SOME FACTORS INFLUENCING THE LEVEL OF REDUCING SUGAR IN THE BLOOD OF BLACK-TAILED DEER

by

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Date May 16, 1966.

ABSTRACT

Some of the factors that influence the blood reducing sugar level in the black-tailed deer Odecoileus hemionus columbianus (Richardson) (Vancouver Island genotype), have been investigated. The distribution of reducing sugar in the blood of these animals was also examined.

It was found that: feed intake during the hour preceeding blood letting, short periods of fast, nature of the feed, and sex of the animal apparently have no effect on the level of blood reducing sugar in deer. Blood samples taken in the evening generally had a higher reducing sugar level than those taken earlier in the day. The means used to restrain the animals during the blood letting procedure was also found to have a marked influence on the level of blood reducing sugar. Deer restrained by physical force exhibited significantly higher and more variable blood sugar levels than those immobilized with succinylcholine. The length of time required to draw a blood sample from an animal also influenced the blood sugar level. The longer the time to let a sample, the higher the blood sugar level in the sample. The results indicate that the degree of excitement, fear, and pain experienced by the animals preceeding and during the blood letting procedure was the principal cause of variability found in the level of blood reducing sugar.

No reducing sugar could be detected in the erythrocytes of these deer.

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INTRODUCTION

INTRODUCTION

Subjective descriptions of the relationship of deer and habitat have dominated game management literature for many years. This material has extended our knowledge of these animals and provided the understanding essential for the development of needed management techniques. However, in keeping with the contemporary movement of research to intensive investigation, deer, more than ever before, are being studied as unique physiological systems manifesting profound cyclic phenomena. Documentations of changes in antler growth, feed intake, body weight, hormonal levels, and other physiological parameters, coincident with the seasonal sexual cycle are becoming priminant in the literature. French et al (1955), Cowan et al (1955), Kitts et al (1956), Magruder et al (1957) have all studied various aspects of the growth and nutrition of deer. Silver et al (1959) reported metabolic rates. Rosen and Bischoff (1952), Kitts et al (1956), Bandy et al (1956), Terri et al (1958), and Youatt et al (1965) have carried out studies on the haematology of deer under various circumstances. Investigations into bone and antler development have been reported by Bernhard et al (1953), Meister (1956) and Long (1959). Robinson et al (1965) studied the reproductive cycle of male white-tailed deer. Investigations into the hormonal system of deer have been reported by Browman and Sears (1956), Grieser and Browman (1956), Tyler (1961), and Dawson (1963). French et al (1960) noted the response of white-tailed bucks to added artificial light.

As one reviews literature of this nature it is apparent that in some cases the investigator has failed to appreciate the full effect of his experimental manipulation on the animal. Techniques of handling,

restraining, and sampling, used with success on domestic animals are often misapplied to wild species. As a consequence results often reflect the state of agitation of the animal rather than its normal, undisturbed, physiological functioning.

Initially this study was intended to explore alterations in the level of blood reducing sugar that might occur in deer during the profound physiological changes that are associated with rut. The findings provide information regarding the factors responsible for variability in the blood reducing sugar levels in deer, and also some indication of the normal range of these levels. They also serve as a caution to those engaged in measuring physiological parameters in wild animals.

Goodwin (1956) pointed out that the blood sugar of adult ruminants is almost exclusively glucose. He suggested, however, that the expression 'blood reducing sugar' is more appropriate as it acknowledges the other reducing sugars (mostly fructose) thought to be present in small amounts. This terminology was adopted and appears throughout the thesis.

Expression of reducing sugar levels as mgm.% in serum rather than as mgm.% in whole blood was done for two important reasons: 1. it is the concentration of sugar in the serum and tissue fluid that the body cells respond to, and 2. changes in the distribution of tissue fluid can occur rapidly, altering the packed cell volume, and subsequently the reducing sugar level if expressed in terms of whole blood.

EXPERIMENTAL

EXPERIMENTAL

1. ANIMALS

Four deer, two male (U16 and U43) and two female (U24 and U34) of the species Odecoileus hemionus columbianus (Vancouver Island genotype) were used for these experiments. The animals were captured as fawns in the spring of 1963 in the vicinity of Courtenay on Vancouver Island, B.C., Canada. Shortly after capture they were shipped, by air, to the Zoology Vivarium on the campus of the University of British Columbia. During the period of experimentation from December 1964 to March 1965 the deer were in the late stages of rut. Normally this is a period when the animals, particularly males, are difficult to handle. However, the close care and attention given them by the experimenter rendered them unusually tractable throughout the experiments.

2. NUTRITION

The experimental animals were fed a commercial milk replacer (Appendix 4) until they had reached a weight of approximately 15 pounds. The weaning process was then initiated and the animals were maintained on the weaning ration (Appendix 5) until early September. They were then placed on the adult ration (Appendix 6) which was fed throughout the remainder of the experimental period. Some modifications were introduced as the investigations directed. Animals U16 and U24 were given chopped alfalfa hay exclusively from January 21 1965 to February 12 1965 and from January 21 1965 to March 23 1965, respectively.

3. HOUSING

The animals used in this study were housed in individual pens. As fawns they were held in 4' by 2' wooden pens located in a ground level steam heated room in the Vivarium. At weaning the deer were moved to larger pens, 3' by 6' in the same room. By October 1963 the size of the deer and the onset of rut necessitated a move to the Wildlife Unit (Wood et al 1961) where they were housed in adult pens. These pens were later modified to include a slatted floor.

4. ANALYTICAL TECHNIQUES

(i) Blood Collection

Blood samples were taken from the jugular vein. Occasionally, when this proved difficult, samples were drawn from the recurrent tarsal vein. Blood was let into untreated, evacuated, 10 cc. vacutainer tubes*. A record was made of the time required for each aspect of every blood drawing operation. '0' time was considered to be the initial intrusion of the technician on the animal i.e. as the pen door was opened. 'Injection' time was then noted as the immobilizing agent was administered. The activity in this interval involved ushering the animal down the hall to the laboratory and the minimal restraint necessary for injection. The succeeding notation came as the animal collapsed due to the action of the drug. During the interval between 'injection' and 'down' time the deer usually stood quietly, apparently undisturbed. Most activity in this period occurred as the animal fought the effects of the drug. The time that blood was first released into the vacutainer and the time the

* B.D. Vacutainer, Becton, Dickinson and Co., Rutherford, N.J., U.S.A.

vacutainer was filled were also noted. See Appendix 10 for an example of the form used.

(ii) Hematocrits

When circumstances allowed, triplicate blood samples were taken in heparinized capillary tubes for the purpose of determining the packed cell volume. These samples were all taken with a single insertion of the vacutainer needle when a sample for glucose analysis was drawn.

(iii) Serum Extraction and Storage

After collection, the blood samples were allowed to stand undisturbed for thirty minutes. The tubes were then 'ringed' with a wood dowel, freeing the clot from the walls of the vessel. They were then placed in a centrifuge* and spun at high speed for ten minutes. Following this the serum was carefully drawn off the clot with a thin bore pipette and placed into clean, dry, test tubes. The serum samples were immediately put in a freezer and stored until analyzed.

(iv) Blood Glucose Determination

Of the methods for determining blood reducing sugar investigated, a Nelson-Somogyi technique modified to suit our requirements was chosen. This method was found to provide accurate reproducible results and is, therefore, described in detail.

0.2 cc. of serum was pipetted into 3.0 cc. of distilled water. Deproteinization with 2.0 cc. of 0.06 N $ZnSO_4$ and 2.0 cc. of 0.06 N $Ba(OH)_2$ followed. It should be noted that the amount and concentration of these solutions is not critical but can be varied to accomodate particular situations (Somogyi 1945). As the amount of glucose in this procedure must not be too high, 2.0 cc. of the serum filtrate was added to 2.0 cc.

* International Model HN, Needham Hts., Mass., U.S.A.

of the combined alkaline tartrate and copper solution (Somogyi 1945). The tartrate and copper solutions were prepared separately and mixed as required as the mixed reagents show some degree of autoreduction. (Somogyi 1952). After heating in a boiling water bath for exactly ten minutes followed by immediate cooling to 20 C in a stream of cold water, the reaction mixture was combined with 2.0 cc. of Nelsons arsenomolybdate reagent (Nelson 1944). The volume was made up to 10 cc. (addition of 4.0 cc. of distilled water) and the color was allowed to develop for at least 15 minutes. The transmittance was then measured with a Beckman Model D.U. Spectrophotometer at 650 mu.

An absorption curve from 400 mu to 1,000 mu of the coloured end product of the procedure was determined and appears in Appendix 7.

Three standard glucose solutions at concentration of 40, 70, and 100 mgm.% were prepared (Natelson 1961). One of the standards was run with every series of unknowns. Ultimately a number of repetitive determinations of each standard solution was made and used to prepare a standard curve. (Appendix 8).

(v) Internal Standard

A standard glucose solution (70 mgm.%) was mixed with a serum sample at three different levels: 2:1, 1:1, and 1:2. The mixtures were frozen and stored for a few days prior to analysis. The results appear in Appendix 9.

EXPERIMENT 1

EXPERIMENT 1

This experiment was designed to investigate the effects of feed intake, means of restraint, sex of the animal, and time of day, on blood reducing sugar levels in deer.

Four animals were placed on a feeding regime such that each was offered 1/4 of 80% of its daily intake (calculated from the average daily intake of the previous week), at each of 0700, 1100, 1500, and 1900 hours. The daily allowance was cut to 80% of normal so that the deer would clean up each feeding almost immediately after it was offered. The animals were allowed a week to become accustomed to this program before the experiment was begun. By this means some control was exercised over the amount and time of feed intake prior to blood letting. Animals 'on feed' were maintained on this regime. Deer 'off feed' received their usual allotment at 1900 hours the day before blood letting. They were given no feed throughout the day that blood was being drawn, the next feeding occurring at 0700 hours the succeeding day. In effect then, blood samples were drawn from these animals after fasts of 12, 16, 20, and 24 hours.

The deer were restrained during the blood letting procedure by one of two means; immobilization with succinylcholine or physical force. When the latter method was used, two or three technicians would sieze the animal (recorded as time 'grabbed'), wrestle it down (time 'held'), and so hold it until the operation was completed.

Blood was drawn at 0800, 1200, 1600, and 2000 hours from the animals as indicated in table 1.

To assess the influence of each variable, i.e. 'on feed' vs. 'off feed' the data was grouped under these headings irrespective of the other

TABLE 1: TREATMENT FOR EXPERIMENT 1

Date	Animals	Restraint	Feeding Regime
16/ 9/64	16.24	physical	on
22/10/64	34.43	physical	on
26/10/64	16.24	succinylcholine	on
27/10/64	34.43	succinylcholine	on
2/11/64	16.24	physical	off
3/11/64	34.43	physical	off
4/11/64	16.24	succinylcholine	off
25/11/64	34.43	succinylcholine	off

variables. In the cases where it was applicable a Student 't' test was applied to the data to determine the significance of any differences noted.

(1) Influence of Feed Intake

As can be seen from table 2 and figure 1 there is no correlation between feed intake the hour prior to blood letting and serum reducing sugar level. That monogastric animals exhibit a postprandial hyperglycemia associated with an increase in glucose absorption through the gut is well documented. Such a rise is not found in cattle (Hodgson et al 1932), or in sheep (Allcroft and Strand 1933). Moreover, it has been shown that the oral administration of glucose in sheep (Schambye 1951) and cattle (Bell and Jones 1945) does not cause an increase in the blood reducing sugar. The explanation for this relative constancy is based on two facts. Firstly, starch and cellulose, the principal carbohydrates found in the diet of ruminants, are rapidly degraded in the rumen by a variety of microorganisms to simple sugars; mostly glucose. These sugars are fermented to short chain volatile fatty acids which are then absorbed into the energy metabolism of the animal. The major source of ruminant blood reducing sugar was once believed to result from acidic and enzymatic digestion of the carbohydrate of ruminal bacteria and protozoa. This degradation occurs in the abomasum and small intestine, and the liberated glucose is subsequently absorbed. Heald (1952), however, investigated this pathway and found, using sheep fed hay, that no more than 20 gms. of glucose per day could be produced in this manner, Later work by Annison et al (1957) supported this finding. Although glucose can be absorbed directly from the rumen as first shown by Rankin (1940), it is unlikely that any great amount

TABLE 2: RESULTS OF EXPERIMENT 1: PART 1

THE EFFECTS OF FEED INTAKE ON SERUM REDUCING SUGAR LEVELS IN DEER.

Feeding Program	Animal	Feed Intake (gms.)	Serum Sugar (mgm.%)
On	U16	426	91.2
		426	90.4
		426	96.8
		426	88.7
		182	88.4
		182	90.1
		182	91.2
		182	87.6
	U24	283	83.6
		198	86.5
		142	100.3
		57	90.9
		340	89.2
		340	81.6
		340	85.9
		266	92.1
	U34	66	102.2
		73	91.5
		87	78.6
		76	109.2
		250	82.9
		250	78.6
		250	93.6
	U43	228	81.9
		226	82.9
		328	90.9
		204	78.1
		68	81.1
		---	79.5
		---	77.0
		---	83.1

Average serum reducing sugar level 'on feed': $\frac{2725.6}{31}$ = 87.9 mgm.%.

TABLE 2: (cont.)

