RELATION OF GALACTOSE FEEDING TO LIVER AND MUSCLE GLYCOGEN IN RATS

by

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INTRODUCTION

Comparative production of liver and muscle glycogen on diets containing galactose and glucose has long been of interest. Cori[1] measured conversion of various sugars to liver glycogen in fasted rats and concluded that galactose was an inefficient glycogen percurser when compared to glucose or fructose. Cori based his conclusions on the fact that the liver is presented with a higher concentration of blood galactose than of blood glucose and attached less importance to urinary losses. Deuel and associates[2] concluded that if allowances were made for the amount of galactose lost in the urine it would compare favorably with glucose as a source of liver glycogen. In nephrectomized rats Bergman and MacKay[3] found that liver glycogen was higher in rats given galactose than in those given glucose. Differences in rates of absorption, large differences in rates of utilization, and the high urinary loss of galactose have made it difficult in such experiments to achieve similar conditions in the metabolism of the two sugars.

Upwards of 80 per cent of fed galactose that is metabolized is removed from the blood by the liver. Small amounts may be utilized by peripheral tissues[4]. It is highly probable that all or nearly all galactose utilized at any site is first converted to glucose.

Tygstrup and Winkler[5] have shown in humans that at levels of blood galactose above about 50 mgs per cent there is no increased rate of metabolism of this sugar. They presented data showing that the liver has the ability to clear all of the galactose from the blood passing through it up to this concentration, but that it is unable to handle higher levels.

Other experiments showed that the amount of galactose metabolized can be increased by increasing the amount of fat in the diet[6]. This is probably because fat slows galactose absorption from the gut and a longer time is available for the liver to handle the same amount of sugar rather than because of a specific effect of fat on galactose intermediary metabolism[7,8]. As nearly all galactose filtered in the kidney is lost in the urine[9], slowing absorption and decreasing blood levels decreases urine loss.

In view of these difficulties it was decided to compare the two sugars when utilized at equal and constant rates along with identical amounts of fat and protein. Ideally the diets would have been administered slowly and evenly throughout the entire 24 hours or each day. As a compromise, the diets were fed in weighed amounts four times each day, and the comparatively high levels of protein and fat were counted upon to keep absorption relatively even.

After several preliminary experiments a diet level was determined that permitted good weight gain and that was eaten within a few minutes of feeding.

EXPERIMENTAL

Male Sprague-Dawley rats kept in individual cages were first fed ground Purina Chow in weighed amounts four times per day (4/24 of the daily ration at 9 am, 4/24 at 1 pm, 6/24 at 5 pm, and 10/24 at 11 pm) for three or more days. Water was allowed ad libitum throughout the experiment. Then after a 32 hour fast (beginning by omitting the 9 am feeding and ending at 5 pm of the following day) rats of 180 to 220 grams fasted weight were fed either a galactose or a glucose diet for one, two, three, or four days. The galactose diet contained: galactose 45, casein 20, Wesson oil 18, cod liver oil 2, brewers yeast 10, and Wesson salt mix 4, all in per cent by weight.

Starting with the end of the fasting period at 5 pm, 15 grams of galactose diet per 200 grams fasted weight rat per 24 hour was given in amounts and times as indicated above and the rats sacrificed for analysis of liver and muscle glycogen.

On the day of sacrifice food was given only at 9 am. Between 5 pm and 8 pm the rats were anesthetized with intraperitoneal pentobarbital, (6 mgs per 100 g)

the right gastrocnemius muscle quickly excised, cleaned of fat and tendon, chopped into fine pieces with a sissors and dropped into 30 per cent KOH. The left gastrocnemius, similarly treated, was placed in ice-cold 10 per cent trichloroacetic acid (TCA). Then the abdomen was opened, the liver removed, blotted and placed in a tissue press where the parenchyma was separated from the capsule and collected in a dry evaporating dish. After a few seconds brisk stirring to assure homogeniety, samples of approximately one gram were placed in 30 per cent KOH and in 10 per cent TCA.

