



6-1957

## Breeding of Jackstock

University of Tennessee Agricultural Experiment Station

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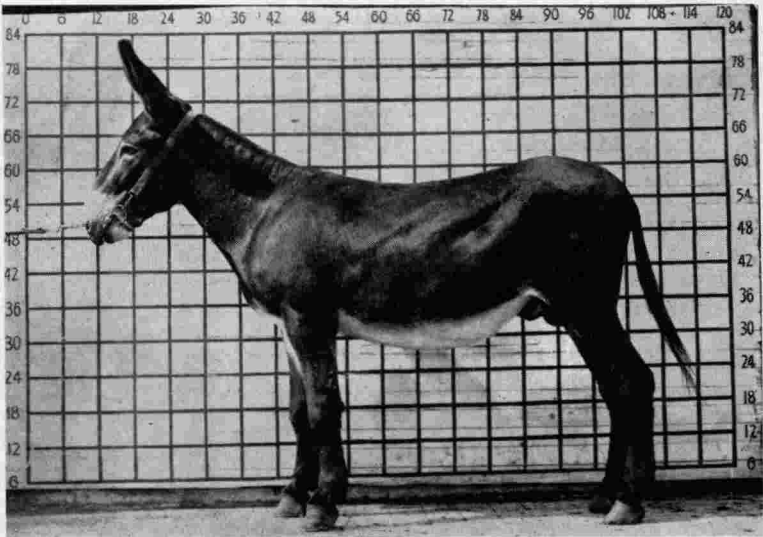
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# BREEDING OF JACKSTOCK

H. R. Duncan  
and  
R. L. Murphree



AGRICULTURAL EXPERIMENT STATION  
THE UNIVERSITY OF TENNESSEE  
KNOXVILLE

## ACKNOWLEDGEMENT

Grateful acknowledgement is hereby made by the authors of this bulletin to the many persons who assisted in the work reported herein.

Dr. M. Jacob, who joined the University staff in 1900, and who served as Dean of the College of Agriculture and Head of the Animal Husbandry Department from 1921 until his death in 1943, initiated the project and supervised it until his death.

Other staff members who gave valuable assistance include Director C. A. Mooers, and Director F. S. Chance, both of whom are now retired; L. R. Neel, former superintendent of the Middle Tennessee Experiment Station, who is now retired; J. A. Ewing, former superintendent of M.T.E.S., who is now Director of the Experiment Station; and the present superintendent of M.T.E.S., E. J. Chapman; J. C. Miller, M. C. Hervey, Dennis Sikes, former members of the Animal Husbandry staff; M. Whitaker, present staff member; and W. K. Dinwiddie, J. N. Cummings, P. Osborne, and George Irwin, the men responsible for the breeding, feeding, management, and mule testing while the project was under way.

E. G. Morrison assisted in the insemination work. The data presented in Part Two of this bulletin are from a thesis submitted by him in partial fulfillment of the requirements for the degree of Master of Science at the University of Tennessee.

The Bureau of Animal Industry at Washington, D. C., and Office of Experiment Stations were represented by Earl B. Krantz, Geo. Haines, Hugh O. McPhee, E. C. Elting, Ralph W. Phillips, H. W. Marston, W. V. Lambert, S. H. Work and T. C. Byerly.

Officers and members of the Tennessee Jack and Mule Breeders Association also gave valuable help in initiating and directing the course of the project.

The late Dean A. E. Trowbridge of the University of Missouri, and his assistants, S. Brody, E. E. Kibler and F. F. McKenzie contributed much to the project, particularly in the testing of mules.

Professor A. B. Caine, Iowa State College, assisted in outlining methods and equipment for performance testing. The dynamometer for testing mule performance was built by W. H. Baskerville, Arthur H. Morgan, and J. W. Clift of the Tennessee Agricultural Engineering staff.

Clarence Dorman, V. R. Burliner and R. H. Menas, of the Agricultural Experiment Station, Mississippi State College, cooperated in the project until the work was discontinued at that station.

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### Cover Picture

U. T. Marvel 35685—A line-bred General Logan jack considered by many observers as ideal for the production of farm mules. This jack was 14½ hands high and weighed 1,050 pounds at maturity.

# BREEDING OF JACKSTOCK

## Part I

**H. R. Duncan**

*Associate Animal Husbandman*

Breeding of Jackstock, and Performance  
Testing of Mules

## Part II

**R. L. Murphree**

*Associate Animal Husbandman*

Sperm Storage and Artificial  
Insemination Studies

AGRICULTURAL EXPERIMENT STATION  
THE UNIVERSITY OF TENNESSEE  
KNOXVILLE

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# PART 1

## Breeding of Jackstock and Performance Testing of Mules

### INTRODUCTION

After a depressing period in the horse and mule industry, it appeared that the reduction in numbers had reached a point in 1937 where the industry might again become stabilized and economically important. There was a general revival of interest, particularly in the raising of mares and colts. Brood mares were in good demand at fair prices.

An acute shortage of jacks was immediately apparent. A survey indicated that the number of jacks in Tennessee had declined from 3,500 in 1920 to 347 in 1935. During this 15-year period of very limited demand for jacks the herds of jackstock had been reduced or depleted to a critical level. The animals were starved to death, fed to hogs, or driven from home to meet the adversities of their time.

If horses and mules were to continue to be of moderate importance, it appeared that something should be done to conserve some breeding stock and to adjust a breeding program to meet a drastically changed situation. Since the demand for mine mules, sugar mules, heavy draft mules, and logging and construction mules had largely disappeared and only a demand for medium-sized farm mules remained, particularly in the South, it seemed important to revamp the breeding program for a very definite type of jack for the types of mares available. Prior to 1920 many types and breeds of jacks had been imported and bred in the leading jack breeding states of Tennessee, Kentucky and Missouri. This heterogeneous lot of jacks was bred to mares which also varied greatly in type and breeding, which resulted in mules of many kinds. As long as there was a demand for practically all types of mules produced, there was not an acute need for a specific type of jack for the production of farm mules. Many breeders had been trying to pursue a breeding program for the improvement of their stock in the time they had been breeding, but the breeders themselves were individualists and had used many different sources of blood and had pursued many different ideas in breeding. Most breeders, however, had been trying to produce big jacks, to produce large draft mules, the type which was in greatest demand. It was, therefore, easy to understand why the existing jackstock produced such a great variety of offspring and failed to meet the need of the situation at that time.

### INITIATION AND OBJECTIVES OF PROJECT

The situation confronting the jack and mule industry seemed of sufficient importance to warrant some constructive assistance. An agreement was reached on November 11, 1937, by the Mississippi and Tennessee Experiment Stations and the Bureau of Animal Industry, United States Department of Agriculture, to undertake a rather extensive experimental program for study and improvement of the industry with these main objectives:

- (1) To determine the merit of, and improve breeding stock for, jackstock and mule production.
- (2) To determine the merit of different types and breeds of mares for mule production.
- (3) To study the physiology of reproduction and the use of artificial insemination in horses and jackstock.
- (4) To develop measures of performance indicative of superior merit in mules.
- (5) To study methods of growing, feeding, and managing horses, mules, and jackstock.

Later the University of Missouri participated in the project, particularly in the field of developing techniques and equipment for testing mules. Mississippi State College decided to abandon work on the project late in 1944. The project was revised in 1948 and 1949 in order to give greater emphasis to the testing of mules and studies designed to extend the use of jack semen. Work was carried on at the Tennessee Station until it became necessary to discontinue the project in December, 1950.

## LAND AND EQUIPMENT

One hundred and fifty acres of land adjacent to the Middle Tennessee Experiment Station was provided for this project. This land was not in a good state of production when purchased, several acres being rocky or having rock close to the surface. Even though it was improved by application of manure, seedings and management practices, the carrying capacity from hay and pasture produced was not high and was insufficient for the load of stock carried. All the concentrates and a considerable amount of hay was bought each year.

The land was fenced, pastures were cleaned up and seeded, alfalfa was sown for hay, and buildings and lots were constructed. The buildings consisted of a large general service barn with several stalls, breeding facilities, a laboratory and a set of scales; a jack barn, mare barn, jennet barn, and several board-fenced lots.

## FOUNDATION STOCK

A total of 17 jennets were bought as foundation for the project, 13 being the initial purchase with 4 added in later years. The initial purchases were made by Dr. M. Jacob, who initiated the project, and Mr. L. R. Neel after they had visited many of the best herds of jennets in Tennessee and Kentucky. An attempt was made to get top jennets of approved blood lines and with production records. Some of the jennets were 10 to 14 years old. The job of assembling these jennets in 1938 was difficult as many herds had passed out of existence or had deteriorated, and the general revival of interest in 1936 to 1938 made it difficult to buy the few top jennets remaining. None of the jennets bought were registered and they had to be registered later on measurements. Most of these jennets carried considerable blood of General Logan, imported Taxpayer and other noted bloodlines. More information was obtained about the breeding of their sires than of their dams.

Two foundation jacks were bought initially for the project. These two were Mammoth Logan or Hunter's Logan, renamed and registered as U. T. Logan 34134, purchased from A. W. Hunter, Lynnville, Tennessee; and Limestone U. T. Monarch, bred by and purchased from L. M. Monsees and Sons, Smithton, Missouri, noted breeders of jackstock.

U. T. Logan was one of the two known living sons of General Logan, the other being Rowdy, owned by Dr. R. B. Gaston, Lebanon, Tennessee. General Logan 4914 (American) and 18831 (Standard) was bred by Gibbs and Makin of Kentucky and known by some as Hampton's General Logan. This jack, however, spent most of his career in Tennessee, making eight seasons in Marshall County. He became noted as a great breeding and show jack, perhaps as noted as his grandsire, Dr. McChord 1766, described in a Marshall County sale catalog in 1915, as "The most wonderful sire living or dead." General Logan was described as "a black jack with white points, 15½ hands high, weight 1,150 pounds, strong conformation, good head and ears, extra heavy bone and wears a number 4 shoe." He was champion at the Kentucky State Fairs. He was defeated only once in class, by a Marshall County jack, at the Tennessee State Fair.

U. T. Logan was 19 years old when bought. He stood 14 hands 1½ inches high, weighed 890 pounds, had an eight-inch front cannon, a good set of legs, large feet and a 32-inch ear spread (Figure 1). He was serviceable two or three years after being bought, leaving eight daughters and one son in the herd. His son, U. T. Logan Again, was used extensively.

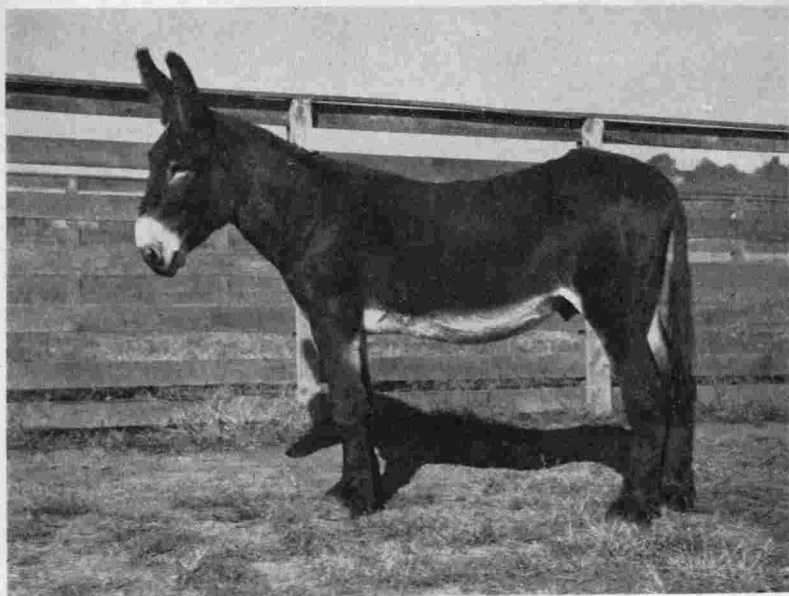


Figure 1—U.T. Logan, a son of General Logan, at 18 years of age. Selected as a foundation jack.

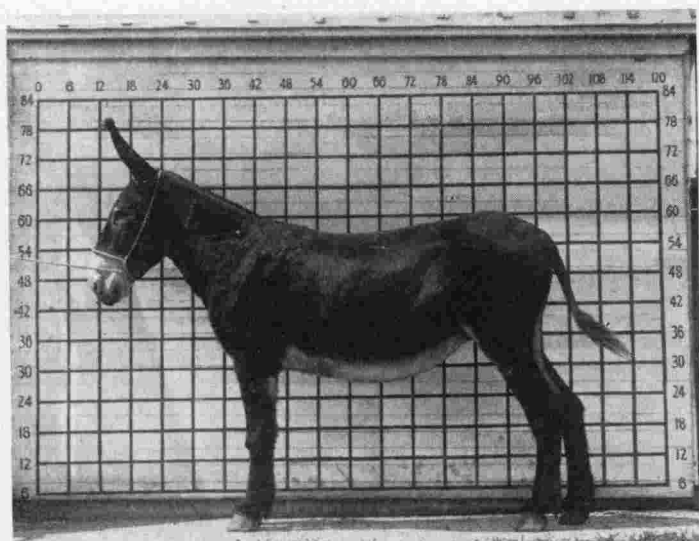


Figure 2.—Miss Derryberry, an outstanding foundation jennet. She stood  $14\frac{1}{2}$  hands high, and weighed 1,000 pounds when fat. She had excellent feet and legs.



Having bought a son of General Logan and several jennets rich in this line, which had been popular and important in producing some of the best jackstock, it was thought desirable to secure another line of breeding which could be used for line breeding or crossing on the Logan line. Since L. M. Monsees and Sons of Smithton, Missouri, had been important breeders in Missouri and had different blood in their herd, the jack Limestone U. T. Monarch was purchased as the number two foundation jack. This jack was short haired and showed a lot of quality in his knees, hocks, cannons and pasterns. In his mature form he stood 14½ hands high, maintained his weight close to 1,050 pounds, and his cannons were as large as those of the other jacks, even though they looked light.

In 1948 two sons of Rowdy, a son of General Logan, owned by Dr. R. B. Gaston of Lebanon, Tennessee, were obtained. One came from Dr. Gaston's Estate and the other from Mr. Howard Hancock, Lebanon. These jacks were registered as U. T. Rowdy and U. T. Rowdy II, respectively.

## MARES FOR MULE PRODUCTION

Four purebred Percheron mares were bought in 1942. These mares were selected to represent a medium-sized mare, but some of them grew to weigh 1,800 pounds in good, but not fat, condition. Four grade draft mares also were bought and used. A group of hot-blooded mares was maintained, usually about seven head, mostly of Tennessee Walking Horse breeding, but with some American Saddle Horse and Standard-bred background.

The mules of various ages raised and maintained inventoried about 35 head in January each year, after full production was reached.

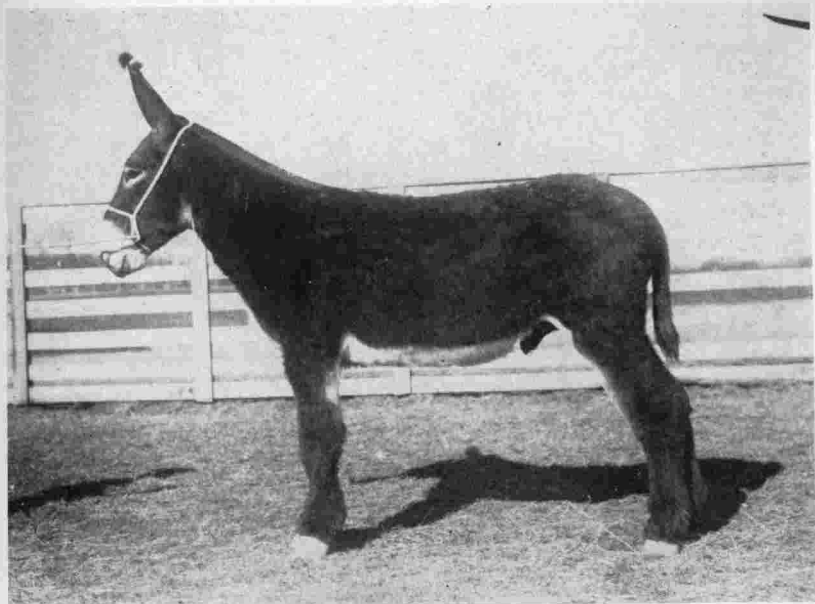
## PROCEDURE AND RESULTS

A policy of mating each jennet to the same sire for two years in succession and then changing sires in order to test the sires' breeding qualities was followed to a considerable extent. The colts were grown out sufficiently to determine their desirability and were then added to the herd, sold, lent to breeders, or discarded. It was impractical to keep all of the fairly normal offspring and bring them into production because of limited facilities, personnel and finances.

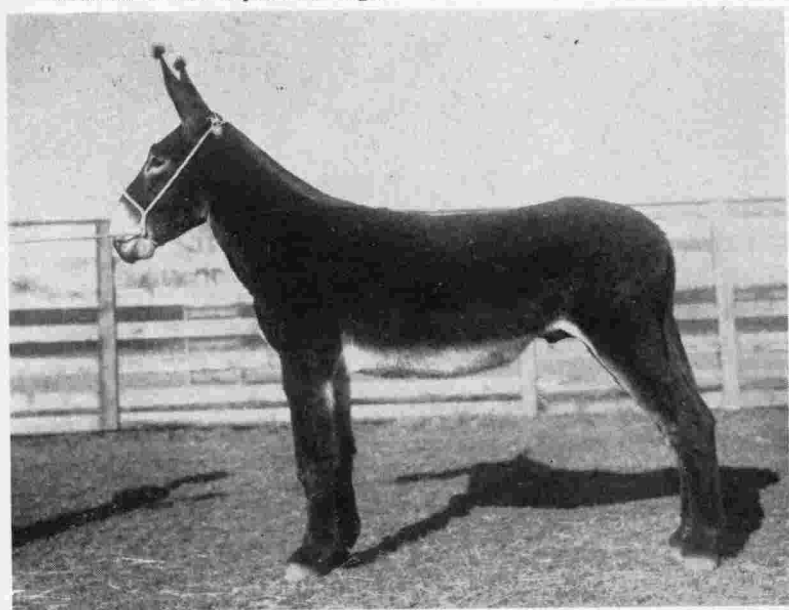
The importance of one of the main objectives, that of improving the uniformity of breeding in jackstock, was evident almost immediately.

In the first crop of jack colts by U. T. Logan there were as many different types of jacks as there were colts. They varied in height at three years of age from 14 to 16½ hands. One was a large, awkward, coarse jack; another was small and refined. There was, however, one outstanding jack in this crop. He was out of Miss Derryberry (Figure 2), considered the top jennet in the herd. This jack, named U. T. Logan Again, matured at 15 hands, had a good body, good head and ear, and an excellent set of feet and legs (Figure 3). He was not extreme in any respect and represented generally the type which those in charge of the project felt should be fixed, if possible, in the breed. This jack and his offspring were used more than any other line in the project until the study was discontinued. A full brother to U. T. Logan Again, U. T. Logan III (Figure 4), a somewhat tall, rangy jack, was 3½ inches higher, 2½ inches larger in heart girth, 2½ inches larger in the knee, 1 7/10 inches greater in fore cannon, two inches higher to floor of chest, and 65 pounds heavier than U. T. Logan Again at the same age—three years.

Miss Derryberry, when mated to Limestone U. T. Monarch (Figure 5), produced U. T. Monarch Again (Figure 6), a very good type of jack which was used as one of the herd sires. But when mated to U. T. Marvel, her grandson, Miss Derryberry produced a dwarfy, too-compact jack which looked good as a colt but failed to grow into a desirably typed jack. This offspring was only 12.3 hands high and weighed only 540 pounds when 22 months old.



Figures 3 and 4.—U.T. Logan Again (shown above) and U.T. Logan III (lower picture), full brothers, out of Miss Derryberry by U-T Logan. These jacks were quite different in type. U.T. Logan III, a tall, rangy jack, was  $3\frac{1}{2}$  inches higher than U-T Logan Again; he was  $2\frac{1}{2}$  inches larger in the knee;  $2\frac{1}{2}$  inches larger in heart girth; and was 65 pounds heavier at three years of age.



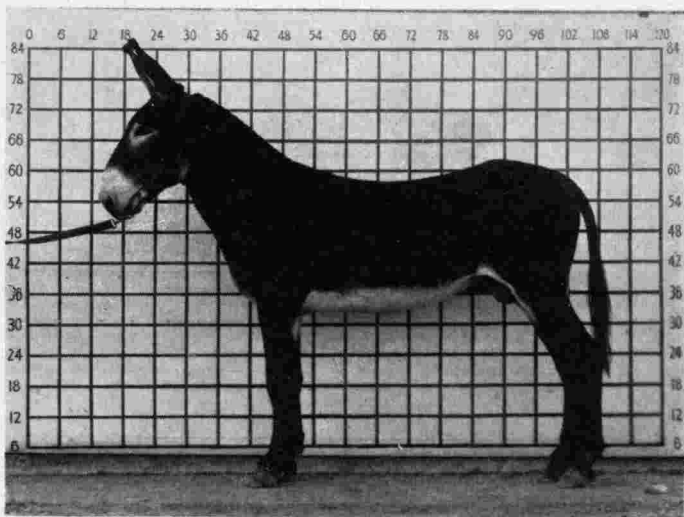


Figure 5.—Limestone U.T. Monarch 32973, a foundation jack purchased from L. M. Monsees and Sons, noted jack breeders of Smithton, Missouri. This jack was short-haired, had a 32-inch ear spread, stood  $14\frac{1}{2}$  hands high, and weighed 1,050 pounds when mature.

