GLUCOSE TOLERANCE IN EQUIDAE

by

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INTRODUCTION

Although glucose has been used as a medicinal food for a long period, it has been given with little knowledge concerning the correct amount to administer to horses. The literature reveals little information on this subject. Some workers have given us valuable information regarding glucose tolerance in man and dogs. Most of the data available concerning this subject in the larger animals are based entirely on clinical results rather than on scientifically conducted experiments.

There are variations in the methods of approach to this problem which may lessen the value of some data. The difficulty encountered in confining large animals so that carefully conducted experiments can be performed is a very important factor to consider. If it were possible to confine horses as one can the smaller animals, more accurate work could be carried out in this connection.

The physical condition of the animals is an important factor, and variations in results may be due to differences in glycogen reserves in the liver and muscles. The functional capacity of the liver and pancreas must also be considered for these organs are the ones most vitally concerned when considering glucose utilization in the body.

The object of the experiments reported in this thesis is to note the rise in the blood sugar level following the intravenous injection of glucose solutions, and to establish the "renal threshold" for this normal constituent of the blood.

It would be desirable to have a method whereby the blood sugar level could be raised slowly and maintained at definite levels for a period of time. By this method one could tell more accurately the exact renal threshold, as the blood sugar could be increased slowly until a trace appeared in the urine.

There was no possibility of error resulting from the use of other drugs in these experiments. Solutions containing the concentrations of glucose commonly used in practice were used in all experiments.

REVIEW OF LITERATURE

Some of the first experiments dealing with the increase in blood sugar in large animals were those of Whitnah and Riddell (1933), but no information is available in regard to the amount of glucose administered or whether any was found in the urine. Using cows in these experiments and giving the glucose via stomach tube directly into the rumen, these investigators found the greatest increase in the blood sugar to be noticed about the fourth hour after giving the glucose. This increase lasted for more than 24 hours.

Janney and Isaacson (1918) administer glucose orally at the rate of 1.75 grams per Kilo of body weight in man. A blood sample is drawn 2 hours after the glucose is given and analyzed for its blood sugar content. Any marked rise in the blood sugar level is indicative of hypoendocrine function.

Using dogs as experimental animals Wierzuchowski (1936) found the renal threshold for blood sugar to be about 200 mgm. per 100 cc. of blood. He used 20 per cent solutions of glucose and continually infused them so as to maintain the blood sugar at definite levels. When administering 1 gram/Kilo/hour no glycosuria resulted, but 2 grams/Kilo/hour resulted in a pronounced glycosuria. The greater the amount of sugar in the blood the greater amount assimilated by the tissues, but after the blood sugar is elevated to about 3 times the normal level, the amount in the urine becomes excessive.

Whitnah and Riddell found the average blood sugar level in cows to be 59 mgm. per 100 cc. blood, and the average level after administering glucose to be 108 mgm. per 100 cc. blood.

EXPERIMENTAL PROCEDURE

Operative Technic

The animals used in these experiments varied in age from 2 years to 17 years. Not only was there an age variation, but also a wide variation in body weights. Although these factors have a tendency to vary the uniformity of results, such information is valuable because the average clinician finds similar variations among his patients.

All the animals were fed the customary ration of hay and grain twice daily. Two of the animals were used on successive days. This gave an opportunity to determine the effects of repeated injections of the sugar. Most of the animals were being treated for some pathological condition, but not of the type which would have much influence on the blood sugar levels. Only one animal was affected by a systemic disturbance which may have had some influence on the glucose tolerance.

It was decided that any procedure which would have a tendency to materially alter the normal body functions should not be used. Therefore, the injections were made by gravitation, the force being regulated according to the desired rate of injection. A simple flutter-value gravitation outfit was used when making the injections. All injections were made into the jugular vein using a 16-gauge needle.

The glucose solutions were prepared from anhydrous glucose (Merck C.P.) and distilled water and sterilized at 16 pounds pressure for 20 minutes. To avoid the possibility of error resulting from the escape of glucose into the subcutaneous tissues when it was being administered, the samples of blood which were drawn for analysis were taken from the jugular vein on the opposite side of the neck.

All instruments were sterilized by boiling and aseptic precautions were used when making the injections or drawing blood samples. Alcoholic sublimate was used as an antiseptic on the skin.

One sample of blood was drawn before the glucose was injected; 10 cc. of blood were drawn for analysis each time. All samples of blood were drawn into clean test tubes which contained 300 mgm. sodium citrate. The urine was removed from the bladder by use of a catheter before any glucose was injected. This urine was always tested for the presence of reducing sugars. Whenever necessary, manual pressure was applied to expel the urine from the bladder. The catheter was left in the urethra to keep the bladder drained so that the urine would not accumulate and become mixed; thus affording an opportunity for confusing results.