Feeding Program	Animal	Hours of Fast	Serum Sugar (mgm.%)
Off	U16	12	85.2
			85.4
	U24		92.1
			75.4
	U34		81.6
			79.0
	U43		81.6
			76.3
	U16	16	83.0
			83.1
	U24		90.4
			80.7
	U34		103.0
			83.6
	U43		102.5
			84.9
	U16	20	87.6
			78.6
	U24		92.1
			75.2
	U34		82.4
			80.9
	U43		79.7
			88.9
	U16	24	86.2
	U24		97.1
	U34		98.4
			87.3
	U43		88.7
			97.4

Average serum reducing sugar level after:

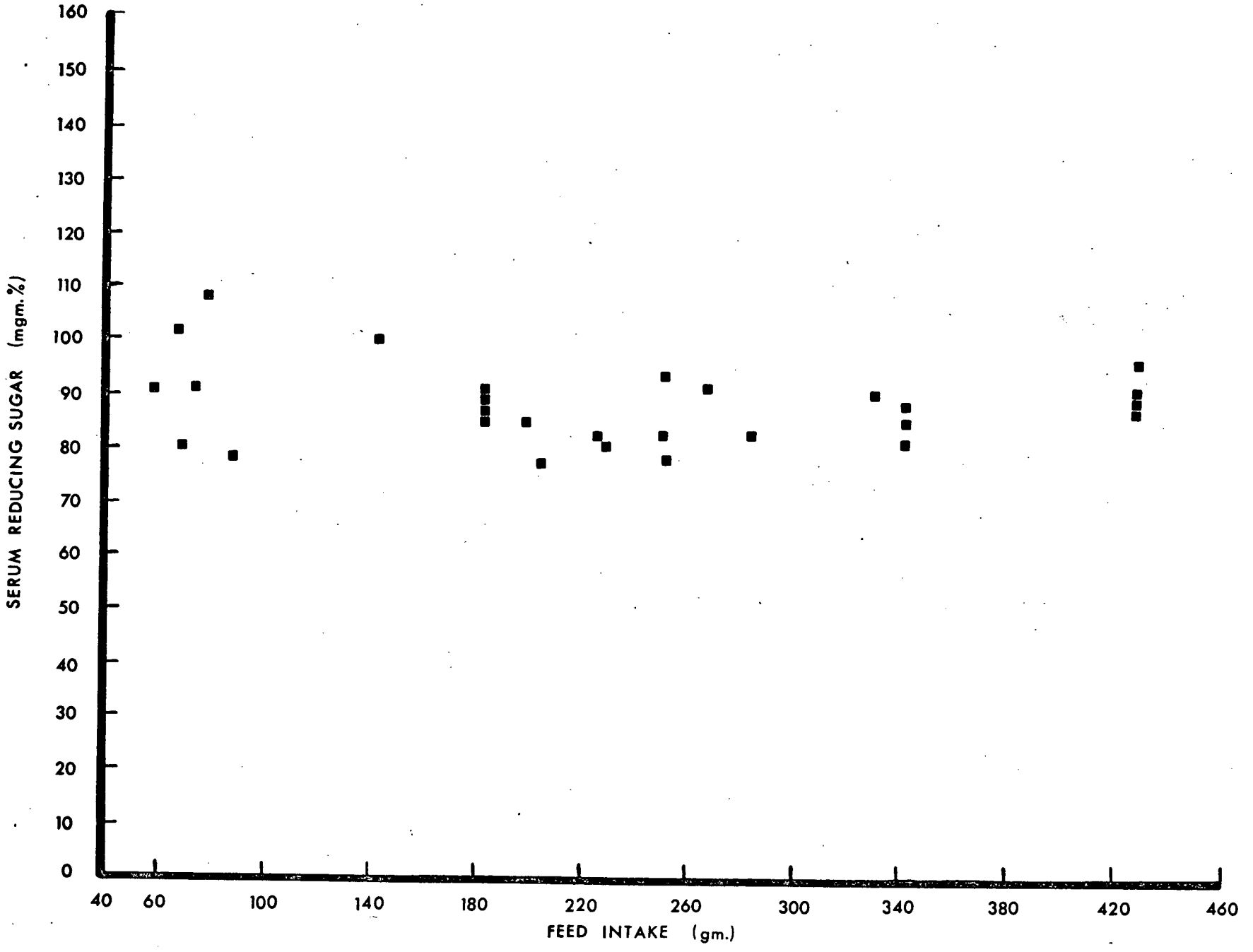
(1) 12 hours fast $\frac{656.6}{8}$ =82.1 mgm.%

(2) 16 hours fast $\frac{711.3}{8}$ =88.9 mgm.%

(3) 20 hours fast $\frac{665.4}{8}$ =83.2 mgm.%

(4) 24 hours fast $\frac{555.1}{6}$ =92.5 mgm.%

FIGURE 1: THE RELATIONSHIP BETWEEN SERUM REDUCING SUGAR LEVEL AND FEED INTAKE DURING THE HOUR PRIOR TO BLOOD SAMPLING.


SERUM REDUCING SUGAR (mgm.%)
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
40
60
100
140
180
220
260
300
340
380
420
460
FEED INTAKE (gm.)

follows this route. The glycemic level of ruminants then is not nearly so much the reflection of exogenous glucose absorption that it is in monogastric animals but is largely a product of gluconeogenesis. Secondly, the large size of the rumen and an associated slow rate of passage results in an even flow of ingesta through the gut. This flow is relatively unaffected by temporary changes in the fill of the rumen. This explains in part why there is no significant difference ($0.200 > P > 0.400$) between blood reducing sugar levels when the animals were on feed as compared to the level after 12, 16, 20, and 24 hours of fast (table 3). Figure 2 illustrates the negligable effect of these relatively short periods of fast on the blood reducing sugar level of deer.

The effect of fasting on blood reducing sugar levels in ruminants is variable. Allcroft and Strand (1933) noted that a seven day fast had little effect on sheep. Magee (1932), on the other hand, recorded a rise in blood reducing sugar in goats during a seven day fast. Robertson (1960) also found a substantial increase in the lactating cow. Hodgson et al (1932), however, noted a considerable decrease in the level in dairy cattle during a seven day fast. Reid (1950) recorded that 45 - 46 hour fasts have little effect on blood reducing sugar levels in sheep but that longer periods produced significant decreases. Had the deer used in this experiment been fasted longer perhaps a similar effect would have been noted.

TABLE 3: RESULTS OF EXPERIMENT 1: PART 1

STUDENT t TEST COMPARING THE SERUM REDUCING SUGAR LEVELS OF FASTED AND NONFASTED DEER.

Animal	Treatment: Nonfasted			Fasted (12-24 hours)		
	X_1	$x_1 = X - \bar{x}$	x_1^2	X_2	$x_2 = X - \bar{x}$	x_2^2
U16	91.2	3.3	10.89	85.2	1.0	1.00
	90.4	2.5	6.25	85.4	.8	.64
	96.8	8.9	79.21	83.0	3.2	10.24
	88.7	.8	.64	83.1	3.1	9.61
	88.4	.5	.25	87.6	1.4	1.96
	90.1	2.2	4.84	78.6	7.6	57.76
	91.2	3.3	10.89	86.2	---	-----
	87.6	.4	.16			
U24	83.6	4.3	18.49	92.1	5.9	34.81
	86.5	1.4	1.96	75.4	10.8	116.64
	100.3	12.4	153.76	90.4	4.2	17.64
	90.9	3.0	9.00	80.7	5.5	30.25
	89.2	1.7	2.89	92.1	5.9	34.81
	81.6	6.3	39.69	75.2	11.0	121.00
	85.9	2.0	4.00	97.1	10.9	118.81
	92.1	4.2	17.64			
U34	102.2	14.3	204.49	81.6	4.6	21.16
	91.5	3.6	12.96	79.0	7.2	51.84
	78.6	9.3	86.49	102.9	16.8	282.24
	109.2	21.3	453.69	83.6	2.6	6.76
	82.9	5.0	25.00	82.4	3.8	14.44
	78.6	9.3	86.19	80.9	5.3	28.09
	93.6	5.7	32.49	98.4	12.2	148.84
				87.3	1.1	1.21
U43	81.9	6.0	36.00	81.6	4.6	21.16
	82.9	5.0	25.00	76.3	9.9	98.01
	90.9	3.0	9.00	102.6	16.4	268.96
	78.1	9.8	96.04	84.9	1.3	1.69
	81.1	6.8	46.24	79.7	6.5	42.25
	79.5	8.4	70.56	88.9	2.7	7.29
	76.9	11.0	121.00	88.7	2.5	6.25
	83.1	4.8	23.04	97.4	11.2	125.44

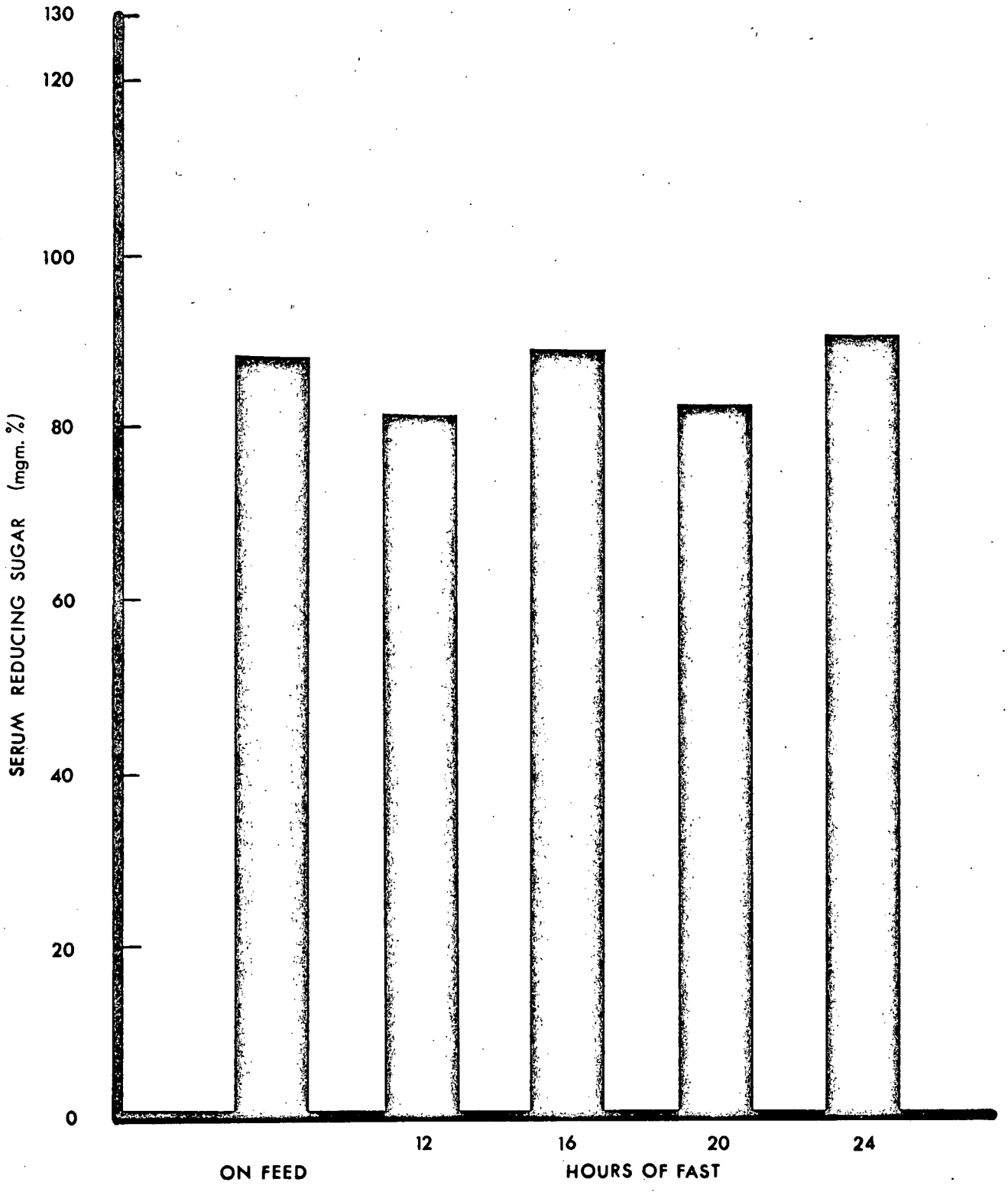
X_1 and X_2 = serum reducing sugar level (mgm.%)

$n_1 = 30$, $n_2 = 31$, $\bar{x}_1 = 86.2$, $\bar{x}_2 = 87.9$, d.f. = 59, $\bar{x}_2 - \bar{x}_1 = 1.7$

$\Sigma x_1^2 = 1,680.80$ $\Sigma x_2^2 = 1,688.75$ $s^2 = 3,369.55/59 = 57.11$

$S_{\bar{x}_1 - \bar{x}_2} = \sqrt{57.11(61)/930} = 1.94$ $t = \frac{1.7}{1.94} = 0.876$ $.400 < P < .200$

FIGURE 2: A COMPARISON BETWEEN THE SERUM REDUCING SUGAR LEVELS OF FASTED AND NONFASTED DEER


130
120
100
80
60
40
20
0
SERUM REDUCING SUGAR (mgm. %)
ON FEED
12
16
20
24
HOURS OF FAST

(2) Means of Restraint

This study was designed to investigate the effect of the means of restraint used to facilitate blood sampling on the level of serum reducing sugar in deer. Two methods of restraint were investigated: physical force, and immobilization with succinylcholine.

Deer physically restrained were thrown and held down by the weight of two or three technicians. The process of throwing the animals generally entailed some fighting, but there was little struggle once they were down and a good hold was secured. It usually took less time from the first intrusion to drawing of the sample using this technique of restraint compared to succinylcholine.

A deer administered a suitable dose of succinylcholine (Cowan 1962), (Nordan, 1962) exhibits a reasonably characteristic response to the onset of paralysis. The 2-3 minutes following injection it remains relatively quiet. Then, just prior to collapse, a brief period of stiff-legged walking, chewing, back-humping and muscle spasms occurs. An inexperienced animal will fight the paralysis and remain standing as long as possible. Its uncontrolled collapse is immediately followed by complete relaxation. An experienced animal, on the other hand, does not fight to the same extent and its collapse is generally more controlled. It is worthy of note that the animals used in this experiment were experienced. Control of the musculature of the jaw, neck, and extremities, usually returned 15-20 minutes after collapse, and the animal would regain its feet apparently unaffected by the paralysis.

The differences in behaviour ellicited by the two methods of restraint are clearly reflected in the response of the blood sugar. As can be seen

from table 4 significantly lower serum reducing sugar levels ($0.001 > P > 0.005$) result when deer are restrained using succinylcholine as compared to when they are restrained using physical force. The difference is illustrated in figure 3. Moreover, it is noteworthy that significantly less variable levels are obtained using succinylcholine. This calculation is presented in table 5.