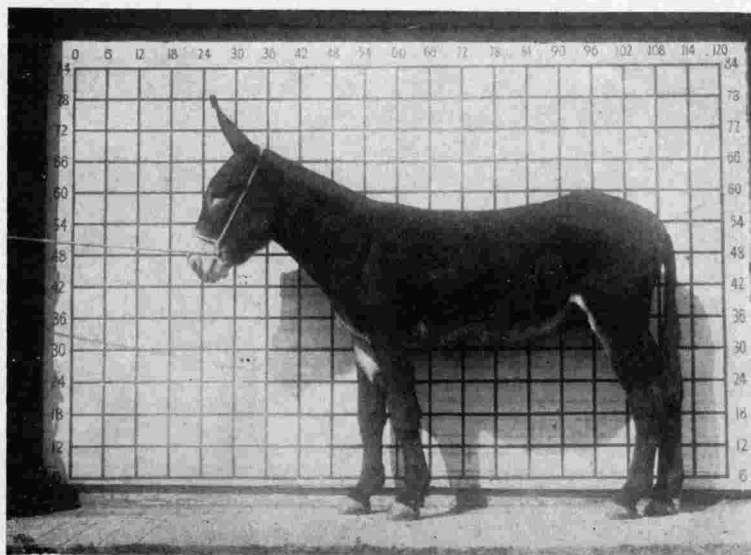
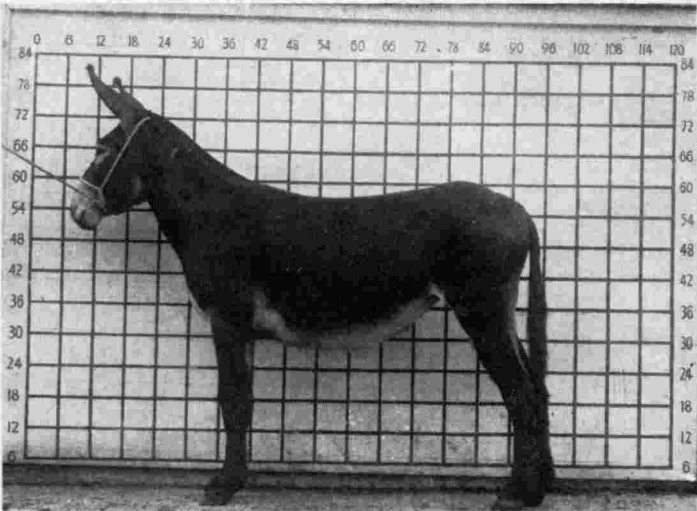
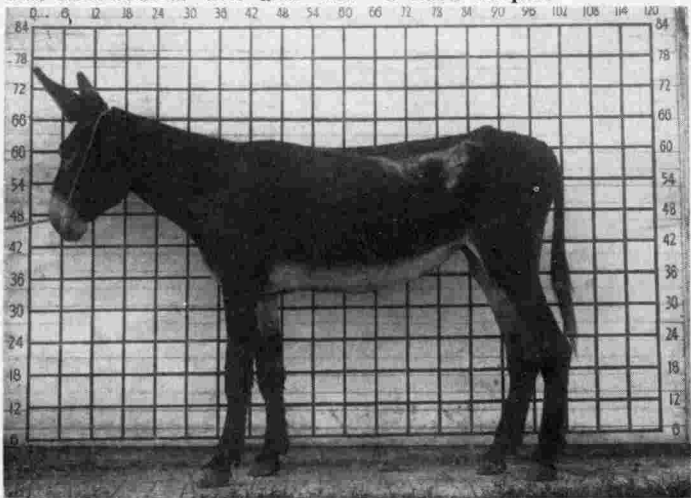


Figure 6.—U. T. Monarch Again 36299, the best son of Limestone U.T. Monarch. His dam was Miss Derryberry, shown in Figure 2. He was  $14\frac{1}{2}$  hands high, and weighed 900 pounds when mature and in average condition.



Figures 7 and 8.—U.T. Theresa 34360 (above) and U.T. Theresa II 34534 (below) were full sisters. Yet they illustrate the lack of uniformity obtained in the breeding project, and the need for a constructive program aimed at production of desirable, uniform types. U.T. Theresa was one of the best daughters of U.T. Logan; and U.T. Theresa II was one of the least desirable. U.T. Theresa II was very tall, rangy, and slim-bodied. She also was "a hard keeper."



Another jennet, Miss Thompson, produced some excellent typed animals as well as some off-typed ones. Several of her female offspring, but no males, were retained in the herd. Her first two daughters, both by U. T. Logan, were extreme in type. U. T. Theresa (Figure 7) was considered one of the best daughters of U. T. Logan, but her full sister, U. T. Theresa II (Figure 8), was a very tall, gangling, poor type which transmitted the same general characteristics. Some of the jacks of this line were also on the rangy order; but one, U. T. Frederick, a linebred, was of the small refined order.

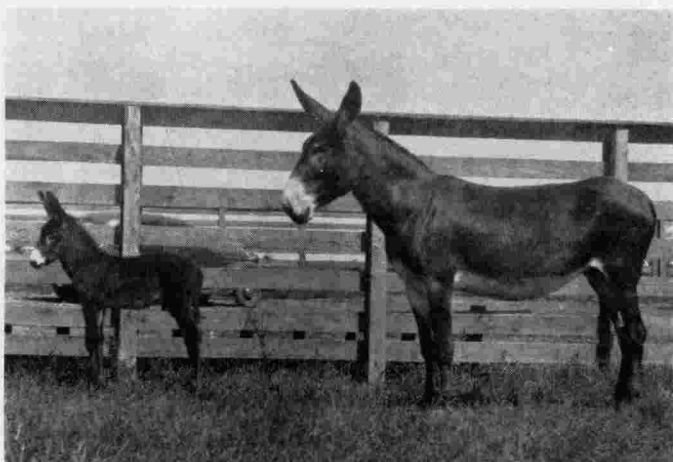


Figure 9.—Young Miss Jordan 34355, a foundation jennet, and her jack foal. This jennet won her class at the Tennessee State Fair in strong competition. She was a large jennet, popular when big jacks were in demand; but considered too big for the production of jacks suitable for siring compact, medium sized farm mules. In good condition, she weighed 1,075 pounds, stood 14 hands  $1\frac{1}{2}$  inches high, had a 35-inch ear spread and an 8-inch fore cannon.

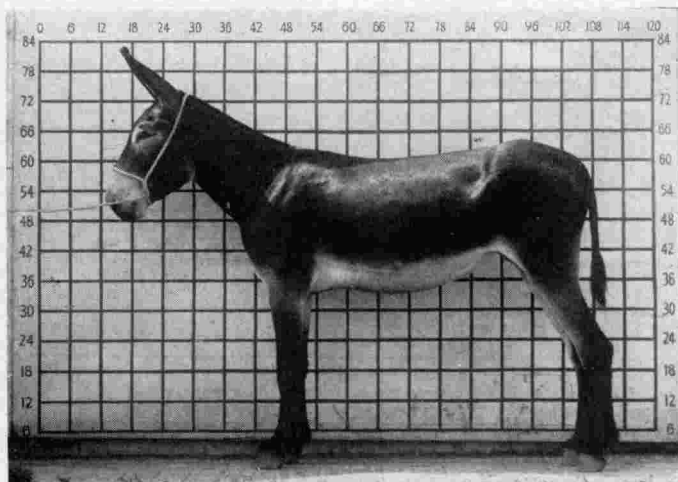


Figure 10.—U.T. Janis 34532, a daughter of Young Miss Jordan by U.T. Logan. While her sire was a  $14\frac{1}{2}$ -hand jack, Young Miss Jordan was taller and rangier than either her dam or sire. She was more than 15 hands high and weighed 1,150 at one time. She was discarded from the herd when she was seven years old because she was considered off-type, and her offspring were too rangy and gangling.

In the heyday of the jack and mule business the highest priced mules were the draft or sugar mules—big, heavy mules. This situation had great influence in the breeding of jacks, and had resulted in establishing the big, weighty, heavy-boned, big-footed jacks as the most desirable type to breed. A few of the foundation jennets were of this order. An example is Young Miss Jordan (Figure 9). This jennet stood 14 hands 1½ inches high, weighed 1,075 lbs., had a 67¼-inch heart girth, an 8-inch fore cannon and a 35-inch ear spread. She was also a show jennet. She won her class at the Tennessee State Fair, which indicated that this type was looked upon with favor. A son of this jennet, by U. T. Logan, developed into a 15-hand jack with fairly acceptable proportions. But a daughter, U. T. Janis (Figure 10) was of the big, tall order. She stood better than 15 hands high and weighed more than 1,100 pounds in good condition. She was well-proportioned for a big jennet, but was considered too big and stretchy for a day when the big mule was out of the picture. Some hope was held for stock of her type for the production of a jack which would give a good sized, rugged mule when mated to a hot-blooded mare. None of this jennet's offspring was retained, even from medium-sized jacks, because of their gangling type and poor feet and legs. Janis herself was eliminated from the herd at seven years of age.

Quality Ezell, one of the foundation Logan-bred jennets, was probably destined to make a lasting contribution to the herd had it been continued. One son, U. T. Marvel (shown on the cover of this bulletin), by the line-bred Logan jack, U. T. Logan Again, was of desirable type, standing nearly 14½ hands high and weighing 1,000 pounds when fat. He had the compactness and size of bone thought desirable to mate with hot-blooded mares for the production of a mule with some size, compactness and scale. He was used extensively as a herd jack. Two daughters by Limestone U. T. Monarch, U. T. Mona and U. T. Mona II, were good-typed jennets. A daughter of U. T. Mona, U. T. Marvel's Mona (Figure 11), by her half-brother, was one of the good jennets at the time the project was discontinued. However, a daughter of Quality Ezell, a full sister to U. T. Marvel, was on the extreme side, being large and coarse.

Two jennets, registered as Miss Gillespie and Young Miss Gillespie, who were half-sisters to Miss Derryberry, were purchased in 1944 for the purpose of having more Logan blood. These jennets through their daughters and granddaughters showed considerable promise of furnishing valuable breeding material. Some of their offspring were not as large as desired but they were generally good in quality and set of feet and legs.

Limestone U. T. Monarch, the Monsees jack, was used freely during the early years of the project; however, not as many of his offspring were retained in the herd as in the case of U. T. Logan line. But because of faults, such as roughness around the eyes, weak loins, light bone, crooked legs and small feet, most of Limestone U. T. Monarch's offspring were discarded from the herd. His best son, U. T. Monarch Again (Figure 6), out of the Derryberry jennet, sired several good colts. There were three of Monarch's daughters and four granddaughters in the herd when it was disbanded.

Since practically all the Logan jacks and jennets used had produced some freaks, along with some desirable offspring, and had failed to produce uniformly, it was felt that additional lines should be secured. Table I shows weights and measurements of representative types of jacks and jennets. After considerable investigation, two sons of Rowdy, the Dr. Gaston son of General Logan, were bought. U. T. Rowdy (Figure 12) was considered to be better than U. T. Rowdy II because he was a rugged, well muscled, heavy boned and good footed jack. U. T. Rowdy II had a better more refined head.

By 1948 it looked as if all the draft-bred mares would disappear and that few, if any, would be bred unless conditions changed materially. Since there were a good many mares of the Tennessee Walking Horse, the Quarter Horse and other hot-blooded types, and there was considerable interest in their breeding, it appeared that this type of mare would be about the only available type left for mule production. In this event a heavy bodied, com-

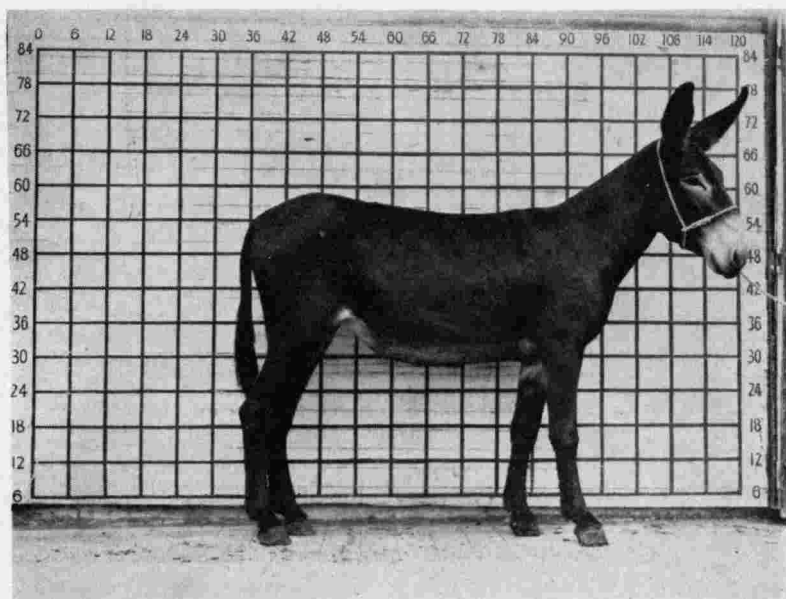


Figure 11.—U.T. Marvel's Mona at four years of age. This was one of the best jennets produced in the latter years of the project. She was sired by U.T. Marvel, out of a daughter of Limestone U.T. Monarch and his granddam. On both sides of her pedigree was the same jennet—Quality Ezell, one of the best-producing foundation jennets.

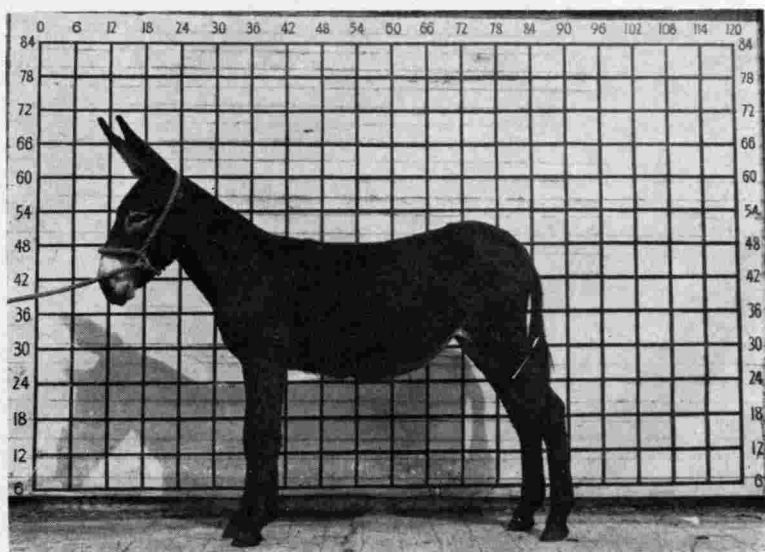


Figure 11a.—A 15-month-old jennet out of Marvel's Mona (shown in Figure 11), sired by U.T. Rowdy (shown in Figure 12).

Table 1—Weights and Measurements of Representative Jacks and Jennets Produced.

Jacks	Date of Birth	Birth		6 Months		1 Year		2 Years		3 Years		4 Years		5 Years	
		Wt.	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.	Ht.
U.T. Logan Again	6—4—39	—	—	350	46.1	510	50.9	815	55.1	925	57.6	935	57.1	925	57.6
U.T. Logan III	12—17—40	190	—	315	47.6	540	54.3	875	59.4	985	60.4	—	—	—	—
U.T. Blaize	7—22—40	90	—	390	50.5	575	56.0	920	60.8	1035	61.0	960	61.2	—	—
U.T. Mack	7—21—39	102	—	380	49.6	650	56.9	1020	64.0	1045	64.4	—	—	—	—
U.T. Marvel	4—19—42	100	—	400	48.7	625	53.2	855	56.8	925	56.8	1000	57.8	1050	57.8
U.T. Monarch Again	3—12—43	95	38.3	400	47.2	540	53.2	765	56.7	800	57.0	900	57.6	880	57.9
U.T. Frederick	9—18—42	75	33.9	320	45.4	470	48.6	685	53.8	—	55.7	—	—	—	—
Miss Derryberry's Small Jack	6—20—47	80	—	280	—	420	47.8	560	50.0	—	51.1	—	—	—	—
U.T. Derryberry's 48 Jack	7—23—48	85	—	360	46.9	550	51.8	790	55.6	—	—	—	—	—	—
U.T. Rowdy	1—5—46	—	—	—	—	—	—	800	57.5	930	59.0	1030	59.0	—	—
Limestone U.T. Monarch	10—13—37	—	—	—	—	—	—	790	56.7	930	58.1	1000	58.4	1035	58.8
<b>Jennets</b>															
U.T. Theresa	6—16—39	60	—	360	45.1	455	42.9	690	55.5	875	56.8	840	57.2	800	57.2
U.T. Theresa II	9—8—40	75	36.5	360	49.2	485	51.7	710	57.8	739	59.6	865	59.7	855	60.1
U.T. Clarice	5—22—40	85	37.2	390	48.6	530	52.3	740	56.3	930	56.5	960	56.6	855	57.1
U.T. Janis	7—25—40	80	37.0	385	49.4	530	53.6	780	58.9	930	60.4	1030	60.6	985	60.6
U.T. Mona	3—22—41	75	37.5	380	47.5	600	54.1	825	56.1	840	56.1	1000	56.5	1000	57.3 <sup>1</sup>
U.T. Ruby	7—12—44	65	—	325	49.0	495	51.3	695	55.2	800	56.8	800	57.4	860	57.9
U.T. Gillespie II	8—6—45	80	—	340	47.0	450	50.2	625	53.3	710	54.1	650	54.9	750	55.8
U.T. Marvel's Mona	5—26—46	90	32.2	315	45.9	420	50.4	630	52.8	850	54.8	840	54.8	—	—
U.T. Gillespie	7—12—45	80	—	330	45.4	455	49.6	590	52.2	680	53.0	620	53.0	680	53.0
U.T. Derryberry	4—13—45	100	—	375	47.8	540	51.4	660	55.3	820	55.7	820	56.5	980	56.7
U.T. Quality	11—8—44	90	—	365	47.7	480	50.7	770	57.0	880	59.0	890	60.4	980	60.3 <sup>2</sup>

<sup>1</sup>Weighed 1130 July 1, 1946 before foaling and 825 one month after foaling.

<sup>2</sup>Weighed 1130 August 1, 1949 before foaling.



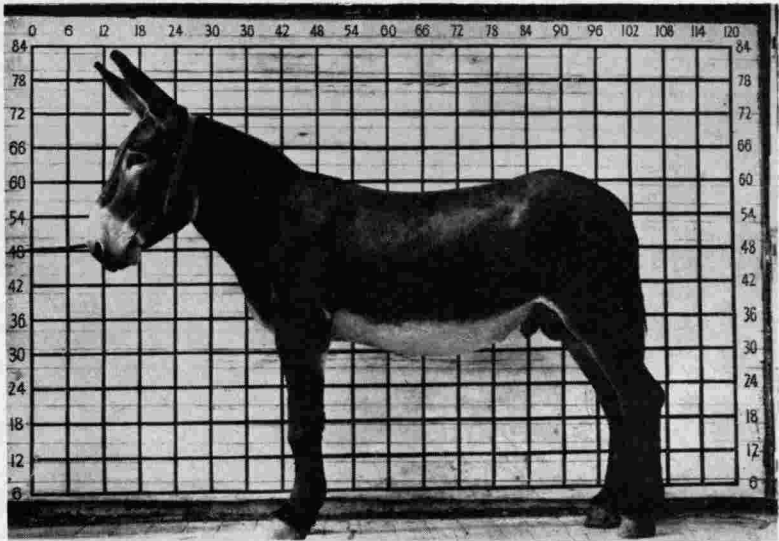


Figure 12.—U.T. Rowdy at four and one-half years of age. This jack was a grandson of General Logan, and was purchased from the Dr. R. B. Gaston estate in 1948 as a good source of blood for the breeding project. At four years of age, U.T. Rowdy stood 14.3 hands high and weighed 1,030 pounds. He had a big foot, heavy bone, good substance, and was of the compact order. It was thought that he was an excellent type of jack to transmit substance, muscling, and compactness to mules from hot-blooded mares. He had sired some excellent foals, both mules and jackstock, when the project was discontinued.

pact, heavy boned, big footed jack probably would be the best type to use for producing a mule out of these mares. U. T. Rowdy had considerable promise as a modern mule jack as well as jennet jack. He resembled U. T. Logan Again and U. T. Marvel in general type but was heavier, better muscled, heavier boned and better footed than either of the other jacks. Twenty-three jack and jennet foals and a number of mule colts were produced in 1949 and 1950 from the two Rowdy jacks. While they were all young when the project was concluded, and it is therefore conjectural on how they would have grown, no freaks or off-typed colts were observed. Thus it appeared that progress was being made in producing a desirable type from this Rowdy line of jacks.

#### FEEDING AND MANAGEMENT STUDIES

The fifth objective—"to study methods of growing, feeding, and managing horses, mules and jackstock"—was accepted initially with considerable hope and enthusiasm as very little information was available in these fields. Jack breeders are, or were, individualists, each with his own ideas, many of which appeared unique, concerning the feeding and management of jackstock. Some breeders would have been considered poor horsemen from the standpoint of present day knowledge. Studies of such matters as: the effects of exercise; types of stalls or barns; effects of pasture on the growth and fertility of jacks and jennets; effects of different levels of feeding on growth and fertility; use of corn as a sole source of grain for breeding jacks; influence of horse companions for young jacks; effects of various rations for nursing jennets; and many other management practices, would have added

much to the project had facilities, finances and personnel been available.