Chemical Procedure

Blood. The procedure used in determining the amount of reducing sugars in blood was the Shaffer-Hartmann-Somogyi method.

The proteins are removed by precipitation by means of zinc sulphate. Other reducing substances such as glutathione, ergothioneine, and uric acid are also removed. The remaining reducing sugars are oxidized with an alkaline copper solution, and the cuprous oxide which is formed by this oxidation is oxidized by dissolving in an acid solution in the presence of free iodine. The excess iodine is determined by titrating with standard sodium thiosulphate.

Eight volumes of special 1.25 per cent zinc sulphate solution are measured into a small flask. One volume of fresh blood is added and mixed until completely laked. One volume of 0.75 N NaOH is added and shaken vigorously and later filtered through quantitative filter paper.

Five cc. of the blood filtrate are put in large Pyrex tubes and 5 cc. of the tartrate-carbonate-copper reagent are added and mixed. A blank is prepared using 5 cc. distilled water in place of the blood filtrate. All tubes are put in a boiling water bath for 15 minutes, after which they are removed and cooled to 35°C. To each tube 2 cc. of 2.5 per cent potassium oxalate in 2.5 per cent potassium iodide solution are added. While being agitated slightly, 5 cc. of N H_2SO_4 are added to each tube to dissolve the cuprous oxide. After 10 minutes, the excess iodine is titrated with 0.005 N sodium thiosulphate solution.

Calculation. Since the blank titration measures the total amount of oxidizing agent used, the difference between this value and the amount left in a sample where some copper has been reduced, gives the amount equivalent to the amount of copper reduced. The difference in cubic centimeters is multiplied by 0.113 mgm. to calculate the amount of glucose in 5 cc. of blood filtrate or 0.5 cc. blood. To calculate the amount of glucose per 100 cc. multiply this amount times 200.

Urine. All samples of urine were tested for the presence of reducing sugars by boiling 2 cc. with an equal volume of Fehling's solution. If a brick red precipitate resulted, a quantitative determination was made. Standardized Fehling's solution was used when making these determinations. Five cc. of Fehling's solution equivalent to 25 mgm. glucose were put in a small flask, diluted with 3 volumes of distilled water and heated to boiling. Urine was added from a buret 1/2 cc. at a time and brought to a boil, after each addition. When all the blue color disappeared from the solution indicating that all the copper had been reduced, the amount of urine which had been added was determined and the amount of glucose per 100 cc. was calculated.

RESULTS

The results from these experiments indicate that most horses can tolerate the intravenous administration of 50 grams of glucose without developing a hyperglycemic condition. The mean variation in the normal blood sugar levels before any glucose was injected was 36.13 mgm. per 100 cc. blood. This appears to be a wide variation, but the low reading, 66.58 mgm. was in a 17 year old horse which was in a debilitated condition; the high reading 102.71 mgm. was in a 2 year old colt in good condition. The average for 5 animals was 82.67 mgm. per 100 cc. blood.

Although the amount of glucose an animal in a debilitated condition can tolerate is much greater than that tolerated by an animal in good condition, the results in these experiments indicate that the "renal threshold" appears to be about the same. The "renal threshold" appears to be about 150 mgm. per 100 cc. of blood.

Rapid administration of comparatively small doses of glucose will produce a temporary hyperglycemia and glycosuria. Since the excess glucose is normally converted into glycogen and stored in the liver and muscles, the rate of this conversion is limited, and rapid administration of even small doses may temporarily exceed the conversion powers of the body. Only a small amount of the blood conveyed to the heart by the anterior vena cava passes through the liver, the organ which regulates the blood sugar, before it goes through other parts of the systemic circulation. If the sugar concentration is above the "renal threshold", glycosuria results.

The administration of glucose on successive days seems to cause a gradual increase in the blood sugar levels. (tables 1 to 6). To what extent this would apply to animals in a diseased condition has not been determined.

Results recorded in tables 1 and 4 indicate that 50 grams of glucose can be injected in 10 minutes without elevating the blood sugar level to the "renal threshold", but 75 grams injected in 17 minutes produced a slight glycosuria in horse No. 1 (table 2). One hundred grams, even when injected very slowly (table 7) produces sufficient hyperglycemia to result in glycosuria. This probably should be considered an excessive dose. A slight amount of sugar being excreted in the urine would not cause a marked increase in the volume of urine excreted.

The size of the animal does not seem to regulate the increase in the blood sugar as much as expected (tables 6 and 7). That is, the response does not seem to be in direct proportion to the weight of the animal.