The difference in blood reducing sugar levels obtained by the two methods of restraint might be accounted for in two ways. Firstly, the degree of muscular energy expended by the animals physically restrained is considerably greater than when succinylcholine immobilization is used. Solandt and Ferguson (1932) investigated the effects of strenuous exercise of short duration on blood reducing sugar in man. They found that 30-45 seconds of standing running at top speed caused a noticable increase in blood reducing sugar five minutes later. Reichard et al (1961) using ^{14}C labled glucose noted that there was an increased uptake of blood sugar by working muscle, compensated by increased hepatic glucose output. The struggle of the physically restrained deer, then, could account in part for the high blood sugar levels recorded. Secondly, though it is impossible to assess with certainty the degree of fright and pain experienced by the animals when restrained by either method, it is undoubtedly true that physical restraint is more traumatic than immobilization with succinylcholine. Therefore, the difference in blood reducing sugar levels obtained by the two methods of restraint, might reflect the degree of stimulation of the sympathicoadrenal complex. The activation of this system has been shown to occur in almost all types of stress situations. The fear and pain associated with both means of restraint, then, trigger the complex resulting

TABLE 4: RESULTS OF EXPERIMENT 1: PART 2

STUDENT t TEST COMPARING SERUM REDUCING SUGAR LEVELS OF DEER IMMOBILIZED WITH SUCCINYLCHOLINE AND THOSE RESTRAINED PHYSICALLY.

Animal	Treatment: Succinylcholine			Physical		
	X_1	$x_1=X-\bar{X}$	x_1^2	X_2	$x_2=X-\bar{X}$	x_2^2
U 16	88.4	4.4	19.36	91.2	1.4	1.96
	90.1	6.1	37.21	90.4	.6	.36
	91.2	7.2	51.84	96.8	7.0	49.00
	87.5	3.5	12.25	88.7	1.1	1.21
	85.4	1.4	1.96	85.2	4.6	21.16
	83.1	.9	.81	83.0	6.8	46.24
	78.6	5.4	29.16	87.6	2.2	4.84
				86.2	3.6	12.96
U24	89.2	5.2	27.04	83.6	6.2	38.44
	81.6	2.4	5.76	86.5	5.3	10.89
	85.9	1.9	3.61	100.3	10.5	110.25
	92.1	8.1	65.61	90.9	1.1	1.21
	75.4	8.6	73.96	92.1	2.3	5.29
	80.7	3.3	10.89	90.4	.6	.36
	75.2	8.8	77.44	92.1	2.3	5.29
				97.1	7.3	53.29
U34	82.9	1.1	1.21	102.2	12.4	153.76
	78.6	5.4	29.16	91.5	1.7	2.89
	93.6	9.6	92.16	78.6	11.2	125.44
	79.0	5.0	25.00	109.2	19.4	376.89
	83.6	.4	.16	81.6	8.2	67.24
	80.9	3.1	9.61	103.0	13.2	174.24
	87.3	3.3	10.89	82.4	7.4	54.76
				98.4	8.6	73.96
U43	81.1	2.9	8.41	81.9	7.9	62.41
	79.5	4.5	20.25	90.9	1.1	1. 21
	76.9	7.1	50.41	78.1	11.7	136.89
	83.1	.9	.81	82.9	6.9	47.61
	76.3	7.7	59.29	81.6	8.2	67.24
	84.9	.9	.81	102.6	12.8	163.84
	88.9	4.9	24.01	79.7	10.1	102.01
	97.4	13.4	179.56	88.7	1.1	1.21

X_1 and X_2 = serum reducing sugar level (mgm.%).

$n_1=29$, $n_2=32$, $\bar{x}_1=84.0$, $\bar{x}_2=89.8$, $\bar{x}_2-\bar{x}_1 = 5.8$

$\Sigma x_1^2 = 928.64$, $\Sigma x_2^2 = 1,973.82$, d.f. = 59

$s^2 = 2,902.46/59 = 49.19$, $S_{\bar{x}_1-\bar{x}_2} = \sqrt{49.19(61)/928} = 1.80$

$t = \frac{5.8}{1.8} = 3.222$ $\quad .005<P<.001$

FIGURE 3: A COMPARISON BETWEEN THE SERUM REDUCING SUGAR LEVELS OF DEER RESTRAINED PHYSICALLY AND THOSE IMMOBILIZED WITH SUCCINYLCHOLINE.

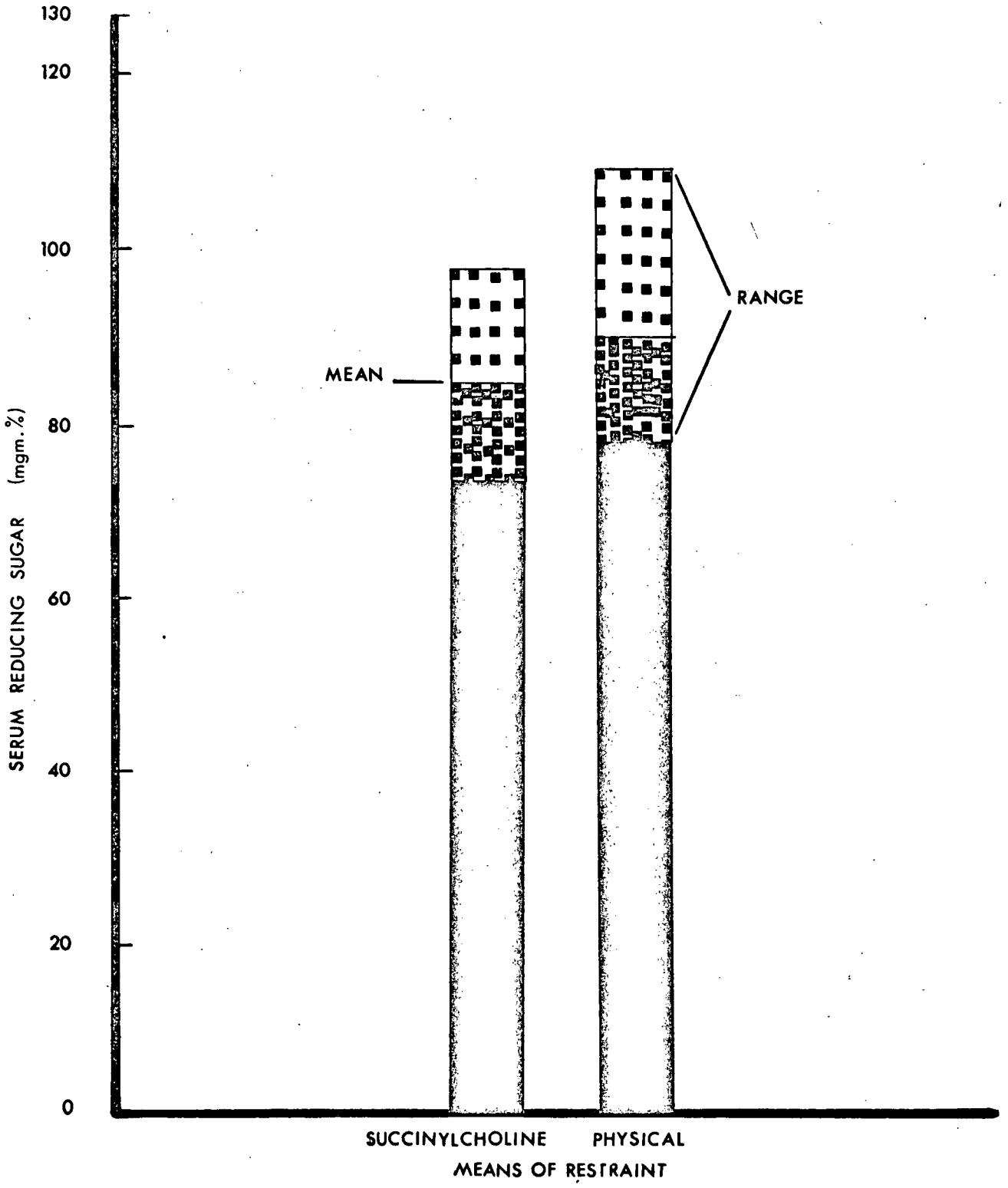

130
120
100
80
60
40
20
0
SERUM REDUCING SUGAR (mgm.%)
MEAN
RANGE
SUCCINYLCHOLINE
PHYSICAL
MEANS OF RESTRAINT

TABLE 5: RESULTS OF EXPERIMENT 1: PART 2

F TEST* FOR DETERMINING THE REPRODUCIBILITY OF SERUM REDUCING SUGAR LEVELS BY THE TWO METHODS OF RESTRAINT.

Animal	X_1	X_1^2	X_2	X_2^2
U16	88.4	7,814.56	91.2	8,317.44
	90.1	8,118.01	90.4	8,172.16
	91.2	8,317.44	96.8	9,370.24
	87.5	7,656.25	88.7	7,867.69
	85.4	7,293.16	85.2	7,259.04
	83.1	6,905.61	83.0	6,889.00
	78.6	6,177.96	87.6	7,673.76
			86.2	7,430.44
U24	89.2	7,956.64	83.6	6,988.96
	81.6	6,658.56	86.5	7,482.25
	85.9	7,378.81	100.3	10,060.09
	92.1	8,482.41	90.9	8,262.81
	75.4	5,685.16	92.1	8,482.41
	80.7	6,512.49	90.4	8,172.16
	75.2	5,655.04	92.1	8,482.41
			97.1	9,428.41
U34	82.9	6,872.41	102.2	10,444.84
	78.6	6,177.96	91.5	8,372.25
	93.6	8,760.96	78.6	6,177.96
	79.0	6,241.00	109.2	11,924.64
	83.6	6,988.96	81.6	6,658.56
	80.9	6,544.81	103.0	10,609.00
	87.3	7,621.29	82.4	6,789.76
			98.4	9,682.56
U43	81.1	6,577.21	81.9	6,707.61
	79.5	6,320.25	90.9	8,262.81
	76.9	5,916.61	78.1	6,099.61
	83.1	6,905.61	82.9	6,872.41
	76.3	5,821.69	81.6	6,658.56
	84.9	7,208.01	102.6	10,526.76
	88.9	7,903.21	79.7	6,352.09
	97.4	9,486.76	88.7	7,867.69

X_1 and X_2 = serum reducing sugar level (mgm.%)

$\leqslant x_1^2 = 205,955.84, \quad \leqslant X_2^2 = 260,346.38$

$(\leqslant X_1)^2 = 5,945,794.56, \quad (\leqslant X_2)^2 = 8,267,925.16, \quad d.f._1=28 \quad d.f._2=31$

$$\phi = \frac{\leqslant X^2 - \frac{(\leqslant X)^2}{n}}{n} \qquad F \equiv \frac{\phi_1}{\phi_2} \qquad F = \frac{63.66}{33.16} = 1.919$$

* Natelson, S. Microtechniques of Clinical Chemistry (1961) pp.493. C.C. Thomas Springfield Illinois, U.S.A.

in manifold physiological responses. For example, heart rate, systolic pressure, cardiac output, blood flow through the liver, brain, kidney, and musculature are undoubtedly increased. On the other hand, the activity of the intestine and genital system are slowed. Of principal importance the adrenal medulla is stimulated to cause an increased release of epinepherine. Epinepherine augments the effects of stimulation of the sympathetic system and ellicits an important hyperglycemic response. It also stimulates the adenohypothesis to release ACTH which increases the release of adrenal cortical hormones, potent in promoting gluconeogenesis from protein (Turner 1960). Reid (1962) attempted to correlate blood sugar and plasma cortisol levels in sheep under stress but was unsuccessful. He assumed that the hyperglycemia noted was an effect mediated by epinepherine rather than by cortisol. Ellis (1956) stated that the metabolic effects of epinepherine on carbohydrate metabolism are far too complex to allow any precise conclusions as to the metabolic significance of the variations in blood sugar recorded.

The data presented in this experiment indicate that the means used to restrain deer during the blood letting procedure has a pronounced effect on the level of serum reducing sugar. Immobilization with succinylcholine results in lower and less variable serum reducing sugar levels.

(3) The Influence of Sex

The data of this experiment (table 6), (figure 4) indicate that there is no significant difference ($.200 > P > .400$) between the serum reducing sugar levels of bucks as compared to those of does. This result is supported by the fact that no difference in blood reducing sugar levels between the sexes has been reported in cattle (Hodgson et al, 1932), in calves (Voelker, 1955), or between wethers and ewes (Reid, 1950). As indicated in table 1 the majority of the blood samples for this experiment were drawn in late October and early November. Although the bucks were entering the rutting period at this time there was little evidence of aggressive behaviour, and no effect on serum reducing sugar levels occurred.

TABLE 6: RESULTS OF EXPERIMENT 1: PART 3

STUDENT t TEST COMPARING THE SERUM REDUCING SUGAR LEVELS OF MALE DEER TO THOSE OF FEMALE DEER.

Male			Female		
X_1	$x_1=X-\bar{X}$	x_1^2	X_2	$x_2=X-\bar{X}$	x_2^2
91.2	5.1	26.01	83.6	4.4	19.36
90.4	4.3	18.49	86.5	1.5	2.25
96.8	10.7	114.49	100.1	12.1	146.41
88.7	2.6	6.76	90.9	2.9	8.41
88.4	2.3	5.39	89.2	1.2	1.44
90.1	4.0	16.00	81.6	6.4	40.96
91.2	5.1	26.01	85.9	2.1	4.41
87.6	1.5	2.25	92.1	4.1	16.81
85.2	.9	.81	92.1	4.1	16.81
83.0	3.1	9.61	90.4	2.4	5.76
87.6	1.5	2.25	92.1	4.1	16.81
86.2	.1	.01	97.1	9.1	82.81
85.4	.7	.49	75.4	12.6	158.76
83.1	3.0	9.00	80.7	7.3	53.29
78.6	7.5	56.25	75.2	12.8	163.84
81.9	4.2	17.64	102.2	14.2	201.64
82.9	3.2	10.24	91.5	3.5	12.25
90.9	4.8	23.04	78.6	9.4	88.36
78.1	8.0	64.00	109.2	21.2	449.44
81.1	5.0	25.00	82.9	5.1	26.01
79.5	6.6	43.56	78.6	9.4	88.36
77.0	9.1	82.81	93.6	5.6	31.36
83.1	3.0	9.00	81.6	6.4	40.96
102.6	16.5	275.25	82.4	5.6	31.36
79.7	6.4	40.96	92.4	4.4	19.36
88.6	2.5	6.25	79.0	9.0	81.00
76.3	9.8	96.04	83.6	4.4	19.36
84.9	1.2	1.44	80.9	7.1	50.41
88.9	2.8	7.84	87.3	.7	.49
97.4	11.3	127.69			

X_1 and X_2 = serum reducing sugar level (mgm.%).