Fairly complete feed records were kept throughout the project, but since it was necessary to group-feed many jacks, jennets, and mules, of different ages, practical interpretation of the records is impossible. Generally, the unavailability of sufficient numbers of jack, jennet or mule colts of the same age or breeding for two or more lots made it questionable whether experimental work should be undertaken involving some of these matters, even if facilities and personnel had been available. It was impossible to execute a breeding schedule with the jennets which would get all the foals dropped at a uniform time. Since the number of breeding jennets was limited, the number of colts of either sex dropped in the spring, summer, or fall was very small. Thus no effective comparison could be made. Some of the jennets were regular breeders, conceiving on the foal heat. Some, however, would not settle until after they weaned their foals. Consequently, foals were dropped in every month of the year, although winter foaling was not encouraged. The matter of running horse colts with the jack colts also interfered with feeding trials, since the horse colts were more ravenous feeders than the jack colts.

The foals were dropped on pasture during the spring and summer, and in large box stalls in the winter and early spring. The mares, jennets, and their foals were run on pasture when it was available and were run on the outside during the winter when the weather was not severe. The weanling jacks, jennets, and mules were grouped and fed in different lots. Each group of jack weanlings were run with a companion horse or filly weanling until they were two years of age in order to acquaint them with horse stock and insure their service on mares when mature. Groups of jack colts had the run of a two-acre bluegrass lot connecting with a stall in the barn during both winter and summer. They were fed about six pounds of mixed grain and all the mixed or legume hay they would eat. This rate of grain feeding was continued until the jacks were three years of age. The mature jacks were fed less grain.

An effort was made to feed the young stock sufficiently well to grow them out in a normal way. Since the pastures varied considerably it was necessary to change the grain rations frequently in order to maintain growth and weight. It was difficult to apply the feed records obtained in determining the amount or cost of feed necessary for any class or age of jackstock or mules.

## BREEDING PRACTICES AND STATISTICS

Most of the jackstock foals were born in the spring and early summer. Some were born in the fall and winter months but during most of the project the jennets were not bred to drop winter foals. Since the jennets did

Table 2—Breeding Performance of Jennets and Mares.

	Jennets	Mares
Number in production	39	18
Number dying during project	6	0
Number production years	162	119
Number births—single or twins	121	108
Number foals born	125	108
Number raised	97	103 <sup>1</sup>
Percent of foals born to production years	77.2 <sup>2</sup>	90.7 <sup>2</sup>
Number dead at birth or lost before weaning	28	5
Percent of colts raised to colts born	77.6	95.4
Percent of colts raised to production years	59.9	88.5
Number abortions observed	7	2
Number twin abortions	4	0
Number twin births, including twin abortions	8	0
Percent of twin births to total births	6.6	0
Number twin births in which both died	2	0
Number twin births in which one was raised	2	0

<sup>1</sup> 99 Mule colts and 4 horse colts

<sup>2</sup> A few mares and jennets which foaled late were intentionally passed over in order to get them on a better breeding schedule.

Table 3—Breeding Performance of Mares Used for Mule Production.

Name of Mare	Breeding	Mature Weight	Years in Project	No. Colts born	No. Lost at Birth or Before Weaning	No. Abortions	No. Weaned
<b>Draft Mares</b>							
Grace	Grade Western	1400	11	11	0	0	11
Duchess	Grade Western	1600	7	6	0	0	6
Miss Evans	Grade Western	1500	7	7	1	0	6
Dinah	Grade Belgian	1600	5	3	1	1	2
Carlaetta	Purebred Percheron	1800	5	5	0	0	5
Mabel	Purebred Percheron	1800	8	7	0	0	7
Pet	Purebred Percheron	1700	8	6	3	0	3
Suetta	Purebred Percheron	1600	8	7	0	0	7
			59	52 <sup>1</sup>	5	1	47
<b>Hot-blooded Mares</b>							
Cherry	Tenn. Walking x Standard	1300	4	4	0	0	4
Blossom	Tenn. Walking	1300	9	9	0	0	9
Gordon	Grade Light Mare	1200	9	9	0	0	9
Gypsy	Tenn. Walking x Am. Saddle	1200	10	10	0	0	10
Natacha	Tenn. Walking x Standard	1400	8	7	0	0	7
Supreme	Tenn. Walking x Standard	1300	8	8	0	0	8
Jane	Grade Standard	1400	4	3	0	0	3
Elsie	Standard x Tenn. Walking	1100	2	2	0	0	2
Fashion	Tenn. Walking x Standard	1200	5	3	0	1	3
Sally Rand	Standard x Tenn. Walking	1100	1	1	0	0	1
			60	56 <sup>1</sup>	0	1	56

<sup>1</sup> A few mares foaling late were intentionally passed over and bred the next spring.

not settle as easily as the mares on the foal heat, they were somewhat irregular in their heat periods and some would not settle until after their foals were weaned. It, therefore, was impossible to maintain a definite breeding schedule.

Insemination for the most part was made artificially, the semen being collected in an artificial vagina. However, in the breaking and management of the jacks some natural services were made. The jennets and mares were inseminated every other day during the heat periods, with some exceptions, during the entire project. The technically and practically trained inseminators maintained a high degree of breeding efficiency in these herds, particularly with the mares. The general health and nutrition, and the close observation and management given to the herds were undoubtedly important influences in the performance. Table 2 gives the overall breeding performance of the jennets and mares during the entire project.

The breeding performance of the mares was phenomenal (Table 3). Twelve of the 18 mares used had perfect records, producing and raising a colt every year. One grade draft mare, Grace, raised 11 colts consecutively. Gypsy Rosalie, a Tennessee Walking mare, raised 10 colts. Most of the losses in colts were from one mare which lost three out of six at birth. These results are much better than those obtained by the average man who stands stallions or jacks where the mares are bred only once during the heat period from semen of an unknown quality and where the mares are not observed carefully and returned for rebreeding. Also many problem or diseased mares are bred. The mule colts apparently were born with that desire to live, and ability to take care of themselves, which characterize them so well in mature life.

The situation was different with the foals from the jennets. Several were born dead or weak, some had difficulty in nursing or would not nurse at all. Many were delicate, and difficult to raise. They were more subject to pneumonia and other troubles than the mule colts (Tables 4 and 5).

Table 4—Sex and Mortality of the Jackstock Offspring.

	Males	Females
Number born—abortions excluded	64	61
Number dead at birth or lost before weaning	18	10
Number weaned	46	51

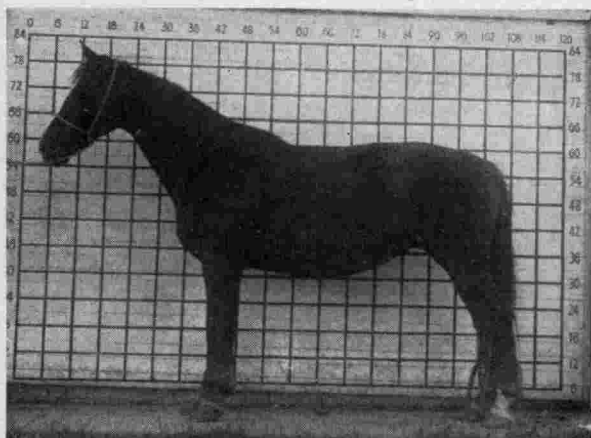
Table 5—Jackstock Offspring of the Herd Sires Used.

Name of Jack	Foals sired
U.T. Logan 34134	18
Limestone U.T. Monarch 32973	19
U.T. Logan Again 34361	27
U.T. Marvel 35685	19
U.T. Blaze 34531	1
U.T. Monarch Again 36299	10
U.T. Rowdy 36972	12
U.T. Rowdy II 37015	9
John Hampton (Owned by K. R. Nelson) 35218	3
Other jacks—service jacks on initial jennets purchased	8

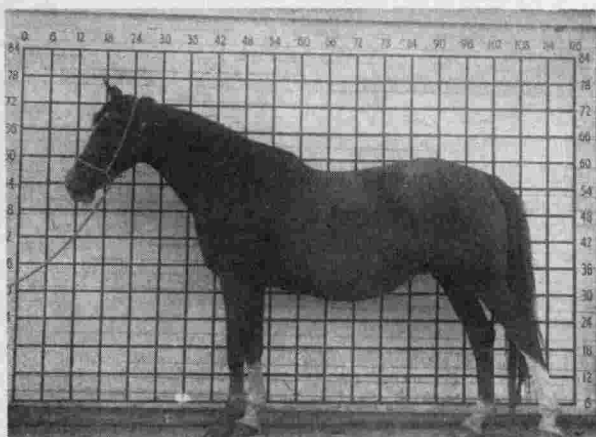
### Mule Production

The group of hot-blooded mares (Figure 13), mostly of the Tennessee Walking Horse breed, but with some American Saddle and Standard breeding, was secured earlier in the project than the draft mares, and more mules were produced from them. Four of the draft mares were purebred Percherons and four were grades (Figure 13A), probably originating in the West. Their breeding performance is shown in Table 3. In Table 6 are given some weight and height records on some of the average and extreme representatives of the draft and hot-blooded mules. In several instances two mules from the same mares and by the same or different sires are shown. These and other records show that as great variations were obtained in the mule as from the jack and jennet offspring. The mules varied in type, weight and temperament and the need for a prepotent jack of desired type was evidenced in the mule breeding phase of the project. In fact, variations in the offspring from certain jacks were noted sooner in his mule than in his jackstock offspring.

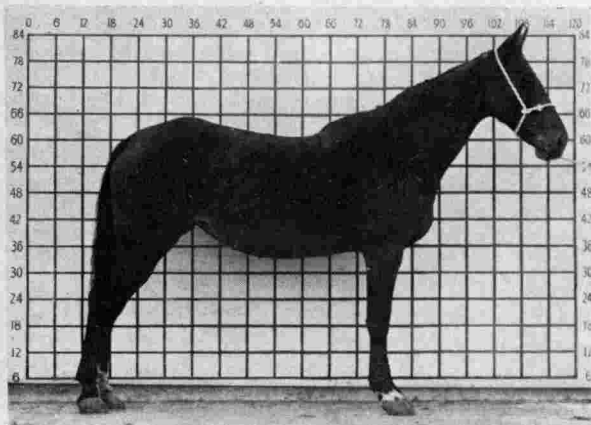
A summary of the weights and heights of the mules by ages is given in Table 7. The appraised values are shown in Table 8. In this table the average appraised value of each mule from each mare is shown regardless of sex. The appraisers usually made a difference of \$75 to \$125 in favor of a mare mule over a horse mule, with a smaller difference for the younger mule. An effort was made to secure practical top prices for each sex each year so that a "percentage of practical top" value could be calculated, thus eliminating to some extent sex differences in the prices of mules. This percentage of practical top value gives a better rating of the market value than the appraised value, because the market fluctuated from year to year and fell very low in 1949 and 1950. Some mares may have had all their mules appraised during the high years while others hit the low years. These values also are influenced by the fact that several of the less desirable light mules were sold at a young age and do not appear in the older age groups.



Blossom, a Tennessee Walking mare, at 8 years of age. She produced nine colts in nine years. Her colts were always "full of ginger," and hard to break.



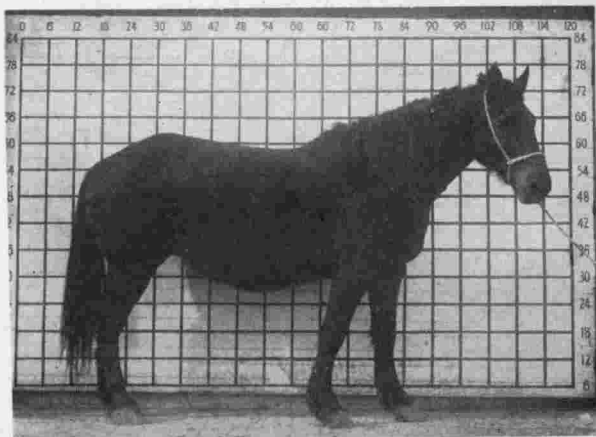
Gypsy at 11 years of age, a Tennessee Walking—American Saddle bred mare. She produced 10 mules in 10 years. Her mules were appraised at 67 percent of practical top values.



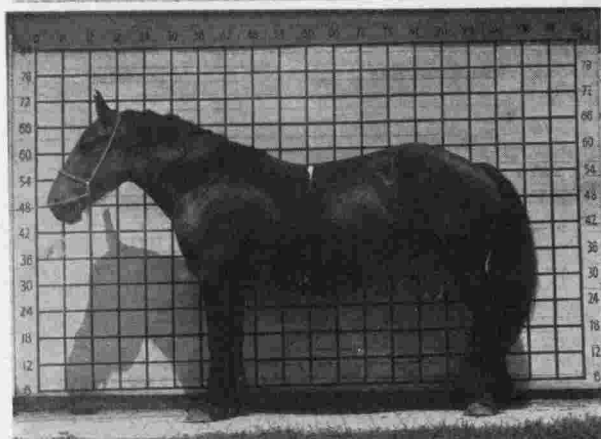
Jane, at 10 years of age. A grade Standard bred mare weighing 1,400 pounds, she produced good-sized but rangy mules.

Figure 13.—Some of the hot-blooded mares used in the breeding project reported in the bulletin.

Grace at 9 years of age. This grade Western mare weighed 1,400 pounds. She produced 11 colts consecutively, some valued as much as \$325 per head.



Duchess, a farm chunky-type mare of the kind desired by some breeders for mule production. She produced some compact and some rangy mules.



Suetta at 3 years of age. A purebred Percheron mare, she was a consistent producer of good mules.

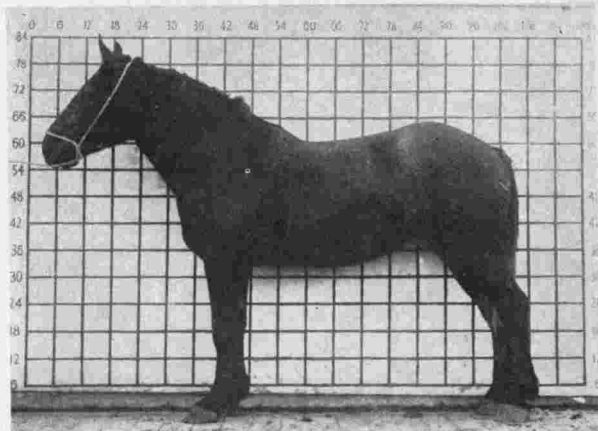


Figure 13a.—Some of the draft mares used in the breeding project.

Table 6—Weights and Heights of Some of the Average and Extreme Mules From the Draft and Hot-blooded Mares.

Name of Mule	Sex and Birth Date	Sire	Birth Weight Lbs.	6 Months Weight Lbs.	6 Months Height In.	1 Year Weight Lbs.	1 Year Height In.	2 Years Weight Lbs.	2 Years Height In.	3 Years Weight Lbs.	3 Years Height In.
<b>Draft Mules</b>											
Duchess '45	F 4—23—45	Logan Again	120	510	50.3	700	54.2	915	58.4	1000	—
Carlaetta '44	F 7—16—44	Logan Again	150	590	54.8	730	58.5	1180	63.2	1500	—
Carlaetta '45	F 7— 8—45	Limestone Monarch	130	550	55.9	820	60.2	1130	64.7	1370	66.0
Dinah '45	F 4— 6—45	Marvel	125	530	50.3	810	54.1	980	58.5	1070	—
Mabel '45	F 3—22—45	Logan Again	125	620	54.7	920	60.0	1185	65.0	1280	—
Mabel '46	F 3— 8—46	Monarch Again	105	520	52.0	730	55.0	940	59.0	990	59.8
Suetta '44	M 3—26—44	Logan Again	125	515	54.0	700	57.0	1044	60.8	1250	—
Suetta '45	M 4— 3—45	Limestone Monarch	100	520	52.5	770	56.8	1060	62.3	1070	—
<b>Hot-blooded Mules</b>											
Cherry '46	F 3—19—46	Monarch Again	105	500	49.9	685	53.0	860	55.4	930	—
Blossom '44	F 4— 4—44	Limestone Monarch	115	490	53.9	580	56.0	850	59.3	1045	61.2
Blossom '45	M 3—18—45	Logan Again	110	505	51.4	770	56.3	975	60.7	1030	—
Gordon '41	M 4—14—41	Limestone Monarch	90	405	50.3	590	—	830	59.1	900	60.6
Gordon '42	F 4— 6—42	Limestone Monarch	100	440	50.6	640	55.0	840	57.5	845	58.3
Gypsy '42	M 3—29—42	Logan Again	95	400	49.1	610	53.1	795	56.2	850	57.8
Gypsy '43	F 3—21—43	Logan Again	100	490	52.6	710	57.3	870	59.0	1045	61.3
Natacha '42	M 8—26—42	Limestone Monarch	90	425	48.6	550	51.8	755	55.2	780	56.0
Supreme '43	M 4—11—43	Logan Again	85	390	48.8	570	54.8	700	56.1	810	57.3
Jane '41	F 4—16—41	Limestone Monarch	120	525	53.7	790	—	1105	62.9	1110	—
Fashion '41	F 4—11—41	Limestone Monarch	95	400	50.4	640	—	900	60.2	940	62.2

Table 7—Weights and Heights of Mules of Different Ages out of Draft Mares and Hot-blooded Mares.

Ages	Draft Mules				Hot-blooded Mules			
	Weight		Height		Weight		Height	
	No. Records	Av. Wt. Lbs.	No. Records	Av. Ht. In.	No. Records	Av. Wt. Lbs.	No. Records	Av. Ht. In.
At birth	35	121	—	—	49	104	—	—
At 6 months	39	545	38	53	46	459	45	53.4
At 1 year	36	758	29	56.9	41	634	29	55.3
At 2 years	31	1001	28	60.8	27	852	22	58.6
At 3 years	24	1173	14	63.1	23	931	19	59.6

### Draft Mules Vs. Hot-Blooded Mules

Some very good general purpose mules were obtained from the hot-blooded mares. But they were generally too small, angular, rangy, light muscled and light-boned to meet market demands which were governed largely by the market dollar sign, rather than the mule's ability to do work. Most of them were high strung and more difficult to break than the draft mules. However, some of them were broken easily and were slow going. In breaking the light mules, their mouths were sometimes cut, which always gave a buyer the idea that they were or had been outlaws. One pair of light mules, Gordon 40 and Gypsy 41, made an excellent team on the University farm where they were used for general farm and plot work. At 12 and 11 years they weighed 1,050 each, were sound, alert, good workers.

The draft mule yearlings were appraised at 85 percent of practical top value in comparison to the light mule yearlings at 70 percent (Figure 14). On this basis, or any other age-appraisal, it was much less profitable to raise a light mule than a draft mule. The average weight of all the three-year-old light mules was 931 pounds, while the draft mules weighed 1,173 pounds. Neither class was mature at these ages, but the difference greatly affected their grades and market values.

If grade draft mares are available they appear to be better mule producers than the light mares (Figure 14A). Light mares bred to heavy boned, compact jacks will produce mules somewhat off type from present standards which will wear collars and do a lot of work. Since the draft mares are disappearing much faster than light mares, light mares may of necessity become the mule producers for the coming decades.

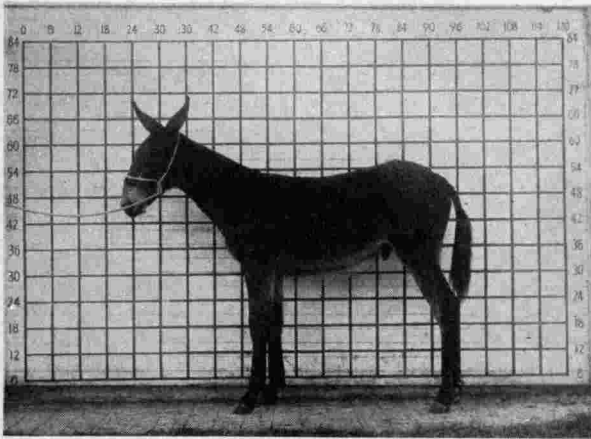
### MULE TESTING

One of the main objectives in the overall project was that of breeding a type of jack which would produce a "tough" mule of a desired size and type for farm work. Some relatively simple and quick measures of a mule were needed before any progress could be made in a scientific way in rating the offspring and in selecting breeding material for such an objective. No such standards or measures had been perfected. Sale values or personal opinions were of little or no value in charting a breeding program. Many mule men contended, for instance, that mules out of hot-blooded mares were much tougher and would do more work than mules out of draft mares; but this could not be proved.