Table 1

Horse 1 (Seventeen years old; weight about 1100 pounds)
Fifty grams glucose in 50% solution
Injection time 15 minutes. March 22, 1938

Time	Minutes after glucose	Mgm./glucose per 100 cc. blood	Grams glucose per 100 cc. urine	Amount of urine in 10 min. period
8:30 A.M.	before	66.58	0.00	26 cc.
9:00 A.M.	15	83.30	0.00	27 cc.
9:15 A.M.	30	74.13	0.00	29 cc.
9:45 A.M.	60	73.47	0.00	24 cc.

Total rise in blood sugar in 15 minutes 16.72 mgms.

Table 2

Horse 1 (Seventeen years old; weight about 1100 pounds)
Seventy-five grams glucose in 50% solution
Injection time 17 minutes. March 23, 1938

Time	Minutes after glucose	Mgm./glucose per 100 cc. blood	Grams glucose per 100 cc. urine	Amount of urine in 10 min. period
1:18 P.M.	before	90.91	0.00	31 cc.
1:40 P.M.	5	153.46	0.91	39 cc.
1:50 P.M.	15	125.60	0.00	34 cc.
2:05 P.M.	30	111.10	0.00	32 cc.
2:35 P.M.	60	101.96	0.00	28 cc.

Total rise in blood sugar in 5 minutes 62.55 mgms.
Total rise in blood sugar in 15 minutes 34.69 mgms.

Table 3

Horse 1 (Seventeen years old; weight about 1100 pounds).
One-hundred grams glucose in 25% solution.
Injection time 20 minutes. March 24, 1938

Time	Minutes after glucose	Mgm./glucose per 100 cc. blood	Grams glucose per 100 cc. urine	Amount of urine in 10 min. period
8:15 A.M.	before	118.75	0.00	38 cc.
8:50 A.M.	15	181.86	1.61	51 cc.
9:05 A.M.	30	166.66	1.19	49 cc.
9:35 A.M.	60	152.10	0.87	47 cc.
10:05 A.M.	90	129.00	0.00	34 cc.
2:00 P.M.	29½	121.59	0.00	27 cc.

Total rise in blood sugar in 15 minutes 63.11 mgms.

Table 4

Horse 2 (Two years old; weight about 700 pounds).
Fifty grams glucose in 25% solution.
Injection time 10 minutes. March 28, 1938

Time	Minutes after glucose	Mgm./glucose per 100 cc. blood	Grams glucose per 100 cc. urine	Amount of urine in 10 min. period
9:00 A.M.	before	102.71	0.00	23 cc.
9:25 A.M.	15	115.51	0.00	23 cc.
9:40 A.M.	30	109.46	0.00	25 cc.
10:10 A.M.	60	100.10	0.00	19 cc.

Total rise in blood sugar in 15 minutes 12.8 mgms.

Table 5

Horse 2 (Two years old; weight about 700 pounds).
Seventy-five grams glucose in 25% solution.
Injection time 15 minutes. March 29, 1938

Time	Minutes after glucose	Mgms./glucose per 100 cc. blood	Grams glucose per 100 cc. urine	Amount of urine in 10 min. period
9:00 A.M.	before	107.00	0.00	26 cc.
9:30 A.M.	15	154.29	0.36	41 cc.
9:45 A.M.	30	137.41	0.00	37 cc.
10:15 A.M.	60	118.16	0.00	36 cc.

Total rise in blood sugar in 15 minutes 47.29 mgms.

Table 6

Horse 2 (Two years old; weight about 700 pounds).
One-hundred grams glucose in 25% solution.
Injection time 20 minutes.

Time	Minutes after glucose	Mgm./glucose per 100 cc. blood	Grams glucose per 100 cc. urine	Amount of urine in 10 min. period
1:40 P.M.	before	101.58	0.00	27 cc.
2:15 P.M.	15	234.19	4.43	62 cc.
2:30 P.M.	30	151.51	0.21	46 cc.
3:00 P.M.	60	153.70	0.24	42 cc.

Total rise in blood sugar in 15 minutes 131.48 mgms.

Table 7

Horse 3 (Seven years old; weight about 1400 pounds).
One-hundred grams glucose in 50% solution.
Injection time 20 minutes. April 14, 1938

Time	Minutes after glucose	Mgm./glucose per 100 cc. blood	Grams glucose per 100 cc. urine	Amount of urine in 10 min. period
1;35 P.M.	before	76.83	0.00	33 cc.
2:00 P.M.	5	185.18	1.34	57 cc.
2:10 P.M.	15	114.85	0.00	42 cc.
2:25 P.M.	30	90.01	0.00	31 cc.
2:55 P.M.	60	86.17	0.00	39 cc.

Total rise in blood sugar in 5 minutes 108.35 mgms.
Total rise in blood sugar in 15 minutes 38.02 mgms.