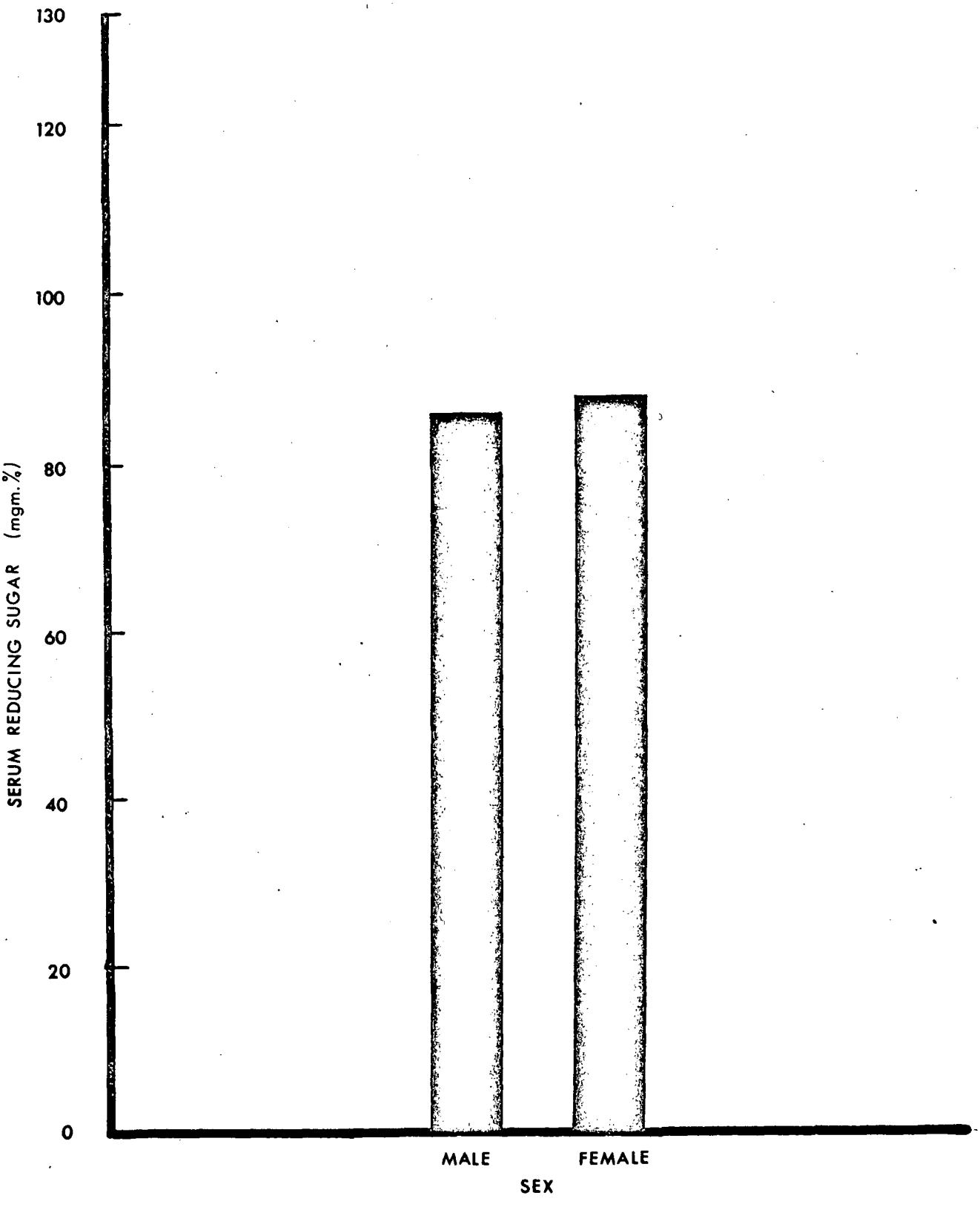
$n_1 = 31, \quad n_2 = 30, \quad \bar{x}_1 = 86.1, \quad \bar{x}_2 = 88.0, \quad \bar{x}_2 - \bar{x}_1 = 1.9$

$\leqslant x_1^2 = 1{,}141.73, \quad \leqslant x_2^2 = 2{,}103.49, \quad$ d.f. $= (31 + 30) - 2 = 59$

$s^2 = 3{,}245.22/59 = 55.0, \quad S_{\bar{x}_1 - \bar{x}_2} \sqrt{55.0(61)/928} = 1.9$

$t = \frac{1.9}{1.9} = 1.0 \qquad .400 < P < .200$

FIGURE 4: A COMPARISON BETWEEN THE SERUM REDUCING SUGAR LEVELS OF MALE AND FEMALE BLACK-TAILED DEER.


130
120
100
80
60
40
20
0
SERUM REDUCING SUGAR (mgm.%)
MALE
FEMALE
SEX

(4) The Influence of Time of Day

This experiment was designed to determine if any change in serum reducing sugar level occurs during the day in deer. The animals were on feed as outlined earlier, and blood samples were let at each of 0800, 1200, 1600, and 2000 hours.

As shown in table 7 and figure 5 the serum reducing sugar levels in samples drawn at 0800, 1200, and 1600 hours are essentially the same. The calculations presented in table 8 show that the slightly higher levels found in samples drawn at 2000 hours are not significantly different from the mean of the levels found at 0800, 1200, and 1600 hours. This elevation at 2000 hours, which was found in the fasted (figure 2) and nonfasted deer (figure 5) might be due to a mild stimulation of the sympathicoadrenal complex. Although the deer used in this experiment were accustomed to considerable activity in the wildlife unit during the day, the disturbance at night (light, talk, movement) associated with blood sampling was unusual and could conceivably ellicited such a response. Reid (1950) also noted in sheep that the blood sugar level was higher in the late afternoon than in the morning but could offer no explanation for this. The findings of this experiment are supported by the investigations of Schuhecker (1925) and by Hitchcock and Phillipson (1946) who found no diurnal variation in the blood sugar of adult ruminants. Teichman (1952) as reported by Reid (1950), and Kennedy et al (1939) reported a marked diurnal variation in the blood sugar levels of calves in response to feeding. Preston and Ndumbe (1961) studied the diurnal variations in the blood sugar of ruminating calves. They noted a postprandial hyperglycemia similar to that found in monogastric animals in calves on a milk

TABLE 7: RESULTS OF EXPERIMENT 1: PART 4

THE INFLUENCE OF TIME OF DAY ON SERUM REDUCING SUGAR LEVELS IN DEER ON FEED.

	Serum Reducing Sugar (mgm.%)			
Time	0800	1200	1600	2000
	91.2	90.4	96.8	88.7
	88.4	90.1	91.2	87.6
	83.6	86.5	100.1	90.9
	89.2	81.6	85.9	92.1
	102.2	91.5	78.6	109.2
	82.9	82.9	78.6	93.6
	81.9	79.5	90.9	78.1
	81.1		77.0	83.1

Average serum reducing sugar level at 0800 = 87.6 mgm.%
1200 = 86.1 mgm.%
1600 = 87.4 mgm.%
2000 = 90.4 mgm.%

FIGURE 5: COMPARING SERUM REDUCING SUGAR LEVELS FOUND IN DEER ON FEED AT DIFFERENT TIMES DURING THE DAY.

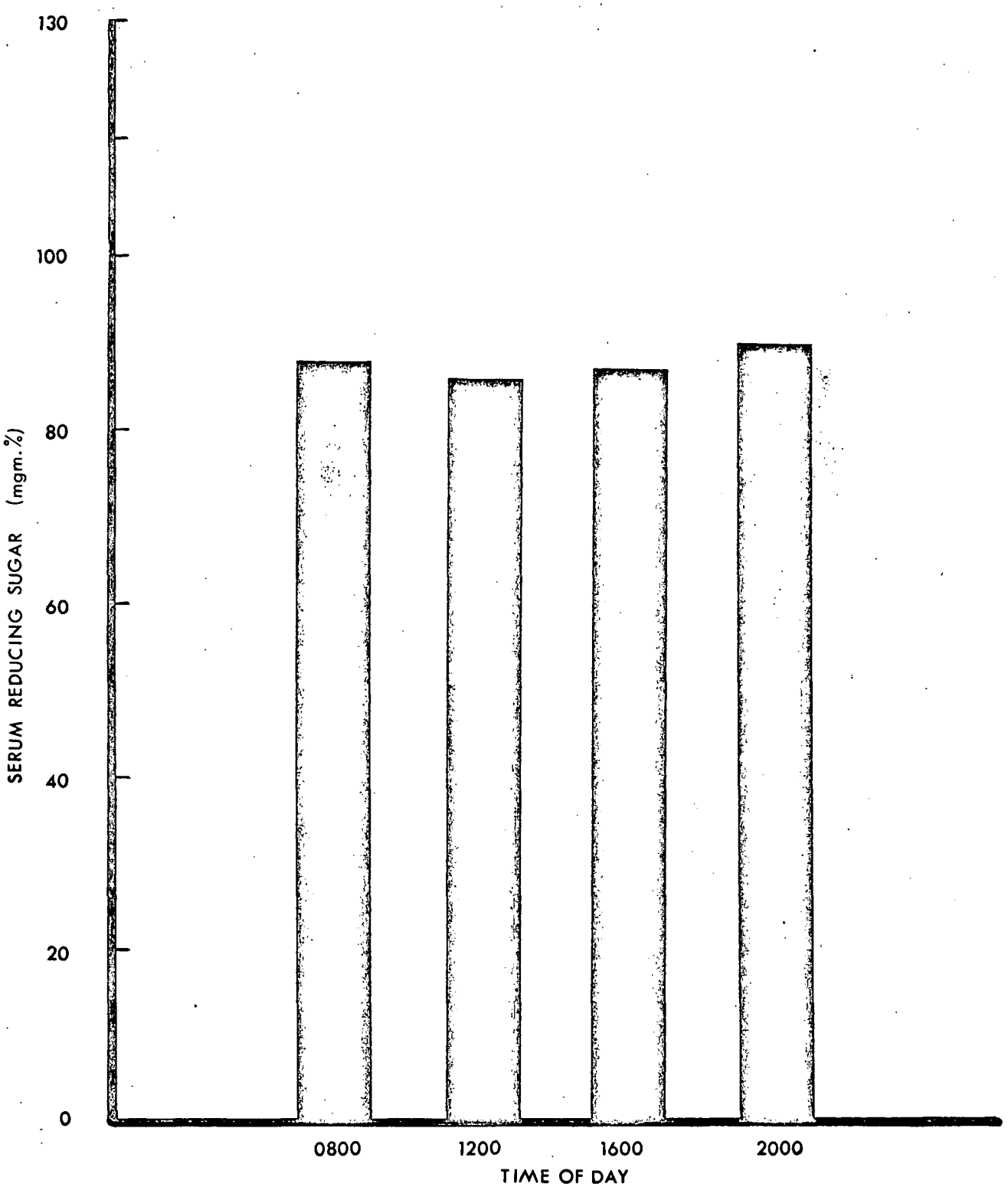

130
100
80
60
40
20
0
SERUM REDUCING SUGAR (mgm.%)
0800
1200
1600
2000
TIME OF DAY

TABLE 8: RESULTS OF EXPERIMENT 1: PART 4

STUDENT t TEST COMPARING SERUM REDUCING SUGAR LEVELS OF DEER SAMPLED AT 0800, 1200, and 1600 HOURS TO THOSE SAMPLED AT 2000 HOURS.