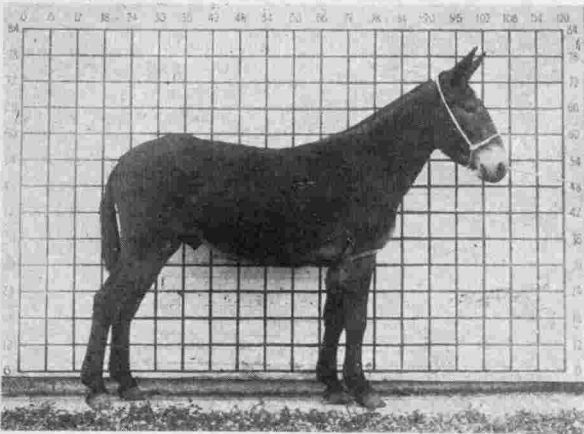
At a conference in Columbia, Tennessee, in September, 1944, a committee composed of V. Berliner, S. Brody, J. N. Cunnings, H. C. McPhee and R. W. Phillips suggested measures of performance involving such features as:

1. The uniform breaking and training of the jacks, jennets, mares, and mules so that tests could be made.

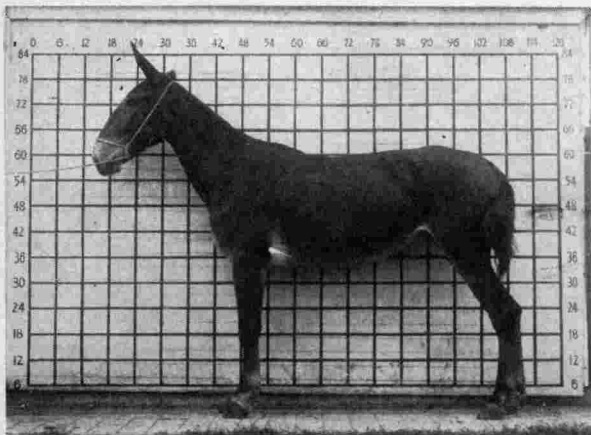




Supreme 45 at six months of age. This mule was rangy and light-boned and was appraised at 66 percent of practical top value as a yearling. He weighed 850 pounds as a 2-year-old.



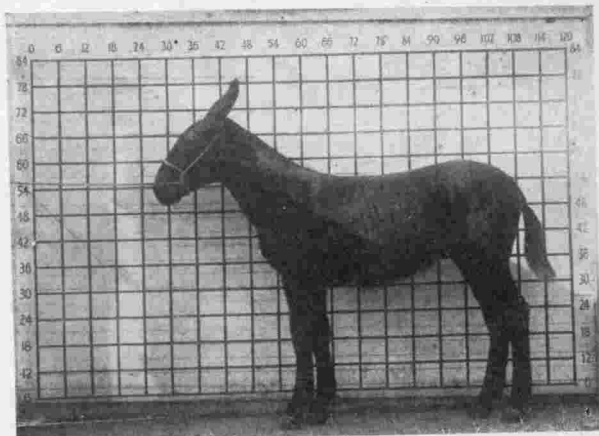
Gypsy 42 at 3 years of age. This mule was appraised at 71 percent of practical top value. He weighed 935 pounds at three and one-half years. He was the top performer of 11 mules tested in 1945.



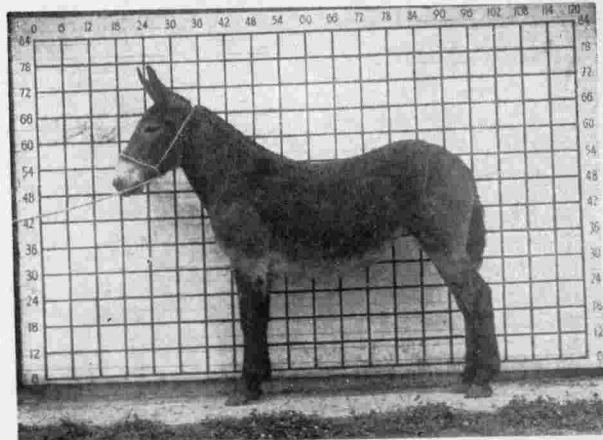
Fashion 41 as a 4-year-old. The market value of this mule was not high, but she had a high rating in harness. She weighed 1,025 as a 4-year-old in hard working condition.

Figure 14.—Examples of some of the mules from hot-blooded mares.

Mabel 45 at six months of age. This mule was appraised at 100 percent of practical top value. She weighed 1,280 pounds as a 3-year-old, and stood better than 16 hands high.



Dinah 45 at one year. This mule was appraised at 113 percent of practical top value. She was considered a modern-typed mule and weighed 1,070 pounds as a 3-year-old.



Suetta 46 at 4 years of age. This mule was appraised at 92 percent of practical top value, and matured at 1,200 pounds in average condition.

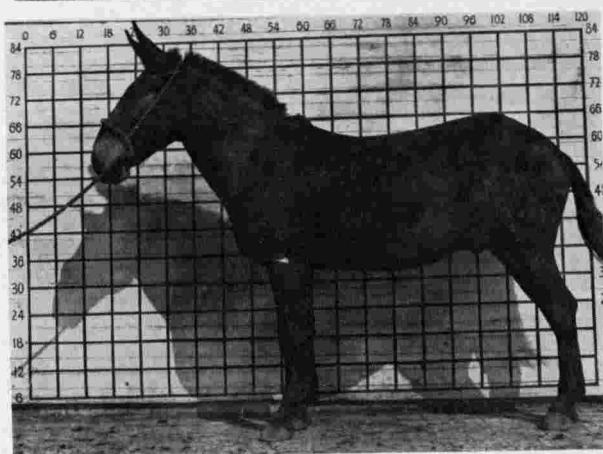


Figure 14a—Examples of mules from draft mares.

Table 8—Appraised Values of the Mules by Mares and by Ages.

Name of Mares	Coming Yearlings			Coming 2 Yr. Olds			Coming 3 Yr. Olds			Coming 4 Yr. Olds			All Ages No. Appr'd	Av. Percent Pract'l Top
	No. Appr'd	Av. Appr'd Value	Av. Percent Pract'l Top	No. Appr'd	Av. Appr'd Value	Av. Percent Pract'l Top	No. Appr'd	Av. Appr'd Value	Av. Percent Pract'l Top	No. Appr'd	Av. Appr'd Value	Av. Percent Pract'l Top		
<b>Draft Mares</b>														
Grace	7	\$ 79	85	7	\$117	88	6	\$275	87	5	\$217	74	10	82
Dinah	2	125	116	2	142	95	2	200	103	1	225	60	2	100
Mabel	6	97	96	5	128	84	4	154	95	2	175	74	6	89
Suetta	6	69	92	5	104	97	4	144	89	3	153	91	6	88
Pet	3	78	94	3	95	71	1	190	63	1	230	85	3	79
Carlaetta	5	96	85	6	137	90	5	166	80	2	232	82	5	83
Duchess	5	59	80	3	112	85	2	162	90	1	110	73	5	78
Miss Evans	5	83	89	3	112	71	2	157	78	—	—	—	5	85
Total & Av.	39	83	90	33	118	86	26	188	87	15	195	78	42	85
<b>Hot-blooded Mares</b>														
Jane				1	140	80	1	275	85	1	190	69	3	78
Gypsy	5	68	67	5	103	73	6	146	72	7	155	64	8	67
Gordon	4	59	61	4	80	63	4	141	74	4	179	59	7	62
Fashion	1	110	73	1	150	86	2	182	81	2	167	71	2	78
Natacha	4	66	70	4	87	73	3	147	87	3	170	71	6	72
Blossom	4	66	80	5	107	81	4	129	87	2	167	64	8	77
Supreme	7	66	80	5	107	81	4	129	87	2	167	64	8	77
Cherry	5	56	65	4	82	73	1	75	88	—	—	—	3	67
Total & Av.	3	57	64	2	87	68	1	125	62	—	—	—	3	66
Total & Av.	29	64	69	26	97	73	22	147	80	19	171	67	40	70

Practical top prices for coming 3-year-olds well broken.

Date	Mare Mule	Horse Mule
12—22—43	\$400	\$275
12—20—44	275	225
1—12—46	245	190
2— 6—47	300	200
1—24—48	250	150
2—16—49	200	125
2—18—50	200	150

2. Record of resting pulse and respiration rates soon after birth and at one-month intervals until four years of age.
3. Record of speed and length of stride of animals when hitched to a light cart with a given load, taken at 2, 2½, 3, 3½ and 4 years of age.
4. Determination of cardio-respiratory capacity at same ages as in No. 3 of animals pulling a load of 30 percent of body weight. Pulse and respiration reading would be taken at rest, at the end of the course, and at intervals after the test until the animals approached normalcy.
5. Analyses and correlation of records with body measurements, weight, height, breeding, etc.

It was believed at this conference that this procedure was a sound approach, but would require more personnel, time, and breeding stock than could be justified or supported.

At a conference in Chicago in November, 1944, attended by representatives of the Missouri, Iowa State College, and Tennessee Experiment Stations, regarding the testing of work stock the following agreements were reached:

1. That the Missouri Experiment Station would continue the testing of mules using the method reported in Missouri Research Bulletin 394 entitled "Field Studies on Cardio-Respiratory Functions and Energy Expenditure During Work and Recovery in Mules;" and in addition, study the rate of recovery of pulse and respiration immediately after work. In the Missouri tests, field trials were conducted with mules pulling a given load in connection with equipment which enabled workers to collect such data as pulse and respiration rates, pulmonary ventilation rate, oxygen consumption, oxygen decrement in expired air, and oxygen debt, all of which were basic in studying endurance or the ability of mules to do work efficiently.
2. That the Tennessee Experiment Station would develop a dynamometer practicable for road tests with which a relatively simple test could be made involving the rate of recovery of pulse and respiration after uniformly conducted tests. It was generally agreed that the height or rate of recovery of pulse and respiration after severe work might give indices of a mule's stamina or ability to work. It was further agreed that mules would be tested by the simple method at the Tennessee Station and tested later by the more exhaustive Missouri test so that correlation of the two tests could be made.

A dynamometer (Figure 15) practicable for testing draft animals on the road, was built with the assistance of the Tennessee Station engineers. It consisted of a gear pump, previously used at the Iowa Experiment Station in testing draft horses, mounted on a frame and geared to the rear axle of an automobile. Oil was used in the pump, and before the vehicle would move it was necessary for the mule to lift a given weight which automatically opened a valve. This permitted the oil to flow through the pump and allow the wheels of the vehicle to move. This machine was calibrated and theoretically was supposed to require the same draft on the level or up and down grade at practicable working speeds.

A course, 1.23 miles long on a smooth, slightly rolling farm road with a chert surface, was selected for the testing ground. After some experimentation, it was decided that a draft of 25 percent of the body weight was sufficient to give a mule a severe workout. When the mules were driven over the course in 30 minutes they were traveling at the rate of 2.46 miles per hour, which was considered a desirable working speed. By placing "five minute stations" on the course it was possible, by the use of a watch, to pace the mules so that the course could be covered in approximately 30 minutes. Driving the mules was very important. Some of them had to be held down while others had to be urged along. Unless an animal stopped momentarily of its own accord, only one stop was made for turning around and reversing the travel to the barn where the pulse and respiration observa-

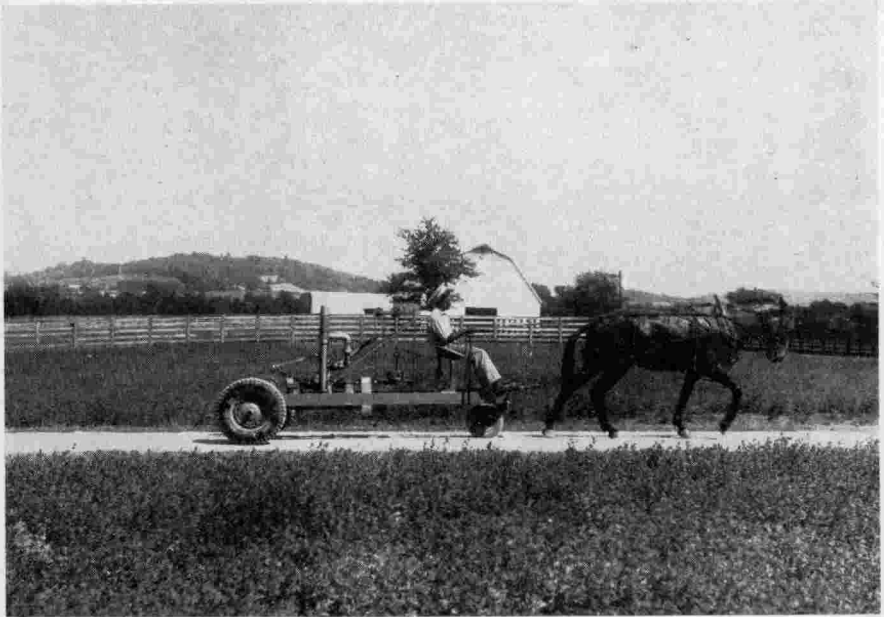


Figure 15.—Gypsy 41 is seen here laboring under the load imposed by the dynamometer, which was built to regulate amount of load. The weight which gave a load equivalent to 25 percent of the mule's body weight is suspended at the mid-point of the machine. In covering the course in 30 minutes the 25 percent load gave the mules a thorough workout, sufficient to stimulate marked heart and lung reactions. The peak and recovery of pulse and respiration were checked at the end of the trial, and at 5-minute intervals for 30 minutes. In the background are jack and mule barns, with one of the two-acre bluegrass paddocks which played an important part in the development and health of the jacks.

tions were taken.

Pulse and respiration readings were made immediately on stopping the mule and at five-minute intervals until 30 minutes had elapsed. The first reading was taken immediately on stopping, and the other readings taken after placing the mule in a large box-stall where conditions for the cooling-off period would be uniform. A stethoscope of the type used by physicians was used to take the pulse. When the breathing was labored, it was easy to count the respirations by observing the movement of the nostril; but for getting the "at rest" or slow respirations a fringed cloth hood placed over the nostrils was found very helpful. "At rest" readings on pulse and respirations were secured for each series of tests. These were generally an average of the low readings secured and were sometimes difficult to obtain. Temperature, time before and after feeding, disposition of the mule on approach or familiarity with the attendant and other factors, affected these readings. The hour of the day, the temperature and the humidity were also taken for each trial.

All the tests made from 1945 to 1950, used the same course, the same percentage load, and the same technique. The help of two to three operators was required. The personnel was not constant through the years, but in no case was an entirely new crew used. Most of the tests were conducted in

Table 9—Extent of Mule Testing 1945-1950

	'45	'46	'48	'50	Years
Number mules from draft mares tested	2	4	6	13	25
Number tests made on draft mules	24	78	50	120	272
Number mules from hot-blooded mares tested	9	2	3	2	16
Number tests made on hot-blooded mules	119	34	35	15	203
Total mules tested	11	6	9	15	41
Total tests made	143	112	85	135	475

the late summer or early fall when the weather was hot. A few tests were made in February, 1950. The first tests were made when the mules were past three years of age. The mules were broken as two-year-olds and an attempt was made to have them equally well broken and conditioned for the tests, though this was not fully accomplished. Table 9 gives information on the numbers and breeding of the mules tested, and the number of tests run on each mule by years.

Tests were conducted in July, August, and September, 1945, on two draft-bred mules and nine hot-blooded mules, 116 individual trials being run. Table 10 gives complete data secured on the four mules tested in the 1945 Tennessee Trials and which were sent to the Missouri station to be tested by the Missouri method. These mules were selected to represent two of the best and two of the poorest performers, based on the July and August tests. These mules varied considerably in breeding, weight, temperament, and pulse and respiration reactions. Before sending these mules to Missouri, investigators decided to run further tests which were made in late September when the humidity was 12 points lower and the temperature 3° higher than when the July and August tests were run. The results obtained in the September tests were somewhat different from those secured in the July and August tests.

The data in Table 10 for the July and August tests along with additional data on seven other mules were statistically analyzed by Messers Brody and Kibler of the Missouri station. Significant differences were found between mules on pulse and respiration rates at the end of the tests and at five, ten, fifteen and thirty-minute intervals after completing the test. Significant differences were also found in most of the above points between the July-August tests and the September tests.

At this time it was apparent that such factors as training of the mule, kind and regularity of previous work, air temperature, humidity, velocity of wind, temperament of the mule, ability of the driver and perhaps other factors, difficult to control, would affect such performance tests.

#### Missouri Tests on Four Tennessee Mules

The equipment and methods used in testing the Tennessee mules were the same as described in Missouri Research Bulletin 394 with the following exceptions: (1) with added respiration and pulmonary ventilation rate recording apparatus and (2) an increase in the load equivalent to 30 percent of body weight.

The Missouri tests were for only nine minutes' duration after a warm-up period. Data on comparable items between the Tennessee July-August and September trials and the Missouri trials are shown in Table 11.

In Table 12 are shown ratings of the four mules based on results secured in the three tests. This is an attempt to apply the data secured without knowing how significant the differences are or which items are of the greatest importance in evaluating the tests.

Because of shifting personnel, finances, or other considerations, the data developed at the Missouri station on the four Tennessee mules have not been completely analyzed. In Table 13 are shown results developed from the Missouri testing apparatus in addition to information shown in Table 10.



Table 10—Continued

## GRACE 42

Trial	1945 Date	Temperature	Humidity	Hour of Day	Load	Length of Course	Time	PULSE							RESPIRATION									
								At rest	End of pull	—5 Min.	—10 Min.	—15 Min.	—20 Min.	—25 Min.	—30 Min.	At rest	End of pull	—5 Min.	—10 Min.	—15 Min.	—20 Min.	—25 Min.	—30 Min.	
1	7—18	72	80	8:58	25%	1.23	24	44	98	90	72	54	60	52	60	14	76	60	58	36	40	40	40	40
2	"	80	50	10:54	"	"	28		100	80	66	56	54	52	50		70	70	74	54	42	48	40	40
3	"	82	61	2:57	"	"	29		112	84	78	72	62	52	58		70	50	32	28	22	20	20	20
4	8—9	70	86	8:35	"	"	30	44	100	72	56	54	54	50	50	14	32	28	26	22	22	22	24	24
5	"	76	74	10:23	"	"	29		98	74	66	58	64	52	54		100	80	52	44	42	32	28	28
6	"	80	64	12:55	"	"	30		106	82	70	62	62	60	58		96	90	86	60	46	46	40	40
7	"	82	61	4:11	"	"	29½		114	78	68	62	60	62	58		100	96	80	70	56	40	32	32
8	8—10	73	86	7:14	"	"	28½	44	104	78	68	60	58	52	58	14	70	64	66	46	46	42	32	32
9	"	78	75	9:35	"	"	30		100	80	68	58	56	56	58		74	74	72	70	60	56	48	48
10	"	82	69	1:00	"	"	29		90	82	70	64	62	62	60		78	78	78	76	50	42	42	42
11	"	83	62	2:45	"	"	30		104	78	66	62	58	62	60		74	68	68	68	44	44	40	40
12	"	83	62	4:29	"	"	30½		106	82	68	64	62	60	64		74	74	80	70	60	42	44	44
Tot.		941	830				347½		1232	960	816	726	712	672	688		914	832	772	644	530	474	430	430
Av.		78.4	69.2				29		102.7	80	68	60.5	59.3	56	57.3		76.2	69.3	64.3	53.7	44.2	39.5	35.8	35.8
13	9—27	77	71	9:24	25%	1.23	29	48	108	80	64	56	56	54	54	22	90	102	90	74	66	50	48	48
14	"	80	68	11:36	"	"	30		102	72	60	54	50	48	48		50	50	42	42	36	32	32	32
15	"	84	49	3:09	"	"	30½		104	76	64	60	56	54	54		40	24	24	22	22	22	20	20
16	"	82	55	5:30	"	"	30		102	70	60	60	50	50	50		40	40	32	30	30	30	28	28
17	9—28	74	74	7:12	"	"	30	48	98	76	68	58	56	58	56	22	82	76	76	54	44	36	38	38
18	"	79	68	9:26	"	"	30		90	72	60	56	54	54	56		78	84	84	78	64	42	42	42
19	"	81	61	11:43	"	"	31		84	74	62	56	56	60	58		80	84	88	70	70	50	44	44
20	"	84	56	2:05	"	"	30		90	74	64	56	54	52	54		40	54	70	70	56	54	44	44
Tot.		641	502				2405		778	594	502	456	432	430	430		500	514	506	440	388	316	296	296
Av.		80.1	62.7				30		92.7	74.2	62.7	57	54	53.7	53.7		62.5	64.2	63.2	55	48.5	39.5	35.7	35.7



Table 10—Continued

## NATACHA 42

Trial	1945 Date	Temperature	Humidity	Hour of Day	Load	Length of Course	Time	PULSE							RESPIRATION														
								At rest	End of pull	—5 Min.	—10 Min.	—15 Min.	—20 Min.	—25 Min.	—30 Min.	At rest	End of pull	—5 Min.	—10 Min.	—15 Min.	—20 Min.	—25 Min.	—30 Min.						
1	7—16			11:49	20%	1.23	25	42	90	60	50	44	54	46		16	40	32	24	22	22								
2	7—17			8:14	25%	"	28		92	72	60	50	48	46	44		46	32	20	18	18	16	16						
3	"	75	65	10:27	"	"	26		98	78	62	54	44	44	42		60	40	30	22	18	20	20						
4	"	80	57	2:15	"	"	25		90	80	74	66	58	50	48		60	40	40	28	24	18	18						
5	"	82	51	4:29	"	"	27		98	76	68	56	46	46	46		64	32	24	18	16	16	14						
6	8—7	80	83	11:46	"	"	28		90	68	68	60	56	54	48		128	120	106	102	90	84	78						
7	"	82	84	2:55	"	"	31½		94	76	68	62	62	54	54		100	82	56	48	42	32	28						
8	"	80	79	5:16	"	"	32½		94	72	62	60	50	56	56		90	52	44	38	30	30	23						
9	8—8	70	81	7:52	"	"	26		94	72	64	54	48	52	50		82	68	52	44	44	38	40						
10	"	74	82	10:08	"	"	30		94	76	66	62	50	52	50		82	64	48	38	34	22	20						
11	"	74	74	1:44	"	"	31		106	74	68	56	44	44	46		80	48	34	34	22	22	16						
12	"	76	74	5:21	"	"	29		78	68	58	54	48	48	46		58	36	34	26	26	22	16						
Tot.		773	730				339		1118	872	768	678	608	592	530		890	646	512	438	386	320	294						
Av.		77.3	73.0				28.2		93.2	72.7	64.0	56.5	50.7	49.3	48.2		74.2	53.8	42.7	36.5	32.2	29.1	26.7						
13	9—27	77	71	8:52	25%	1.23	28	42	110	90	82	76	72	64	62		22	114	108	94	84	72	64	46					
14	"	80	68	11:01	"	"	30		106	86	78	70	66	64	62		108	84	62	64	54	46	40						
15	"	84	56	2:34	"	"	30		106	90	82	74	66	60	60		106	90	66	58	46	40	34						
16	"	84	49	4:53	"	"	32½		100	80	72	66	54	54	50		88	60	42	40	40	34	26						
17	9—28	75	74	8:19	"	"	28	42	100	84	74	68	62	58	54		22	96	92	86	86	82	76	70					
18	"	80	68	10:35	"	"	31		92	74	68	62	56	58	58		112	96	90	90	80	80	70						
19	"	83	56	12:53	"	"	29½		98	88	76	70	66	62	62		120	116	96	92	80	72	66						
20	"	83	56	3:09	"	"	31		84	72	70	62	60	60	60		106	100	100	90	80	66	64						
Tot.		646	493				2400		796	664	602	548	502	480	468		850	746	636	604	534	478	416						
Av.		80.7	62.2				30.0		99.5	83.0	75.2	68.5	62.7	60	58.5		106.2	93.2	79.5	75.5	66.7	59.7	52.0						