Table 8

Horse 4 (Four year old stallion; weight 1600 pounds).
One-hundred fifty grams glucose in 50% solution.
Injection time 25 minutes. April 21, 1938

Time	Minutes after glucose	Mgm./glucose per 100 cc. blood	Grams glucose per 100 cc. urine	Amount of urine in 10 min. period
1:45 P.M.	before	90.90	0.00	41 cc.
2:15 P.M.	5	182.65	2.29	62 cc.
2:25 P.M.	15	138.16	0.17	45 cc.
2:40 P.M.	30	111.73	0.00	36 cc.
3:10 P.M.	60	99.46	0.00	42 cc.

Total rise in blood sugar in 5 minutes 91.75 mgms.
Total rise in blood sugar in 15 minutes 47.26 mgms.

Table 9

Horse 5 (Six years old; weight about 1600 pounds).
One-hundred twenty-five grams glucose in 25% solution.
Injection time 12 minutes. April 28, 1938

Time	Minutes after glucose	Mgm./glucose per 100 cc. blood	Grams glucose per 100 cc. urine	Amount of urine in 10 min. period
1:20 P.M.	before	76.34	0.00	23 cc.
1:47 P.M.	15	151.51	0.12	29 cc.
2:02 P.M.	30	123.04	0.00	31 cc.
2:32 P.M.	60	101.26	0.00	22 cc.

Total rise in blood sugar in 15 minutes 75.17 mgms.

SUMMARY

It is the author's opinion that large amounts of glucose might have a tendency to dehydrate the body instead of combating a hypohydrated condition for which it is often administered. The animals were kept under ordinary conditions which should make the results fairly accurate because no unusual handling or care entered into the procedure. No anesthetics were used and the only disturbance caused to the animals were the veni-punctures made when drawing blood samples and making the injections of glucose solutions.

The amount of increase in the blood sugar levels seem to be directly proportional to the amount of glucose injected, rate of injection, and condition of the animals. The concentration of the glucose solutions injected did not seem to have much effect on the increase in the blood sugar level, but the total amount of glucose by weight and the amount of time taken for making the injections are the governing factors.

CONCLUSIONS

1. The renal threshold for blood sugar in horses is about 150 mgm. per 100 cc. blood, and
2. The average amount of glucose tolerated by most horses, as evidenced by these experiments is 75 grams, and
3. The age and condition of the animal are very important factors in governing the amount of glucose it can tolerate, and
4. Rapid administration of even small amounts of glucose will cause a temporary hyperglycemia, and glycosuria, and
5. The size of the animal does not always govern the amount of glucose the animal can tolerate, and
6. The rate of injection and total amount of glucose given largely influence the amount of rise in the blood sugar, and
7. The blood sugar level returns to normal slowly.

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Abstract of Thesis

GLUCOSE TOLERANCE IN EQUIDAE

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This experimental work was carried out in an attempt to determine the correct amount of glucose to administer to sick animals. Although this does not deal with the effects of the drug, it has given us information concerning the proper amount to give at one time.

Glucose is a normal constituent of the blood and the amount of it per 100 c.c. of blood can vary within certain limits. When the amount of glucose gets to a high level in the blood, a certain amount of it is excreted in the urine. It is, therefore, an undesirable practice to give a sufficient amount of this material to cause glycosuria, for this has a tendency to dehydrate the body. While this may be desirable in some cases, most sick patients need more instead of less fluid.

There is a variation among animals regarding the concentration of sugar in the blood necessary to cause the excretion of it in the urine. The "renal threshold" for man appears to be about 180 mgs. per 100 c.c. blood; for dogs about 200 mgs. per 100 c.c. blood, but horses seem to excrete glucose if the concentration gets above 150 mgs. per 100 c.c. blood.

Results obtained in these experiments indicate that most horses can utilize as much as 75 grams of glucose providing it is not injected too rapidly. To give an average size horse this amount of glucose intravenously in 10 minutes would cause a temporary glycosuria, but if the injection time is prolonged to 15 minutes, very little, if any, glycosuria results.

Doses of 50 grams of glucose when given intravenously do not produce a marked increase in the blood sugar of most horses. However, does of 100 grams produce sufficient hyperglycemia to result in glycosuria even though the rate of injection is very slow. This amount seems to be greater than the body can utilize, although the liver and pancreas may be functioning normally.

Although these experiments are very limited, it appears that the "renal threshold" for blood sugar in horses is about 150 mgs. per 100 c.c. blood, and that the maximum amount of glucose, when injected intravenously without producing glycosuria is about 75 grams. This amount will increase the blood sugar level sufficiently for it to be elevated for 24 hours after the administration.