0800, 1200, 1600 Hours			2000 Hours		
X_1	$x_1=X-\bar{x}$	x_1^2	X_2	$x_2=X-\bar{x}$	x_2^2
91.2	4.2	17.64	88.7	1.7	2.89
88.4	1.4	1.96	87.6	2.8	7.84
83.6	3.4	11.56	90.9	.5	.25
89.2	2.2	4.84	92.1	1.7	2.89
102.2	5.2	27.04	109.2	18.8	353.44
82.9	4.1	16.81	93.6	3.2	10.24
81.9	5.1	26.01	78.1	12.3	151.29
81.1	5.9	34.81	83.1	7.3	53.29
90.4	3.4	11.56			
90.1	3.1	9.61			
86.5	.5	.25			
91.5	4.5	20.25			
82.9	4.1	16.81			
79.5	7.5	56.25			
96.8	9.8	96.04			
91.2	4.2	17.64			
100.1	13.1	171.61			
85.9	1.1	1.21			
78.6	8.4	70.56			
78.6	8.4	70.56			
90.9	3.9	15.21			
77.0	10.0	100.00			
81.6	5.4	29.16			

X_1 and X_2 = serum reducing sugar (mgm.%)

$n_1 = 23$, $\bar{x}_1 = 87.0$, $\Sigma x_1^2 = 827.39$, $\bar{x}_2-\bar{x}_1 = 3.4$, d.f. = 29

$n_2^2 = 8$, $\bar{x}_2 = 90.4$ $\Sigma x_2^2 = 582.13$,

$s^2 = 1{,}409.52/29 = 48.6$

$S_{\bar{x}_1-\bar{x}_2^2} = \sqrt{48.6(31)/184} = \sqrt{8.18} = 2.86$

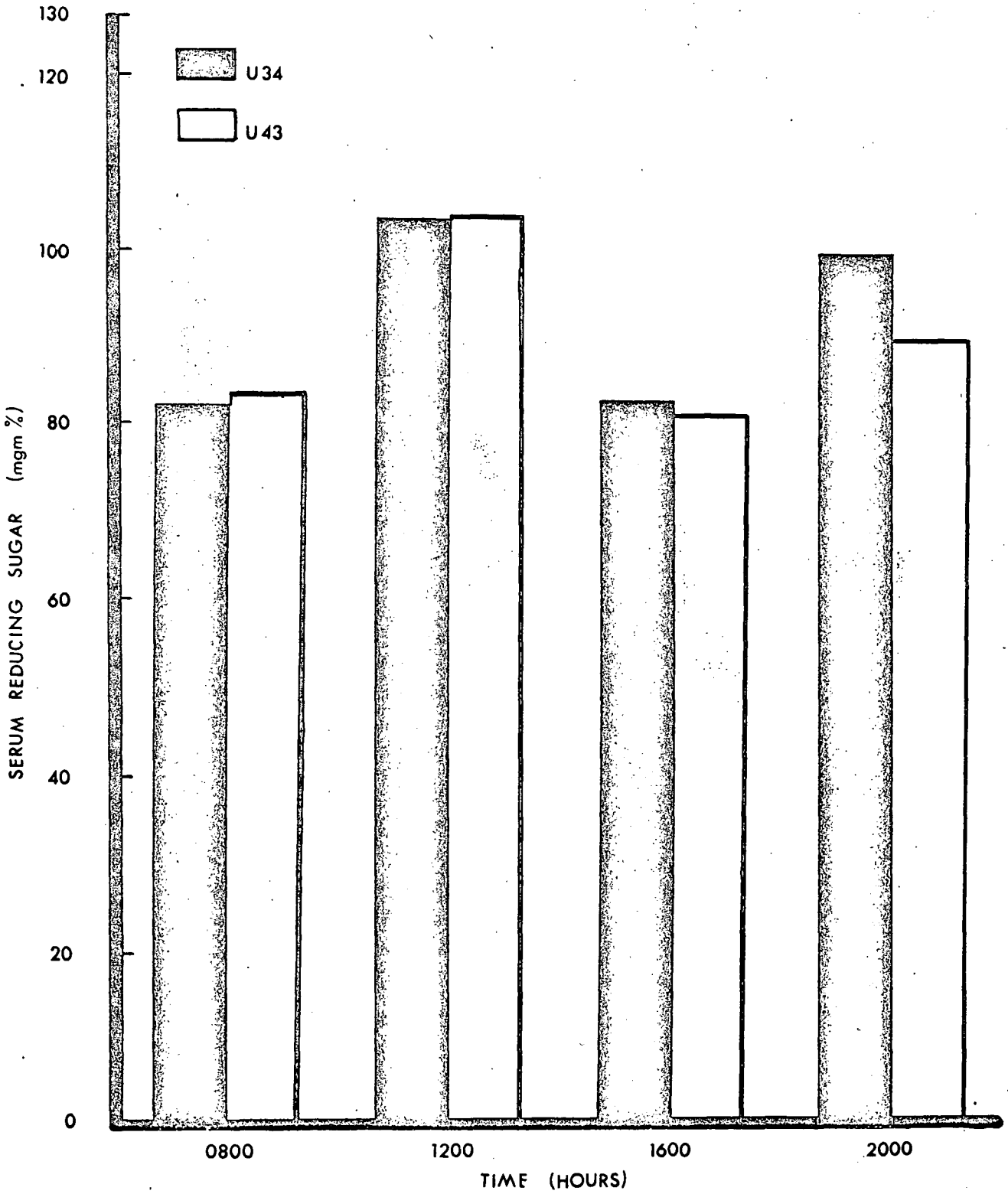
$t = \frac{3.4}{2.86} = 1.188$ $.400 < P < .200$

diet. Calves on dried grass, however, showed no response to feeding. Another group on a concentrate ration exhibited a postprandial hypoglycemia, presumably due to active fermentation in the rumen immediately following feeding. The results of these studies on calves do not detract from the findings of this experiment on deer. It is unlikely that the energy metabolism of calves, even ruminating calves, as reflected by blood reducing sugar levels can be compared to any advantage to that of adult deer.

It is pertinent to note that blood sugar levels of animals sampled on the same day at the same time occasionally varied in the same direction. Figure 6 shows the blood reducing sugar levels of U34 and U43 during the fasting experiment. Both deer were sampled at 0800, 1200, 1600, and 2000 hours, in every case U34 was sampled first. In this particular example the reducing sugar levels of both deer are almost identical and their coincident variation is striking. In other instances, although the reducing sugar levels of the deer are different the same coincidence of variation occurs. This phenomena is likely due to excitement of the animals preceeding blood sampling. If, for example, the deer were undisturbed the hour or so prior to blood sampling the blood sugars of both would be relatively low. Had some unusual disturbance occurred, on the other hand, then the blood sugars of the deer would be correspondingly higher. Under the conditions of the experiment it was impossible to control this 'extraneous disturbance' factor. In this regard it is noteworthy that the second animal sampled each day did not show consistently higher serum reducing sugar levels as compared to the first deer sampled. This can be accounted for in two ways. Firstly, and most important, the animals were accustomed to some

disturbance during the day and blood sampling was carried out quietly in a laboratory somewhat removed from the other animals in the unit. Secondly, it is impossible to assess the starting or normal blood reducing sugar level, therefore, if the second animal had a lower normal level than the first any increase due to excitement could be masked.

FIGURE 6: ILLUSTRATING THE RELATIONSHIP THAT CAN OCCASIONALLY OCCUR BETWEEN THE SERUM REDUCING SUGAR LEVELS OF TWO ANIMALS SAMPLED AT THE SAME TIMES ON THE SAME DAY.


130
120
100
80
60
40
20
0
U34
U43
SERUM REDUCING SUGAR (mgm %)
0800
1200
1600
2000
TIME (HOURS)

EXPERIMENT 2

EXPERIMENT 2

This experiment was designed to determine if a change in diet from a concentrate ration to chopped alfalfa hay would alter the blood reducing sugar levels found in deer.

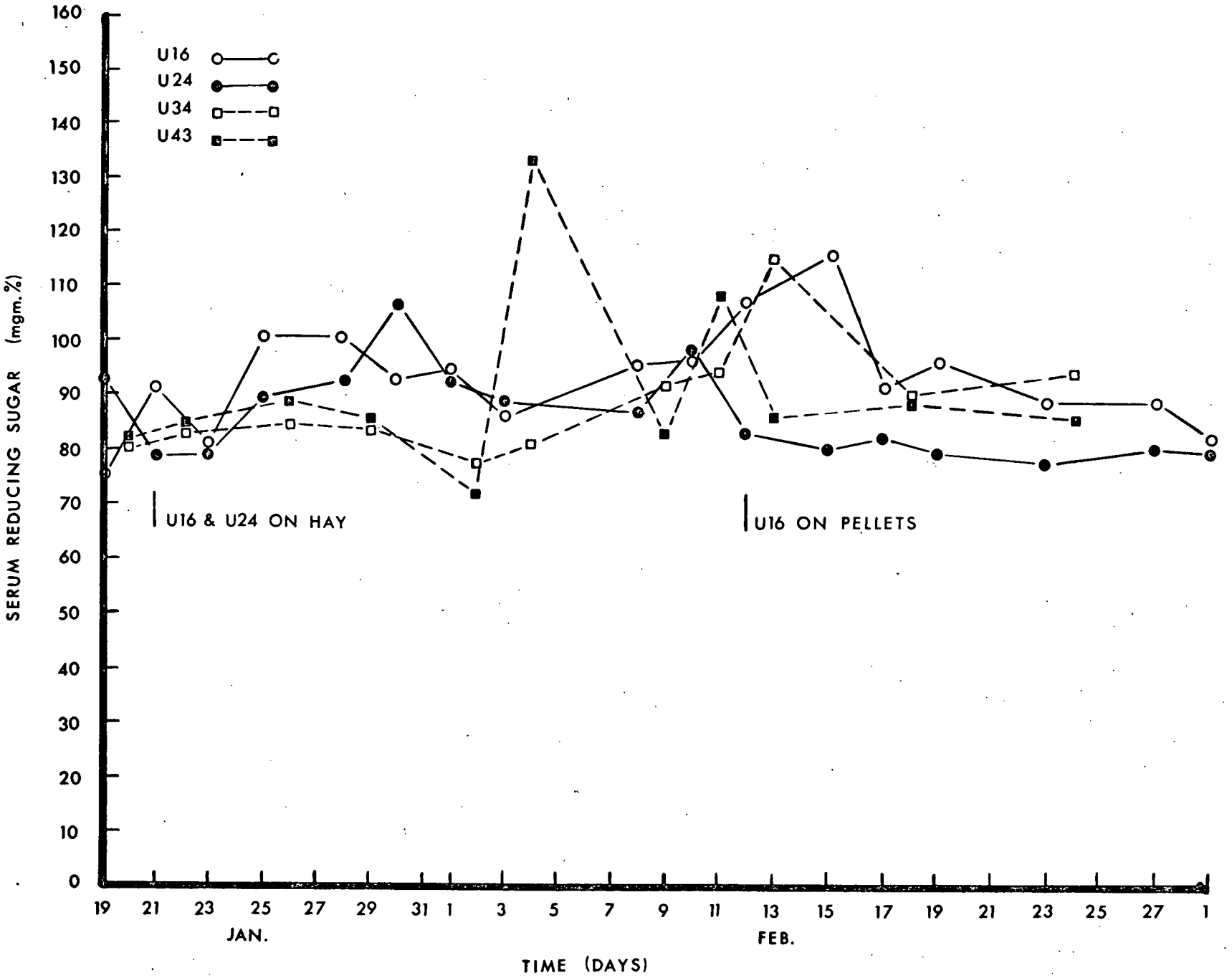
As in the first experiment the animals were placed on a feeding program such that each was offered 1/4 of 80% of its daily intake (calculated from the daily intake of the previous week) at each of 0700, 1100, 1500, and 2000 hours. As indicated earlier some control was thus exercised over the amount and time of feed intake the hour prior to blood letting. A week was allowed for the deer to become accustomed to this regime before the experiment was begun. All blood samples were drawn about 0800 hours, one hour after feeding. The remaining feed of the animal being sampled was weighed back and a record was kept of the feed intake the hour prior to blood letting.

On the basis of the results of experiment 1 all animals were immobilized with succinylcholine before blood samples were drawn.

10 cc. samples were let from all deer once they were accustomed to the new feeding regime. Following this U16 and U24 were taken off feed for two days and then offered chopped alfalfa hay on the same schedule that the concentrate ration had been given. U34 and U43 were treated as controls, remaining on concentrate throughout the experiment. Blood samples were drawn every second day, on alternate days, from both animals in each group. The deer were weighed regularly throughout the experiment.

Figure 7 illustrates the considerable variation found in the serum reducing sugar levels of the deer during the experimental period. As can

FIGURE 7: ILLUSTRATING THE CHANGES IN SERUM REDUCING SUGAR LEVELS THAT OCCURRED IN DEER ON PELLETS AND THOSE ON HAY.


U16
U24
U34
U43
SERUM REDUCING SUGAR (mgm.%)
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
U16 & U24 ON HAY
U16 ON PELLETS
19 21 23 25 27 29 31 1 3 5 7 9 11 13 15 17 19 21 23 25 27 1
JAN.
FEB.
TIME (DAYS)

be seen from tables 8 and 9 no clearly defined trends are apparent in the levels of reducing sugar in the control animals. Both deer exhibit levels ranging quite consistently between 80 mgm.% - 90 mgm.%. U43 showed the greatest extremes in levels with a low of 72 mgm.% and a high of 133 mgm.%. The serum reducing sugar levels of the experimental deer (U16 and U24) as shown in tables 10 and 11, remained reasonably constant throughout the experiment and were generally within the same range as those of the controls. The data for U24 (table 11) might indicate a slight increase in serum reducing sugar level during the first week on hay. Only three samples, however, contribute to the higher level and considering the general variability encountered it is unlikely that this is significant. The relatively low levels obtained toward the end of the experiment (see figure 7) are no lower than those exhibited by this animal on the pelleted ration in earlier experiments (see table 3). There was, therefore, no significant change in the levels of serum reducing sugar in this deer after a period of six weeks on hay. The range in the level of reducing sugar (85 mgm.% - 95 mgm.%) of U16 is generally higher than that of the other animals. As indicated in table 10, however, this deer showed no remarkable change in level of serum reducing sugar despite his serious loss of body weight on hay. The feed intake of the control animals (tables 8 and 9) was relatively constant and apparently sufficient as they maintained their weight with only minor fluctuations throughout the experiment. The hay intake of U16 and U24 was also reasonably constant (tables 10 and 11), both however, suffered considerable weight loss. In the case of U16 this was serious enough to force his return to the pelleted ration after only three weeks on hay. During the last few days on this diet U16 was so weak and

TABLE 8: FEED INTAKE, SERUM REDUCING SUGAR, AND WEIGHT DATA OF U34 THROUGHOUT EXPERIMENT 2.

Date	Feed Intake (gms.pellets)	Reducing Sugar (mgm.%)	Weight (lb.)
19/1/65	834	---	---
20	902	82.4	---
21	963	---	950
22	753	82.9	---
23	753	---	94.5
24	743	---	---
25	755	---	94
26	858	84.4	---
27	865	---	95
28	825	---	---
29	946	84.1	94.5
30	427	---	---
31	747	---	---
1/2/65	699	---	93
2	811	77.4	---
3	815	---	93
4	806	80.9	---
5	881	---	92.5
6	806	---	---
7	515	---	---
8	629	---	---
9	847	91.8	---
10	761	---	93
11	805	94.2	---
12	906	---	93
13	714	115.7	---
14	578	---	91
15	772	---	---
16	946	---	92
17	860	---	---
18	914	90.4	93
19	868	---	---
20	669	---	---
21	829	---	92
22	728	---	---
23	859	---	93
24	1,004	94.9	---
25	1,004	---	93.5
26	890	---	---
27	562	---	---
28	516	---	93
1/3/65	875	---	---

TABLE 9: FEED INTAKE, BLOOD REDUCING SUGAR, AND WEIGHT DATA OF U43 THROUGHOUT EXPERIMENT 2.