Table 10—Continued

GORDON 41

Trial	1945 Date	Temperature	Humidity	Hour of Day	Load	Length of Course	Time	PULSE							RESPIRATION										
								At rest	End of pull	—5 Min.	—10 Min.	—15 Min.	—20 Min.	—25 Min.	—30 Min.	At rest	End of pull	—5 Min.	—10 Min.	—15 Min.	—20 Min.	—25 Min.	—30 Min.		
1	7—16	78		4:35	25%	1.23	24	42	84	74	64	52	48	48	50		50	42	34	28	24	18			
2	7—17	68	75	8:48	"	"	26		86	68	55	52	50	56	54		56	38	24	22	20	24	22	20	22
3	"	76	66	10:59	"	"	27		86	70	60	54	50	50	50		68	46	36	32	22	20	20	20	20
4	"	80	50	2:48	"	"	28	42	90	70	64	52	56	56	56	16	70	66	44	40	34	24	24	18	18
5	"	82	51	5:03	"	"	26		88	68	64	54	50	50	50		70	48	36	28	28	20	20	20	20
6	8—7	77	91	10:12	"	"	26		100	80	76	72	66	62	56		112	102	100	90	86	72	60	60	60
7	"	82	84	1:06	"	"	29 $\frac{1}{2}$		92	76	74	68	66	64	64		112	124	124	120	106	104	84	84	84
8	"	82	76	3:30	"	"	29	42	92	80	70	64	56	56	50		100	92	86	76	66	50	34	34	34
9	8—8	70	90	8:59	"	"	30		90	72	64	52	50	46	48		100	80	60	42	36	24	28	28	28
10	"	73	86	11:19	"	"	29		88	70	60	54	48	50	50		98	74	64	38	33	32	28	28	28
11	"	75	74	3:02	"	"	30		94	80	64	58	54	54	52		90	70	54	32	28	30	26	26	26
12	8—10	75	78	9:00	"	"	27		90	78	66	60	50	54	52		110	92	96	80	68	60	44	44	44
Tot.		918	821				331 $\frac{1}{2}$		1080	886	781	692	644	646	632		1036	874	758	628	556	478	384	384	384
Av.		76.5	74.6				27.6		90	73.8	65.1	57.7	53.7	53.8	52.7		86.3	72.8	63.2	52.3	46.3	39.8	34.9	34.9	34.9
13	9—27	76	74	8:17	25%	1.23	29		92	82	76	66	60	54	54		92	76	72	60	48	40	32	32	32
14	"	80	68	10:32	"	"	27		92	80	72	68	66	62	62		58	58	80	68	66	56	56	56	56
15	"	83	56	2:00	"	"	29 $\frac{1}{2}$		90	76	70	64	62	58	56		64	50	40	36	34	30	24	24	24
16	"	84	49	4:19	"	"	30		88	76	62	58	54	52	50		74	56	46	46	44	40	30	30	30
17	9—28	74	74	7:46	"	"	28 $\frac{1}{2}$		96	76	66	62	58	54	52		96	76	76	66	66	62	56	56	56
18	"	79	68	10:01	"	"	29 $\frac{1}{2}$		94	74	64	62	66	66	68		104	98	88	80	70	64	64	64	64
19	"	82	61	12:18	"	"	29 $\frac{1}{2}$		94	80	76	70	64	64	62		96	88	90	76	74	60	56	56	56
20	"	84	56	2:33	"	"	30		82	76	70	64	64	60	60		82	70	64	60	46	40	32	32	32
Tot.		642	506				2330		728	620	556	514	492	470	464		666	572	556	492	448	392	350	350	350
Av.		80.2	63.2				29.1		91.0	77.5	69.5	64.2	61.5	58.7	58.0		83.2	71.5	69.5	61.5	56.0	49.0	46.7	46.7	46.7

Table 11—Results of the Tennessee and Missouri Tests on Four Mules.

	MULES			
	Natacha 42	Gypsy 42	Gordon 41	Grace 42
Age of mules — years				
Body weight — Tenn., July, Lbs.	3½	3½	4½	3½
Body weight — Mo., Oct., Lbs.	800	860	955	1120
Pulse rate at rest per min., Tenn., July-Aug.	844	913	980	1173
Pulse rate at rest per min., Tenn., Sept.	42	42	42	44
Pulse rate at rest per min., Mo., Oct.-Nov.	42	44	42	48
Pulse rate at end of work (30 min) Tenn., July-Aug.	35.0	35.0	35.5	37.0
Pulse rate at end of work (30 min) Tenn., Sept.	93.2	88.7	90.0	102.7
Pulse rate at end of work (9 min) Mo., Oct.-Nov.	99.5	92.7	91.0	97.2
Pulse rate 5 min. after work, Tenn., July-Aug.	86.4	85.6	80.3	94.6
Pulse rate 5 min. after work, Tenn., Sept.	72.7	56.8	73.8	80
Pulse rate 5 min. after work, Mo., Oct.-Nov.	83.0	78.5	77.5	74.4
Percent increase in pulse due to work, Tenn., July-Aug.	61.6	58.2	60.0	67.0
Percent increase in pulse due to work, Tenn., Sept.	222	211	214	233
Percent increase in pulse due to work, Mo., Oct.-Nov.	237	220	216	221
Percent recovery of pulse in 5 min., Tenn., July-Aug.	247	247	226	256
Percent recovery of pulse in 5 min., Tenn., Oct.	40	49	34	39
Percent recovery of pulse in 5 min., Mo., Oct.-Nov.	29	30	28	41
Percent recovery in 20 min. after work, Tenn., July-Aug.	48	54	45	48
Percent recovery in 20 min. after work, Tenn., Oct.	86	83	76	74
Respiration rate at rest per min., Tenn., July-Aug.	64	68	60	86
Respiration rate at rest per min., Tenn., Sept.	16	14	18	14
Respiration rate at rest per min., Mo., Oct.-Nov.	22	24	24	22
Respiration rate at end of work per min., Tenn., July-Aug.	17.8	19.0	17.0	15.0
Respiration rate at end of work per min., Tenn., Sept.	74.2	61.8	86.3	76.2
Respiration rate at end of work per min., Mo., Oct.-Nov.	106.2	82.1	72.8	62.5
Respiration rate 5 min. after work, Tenn., July-Aug.	52.9	71.4	53.6	48.6
Respiration rate 5 min. after work, Tenn., Sept.	53.8	36.7	72.8	69.3
Respiration rate 5 min. after work, Mo., Oct.-Nov.	93.2	73.0	71.5	64.2
Percent increase in respiration due to work, Tenn., July-Aug.	40.0	30.1	33.9	24.7
Percent increase in respiration due to work, Tenn., Sept.	464	441	421	544
Percent increase in respiration due to work, Mo., Oct.-Nov.	663	586	520	446
Percent recovery in respiration in 5 min., Tenn., July-Aug.	298	360	315	324
Percent recovery in respiration in 5 min., Tenn., Sept.	35	53	20	11
Percent recovery in respiration in 5 min., Mo., Oct.-Nov.	15	16	19	—2
Percent recovery in respiration in 20 min., Tenn., July-Aug.	37	80	54	71
Percent recovery in respiration in 20 min., Tenn., Sept.	72	87	60	51
	47	46	46	35

Table 12—Comparative Ratings of Four Mules on Tennessee July-August and September Tests and on October and November Tests at Missouri.

(Ratings based entirely upon rise and recovery in pulse and respiration during and after work.)

	Natacha 42			Gypsy 42			Gordon 41			Grace 42		
	Tenn	Tenn	Mo	Tenn	Tenn	Mo	Tenn	Tenn	Mo	Tenn	Tenn	Mo
	July & Aug	Sept	Oct & Nov	July & Aug	Sept	Oct & Nov	July & Aug	Sept	Oct & Nov	July & Aug	Sept	Oct & Nov
Pulse rate immediately after work	3	4	3	1	2	2	2	1	1	4	3	4
Pulse rate 5 min. after work	2	4	3	1	3	1	3	2	2	4	1	4
Percent recovery in pulse rate in 5 min.	2	2	2.5	1	1	1	3	4	4	4	4	2.5
Respiration rate at end of work	2	4	2	1	3	4	4	2	3	3	1	1
Respiration rate 5 min. after work	2	4	4	1	3	2	4	2	3	3	1	1
Percent recovery in respiration in 5 min.	2	3	4	1	2	1	3	1	3	4	4	2
Average rating	2	4	4	1	2 or 3	1	3 or 4	1	3	4 or 3	2 or 3	2

Table 13—Air Per Respiration, Ventilation Rate, and Oxygen Pulse as Determined from the Missouri Trials on the Four Tennessee Mules.

	9 Natacha 42	9 Gypsy 42	9 Gordon 41	9 Grace 42
Pulmonary ventilation rate — cu. ft. min. during 8th to 9th minute of work	17.4	22.9	20.0	22.7
Pulmonary ventilation rate for above period per 100 lbs. live weight (cu. ft.)	2.06	2.51	2.04	2.00
Air per respiration at rest cu. ft. min.	.116	.121	.139	.196
Air per respiration during 8th to 9th minute of work cu. ft. per min.	.329	.321	.373	.467
Oxygen pulse kg. of body wt. ce <sup>1</sup>	.508	.555	.551	.488

<sup>1</sup>Oxygen pulse — amount of oxygen consumed by body from the blood of one systolic discharge of the heart. A high oxygen pulse is desirable.

Table 14—Summary of Mule Tests by Mares.

	Grace	Dinah	Duchess	Miss Evans	Mabel	Carlaetta	Suetta	Pet
<b>Draft Mares</b>								
Number of different mules tested	6	1	1	1	3	4	4	1
Number of individual tests made	77 <sup>1</sup>	17	8 <sup>2</sup>	8	33 <sup>1</sup>	43 <sup>1</sup>	60 <sup>3</sup>	69 <sup>1</sup>
Av. age of mules when tested yr.	3.5	3	4	3	3.25	3.2	4.1	5.3
Av. temperature at time of tests	72	68	48	69	61	70	64	69
Av. humidity at time of test	65	63	66	67	65	56	56	59
Av. time in covering course of 1.23 miles (min.)	30	30	30	31	30	30	30	30
<b>Pulse:</b>								
Av. pulse at rest (min.)	41	40	38	46	41	41	39	39
Av. pulse at end of trial	97	100	91	121	100	113	105	114
Av. percent recovery in 5 min.	39	42	53	36	41	42	45	44
Av. percent recovery in 20 min.	79	73	85	72	85	78	85	84
Av. percent recovery in 30 min.	82	—	92	84	—	—	92	—
<b>Respiration:</b>								
Av. respiration at rest (min.)	17	20	18	18	15	17	19	20
Av. respiration at end of trial	78	61	73	89	80	84	76	103
Av. percent increase during work	359	205	305	394	433	394	200	415
Av. percent recovery in 5 min.	14	0	45	17	25	9	32	44
Av. percent recovery in 20 min.	52	56	80	58	74	57	79	89
Av. percent recovery in 30 min.	72	—	89	72	—	—	89	—
							<b>Av. Draft Bred</b>	<b>Av. Hot-B.</b>
<b>Hot-blooded Mares</b>	<b>Fashion</b>	<b>Blossom</b>	<b>Gordon</b>	<b>Gypsy</b>	<b>Natacha</b>	<b>Cherry</b>		
Number of different mules tested	1	2	4	6	2	1		
Number of individual tests made			49	68 <sup>1</sup>	27	7 <sup>2</sup>		
Av. age of mules when tested yr.	4	3.3	4	4.6	3.3	4	3.5	3.8
Av. temperature at time of tests	84	76	79	73	81	48	65	73
Av. humidity at time of test	61	54	66	56	66	55	62	60
Av. time in covering course of 1.23 miles (min.)	30	30	29	29	29	30	30.1	29.5
<b>Pulse:</b>								
Av. pulse at rest (min.)	42	45	42	42	41	46	41	43
Av. pulse at end of trial	90	94	94	92	95	117	105	97
Av. percent recovery in 5 min.	50	43	38	44	38	48	43	44
Av. percent recovery in 20 min.	79	73	75	82	76	83	80	78
Av. percent recovery in 30 min.	—	—	79	—	81	93	87	84
<b>Respiration:</b>								
Av. respiration at rest (min.)	20	17	16	19	19	20	18	18
Av. respiration at end of trial	78	71	78	77	90	85	80	80
Av. percent increase during work	290	318	387	305	374	325	344	344
Av. percent recovery in 5 min.	22	24	26	26	20	38	23	26
Av. percent recovery in 20 min.	52	57	58	62	54	71	68	59
Av. percent recovery in 30 min.	66	—	68	69	69	82	79	71

<sup>1</sup>Includes 8 trials made Feb. 1950

<sup>2</sup>All trials made Feb. 1950

<sup>3</sup>Includes 24 trials made Feb. 1950

Table 15—Variations in Results Obtained in Three Series of Tests with Mabel 43 and Dinah 43.

(When these tests were made, both mules were 3 years old; they weighed 1,140 pounds, were well-broken and were worked regularly at farm work.)

Date of Tests	No. Tests	Average Temperature	Average Humidity	% Load	Average Time	At Rest	PULSE					% Recovery 20 Min.	RESPIRATION					% Recovery 20 Min.	
							At End of Pull	—5 Min.	—10 Min.	—15 Min.	—20 Min.		At Rest	At End of Pull	—5 Min.	—10 Min.	—15 Min.		—20 Min.
<b>Mabel 43</b>																			
Sept. '46	9	72	65	25	30'	40	101.5	76.0	62.8	55.8	50.6	82.7	14	87.7	85.8	73.8	60.0	46.4	56.0
Oct. '46	4	64	65	25	30'	40	90.5	66.0	55.5	46.0	42.5	91.1	14	48.0	31.5	33.5	25.5	21.0	79.4
Nov. '46	4	61	54	25	29.1'	40	86.0	71.0	63.0	55.5	50.5	77.1	14	67.5	59.0	42.5	33.0	27.5	74.8
Weighted Av.		67.7	62.2		29.8'		95.3	72.5	61.2	53.4	48.7	84.3		73.6	66.7	56.9	45.3	36.0	63.1
<b>Dinah 43</b>																			
Sept. '46	9	73	64	25	30'	40	102.0	76.0	65.5	58.8	56.2	73.9	20	72.6	60.9	51.8	45.5	37.7	66.3
Oct. '44	4	64	62	25	30'	40	98.0	70.5	62.5	57.5	53.5	76.7	20	49.5	47.0	34.5	31.0	29.0	69.5
Nov. '46	4	59	59	25	28'	40	96.5	77.0	67.0	62.5	59.0	66.4	20	66.0	77.0	68.0	55.0	45.0	45.7
Weighted Av.		67.0	62.7		29.4'		99.7	74.9	65.2	59.4	56.2	72.9		60.3	61.4	51.4	44.3	37.4	56.8

A summary of all the Tennessee tests by mares is given in Table 14. This summary shows rather marked differences between mares but an attempt to interpret them is difficult because of the condition and training of the mules, time the tests were run, weather, humidity, etc. Though this is not shown in the table, recovery of pulse and respiration was much quicker or more complete in the February tests than in the tests run in the summer and fall. It also appears that there are greater variations in the rise and recovery of respiration than in pulse. Or in the simple language of the horse-men, they differed in their "wind." In the eyes of the horse-men, the kind of "wind" an animal has is the most important indicator of a work animal's ability. The mule or horse which pants all day in the shade never has been considered desirable. "Wind" no doubt is associated with the heart in getting a large volume of blood to the lungs in order to pick up the oxygen from the inhaled air. This tabulation (Table 14) shows that the draft mules recovered more completely in their respiration than the hot-blooded mules. This may have been due to the fact that the light mules were not as well conditioned as the draft mules since more of the draft mules tested had been worked more regularly on the farm. At any rate the hot-blooded mules did not show any superiority over the draft mules in these tests.

Table 15 and Figure 16 present results secured with Dinah 43 and Mabel 43, to illustrate some of the complications and problems experienced in making these tests. These two mules were the same age, were worked regularly together as a team at farm work, weighed the same and appeared to be in good physical condition. They were tested the same number of times on the same days, yet in nine trials in September, 1946, four trials in October, 1946, and four trials in November, 1946, they gave different performances particularly in regard to respiration. Each mule had off days when he did not do well. Throughout the trials it was observed that the mules varied considerably in some of their trials. Sometimes a mule would go out and do the job and come in cool and unconcerned; at other times he would come in dripping wet and in an excited and exhausted condition.

## CONCLUSIONS

### Jack Breeding

Since interest in mules has dropped to a low point it may appear to many that this report is of limited value. Only time will tell whether or not the mule gains or maintains any degree of importance in this country. The results of the tests are reported as a matter of record to be available should interest in mules revive.

In a breeding project as short lived as this one, no very definite conclusions are possible. However, the following remarks seem justified.

1. With the heterogenous foundation stock secured, great variation was observed in the offspring from the same or different matings. Several very desirable typed jacks and jennets as well as some freaks, or off type animals, were produced (Figures 17 and 18). Since the same situation was found in the mule phase also, it is evident that a breeding program for the production of jackstock, which would produce good uniform mules, is still incomplete. It would probably take many years to determine such a breeding program. It seemed that some progress was made during the 13 years the project was in effect, particularly in uniformity of the stock, including their feet and legs.

2. Several overshot or undershot jaws were observed in several lines of the jackstock. All animals in which this defect was noticeable were discarded. It has been observed that parrot mouthed jacks have produced parrot mouthed mules, some of which were not able to feed sufficiently well to stay in good condition (Figure 19).

3. Some eye trouble and blindness was experienced. U. T. Logan had a cataract on one eye when bought at 18 years of age and was practically blind when disposed of three years later. His half brother, Rowdy, used in

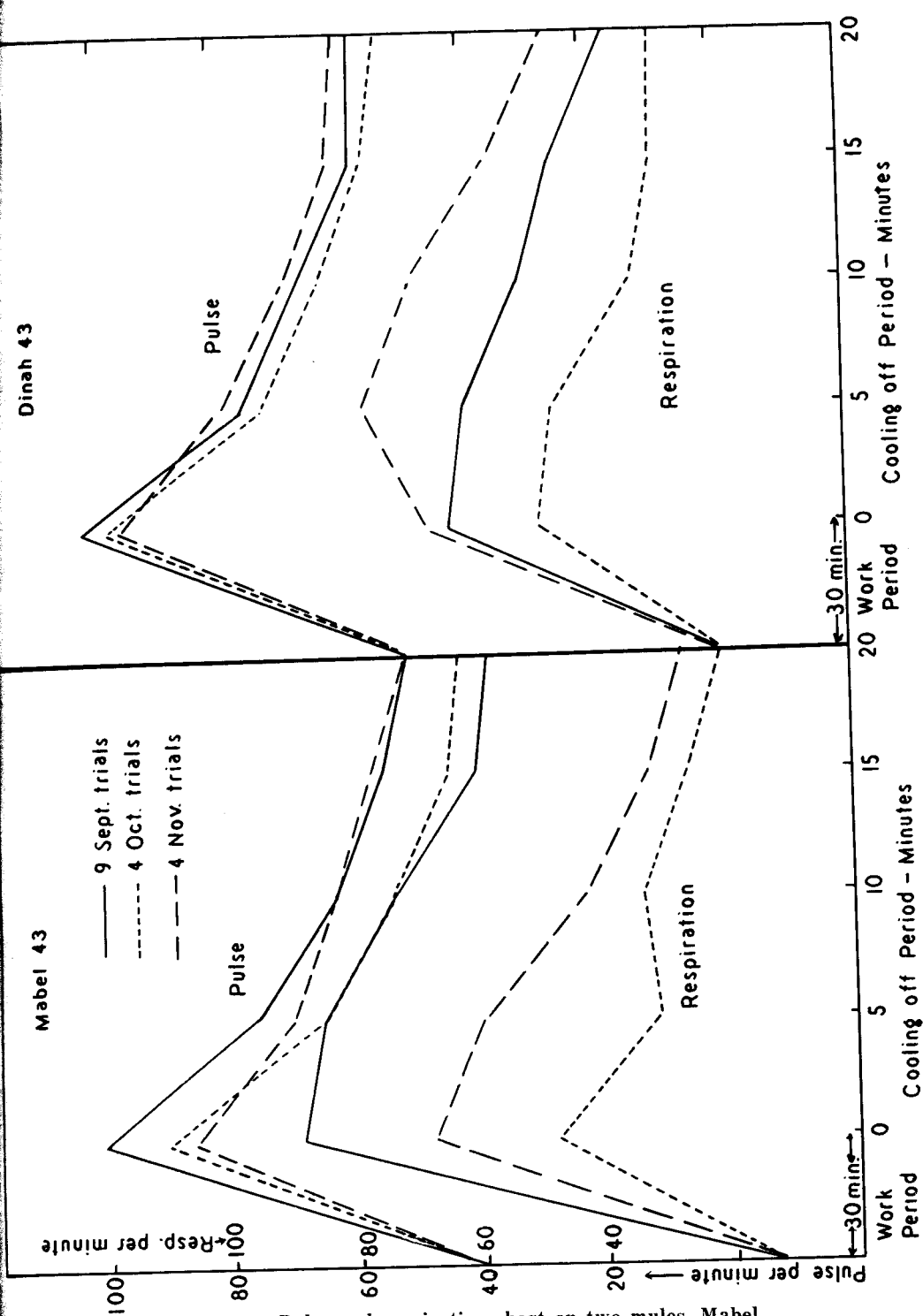


Figure 16.—Pulse and respiration chart on two mules, Mabel 43, and Dinah 43, showing their rates of recovery after the series of performance tests.



