
Genotype	2	478	5.80**
Time	5	13622	165.17**
Level	1	4933	59.82**
Genotype x time	10	379	4.60**
Level x Genotype	2	317	3.85*
Time x level	5	1562	18.94**
Level x time x genotype	10	169	2.05*
Within	828	82	
Total	863		

* Probability of chance occurrence less than .05.

** Probability of chance occurrence less than .01.

tween time intervals, dosage level and genotype. A significant interaction ($P < .05$) was found between insulin dosage level and genotype.

These results further substantiate the previous results that dwarfs and carrier animals were more sensitive to a high dosage of insulin (0.8 unit per kilogram of body weight) than were pedigree-clean individuals. At the lower dosage of insulin (0.3 unit per kilogram of body weight) the dwarfs were considerably more sensitive than cattle of the other two genotypes but the carriers were slightly less sensitive than the pedigree-clean individuals.

SUMMARY

Previous studies at the Missouri Agricultural Experiment Station had indicated that dwarf beef animals did not have the ability to return their blood sugar levels to normal following an insulin injection as quickly as normal appearing animals. To better evaluate this, an insulin tolerance test was conducted on 12 pedigree-clean, 12 known carrier, and 12 dwarf individuals. This test involved the measurement and comparison of insulin-induced hypoglycemia and its duration at two different insulin dosage levels.

Blood sugar values were determined for each blood sample by the Nelson-Somogyi method of analysis, which is supposed to measure glucose only, and the Folin-Wu method, which measures non-sugar reducing substances such as glutathione as well as glucose. A highly significant difference ($P<.01$) was found between blood sugar values determined by the two methods. The values obtained with the Folin-Wu method were usually larger than those obtained with the Nelson-Somogyi method. It was found, however, that this was not true for the dwarf animals. This indicates that perhaps the blood of the normal appearing animal contains some substance not present in dwarf blood.

Highly significant differences were found between genotypes in their response to insulin. This was not due to the effect of a great dfference in one genotype since at the higher dosage level highly significant differences were found for the mean values of all genotypes regardless of the method used for the blood sugar determinations. At the lower insulin dosage the dwarf animals differed significantly from those which were phenotypically normal. However, a significant difference was not found between the pedigree-clean and carrier animals with the Nelson-Somogyi method. A difference significant at the 5 percent level did exist between the pedigree-clean and carriers animals when the readings were obtained by the Folin-Wu method of glucose determination.

Highly significant differences were also observed in the changes in blood sugars between the insulin dosage levels studied as well as for the various time intervals after insulin was injected. Interactions in which genotypes were involved were usually significant.

The area of glucose change after insulin was injected was calculated for each animal. This blood glucose change index was based upon the up and down fluctuations above and below the base line or the initial blood sugar reading. When the accumulated index calculated from both methods of sugar analysis was considered, highly significant differences were obtained between genotypes, time intervals after insulin injections, insulin dosage level and most interactions in which genotypes were involved.

These results lead to the conclusion that the genes responsible for "snorter" dwarfism in cattle are in some way concerned with the anterior pituitary, since dwarfs, like hypophysectomized animals, are hypersensitive to the hypoglycemic effects of insulin. It is not known if this is a direct or indirect physiological effect of the gene for dwarfism.

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