Date	Feed Intake (gms.pellets)	Reducing Sugar (mgm.%)	Weight (lb.)
19/1/65	1,100	---	---
20	1,100	81.1	---
21	1,100	---	126.5
22	825	83.4	---
23	825	---	128
24	1,011	---	---
25	1,100	---	129
26	1,100	88.9	---
27	1,100	---	128
28	1,056	---	---
29	1,100	85.2	128
30	550	---	---
31	880	---	---
1/2/65	1,100	---	128
2	1,100	71.9	---
3	1,100	---	126
4	1,100	133.3	---
5	1,100	---	124.5
6	1,100	---	---
7	905	---	---
8	1,100	---	---
9	943	83.1	---
10	1,100	---	126
11	1,100	108.8	---
12	1,100	---	126.5
13	825	85.9	---
14	970	---	124
15	1,032	---	---
16	1,100	---	125
17	1,100	---	---
18	1,100	88.9	126.5
19	1,100	---	---
20	1,100	---	125.5
21	1,100	---	---
22	1,082	---	127
23	1,100	---	---
24	1,100	85.9	127.5
25	1,100	---	---
26	1,100	---	---
27	852	---	---
28	536	---	126.5
1/3/65	1,235	---	---

TABLE 10: FEED INTAKE, BLOOD REDUCING SUGAR, AND WEIGHT DATA OF U16 THROUGHOUT EXPERIMENT 2.

Date	Feed Intake (gms. hay)	Reducing Sugar (mgm.%)	Weight (lb.)
19/1/65	----	92.7	----
20	----	----	----
21	285	79.2	109
22	336	----	----
23	349	79.7	108.5
24	350	----	----
25	472	100.8	----
26	482	-----	----
27	631	-----	105
28	580	100.8	----
29	810	----	105
30	403	92.7	----
31	756	----	----
1/2/65	648	94.6	103.5
2	500	----	----
3	333	85.7	102.5
4	246	----	----
5	231	----	99.5
6	420	----	----
7	274	----	----
8	602	95.8	----
9	702	----	----
10	622	96.4	97
11	590	----	----
12	162/549*	107.6	97
13	819	----	----
14	1070	----	97.5
15	721	115.7	----
16	1243	----	96
17	1168	92.1	
18	1268	----	91
19	1646	96.4	----
20	1865	-----	94
21	1544	----	----
22	1805	----	96
23	1968	88.9	----
24	2268	----	96.5
25	2198	----	----
26	1837	----	100.5
27	2276	88.9	----
28	1969	----	99.5
1/3/65	2276	82.6	----

* returned to pellets

TABLE 11: FEED INTAKE, BLOOD REDUCING SUGAR, AND WEIGHT DATA OF U24 THROUGHOUT EXPERIMENT 2.

Date	Feed Intake (gms.hay)	Reducing Sugar (mgm%)	Weight (lb.)
19/1/65	---	75.2	---
20	---	---	---
21	234	91.2	107
22	235	---	---
23	389	80.9	105.5
24	464	---	---
25	414	89.2	---
26	372	---	---
27	737	---	105.5
28	632	92.7	---
29	699	---	106.5
30	344	106.8	---
31	667	---	---
1/2/65	499	92.7	104.5
2	416	---	---
3	570	88.9	103
4	452	---	---
5	630	---	101.5
6	669	---	---
7	478	---	---
8	676	87.0	---
9	564	---	---
10	559	97.8	101
11	610	---	---
12	774	83.1	100.5
13	602	---	---
14	773	---	---
15	947	80.4	---
16	507	---	98.5
17	901	82.4	---
18	831	---	100.5
19	939	79.7	---
20	845	---	98
21	1,059	---	---
22	962	---	100
23	958	77.9	---
24	891	---	99.5
25	756	---	---
26	726	---	97.5
27	756	80.7	---
28	479	---	97
1/3/65	1,112	79.5	---

lethargic that he could not walk to the scale but had to be carried. U24 lost weight consistently during the first three weeks of the experiment, thereafter she maintained weight stasis.

There are three possible explanations for the inability of these animals to maintain their weight during the early part of the experiment. Firstly, inadequate hay intake, secondly, the poor quality of the hay, and thirdly, slow development of a rumen microflora capable of handling the hay. Although no attempt was made to assess in detail the nutritive quality of the hay it was apparent from the considerable amount of coarse material left by the deer that it was poor. This and the physical limitations of the feeding arrangements undoubtedly resulted in insufficient intake. It is well estbalished that a considerable change in rumen microflora is associated with any marked alteration in diet. The time required to effect this change is variable, being dependent on a multitude of factors. Warner (1962) noted that in a sheep switched from a diet of hay alone to one of hay plus concentrate all major changes in rumen microflora were completed by ten days. He points out that this is usually the period needed for adaptation to a new diet in ruminants. Gouws (1965) studied the alteration in cellulytic bacterial species in sheep associated with a change from lucerne to teff hay. He stated that the time lapse between a change of diet and the attainment of a balance characteristic of the new diet varied from animal to animal. In one case he studied, the change was complete in a week, in another there was no change after four weeks. Although it is attractive to account for the loss in body weight of the deer during the first three weeks as being due to the development of a rumen microflora capable of fermenting hay, to do so is complete conjecture.

The principal object of this experiment was to determine the effect of a change in ration on the serum reducing sugar level in deer. It is apparent from the data (tables 8, 9, 10, and 11) that there was no change in this parameter associated with the alteration in diet. This finding is supported by that of Hibbs (1956) who noted that calves fed various ratios of hay to grain had essentially the same level of blood reducing sugar. Lambert (1955) reported similar results with calves fed various ratios of calf starter and alfalfa.

There were two factors inherent in the experiment that might have worked to alter the serum reducing sugar levels of the deer. One was the nature of the diet. Annison et al (1959) found that when sheep on a diet of hay plus additives were turned out to lush spring grass their blood reducing sugar levels rose from 45 mgm.% to 60 mgm.%. He attributed this increase to either or both the increased availability of proportionate or the abundant supply of lactate. Presumably the hay used in this experiment did not provide a sufficient alteration in nutrients to cause a change in the reducing sugar level. The second factor that might have led to a change in the level of serum reducing sugar was the stress that could have been imposed on the deer by the substitute regime. Reid (1950) and Wright (1962) showed that underfeeding can produce a fall in blood sugar levels in pregnant ewes. They also noted that blood reducing sugar levels fall during lactation in these animals. Their explanation for the decrease in levels found was that the demands of, in the first case the feotus, and in the second, milk production, outstripped the ewes exogenous and endogenous sources of glucose. During the experiment reported here, a stress, as reflected by the loss in body weight suffered by the deer, particularly U16, was undoubtedly imposed.

However, sufficient exogenous and endogenous material must have been present to maintain the serum reducing sugar level as no decrease was found.

EXPERIMENT 3

EXPERIMENT 3

This experiment was designed to explore the possibility that the factor of time during the actual drawing of the blood sample had an influence on the blood reducing sugar levels determined.

On the basis of the results of experiment 1 no control of feed intake was exercised.

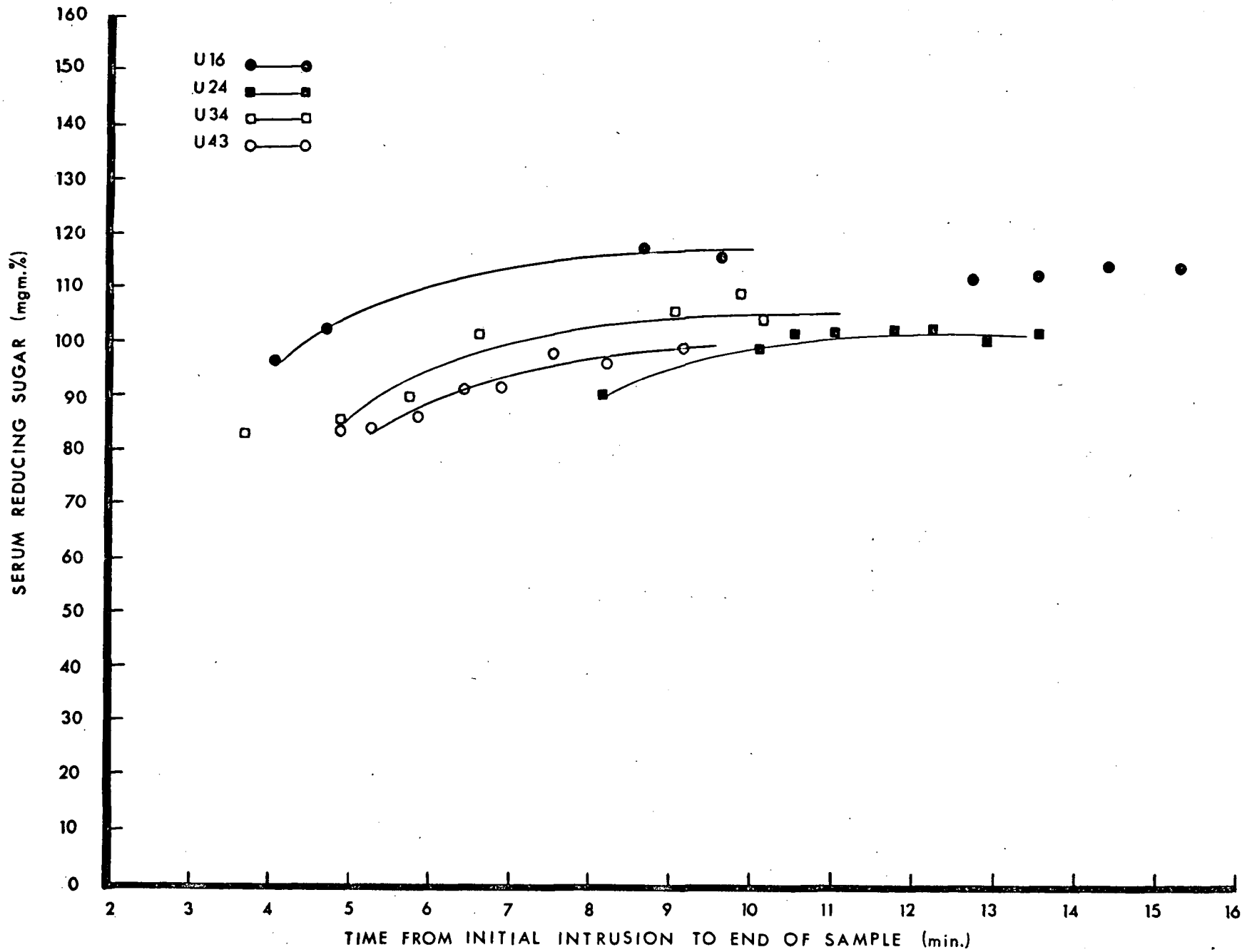
The deer were immobilized with succinylcholine before blood samples were drawn. Each animal was immobilized on separate days generally in the morning. As outlined earlier accurate accounts of the time required for each aspect of every blood letting were made. In this experiment sequential rather than single samples were drawn from each animal. Once the deer was immobilized and the vacutainer needle seated, a series of samples were released at roughly 30 second intervals into separate, untreated vacutainer tubes.

It is apparent from the data presented in table 12 that the time lapse between initial intrusion on the animal and actual drawing of the blood sample has a profound effect on the level of serum reducing sugar. As shown in figure 8 all the deer exhibit the same general pattern although the results are displaced one from the other both in time and in reducing sugar level. The data for U34 represents the trend and presents an almost complete picture. The first sample was drawn from this doe only three minutes and forty seconds after the initial intrusion of the technician. This is close to the minimal amount of time necessary to inject the animal, have it collapse, seat a vacutainer needle, and draw a blood sample. The second sample let slightly over a minute later showed essentially the same

TABLE 12: REDUCING SUGAR LEVELS IN SEQUENTIAL BLOOD SAMPLES.

'Time' is the interval (in minutes and seconds) from initial intrustion of the technician on the animal to the end of that particular sample.

Animal	Time	Reducing Sugar (mgm.%)
U16	4:02	97.1
	4:40	102.6
	8:41	118.1
	9:34	117.4
	12:45	113.0
	13:30	113.5
	14:21	115.3
	15:14	114.8
U24	8:08	90.9
	10:03	99.4
	10:30	101.9
	11:00	102.2
	11:45	102.6
	12:13	103.3
	12:51	101.2
	13:30	102.6
U34	3:40	83.6
	4:52	85.4
	5:42	90.1
	6:35	102.2
	9:01	105.6
	9:50	110.0
	10:07	105.2
	11:10	105.2
U43	4:52	844.4
	5:17	83.6
	5:50	86.2
	6:24	91.5
	6:52	91.8
	7:30	98.1
	8:11	97.1
	9:07	99.7


U16
U24
U34
U43
SERUM REDUCING SUGAR (mgm.%)
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
TIME FROM INITIAL INTRUSION TO END OF SAMPLE (min.)

level of reducing sugar. It can be concluded that these levels are reasonably close to the normal for this deer. The following three samples, drawn in the interval from five minutes to nine minutes after initial intrusion are typical of the rapid increase that occurred in all the deer with the exception of U24. During this interval U34 exhibited a 25.8% increase over the initial serum reducing sugar level. The levels in blood samples drawn after ten minutes were generally constant in all the deer.

The change in the serum reducing sugar levels during the blood sampling procedure found in this experiment might be due to three factors: firstly to the excitement and anticipation experienced by the animals as they were moved into the laboratory, secondly to the fear and pain associated with injection, immobilization, and withdrawal of the blood samples, and thirdly to the muscular energy expended by the deer throughout the procedure. Unfortunately the relative contributions of these factors cannot be determined from this data. The effects of all are mediated through the sympathico-adrenal complex. As pointed out earlier, the speed and extent of the mobilization of glucose, as reflected in the serum reducing sugar level, depends on the degree of stimulation of this complex. That excitement might have an effect on blood reducing sugar levels has been suggested by others; Hodgson et al (1932), in cattle Reid (1962) in sheep, Wing (1955) in calves, and Bandy (1957) and Youatt et al (1965) in deer. No work has been done, however, to determine the rate and magnitude of the change that excitement during blood sampling can ellicit. The response of the deer in this experiment was undoubtedly minimal as they had been handled extensively and were relatively accustomed to immobilization. The pattern for less

experienced animals could show a curve attaining higher levels in a much shorter time. Moreover there might be considerable interspecies variation in the rate and magnitude of change in the blood sugar. Domestic species, for example, might exhibit a slower and smaller increase under a particular blood sampling.procedure than a wild species. The findings here add a new element to the factors that must be controlled if useful blood sugar values are to be obtained.