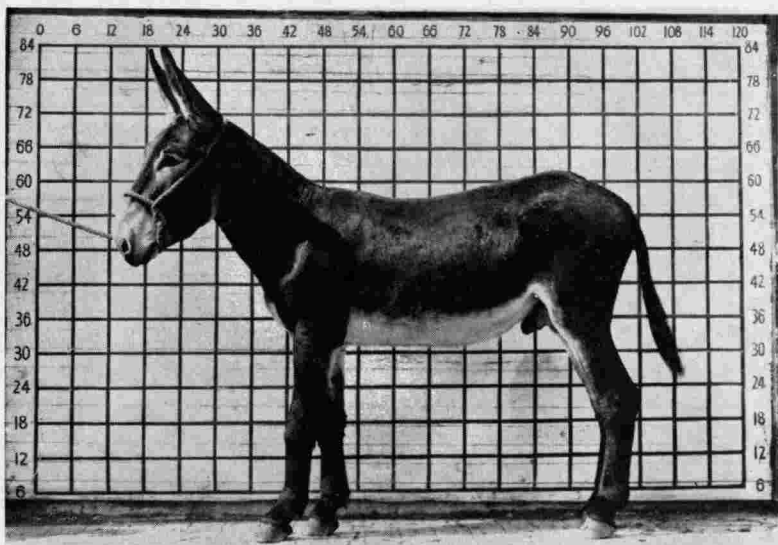


Figure 17.—U.T. Derry, at 27 months of age, was one of the best jacks produced in the late years of the investigations reported in this bulletin. Sired by U.T. Monarch Again out of U.T. Derryberry, he was a grandson of Miss Derryberry through both his sire and dam. This jack had good feet, was sturdy of bone, and was reasonably straight of leg.

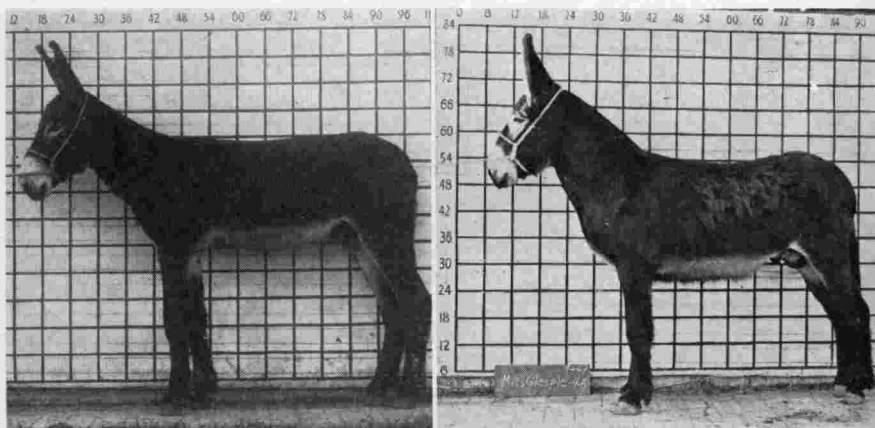


Figure 18.—Two extremes in type. Left is a 15-hand, 600-pound jack at 19 months of age. He is a son of U.T. Janis. At right is a 3-year-old 12½-hand, 800 pound jack, a son of Miss Gillespie. The little jack (right) had an excellent set of feet and legs and good body proportions, but he failed to grow out as expected.



Figure 19.—Here is an example of a badly overshot lower jaw. This foal was weak and could not nurse. This deformity, either in the overshot or undershot lower jaw, was noted in several of the jackstock offspring. The deformity appeared to be hereditary.

Dr. Gaston's herd, was blind for years and two of his sons—U. T. Logan Again and U. T. Blaze—went blind sometime from three to eight years of age. Some of this trouble was diagnosed as periodic ophthalmia, supposedly due to faulty nutrition. U. T. Logan Again, however, had had good legume hay and had access to green grass practically every day of his life. Blindness in jacks has been quite common but a blind mule is a rarity even in the day when there were many blind or weak-eyed mares.

4. Jack sores caused very little trouble in this project. Occasionally, eruptions on the cannons or hocks would break and run some, but these breaks always healed when the grain rations were reduced. There was not a single chronic case of jack sores during the project except on one of the foundation jennets (Figure 20). A light grain ration, grass and exercise kept jacks and their legs in good physical trim.

#### Mule Production

1. With good technique in breeding and management it was possible to maintain a high degree of efficiency in mule production, much higher than with the jackstock where the same technique was used.

2. The mules from the hot-blooded mares, as judged by present day standards, lacked the temperament, weight, and conformation to make them as valuable for market or draft purposes as mules from the draft mares.

3. If draft mares disappear, it should be possible to produce mules from the hot-blooded mares which will be fairly satisfactory for light or general purpose work on farms where work stock is wanted.

#### Mule Testing

1. With the dynamometer and methods used it was possible to detect differences, which appeared to be significant, in mules in their pulse and respiration reactions.

2. Sufficient records are presented herein to show the nature, difficulties and results in this major initial attempt to test a mule's ability to do work. Such records need to be checked or correlated with more comprehensive tests such as those conducted at the Missouri Experiment Station before significant indices can be determined.

3. The mule testing program was not carried through a sufficient span of years or developed sufficiently to have found lines of jacks, jennets, or mares which transmitted superior qualities to their offspring intended for draft purposes.

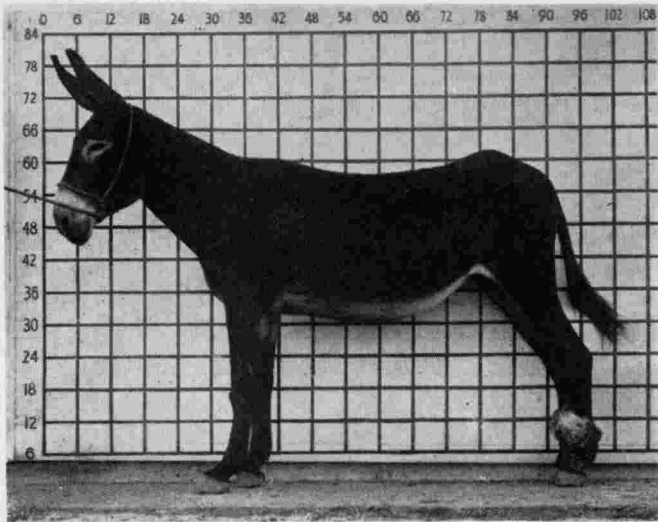


Figure 20.—“Jack sores” are somewhat common in jackstock, appearing on either the fore or hind legs at various places. The typical case shown here was on Miss Sory, one of the foundation jennets. She eventually had to be destroyed because of this sore. Exercise, early attention, and light grain feeding usually eliminated such trouble.

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# PART 2

## Sperm Storage and Artificial Insemination Studies

### INTRODUCTION

One of the objectives of the jackstock research project as approved in 1937 was "to study the physiology of reproduction and the use of artificial insemination in horses and jackstock." Artificial insemination was used in breeding mares and jennets in the Tennessee Station herd during the early years of the breeding program. Intensive studies of the problems of field use of artificial insemination were initiated in 1948.

No attempt has been made to review all of the literature pertaining to jack or stallion sperm physiology and the use of these sperm in artificial insemination. Anderson (1945) reviewed the literature in that field. Only selected references to the work of others will be made herein.

In a review of some of the problems of artificial insemination in horse and mule production, Berliner (1941) briefly described some of the differences in physiology of ruminant and horsestock sperm. The sperm have both anaerobic metabolism, using carbohydrates as sources of energy resulting in accumulation of lactic acid in the semen; and an aerobic metabolism with  $\text{CO}_2$  as the end product. In bull semen this metabolism is very rapid due to its high sperm concentration and content of glucose and glycogen; as a consequence, the semen rapidly becomes acid and as acidity increases, metabolism and motility of the sperm are slowed down. Thus, the sperm go into a reversible resting stage called anabiosis. Anabiosis also can be brought about through cooling. On rewarming and addition of buffers, the sperm again become mobile. Of course, there is a limit to this process—after prolonged storage the sperm die through aging. This anabiosis of bull sperm, that is so favorable for storage, cannot be produced as effectively in the semen of jacks and stallions. Their semen contains far less sugars; their sperm concentration is considerably lower (100-500 million in jack vs. 800 million to 2 billion in bull semen). Jack semen contains large amounts of seminal fluid coming from the accessory glands. This large amount of fluid accounts for the low sperm concentration and it influences the chemical picture of the semen. These fluids contain a high concentration of bases giving the semen a neutral or even alkaline reaction. For this reason, the quantity of acids produced is too low and rate of production is too slow to cause anabiosis. It has been observed that stallion semen on standing becomes alkaline due to the production of ammonia, as a by-product of protein metabolism, and this counteracts the onset of anabiosis. As a consequence, stallion and jack sperm do not go into a state of rest, but continue to swim around until exhausted. Further, the electrolytes affect the electric charge carried by the individual sperm, stimulating motility, but at the same time stimulating destruction of the protective lipoid capsule of the sperm head. There may be further detrimental effects due to the large amount of electrolytes in the accessory fluids.

Various methods have been tried to overcome these unfavorable conditions. In general, all methods have been based on changing the environmental medium of the sperm by adding certain ingredients that would make the seminal fluid less harmful and at the same time make the sperm more resistant to destruction by strengthening the protective capsule against the influence of the electrolytes. This led to the development of the artificial media now known as semen dilutors. The early dilutors were developed by Russian workers.

Milovanov (1934, 1936) suggested several dilutors for all species of farm animals that were made up according to the physiological and chemical specificity of the different semen types. In one series of stallion semen

dilutors, called TGL, Milovanov combined glucose with sodium-potassium tartrate (Rochelle Salt), and peptone.

Berliner (1942) compared various modifications of the TGL series and reported that a dilutor prepared by dissolving a ½-ounce gelatin capsule containing 5.75 grams glucose and 0.67 grams Rochelle salt in 100 cc water and adding one egg yolk, give satisfactory results for immediate use and for storage. Pregnancies were obtained with semen that was stored for 24 and 48 hours.

Bogart and Mayer (1950) recommended that the combination of egg yolk with an isotonic solution either of sucrose or of glucose be used in the dilution of stallion semen for optimum survival of sperm during storage.

The ultimate test of the efficacy of any method of prolonging sperm survival is the fertility record in mares inseminated with stored semen. Berliner *et al.* (1938) reported three mares were diagnosed as pregnant following insemination with diluted semen which had been stored for 6, 13, and 15 hours, respectively, prior to use. They also state that the six-hour old semen was transported by car to a mare approximately 85 miles from the Station. Walton (1938) reported obtaining pregnancies in three of six mares inseminated only once during heat with fresh semen, one of seven mares inseminated one time during heat with 24-hour old semen, three of seven mares inseminated more than once during heat with 24-hour old semen, and none in 20 mares inseminated only once during heat with 48-hour old semen.

McKenzie *et al.* (1939) obtained pregnancies in two mares inseminated with 20-hour old semen. In one case, the semen was collected at Beltsville, Maryland, and shipped to Miles City, Montana, to inseminate the mare. In both cases, the sperm were washed and centrifuged prior to storage of the sperm mass under oil. Immediately before use, the sperm were warmed to 38°C and diluted with the original supernatant fluid.

Berliner *et al.* (1940) reported the birth of two colts following insemination of the mares with 24-hour old semen. One of the mares was inseminated only one time during the heat period. However, manual palpation of the ovaries indicated that ovulation occurred within 12 hours of the time of insemination. The second mare was inseminated on two successive days with 24-hour old semen. Conception was reported in two other mares, both in foal heat on the same day, that were inseminated with semen which had been stored for 24 hours, and then inseminated again on the two following days with fractions from the original ejaculate.

Chang (1943) obtained pregnancies in two of 14 mares inseminated with 24-hour old semen. No mention was made of the stage of the heat period at the time of insemination for any of these mares.

The fertility results with stored semen were obtained with a variety of dilutors and with the sperm processed in a number of different ways. In general, the tests were made under laboratory conditions, i.e., the mares were maintained under close supervision prior to and after insemination.

In the studies reported here, the emphasis was upon finding or developing a dilutor which would prolong sperm survival to the point that successive inseminations could be made at 24 hour intervals for two or more days from the same ejaculate.

## EXPERIMENTAL PROCEDURE AND RESULTS

The semen used in this study was obtained from two jacks. The animals were run in separate outside grass paddocks as much as weather permitted. They were fed a ration consisting of one part shelled corn and two parts oats with free access to grass hay while in the barn.

Weekly semen collections, using an artificial vagina (Missouri type) were obtained from each jack. After the semen had been obtained, the bottle with its protective cover was further wrapped with paper towels to prevent marked temperature changes during transportation to the laboratory. The elapsed time between collection and dilution was 30 to 45 minutes.

The volume of the ejaculate was measured in a graduated cylinder and

the sperm concentration was determined by counting in a hemocytometer. Immediately after arrival at the laboratory, the percentage of live sperm was estimated, using the Fast Green-Eosin staining technique (Mayer *et al.* 1951). Two hundred sperm were counted in determining the percentage of live sperm. Determinations of pH values were made with a model G. Beckman potentiometer with a glass electrode. All pH readings were recorded to the nearest tenth.

All dilutors were freshly prepared just prior to the time of semen collection and allowed to stand at room temperature until used. Fresh eggs were obtained from the University Poultry Farm. In no case were the eggs more than 24 hours old at the time of use. After separation of the albumin and yolk, the yolk membrane was ruptured with a glass stirring rod and the yolk collected in a small beaker. The yolk and glucose solutions were then mixed in the desired proportions in a graduated cylinder.

Initial sperm motility was determined immediately upon arrival at the laboratory and prior to dilution. Semen dilutions were made in a graduated cylinder and mixing was accomplished by stirring with a glass rod. A dilution rate of 1:1 (one part of semen to one part of dilutor) was used in all cases. Stopped glass tubes containing 10 ml. of diluted semen were placed in a beaker of tap water at room temperature (approximately 26°C). The beaker containing the samples was then placed in a refrigerator with a temperature of about 8°C. The cooling of the water slowed the cooling rate of the semen and presumably reduced the chances of temperature shock to the sperm during cooling.

At 24 hour intervals for 240 hours, or until the sperm showed no motility, each semen sample was examined microscopically to determine the degree of sperm motility. Motility was determined by placing a drop of semen on a clean glass slide, covering the drop with a cover slip, and examining under the low power objective (100 x magnification). To avoid variations in temperature, the slides were held in a warming stage at 37°C. Motility was rated on a numerical scale from 0 to 5, 0 denoting no motility and 5 maximum motility.

The data on sperm motility at the end of 96 hours of storage was subjected to statistical analysis by the techniques of analysis of variance and the "t" test for paired differences (Snedecor, 1946). The reasoning behind the choice of this interval was that any semen treatment which would maintain a satisfactory degree of motility for 96 hours would permit several successive inseminations from the same shipment of semen.

The final test of the efficacy of any semen dilutor is conception in mares inseminated with semen diluted with that dilutor. Because of inadequate numbers of mares in the Experiment Station herd to test any single dilutor, an effort was made to enlist the cooperation of farmers in the Knoxville area. Mare owners within a 45-mile radius of the main Station, Knoxville, were contacted. Cooperators agreed to notify the inseminator when a mare was first observed in heat. Additionally, arrangements were made to ship semen to the Middle Tennessee Experiment Sub-Station (MTES) Columbia, to be used for insemination of mares. Following notification that a mare was in heat, the semen was prepared for transportation by wrapping glass tubes, containing diluted semen, in paper towels. The tubes were then placed in a thermos bottle containing cracked ice. Upon arrival at the farm, two one-ounce gelatin capsules were filled with semen and deposited in the uterus of the mare. Mares were inseminated only one time in a heat period.

For shipment to MTES the semen was collected between 8 and 10 A. M. It was immediately diluted and cooled to refrigerator temperature as previously described. Tin cans, previously filled with water, sealed and frozen, were wrapped in several layers of paper towels. The tubes containing the semen were then individually wrapped in paper towels and taped to the sides of the cans of ice. The cans and tubes were further wrapped in paper towels and placed in double-walled cardboard shipping boxes. The semen was shipped via airmail from Knoxville to Nashville and thence by train

to Columbia, a distance of approximately 250 miles. The semen was used to inseminate the mares shortly after the semen arrived. It was approximately 24-hours old at the time of use.

## RESULTS

The average volume (73.3 ml) of semen and initial percentage of live sperm (86 percent) as shown in Table 16, are in agreement with data reported by other workers. However, the average concentration (492,391/mm<sup>3</sup>) is somewhat higher than in other reports.

Table 16—Volume of Jack Semen, Sperm Concentration and Percentage of Live Sperm

	No. of Ejaculates	Mean	Range
Volume (ml)	123	73.3	40-135
Concentration (1000's per mm <sup>3</sup> )	133	492.4	140-980
Initial per cent live sperm	100	86.0	55-94

Table 17—pH of Jack Semen

No. of Ejaculates	Age of Semen (hrs.)	Average pH	Range
Undiluted Semen			
14	1	7.20	7.00-7.85
15	24	7.35	6.95-7.90
11	48	7.45	7.00-7.90
13	72	7.42	7.00-7.85
Diluted Semen*			
10	1	6.71	6.65-6.80
12	24	6.65	6.50-6.80
11	48	6.62	6.40-6.80
6	72	6.56	6.40-6.75

\*Semen diluted with egg yolk-glucose dilutor in a 1:1 ratio

The pH of diluted semen decreased slightly during the first three days of storage while the pH of undiluted semen increased slightly during the same interval (Table 17). This is in agreement with Davis and Cole (1939) that little, if any, lactic acid is produced in stallion semen. These results confirm the suggestion (Bogart and Mayer, 1950) that a dilutor for jack semen need not contain a buffer for good storage since the diluted samples contained no buffer. All diluted samples maintained motility for several days while the undiluted samples showed no motility at the end of the first 24 hours of storage.

### The Effect of Egg Yolk and Various Amounts of Glucose on Sperm Motility

Ten ejaculates were used in comparing five different dilutors with an undiluted control. The components of the five dilutors were as follows: (1) five percent glucose solution; (2) one part of egg yolk to one part of five percent glucose solution; (3) one part of egg yolk to four parts of five percent glucose solution; (4) one part of egg yolk to five parts of five percent glucose solution; and (5) one part of egg yolk to ten parts of five percent glucose solution. Fresh semen was diluted with an equal volume of each of these dilutors.

The data show that dilutors are necessary for maintaining the motility of sperm since the undiluted samples showed no motility the day after collection (Figure 21). In undiluted samples examined approximately six hours after collection only a few weakly motile sperm were observed. Motility must be maintained if any degree of success is to be expected in producing pregnancy with stored semen. It was quite obvious that the dilutors containing egg yolk were superior to those without egg yolk (Figure 21 and Table 18). One part of egg yolk to four, five, or ten parts of the glucose.

#### LEGEND

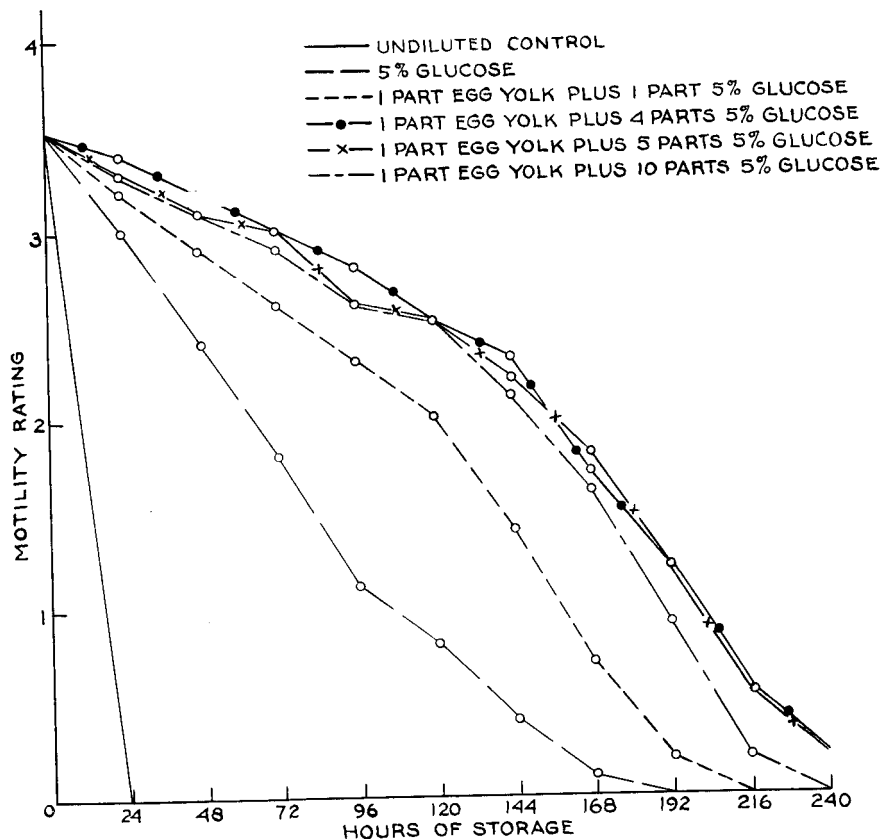


Figure 21.—The effect of egg yolk and various amounts of glucose on sperm motility.