EXPERIMENT 4

EXPERIMENT 4

This experiment was carried out to determine the distribution of reducing sugar in the blood of deer.

On the basis of the results of earlier experiments no control of feed intake was exercised, all the deer were on pellets ad lib.

The deer were immobilized with succinylcholine before blood was drawn. Each animal was immobilized on a different day, generally in the morning. Two blood samples were let from each animal into separate test tubes, the first being followed immediately by the second. The first tube was untreated, and serum was extracted as outlined earlier. The second tube was heparinized. Within minutes of being drawn, 0.2 cc. of whole blood from this tube was pipetted into 3.0 cc. of distilled water, thus laking the cells. The level of blood reducing sugar in this sample was then determined in the usual manner.

The results of the experiment appear in table 13.

Although only a single determination per deer was done it appears that no reducing sugar occurs in the erythrocytes of these animals. Goodwin (1956) using the same method of calculation, found that in sheep the corpuscular/plasma glucose concentration was 23.5%. This ratio in cattle was roughly the same, being 24.2%. In other words, he found that there was a considerable amount of reducing material in the erythrocytes of these animals. In vitro permeability studies, however, have shown that the erythrocytes of most nonprimate adults are impermeable to glucose. To rationalize this inconsistancy Somogyi (1933) suggested that the corpuscular glucose of sheep and cattle is due to the plasma retained between the packed cells. Andreen-Svedberg (1933) on the other hand, considered the glucose to be absorbed on the cells.

TABLE 13: THE DISTRIBUTION OF REDUCING SUGAR IN THE BLOOD OF DEER

Animal	Serum Level (mgm.%)	Hematocrit	Whole Blood (mgm.%)	
			Calculated	Actual
U16	96.1	42.5%	55.3	56.5
U24	84.6	46.5%	45.3	49.9
U34	96.4	48.2%	49.9	47.6
U43	120.1	49.2%	61.0	59.6

Sample Calculation:

serum reducing sugar level = 96.0 mgm.%

hematocrit of 45% (therefore 55% is plasma)

$\frac{96 \text{ mgm.\%} \times 55\%}{100}$ = the amount of reducing sugar expected in whole blood sample assuming that all the sugar occurs in the serum.

RESUME

RESUME

This investigation has shown that feed intake during the hour preceeding blood sampling, short periods of fast, nature of the diet, and sex of the animal, apparently have no effect on blood reducing sugar levels in deer. It was also found that blood samples taken in the evening had a slightly higher reducing sugar level than those drawn earlier in the day. No reducing sugar was found to occur in the erythrocytes of these deer.

Of the factors studied that could possibly influence reducing sugar levels, only two, the means of restraint, and the time required to draw samples were found to have any effect. Deer physically restrained exhibited higher and more variable reducing sugar levels than those immobilized with succinylcholine. The animals also showed a precipitous increase in reducing sugar level during the blood sampling operation. It is interesting that these two factors, means of restraint, and time required to draw a sample, are rarely noted by investigators reporting sugar levels in animals. Table 14 presents a comparison of the blood sugar levels that have been reported for Odecoileus hemionus species. In every case the deer were restrained by force, and blood sugar values were reported as mgm.% in whole blood. Bandy (1957) found a significant difference between the blood sugar levels of yearling deer on a high plane of nutrition and those on a low plane. He also noted that the blood sugar levels of fawns (20 - 100 days old) and those of adult deer were significantly different. Bandy restrained the deer with physical force, but did not note the time required to draw samples. The blood sugar levels and variation that he found in adult animals are very close to those reported in this experiment.

TABLE 14: COMPARISON OF BLOOD SUGAR VALUES REPORTED FOR DEER.

Investigator	Animal	Restraint	# Det.	Blood Sugar (mgm.%)
Bandy, P.J. et al (1957)	Odecoileus hemionus columbianus	Physical	---	37.2 ± 5.4 (whole blood)
Terri, A. et al (1958)	Odecoileus hemionus virginianus	Physical	12	66.9 ± 12.9 (whole blood)
Youatt, W.G. et al (1965)	Odecoileus hemionus virginianus	Physical	20	91.3 ± 8.1 (whole blood)
This report	Odecoileus hemionus columbianus	Physical	32	44.0 ± 4.0* (whole blood)
This report	Odecoileus hemionus columbianus	Succinyl-choline	29	42.0 ± 2.9* (whole blood)

* Calculated from serum values on the basis of a hematocrit of 50%.

Terri et al (1958) reported blood sugar values for deer in 'nutritionally poor status'. The animals were thrown and blood samples were let from the jugular vein. Terri noted that the deer were extremely nervous, frothing at the mouth, and that occasionally some were fatally injured by this procedure. The levels and variation that he found likely reflect the fear and pain experienced by the deer during blood sampling. The blood sugar levels reported by Youatt et al (1965) are even higher and more variable than those reported by Terri et al (1958). Youatt captured his animals with a net and shackled them to a restraining board during blood sampling. He noted that the deer were very excited breathed heavily, and became exhausted fighting this procedure.

On the basis of the results reported in this thesis the value of the blood sugar levels obtained by these methods of restraint are open to question. They most assuredly are not normal levels, therefore their comparative value is doubtful, moreover, any changes recorded as being due to experimental manipulation are likely fortuitous. It is apparent then, that the blood reducing sugar levels reported for deer to date reflect the degree of excitement, fear, and pain experienced by the animals during the blood sampling procedure employed. It is pertinent once again to point out the relatively low and consistent blood sugar levels that result when succinylcholine is used to immobilize the deer during blood sampling (table 13). The use of succinylcholine, however, does not guarantee consistent, normal, blood sugar levels. A number of other factors must be considered: the deer must be completely familiar with those involved in the blood sampling operation; records should be made of the time required for each aspect of every blood sampling, and of the activity of the deer throughout the operation; and the

animals should be housed such that extraneous disturbance is minimized. Only through the adoption of such an intensive program will worthwhile data relating blood sugar to experimental manipulation be realized.

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APPENDICES

APPENDIX I

Glucose Metabolism

Glucose is the major end product of carbohydrate digestion in the monogastric animal, and plays an important role in its metabolic processes. It is essential for the maintenance of cells both as a precursor to many cellular components and as a source of energy. In the lactating animal it is a precursor of lactose. Glucose also provides 2-carbon fragments for fat synthesis and the necessary reduced coenzyme (NADPH) for the incorporation of the 2-carbon units into long chain fatty acids (Armstrong 1965). It has long been considered that glucose metabolism is quantitatively less important in ruminants as compared to non-ruminants. (Baxter et al 1955). The fact that little glucose is absorbed from the digestive tract of the ruminant, (Schambye 1951) and that it appears in low concentrations in the blood of these animals supports such a contention. Utilization rates measured by ^{14}C-glucose infusion, and expressed per unit of metabolic body size, however, indicate that the rate of utilization of glucose in ruminants is essentially the same as it is in simple-stomached animals. (Armstrong 1965). Glucose, then, is as important in the metabolism of the ruminant as it is the non-ruminant. (Lindsay 1959).

In ruminants dietary carbohydrates are fermented rather than digested in the rumen. The ultimate products of this fermentation are the volatile fatty acids, acetic, proprionic, and butyric. These are absorbed into the portal blood system through the rumen wall and play a major role in the energy metabolism of the animal. Some of the acetate is oxidized in the liver, but the majority is passed into the peripheral circulation where it is utilized by the tissues as a source of energy. It is interesting to note

that many tissues can derive energy from oxidizing fatty acids and ketone bodies, but nervous tissue can utilize only glucose as an energy source. (Krebs 1964). Proprionic acid is either oxidized or converted to glucose in the liver. Little butyric acid is carried to the liver, the majority is converted to ketone bodies in the rumen epithelium. (Armstrong 1965).

Glucose in the ruminant is derived from non-carbohydrate sources by the process of gluconeogenesis. Proportionate and amino acids are the principal precursors. The glucose formed is metabolized in essentially the same manner as it is in monogastric animals. Little, however, is utilized in the elaboration of long chain fatty acids. The abundant supply of readily activated acetate is the primary source of these in the ruminant. It has also been shown that glucose is involved in the synthesis of lactose, glycerol, milk citrate, and some non-essential amino acids in ruminants. (Armstrong 1965).

APPENDIX 2

Hormonal Regulation of Blood Reducing Sugar

The maintenance of normal levels of reducing sugar in the blood of mammals is a finely regulated homeostatic mechanism. The liver plays an essential role as it functions both to remove and to add sugar to the blood. The activity of the liver in maintaining normal levels of sugar in the blood is influenced by a number of hormonal factors.

1. Insulin

Insulin is a secretion fo the B-cells of the islets of Langerhans. It functions to lower the blood reducing sugar level by promoting peripheral utilization of glucose, glycogenesis in muscle and liver, and lipogenesis. The mechanism of insulin action remains open to discussion, however, experimental evidence suggests that it acts by affecting 1. membrane transport phenomena, and 2. oxidative phosphorylation reactions. (Bard 1961).

That injection of insulin lowers the level of blood reducing sugar in sheep was shown by Reid (1951) and (1952). The rate of fall, however, was considerably slower than that reported for non-ruminants, and the level did not fall below 5 mgm.%. Moreover, further injection of large doses of insulin (10 U/Kg.) did not cause a further reduction in blood sugar, but merely prolonged the existing hypoglycemia. It is noteworthy that spontaneous diabetes is uncommon in ruminants.

2. Epinepherine

Hyperglycemia has been reported in sheep (Satchell and McClymont 1955) and in cattle (Garner 1952) when injected with epinepherine. The importance of this secretion of the adrenal medulla becomes apparent when

a splanchnic section (Potter 1952) is carried out on a sheep - the blood sugar level falls to nothing.

3. Glucagon

Glucagon is a secretion of the α cells of the pancreas that operates to raise the level of blood sugar by promoting glycogenolysis. The site of action of glucagon is apparently the same as that of epinepherine. The hyperglycemic reaction of glucagon administration has been demonstrated in sheep (Ho and Reber 1957).

4. Growth Hormone

In monogastric animals, growth hormone acts to decrease peripheral utilization of blood sugar and to increase glycogenolysis in the liver. The net effect, then, is to raise the level of blood reducing sugar. This effect was noted in sheep (Kronfeld 1957), however, the elevated level did not persist despite repeated injection of the hormone.

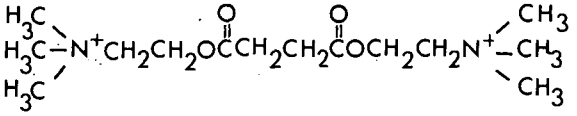
5. ACTH and Cortisone

ACTH undoubtedly exerts its effect indirectly via increased release of 11-oxycorticoids from the adrenal cortex. The 11-oxycorticoids exert their diabetogenic effect through increased gluconeogenesis from amino acids in the liver. These hormones are also insulin antagonists. Satchell and McClymont (1955) in sheep and Shaw (1955) in cattle produced hyperglycemia by injecting ACTH and cortisol.

APPENDIX 3

Succinylcholine Chloride

Hunt (1906) was the first to synthesis succinylcholine. The immobilization action of the drug remained undiscovered for forty years. Castillo and deBeer (1950) first documented this and since then numerous reports of its use in veterinary and human medicine have appeared. The structure of succinylcholine, which appears below, is very similar to that of acetylcholine.



The mechanism of action of succinylcholine is believed to be enzymatic inhibition at the myoneural junction (Pistey and Wright 1961). The succinylcholine inhibits the action of acetylcholinesterase resulting in an accumulation of acetylcholine. This accumulation lowers the resting potential of the muscle, reducing its excitability and the muscle is effectively paralyzed (Ochs 1960). Pseudocholinesterase, found in the plasma, hydrolyzes succinylcholine into succinylmonocholine and choline. The succinylmonocholine is then degraded into succinic acid and choline chloride (Bovet et al 1949 as reported by Pistey and Wright 1961). Acetylcholinesterase then acts to degrade the accumulated acetylcholine, conduction is resumed and the paralysis is terminated.

Mayrhofer (1952) induced self paralysis with succinylcholine. Muscle weakness, initially of the neck, jaw and diaphragm, painful muscle twitching, and double vision, characterize the sensations that he attributed to the action of the drug. At no time did he become unconscious, and no change in pulse rate or blood pressure was recorded. Moreover, no after or side effects were experienced.

APPENDIX 4

Peebles V'LER Milk Replacer

Ingredients	Guaranteed minimum analysis	
Dried skim milk	crude fat	16.0%
Dried butter mil	crude protein	24.0%
Dried whey-product	Vit A	1,500 U/lb
Lecithin	Vit D_2	3,000 U/lb
Sodiumbenzoate	Vit B_1	11 mgm.
Magnesium carbonate	Vit B_2	11 mgm.
Dicalcium phosphate		
Iron sulphate		
Antibiotic supplement (oxytetrcycline and terramycin)		
Vit A palmitate		
Vit D_2		
Vit B_1 (thiamine)		
Vit B_2 (riboflavine)		

APPENDIX 5

Weaning Ration

Ingredient	lbs/ton
Ground barley	200
Ground wheat	585
Oat groats	390
Wheat bran	130
Herring meal	200
Soya meal	100
Lysine	---
Methionine	---
Skim milk powder	200
Brewers yeast	20
Irradiated yeast	2
Distillers solubles	---
Dehydrated grass meal	150
Vit D_2	---
Dicalcium phosphate	10
Iodized salt	10
Manganous sulphate	---
Zinc sulphate	---
Chromic oxide	1

APPENDIX 6

Adult Ration (36-57)

Ingredients	lbs/ton
Corn meal	600
Ground wheat	250
Bran	275
Beet pulp	200
Vitagrass	200
Soyabean meal	175
Herring meal	110
Bone meal	20
Iodized salt	20
Molasses	150

APPENDIX 7

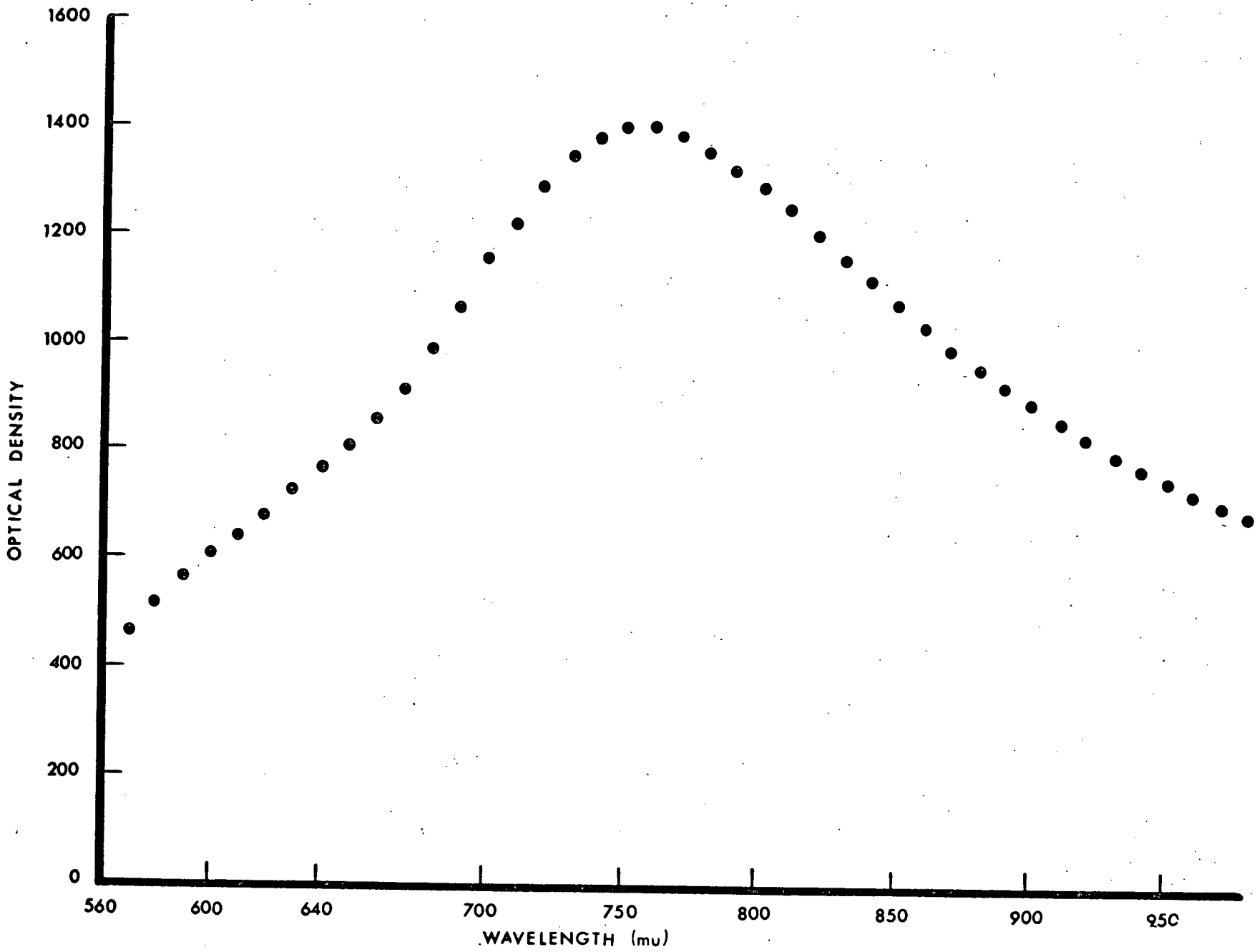
Absorption Curve for the Nelson-Somogyi Method of Determining Blood Glucose.

Beckman Model DU Spectrophotometer

Standard Glucose Solution 100 mgms.%

Wavelength	Slitwidth	O.D.	Wavelength	Slitwidth	O.D.
400	1.95	.0752	675	0.065	.9508
410	1.15	.0809	680	0.065	.9914
420	0.68	.0894	685	0.065	1.0269
430	0.44	.1007	690	0.065	1.0706
440	0.30	.1124	695	0.065	1.1079
450	0.22	.1278	700	0.065	1.1549
460	0.158	.1421	710	0.065	1.2218
470	0.150	.1586	720	0.065	1.2924
480	0.150	.1733	730	0.065	1.3468
490	0.140	.1898	740	0.065	1.3768
500	0.140	.2083	750	0.065	1.4089
510	0.140	.2291	760	0.065	1.4089
520	0.065	.2557	770	0.065	1.3979
530	0.065	.2865	780	0.065	1.3768
540	0.065	.3242	790	0.065	1.3279
550	0.065	.3675	800	0.065	1.3010
560	0.070	.4179	810	0.065	1.2596
570	0.080	.4660	820	0.065	1.2076
580	0.090	.5186	830	0.065	1.1675
590	0.110	.5686	840	0.065	1.1249
600	0.14	.6126	850	0.065	1.0809
605	0.17	.6308	860	0.065	1.0410
610	0.22	.6478	870	0.065	1.0000
615	0.28	.6676	880	0.065	.9626
620	0.32	.6799	890	0.065	.9318
625	0.40	.6946	900	0.065	.9031
630	0.075	.7305	910	0.065	.8665
635	0.075	.7471	920	0.065	.8356
640	0.065	.7696	930	0.065	.8097
645	0.065	.7878	940	0.065	.7852
650	0.065	.8069	950	0.065	.7595
655	0.065	.8327	960	0.065	.7375
660	0.065	.8539	970	0.065	.7122
665	0.065	.8827	980	0.065	.6946
670	0.065	.9136	990	0.065	.6757
			1000	0.065	.6576

FIGURE 9: ABSORPTION SPECTRUM: NELSON-SOMOGYI METHOD OF DETERMINING BLOOD GLUCOSE


1600
1400
1200
1000
800
600
400
200
0
OPTICAL DENSITY
560
600
640
700
750
800
850
900
950
WAVELENGTH (mu)

APPENDIX 8

Standard Curve for the Nelson-Somogyi Method of Determining Blood Glucose.

Beckman Model DU Spectrophotometer

Slitwidth 0.065

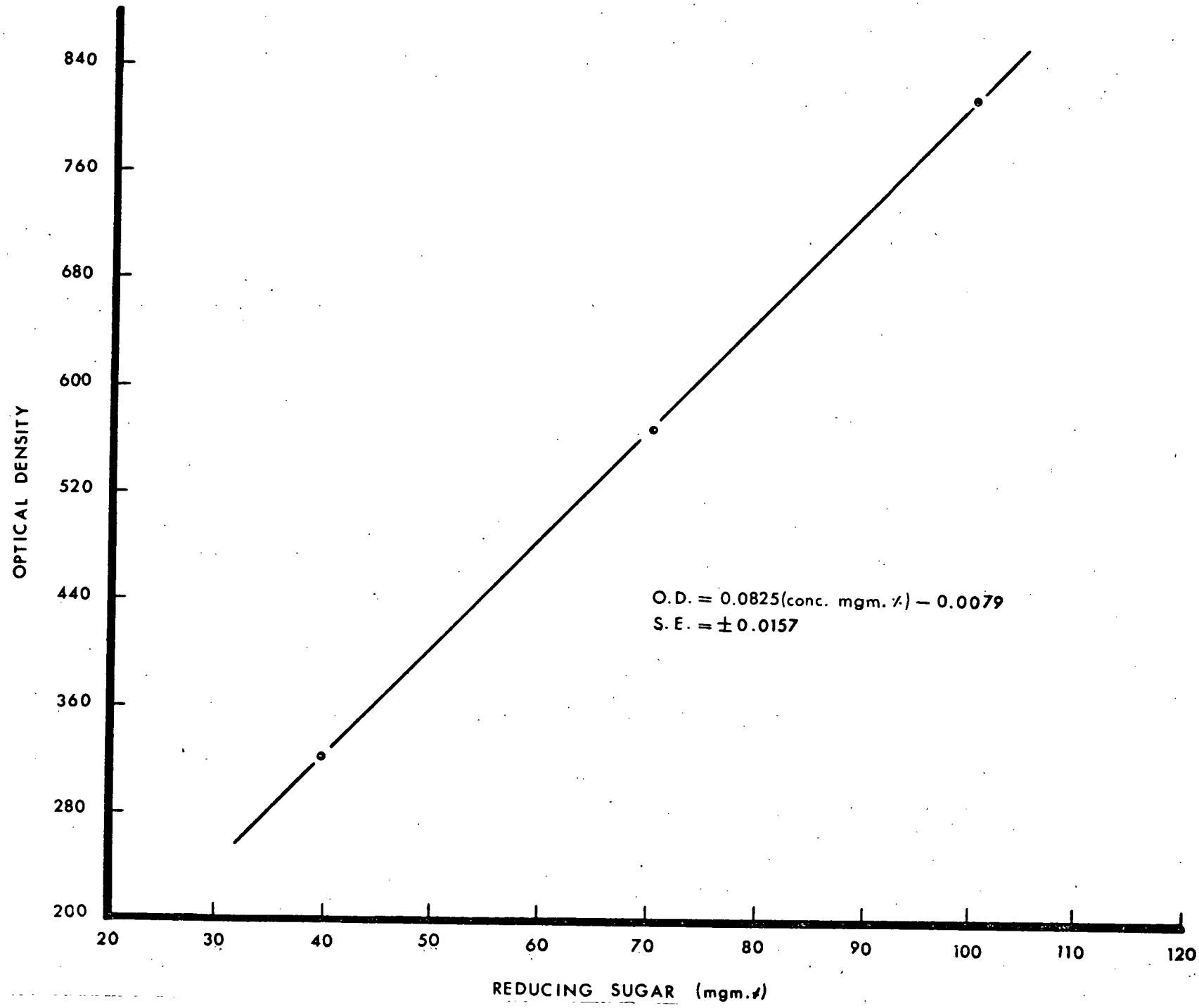
Wavelength 650 mu

Concentration	O.D.	Concentration	O.D.	Concentration	O.D.
100	.8539	100	.8239	100	.8125
	.8539		.8239		.8125
	.8477		.8239		.8097
	.8477		.8239		.8041
	.8477		.8239		.8041
	.8477		.8239		.8041
	.8477		.8239		.8041
	.8477		.8239		.8041
	.8477		.8210		.8041
	.8416		.8210		.8041
	.8416		.8210		.8013
	.8416		.8210		.8013
	.8416		.8210		.7986
	.8416		.8210		.7986
	.8386		.8210		.7986
	.8386		.8182		.7986
	.8386		.8182		.7986
	.8386		.8182		.7986
	.8386		.8182		.7959
	.8386		.8182		.7959
	.8356		.8182		.7959
	.8297		.8153		.7905
	.8268		.8153		.7878
	.8268		.8153		.7878
	.8268		.8153		.7878
	.8268		.8153		.7852
	.8239		.8153		.7852
	.8239		.8153		.7852
	.8239		.8153		.7825
	.8239		.8125		.7747
	.8239		.8125		

APPENDIX 8 (cont.)

Concentration	O.D.	Concentration	O.D.	Concentration	O.D.
70 mgm.%	.6055	70 mgm.%	.5654	40 mgm.%	.3270
	.6055		.5654		.3270
	.5918		.5638		.3261
	.5918		.5638		.3261
	.5969		.5622		.3261
	.5969		.5607		.3251
	.5982		.5607		.3251
	.5901		.5591		.3251
	.5884		.5591		.3251
	.5850		.5560		.3251
	.5850		.5560		.3233
	.5834		.5575		.3233
	.5834		.5544		.3233
	.5834		.5513		.3224
	.5817		.5513		.3215
	.5800		.5513		.3215
	.5751		.5513		.3206
	.5735		.5498		.3197
	.5735		.5492		.3188
	.5735		.5452		.3188
	.5719		.5376		.3179
	.5602	40 mgm.%	.3468		.3170
	.5602		.3382		.3152
	.5670		.3382		.3152
	.5686		.3372		.3152
	.5686		.3354		.3143
	.5686		.3344		.3143
	.5670		.3344		.3143
	.5654		.3335		.3125
	.5654		.3316		.3116
	.5654		.3316		.3116
	.5654		.3316		.3116
	.5654		.3307		.3107
	.5654		.3298		.3054
	.5654		.3279		.3045
					.3028

FIGURE 10: STANDARD CURVE: NELSON-SOMGYI METHOD OF DETERMINING BLOOD GLUCOSE.


840
760
680
600
520
440
360
280
200
OPTICAL DENSITY
20
30
40
50
60
70
80
90
100
110
120
REDUCING SUGAR (mgm.%)
O.D. = 0.0825(conc. mgm.%) − 0.0079
S.E. = ±0.0157

APPENDIX 9

Recovery of Glucose from Serum.

Sample	Recovery	Calculated	% Recovered
70 mgm.%	69.9	70.0	99.9
2:1	84.1	82.0	102.6
1:1	87.3	88.0	99.2
1:2	89.5	94.0	95.2
serum	106.0		

APPENDIX 10: BLOOD SAMPLING RECORD FORM.

BLOOD ANALYSIS DATA

DATE _____ RESTRAINT _____ ANECTINE DOSE _____

ANIMAL _____ WEIGHT _____ SEX _____

TIME	0800	lapse	1200	lapse	1600	lapse	2000	lapse
Time approached								
Time grabbed/injected								
Time held/down								
Sampling begun								
Sampling ended								
Sample ringed								
Sample centrifuged								
Sample in freezer								
COMMENTS								

GLUCOSE ANALYSIS

Time	Date	% T.	O.D.	mgm. % Glucose
0800 1				
2				
3				
1200 1				
2				
3				
1600 1				
2				
3				
2000 1				
2				
3				

CALCIUM ANALYSIS

Time	Date	% T.	O.D.	mgm. % Calcium
0800 1				
2				
3				
1200 1				
2				
3				
1600 1				
2				
3				
2000 1				
2				