Table 18—Analysis of Variance of Motility Ratings of 96 Hour Old Sperm Diluted with Various Levels of Egg Yolk-Glucose.

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Squares
Total	59	71.40	
Between subclasses	5	62.00	12.40**
Undiluted vs. diluted	1	43.32	43.32**
Diluted with egg yolk vs. diluted without egg yolk	1	17.40	17.40**
Between levels of egg yolk-glucose	3	1.28	0.43
Within subclasses	54	9.40	0.17

\*\*Highly significant.  $P < .01$ .



solution gave better results in maintaining motility than equal parts of egg yolk and glucose solution, however, the differences were not significant. Due to the slight advantage shown by the dilutor containing four parts of the glucose solution, it (one part of egg yolk to four parts of five percent glucose solution in a 1:1 ratio with semen) was used as the control dilutor in subsequent studies.

### The Effect of Sodium Potassium Tartrate-Egg Yolk-Glucose and Glucose-Egg Yolk-Phosphate on Sperm Motility

Thirteen ejaculates were used in this study. The dilutors were prepared as follows: (1) control: One part of egg yolk to four parts of five percent glucose solution; (2) TGL: 5:76 g. of glucose plus 0.67 g. of Rochelle Salt in a one-half ounce gelatin capsule dissolved in 100 ml. of distilled water with one egg yolk added (Berliner, 1942); (3) GYP: One part of GYP (0.793 g. of  $\text{Na}_2\text{HPO}_4$ , and 5 g. of glucose dissolved in 100 ml. of distilled water) plus four parts of egg yolk (Berliner, 1942). Fresh semen was diluted with an equal volume of these dilutors.

Sperm motility was not maintained as satisfactorily in either TGL or GYP as in the control dilutor (Figure 22). The GYP dilutor was less satisfactory than the TGL dilutor. All differences were highly significant (Table 19). However, it was difficult to see the individual sperm in the TGL dilutor and the motility estimates were harder to make.

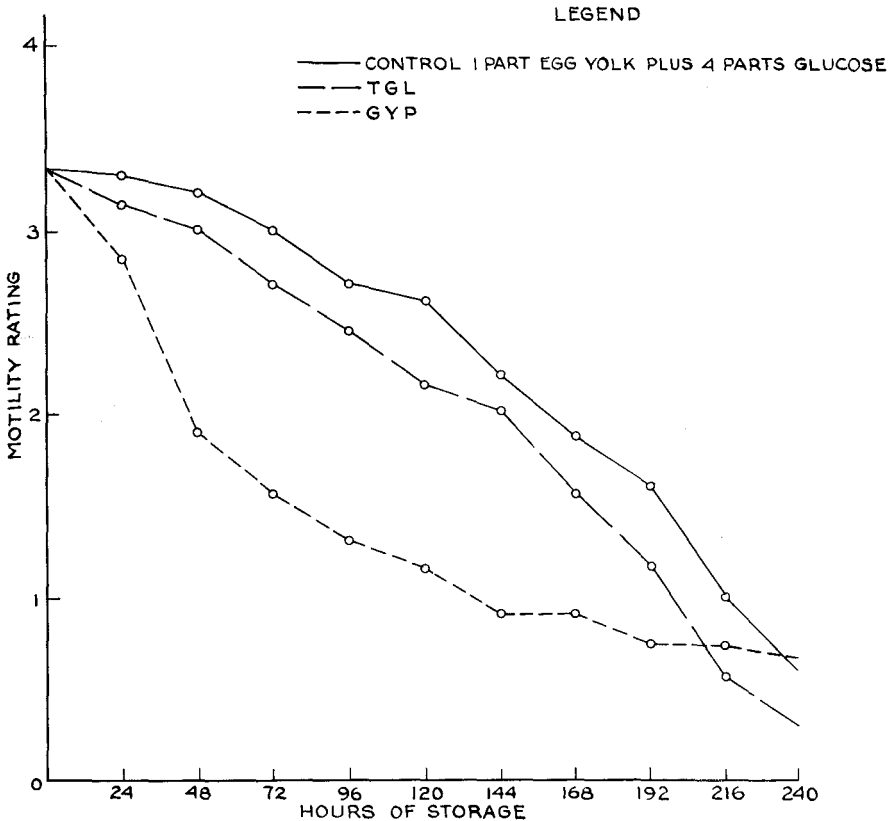


Figure 22.—The effect of TGL and GYP on sperm motility.

Table 19—Analysis of Variance of Motility Ratings of 96 Hour Old Sperm in Egg Yolk-Glucose, TGL and GYP Dilutors.

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Squares
Total	38	23.08	
Between subclasses	5	15.14	3.03**
Egg yolk-glucose vs. TGL and GYP	1	5.66	5.66**
Between jacks	1	0.79	0.79*
Interaction jack and (TGL, GYP)	1	0.02	0.02
Between TGL and GYP	1	8.65	8.65**
Interaction jack and TGL and GYP	1	0.02	0.02
Between ejaculates of same jack	11	4.25	0.39 <sup>p</sup>
Interaction (TGL, GYP) and ejaculate (error)	22	3.69	0.17

\*Significant,  $P < .05$ .

\*\*Highly significant,  $P < .01$ .

### The Effect of Various Amounts of Ascorbic Acid in Combination With Egg Glucose on Sperm Motility

Although Soliven and Gonzaga (1940) found no significant influence on stallion sperm longevity in storage when vitamins A, B, C, and D were added singly, combinations of these vitamins did favorably influence maintenance of motility. Consequently, it was decided to study the effect, on jack sperm motility during storage, of some vitamins known to be involved in cellular metabolism. The possibility was also considered that the vitamins might exert varying effects when used in different dilutors. Soliven and Gonzaga used dilutors different from the ones used in this study.

The role of ascorbic acid is not completely understood. The most obvious property of ascorbic acid is the reversible oxidation and reduction capacity. It also serves as a hydrogen-transport agent between unidentified metabolites and other carriers or molecular oxygen, by the way of two or more oxidase systems (Rosenberg, 1945).

Ten ejaculates were used in this study in comparing the effects of the addition of various amounts of ascorbic acid to diluted semen. Semen samples diluted with egg yolk-glucose containing one, three, five, and ten mg. of added ascorbic acid were compared with a diluted control containing no additional ascorbic acid.

The results showed no effects attributable to vitamin C during the first 96 hours of storage (Figure 23). Three and ten mg. apparently exerted some stimulatory effect during longer intervals of storage; however, the differences were not significant (Table 20). It was not clear why the samples containing five mg. had a lower motility through the major part of the storage period than did the samples containing three or ten milligrams.

### The Effect of Various Amounts of Thiamine in Combination with Egg Yolk-Glucose on Sperm Motility

Thiamine is closely associated with carbohydrate metabolism, apparently forming part of an enzyme system which is necessary for the decarboxylation of pyruvic acid, an intermediate compound of carbohydrate metabolism (Rosenberg, 1945).

Fifteen ejaculates were used in this study. Samples containing one, three, five, ten, and twenty mg. of added thiamine hydro-chloride were compared with a diluted control containing no additional thiamine.

All samples containing thiamine except the 20 mg. sample showed higher motility than the control from the 48th to the 120th hours of storage (Figure 24). The differences between all levels of thiamine were highly significant at 96 hours of storage; however, this was due to the low motility in the 20 mg. sample (Table 21). The "t" test for paired differences showed no level of thiamine to be significantly better than the control in maintaining motility.

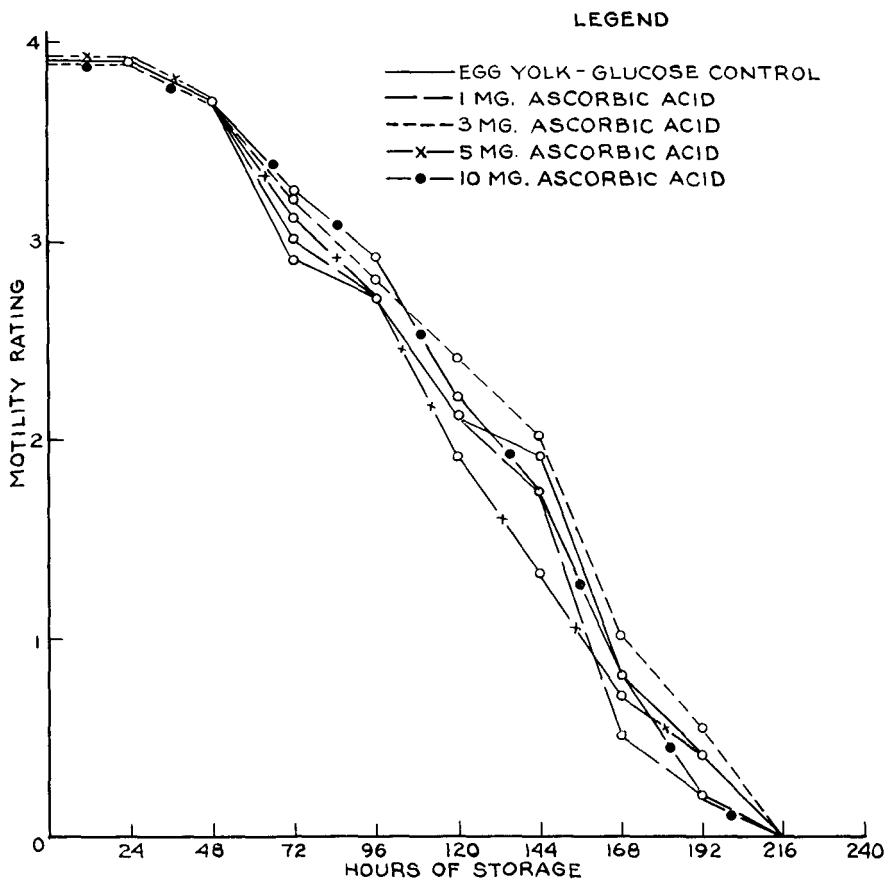


Figure 23.—The effect of various amounts of ascorbic acid in combination with egg yolk-glucose on sperm motility.

Table 20—Analysis of Variance of Motility Ratings of 96 Hour Old Sperm Diluted with Egg Yolk-Glucose and Varying Levels of Ascorbic Acid.

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Squares
Total	49	41.12	
Between subclasses	9	0.72	0.08
Control vs. treated	1	0.04	0.04
Between jacks	1	0.08	0.08
Interaction jack and treated	1	0.05	0.05
Between levels	3	0.28	0.09
Interaction jack and level	3	0.27	0.09
Between ejaculates of same jack	8	37.84	4.73**
Interaction treated and ejaculates (error)	32	2.56	0.08

\*\*Highly significant,  $P < .01$ .

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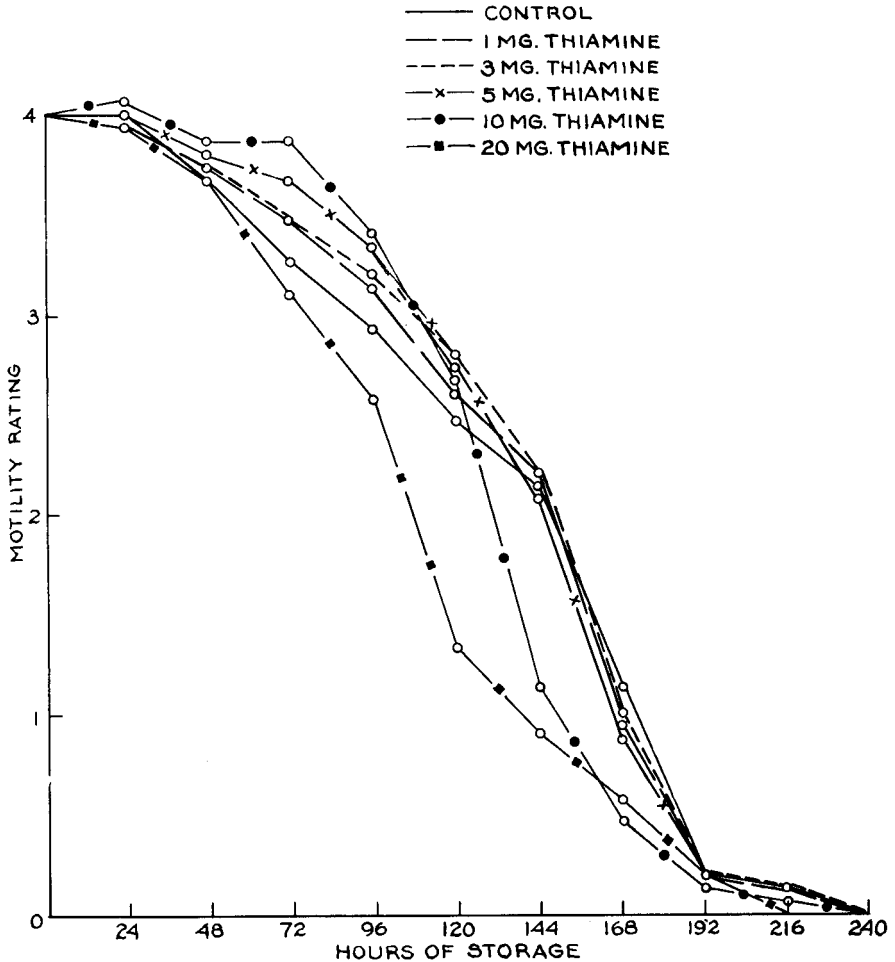


Figure 24.—The effect of various amounts of thiamine in combination with egg yolk-glucose on sperm motility.

Table 21—Analysis of Variance of Motility Ratings of 96 Hour Old Sperm Diluted with Egg Yolk-Glucose and Varying Levels of Thiamine.

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Squares
Total	89	58.10	
Between subclasses	11	10.06	0.91**
Control vs. treated	1	0.50	0.50
Between jacks	1	0.35	0.35
Interaction jack and treated	1	0.32	0.32
Between levels	4	6.01	1.50
Interaction jack and level	4	2.88	0.72
Between ejaculates of same jack	13	29.29	2.25**
Interaction treated and ejaculates (error)	65	18.75	0.29

\*\*Highly significant, P < .01.

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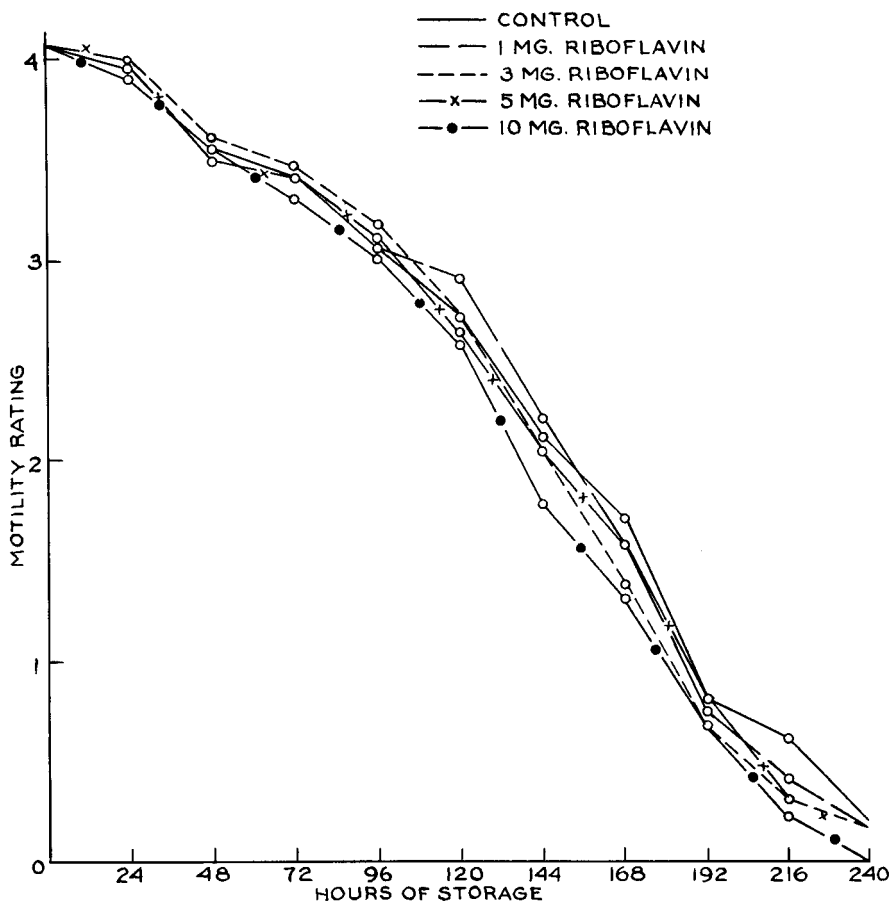


Figure 25.—The effect of various amounts of riboflavin in combination with egg yolk-glucose on sperm motility.

Table 22—Analysis of Variance of Motility Ratings of 96 Hour Old Sperm Diluted with Egg Yolk-Glucose and Varying Levels of Riboflavin.

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Squares
Total	99	38.51	
Between subclasses	9	5.61	0.62**
Control vs. treated	1	0.01	0.01
Between jacks	1	4.41	4.41**
Interaction jack and treated	1	0.49	0.49*
Between levels	3	0.25	0.08
Interaction jack and level	3	0.45	0.15
Between ejaculates of same jack	18	26.50	1.47**
Interaction treated and ejaculates (error)	72	6.40	0.09

\*Significant,  $P < .05$ .

\*\*Highly significant,  $P < .01$ .

### **The Effect of Various Amounts of Riboflavin in Combination with Egg-Yolk Glucose on Sperm Motility**

The fundamental action of riboflavin in living tissue is to take part in enzyme systems which regulate cellular oxidations (Rosenberg, 1945).

Twenty ejaculates were used in this study. Samples containing one, three, five, or ten mg. of added riboflavin were compared with a diluted control with no additional riboflavin. The data showed no beneficial effect on sperm motility due to the addition of riboflavin at any level to diluted semen (Figure 25 and Table 22). The riboflavin had a low solubility and the individual sperm were difficult to see in the samples containing five and ten mg. of added riboflavin.

### **The Effect of Various Amounts of Pyridoxine in Combination with Egg Yolk-Glucose on Sperm Motility**

Little is known about the function of pyridoxine, although research has shown that it plays a role in the metabolism of proteins (Rosenberg, 1945).

Twenty ejaculates were used in this study. Samples containing one, three, five, and ten mg. of added pyridoxine hydrochloride were compared with a diluted control with no additional pyridoxine.

Pyridoxine added to diluted semen had a stimulating effect on sperm motility during the first 72 hours of storage (Figure 26). The differences between all levels of pyridoxine were highly significant at 96 hours of storage; however, this was due to the lower motility in the 10 mg. sample (Table 23). The samples containing three or five mg. of additional pyridoxine showed a highly significant higher degree of motility than did the control sample at the end of 96 hours of storage ("t" test).

### **The Effect of Various Amounts of Niacin in Combination with Egg Yolk-Glucose on Sperm Motility**

Niacin plays an important role in carbohydrate metabolism. Coenzyme I and Coenzyme II, both of which are involved in carbohydrate metabolism, are derivatives of nicotinic acid amide, the amide of niacin (Harrow, 1943).

Five ejaculates were used in this study. Samples containing one, three, five, and ten mg. of added niacin were compared with a diluted control with no added niacin.

The results show that the addition of small amounts of niacin to diluted semen had a stimulating effect on sperm motility at the end of 96 hours of storage (Figure 27). Although the differences were not significant (Table 24), the fact that all samples containing added niacin showed a higher level of sperm motility than the control suggest that niacin was beneficial. It is possible that had more ejaculates been studied, a significant effect would have been found, especially at the five and ten mg. levels.

### **The Effect of Potassium Permanganate in Combination with Egg Yolk-Glucose on Sperm Motility**

Patrick (1949) and Good (1950) found that by using a final concentration of 1:25,000 potassium permanganate in jack semen diluted with egg yolk-glucose, the sperm motility was increased above that of semen diluted in the same manner, but without potassium permanganate. This study was undertaken to see if potassium permanganate, when added after 48 or 72 hours of the storage period, would maintain a higher level of sperm motility for a longer period than when the permanganate was added at the time of initial duration.

Fourteen ejaculates were used in this study. The samples were made up as follows: (1) a control sample diluted with egg yolk-glucose; (2) a sample of egg yolk-glucose diluted semen containing a final concentration of 1:25,000 potassium permanganate added at the time of initial dilution; (3) a sample of egg yolk-glucose diluted semen containing a final concentra-

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- CONTROL
- 1 MG. PYRIDOXINE
- - - 3 MG. PYRIDOXINE
- - - 5 MG. PYRIDOXINE
- 10 MG. PYRIDOXINE

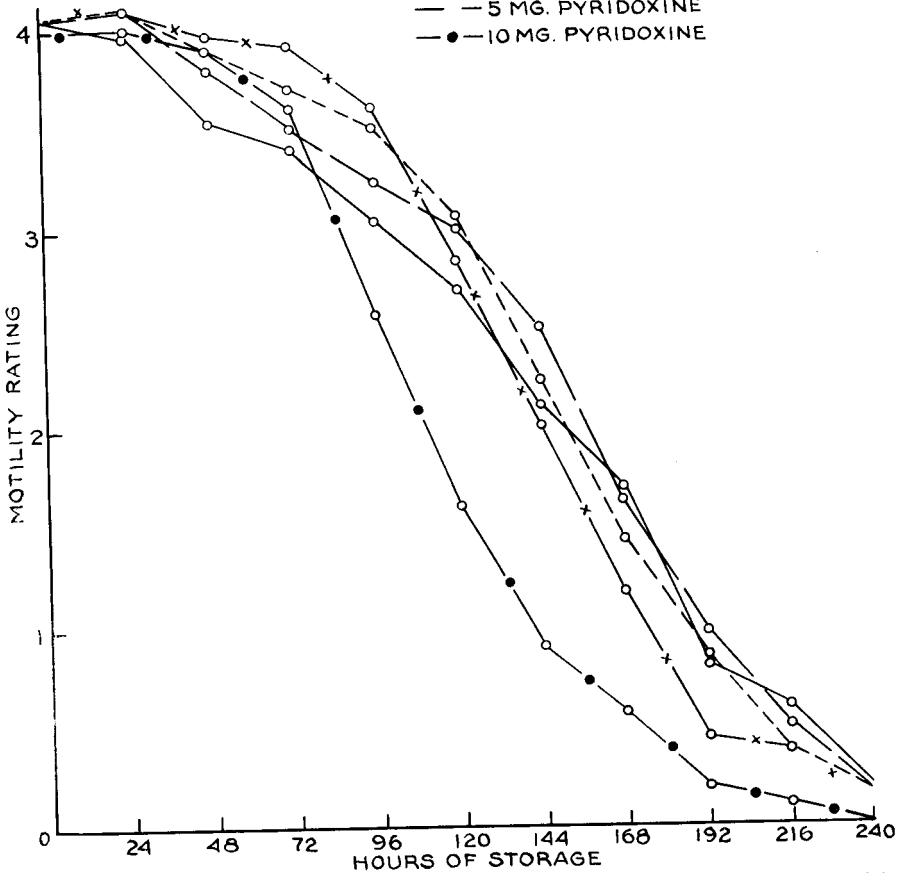


Figure 26.—The effect of various amounts of pyridoxine in combination with egg yolk-glucose on sperm motility.

Table 23—Analysis of Variance of Motility Ratings of 96 Hour Old Sperm Diluted with Egg Yolk-Glucose and Varying Levels of Pyridoxine.

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Squares
Total	99	61.39	
Between subclasses	9	20.29	2.25**
Control vs. treated	1	0.49	0.49
Between jacks	1	2.89	2.89*
Interaction jack and treated	1	0.81	0.81
Between levels	3	13.90	4.63**
Interaction jack and level	3	2.20	0.73
Between ejaculates of same jack	18	10.10	0.56
Interaction treated and ejaculates (error)	72	31.00	0.43

\*Significant,  $P < .05$ .

\*\*Highly significant,  $P < .01$ .

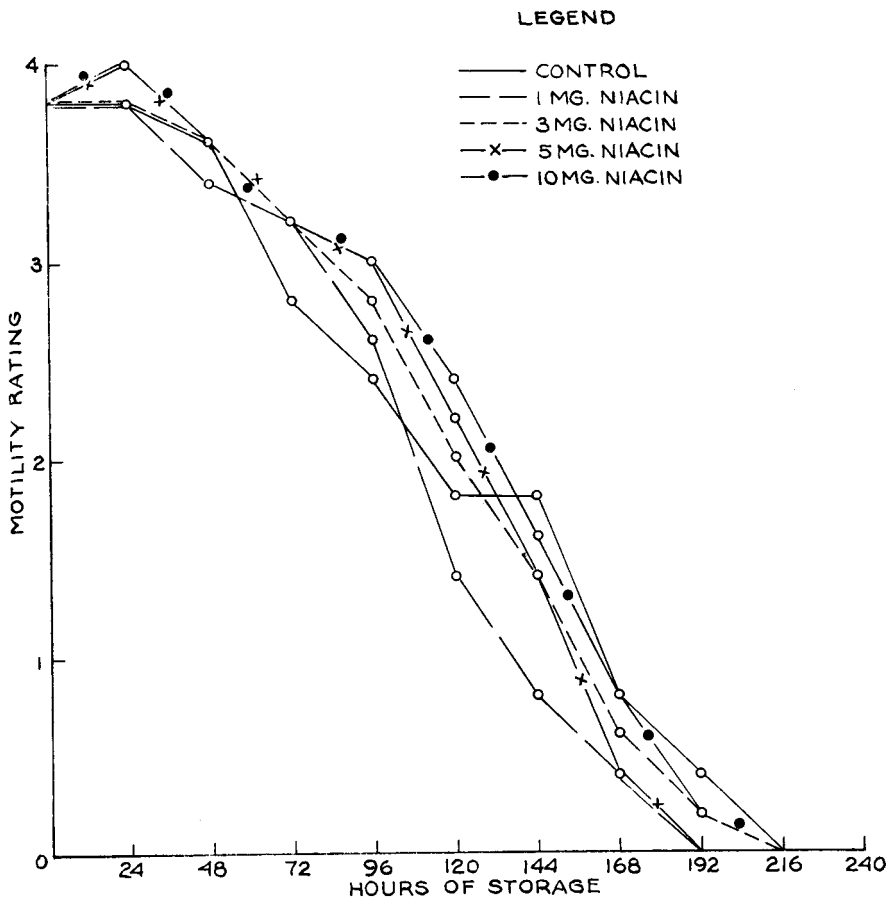


Figure 27.—The effect of various amounts of niacin in combination with egg yolk-glucose on sperm motility.

Table 24—Analysis of Variance of Motility Ratings of 96 Hour Old Sperm Diluted with Egg Yolk-Glucose and Varying Levels of Niacin.

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Squares
Total	24	26.56	
Between subclasses	4	1.36	0.34
Control vs. treated	1	0.81	0.81
Between levels	3	0.55	0.18
Within subclasses	20	25.20	1.26



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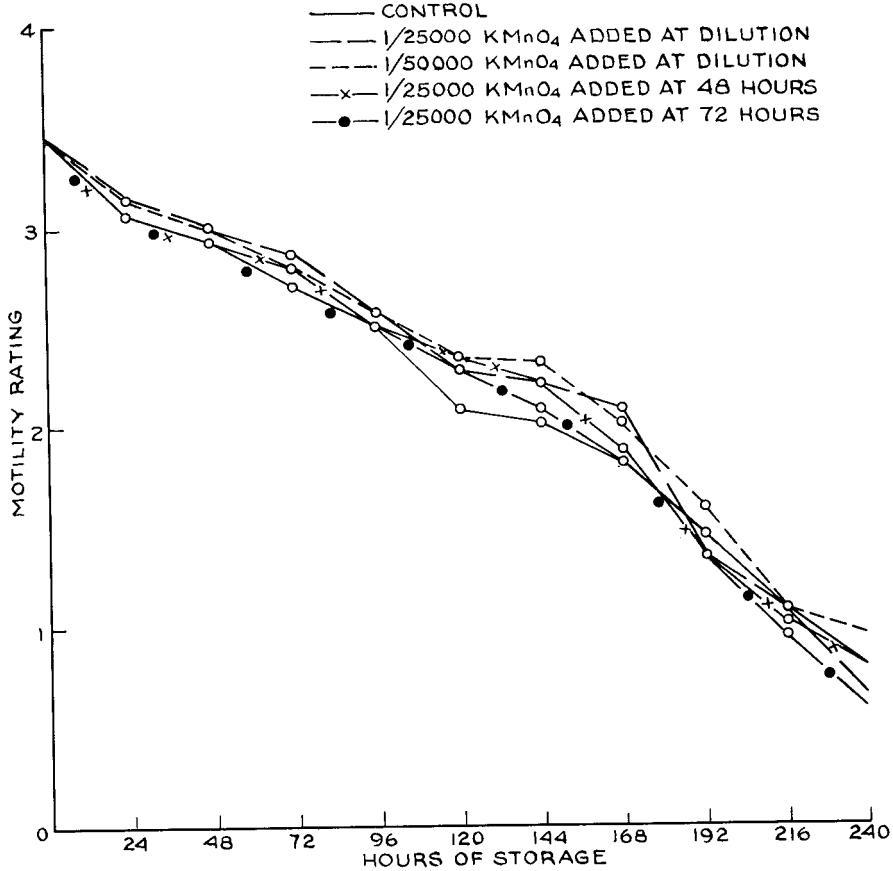


Figure 28.—The effect of potassium permanganate in combination with egg yolk-glucose on sperm motility.

Table 25—Analysis of Variance of Motility Ratings of 96 Hour Old Sperm Diluted with Egg Yolk-Glucose and Potassium Permanganate.

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Squares
Total	69	17.44	
Between subclasses	9	0.34	0.04
Control vs. treated	1	0.01	0.01
Between jacks	1	0.20	0.20
Interaction jack and treated	1	0.01	0.01
Between treatments	1	0.07	0.02
Interaction jack and treatments	3	0.05	0.02
Between ejaculates of same jack	12	12.84	1.07**
Interaction treatments and ejaculates (error)	48	4.26	0.09

\*\*Highly significant, P < .01.

tion of 1:50,000 potassium permanganate added at the time of initial dilution; (4) a sample of egg yolk-glucose diluted semen containing a final concentration of 1:25,000 potassium permanganate added to 48-hours old diluted semen; and (5) a sample of egg yolk-glucose diluted semen containing a final concentration of 1:25,000 potassium permanganate added to 72-hour old diluted semen.

The results show that samples of diluted semen with addition of either 1:25,000 or 1:50,000 concentrations of potassium permanganate maintained a higher level of sperm motility throughout most of the storage period than did the control sample with no additional permanganate; however, the differences at the end of 96 hours of the storage period were not significant (Figure 28 and Table 25).

After 120 hours of storage, all samples containing permanganate showed a significantly higher degree of motility than the control (Table 26). Using the "t" test, only the samples containing permanganate added to a final concentration of 1:50,000 at the time of initial dilution and 1:25,000 added at 48 hours after initial dilution showed a significant advantage over the control.

### **The Effect of Potassium Permanganate and Some Vitamins in Combination with Egg Yolk-Glucose on Sperm Motility**

Since some of the vitamin compounds had a stimulating effect on sperm motility, the possibility was considered that vitamins together with potassium permanganate, when added to diluted semen, would have an even greater stimulatory effect on sperm motility than either of the compounds when added singly.

Ten ejaculates were used in comparing the following dilutors: (1) a control diluted with egg yolk-glucose; (2) the addition of 1:50,000 concentration of potassium permanganate plus five mg. of thiamine hydrochloride to diluted semen at the time of dilution; (3) the addition of a 1:50,000 concentration of potassium permanganate plus ten mg. of thiamine hydrochloride to diluted semen at the time of dilution; and (4) the addition of a 1:50,000 concentration of potassium permanganate plus three mg. of ascorbic acid to diluted semen at the time of dilution.

The data show that all samples containing either five or ten mg. of thiamine or three mg. of ascorbic acid and a final concentration of 1:50,000 potassium permanganate maintained a higher level of sperm motility during the early part of the storage period (Figure 29); however, the differences were not significant after 96 hours of storage (Table 27).

### **The Effect of Various Other Substances on Sperm Motility**

In addition to the diluents previously discussed, the effects of the following combinations were also studied: (1) egg yolk and cane sugar; (2) egg yolk and sodium citrate; (3) egg yolk-glucose and sodium citrate; (4) egg yolk and gum arabic; (5) egg yolk and gum ghatti; (6) glucose and gum arabic; (7) glucose and gum ghatti; (8) glucose and lecithin; (9) varying quantities of lecithin; and (10) the addition of potassium permanganate to the TGL and GYP dilutors. Although in diluents (3), (4), (5), and (10) sperm motility was maintained longer than in the undiluted semen, none were as satisfactory as the egg yolk-glucose dilutor.

### **The Effect of Centrifugation on Sperm Motility**

Several workers (Walton, 1938; Camici and Polestra, 1939; Chang, 1943) obtained better storage results with stallion sperm by centrifuging and diluting, and removing a part of the seminal fluid, than by diluting uncentrifuged semen. An attempt was made to see if the same was true for jack sperm.

Semen was collected and diluted with egg yolk-glucose as previously

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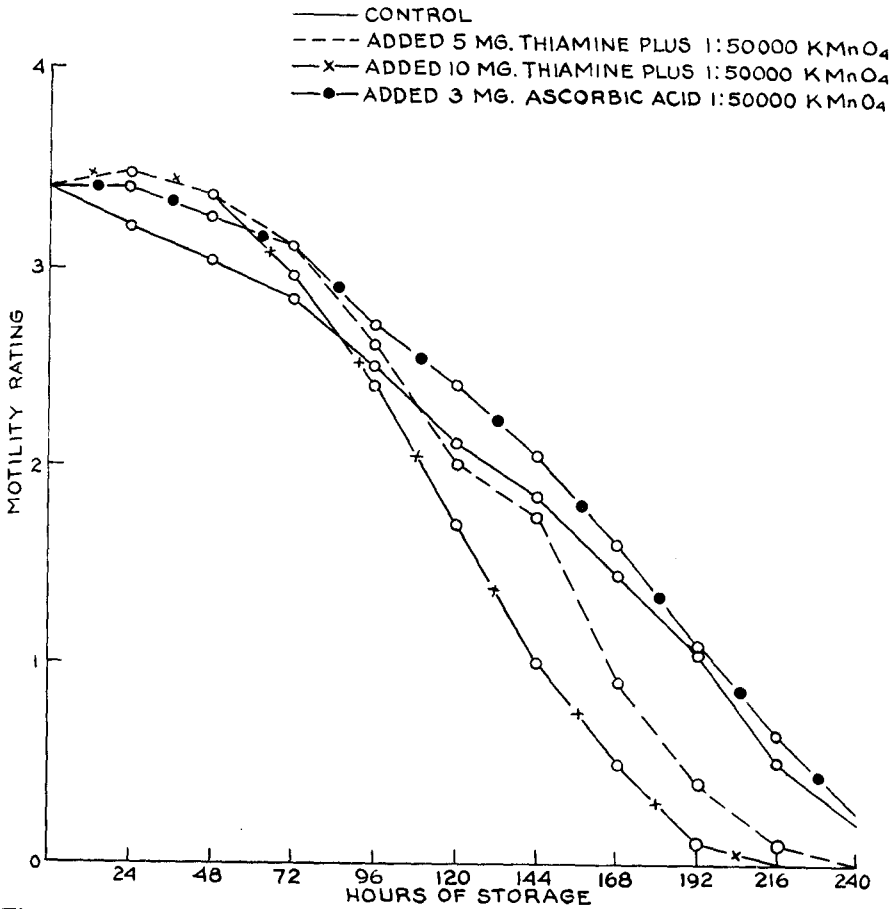


Figure 29.—The effect of potassium permanganate and some vitamins in combination with egg yolk-glucose on sperm motility.

Table 26—Analysis of Variance of Motility Ratings of 120 Hour Old Sperm Diluted with Egg Yolk-Glucose and Potassium Permanganate.

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Squares
Total	69	19.84	
Between subclasses	9	2.51	0.28*
Control vs. treated	1	0.70	0.70*
Between jacks	1	0.89	0.89**
Interaction jack and treated	1	0.06	0.06
Between treatments	3	0.07	0.02
Interaction jack and treatments	3	0.79	0.26
Between ejaculates of same jack	12	12.55	1.05**
Interaction treatments and ejaculates (error)	48	4.78	0.0996

\*Significant,  $P < .05$ .

\*\*Highly significant,  $P < .01$ .

Table 27—Analysis of Variance of Motility Ratings of 96 Hour Old Sperm Diluted with Egg Yolk-Glucose, Vitamins, and Potassium Permanganate.

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Squares
Total	39	21.98	
Between subclasses	7	4.78	0.68
Control vs. treated	1	0.08	0.08
Between jacks	1	0.63	0.63
Interaction jack and treated	1	0.07	0.07
Between treatments	1	2.60	1.30
Interaction jack and treatments	2	1.40	0.70
Between ejaculates of same jack	8	7.10	0.89
Interaction treatments and ejaculates (error)	24	10.10	0.42

described. In the first method tried, the same semen samples were centrifuged after 24, 48, 72, and 96 hours of storage, the supernatant liquid removed and a sufficient amount of fresh egg yolk-glucose diluent to maintain the original volume was added after each centrifugation.

In the second method, the effect of a single centrifugation after varying periods of storage was studied; i.e., samples centrifuged after 24, 48, or 72 hours of storage. After centrifuging and removing the supernatant fluid, a sufficient amount of fresh dilutor to maintain the original volume was added to each sample. A noncentrifuged sample diluted in the same ratio as the centrifuged samples served as a control. In no case did the centrifuged sperm maintain a level of motility as high or for as long a period as did the control. A majority of the centrifuged samples maintained a fair degree of motility for 72 or 96 hours but showed none thereafter.

#### CONCEPTION IN MARES INSEMINATED WITH DILUTED SEMEN

Forty-nine mares were inseminated with diluted jack semen that ranged from two to 67 hours of age at the time of use. Seven of 13 (54%), inseminated within two to four hours after collection of the semen, conceived and dropped foals. Twelve mares of 27 (44%), inseminated with 24-hour old semen, foaled. One of two mares inseminated with 48-hour old semen foaled. The one mare inseminated with 67-hour old semen did not conceive.

Due to the limited number of mares available for insemination, the conception data did not permit a satisfactory comparison of the efficacy of the different dilutors used. The conditions under which the data on conception were obtained were far from ideal. The mares were not concentrated in one area. For the most part, a single man owned no more than three, and many owned only one mare. The majority of the mares were not used for draft purposes and were not observed daily. Few of the owners had any way of determining whether the mare was in heat except by observation. On a number of occasions the inseminator found, on arrival at the farm and examination of the mare, that she obviously was not in heat. In other cases, questioning of the owner or farm tenant revealed, in a number of cases, that they had no idea as to how long the mare may have been in heat prior to the time of insemination. In other cases, the owner moved out of the area, the mare was sold, or for some other reason the insemination results were not available.

#### DISCUSSION

Based on the findings of a number of studies as reviewed by Andrews and McKenzie (1941) it seems to be the consensus of the investigators that ovulation in the mare occurs at some time during the last 48 hours of the heat period or occasionally after the end of heat. Furthermore, that for best breeding results the interval between service and ovulation should not be longer than 24 to 48 hours as few stallions produce sperm of sufficient vigor and vitality to survive a longer interval (Berliner, 1946). The length

of the heat period is long, compared to other farm animals. As a rule, it lasts five to eight days. Thus, many times it is impossible to time accurately the end of the heat period in order to inseminate the mare within the optimum period. The use of previously stored semen further complicates the problem in that, in all probability, the stored sperm do not live as long in the reproductive tract of the mare as do fresh sperm.

If artificial insemination of mares, under field conditions, is to result in a satisfactory conception rate the following conditions are highly desirable: (1) a single insemination should be timed with reference to the end of the heat period; or (2) successive inseminations should be made on alternate days during the heat period with fresh semen; or (3) successive inseminations should be made daily during the heat period with semen from a single ejaculate. The latter condition would be possible only if satisfactory sperm motility was maintained for a period of four or more days. A **prior** reasoning would lead one to believe that storage conditions which would satisfactorily maintain sperm motility for four days would also be satisfactory at shorter intervals of storage. For these reasons, primary emphasis in the laboratory studies and statistical analyses of the data were on the levels of sperm motility at the end of four days of storage.

### SUMMARY

In undiluted jack semen, the sperm lose their motility within a few hours after collection. Some method of prolonging sperm survival is necessary if artificial insemination is to be successfully used in horse stock. The effects of dilutors containing egg yolk and varying quantities of a glucose solution on sperm motility have been evaluated. Based on the results obtained in this study, a dilutor composed of one part egg yolk to four parts of five per cent glucose solution mixed in equal quantity with the semen did significantly prolong the survival time of the sperm. The sperm in semen diluted with more complex dilutors containing B vitamins or ascorbic acid showed a higher degree of motility after 96 hours of storage. However, the necessity of accurately controlling the quantities of these compounds that give optimum results would seem to preclude their use in jack semen dilutors under most field conditions.

Due to the limited number of mares available for insemination, a test of the efficacy of the different dilutors was not possible. Foaling data were available on 49 mares inseminated with diluted semen that ranged from two to 67 hours of age at the time of use. Seven of 13 (54%) inseminated within two to four hours after collection of the semen dropped foals. Twelve of 27 (44%) inseminated with 24-hour old semen foaled. One of two mares inseminated with 48-hour old semen foaled. The one mare inseminated with 67-hour old semen did not conceive.

By use of glucose-egg yolk diluted semen successive inseminations of mares at 12-24-hour intervals for three or more days from the same ejaculate would be practical and should materially increase the chances of conception in mares.

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