Glycogen was determined on the 30 per cent KOH samples by the method of Good, Kramer, and Somogyi[10], and on the TCA samples by the method of Bloom and associates[11]. The difference obtained by subtracting the TCA extractable glycogen from the KOH extractable glycogen is termed residual glycogen.

In both cases the precipitated glycogen was washed once with 70 per cent ethanol, centrifuged, allowed to dry and hydrolyzed in N sulfuric acid for three hours. After neutralization to phenol red, glucose was determined by the method of Somogyi[12].

Four rats were placed in individual metabolism cages and urine collected under toluene for each of four consecutive 24 hour periods. Samples were frozen

and stored until analyzed. They were thawed, aerated to remove toluene, and a Somogyi filtrate[13] prepared. Urine galactose was determined for each 24 hour period, the total amount of galactose excreted by each rat in four days computed and a correction of 0.150 g added for the amount of galactose estimated to be present in body water at the time of sacrifice. This total, taken as the amount of non-utilized galactose, was subtracted from the total amount fed, and the difference, divided by four, taken as the amount of galactose metabolized per rat per day. A grand average was then computed for the total of four rats.

A glucose diet was then prepared so that each rat would receive glucose equal in weight to the average amount of galactose utilized by the galactose-fed rats. Wesson oil, cod liver oil, salts, casein and yeast were included in amounts equal to those fed the galactose-fed rats. It was necessary to add about 5 per cent of Sulkafloc, a cellulose floc, to gain sufficient granularity to permit accurate weighing. This glucose diet was then fed to other rats under the same conditions as described for the galactose-fed rats.

It was found that the glucose diet should contain: glucose 18.0 per cent, casein 30.4 per cent, Wesson oil plus cod liver oil 30.4 per cent, yeast 15.2 per cent

and Wesson salts 6.0 per cent. 5 g of sulfafloc were added per 100 g of ration. A total of 9.88 g of nutrients per 200 g fasted weight per 24 hours was given. This contained: glucose 1.78 g, casein 3.0 g, fat 3.0 g, yeast 1.5 g, and salts 0.6 g.

Experiments using the galactose diet were done in November and December of 1957 and during January of 1958. Experiments using the glucose diet were done in February, March, and April of 1958. The temperature in the room housing the experimental animals was possibly 1-2° C higher during some of the glucose experiments, due to modification of the room's ceiling and changes in heating facilities.

RESULTS AND DISCUSSION

It is seen on inspecting Figures 1 and 2 that the curves representing total liver and muscle glycogen have the same general shape. There is a sharp increase in both liver and muscle glycogen after one day of feeding. As this same level of feeding is continued for two days there is a decline in both liver and muscle glycogen to a level which remains relatively constant for the next two days.

Excepting a significant tendency for muscle glycogen to decline to lower levels in galactose-fed animals on days two, three, and four, there is no significant

difference in the total liver or total muscle glycogen patterns on the two diets. In particular there is no evidence for a difference of liver glucose derived from dietary galactose as against that derived from dietary glucose as a glycogen former.

The shape of these curves indicate fasting has activated factors that cause higher levels of both liver and muscle glycogen than would occur at the same feeding levels in non-fasted animals. Prolonged fasting is known to increase the conversion of proteins to carbohydrates. Liver glycogen levels tend to rise somewhat following prolonged fasting. Presumably, feeding would decrease the conversion of protein to carbohydrate. It is improbable that much of the increased glycogen found on the first day is derived from endogenous protein. Preliminary feeding experiments at decreased food intakes resulted in much lower glycogen levels throughout, with a corresponding decrease in difference between the first and following days, though the percentage decrease was roughly the same.

More probably the high levels of glycogen are due to increased production of glycogen from substances in the diet, either from protein or from carbohydrate or both.

It is of interest to note that body weight usually

decreased during the first day of feeding and weight gain became apparent usually only after the end of two days, coinciding with a drop in liver and muscle glycogen. This is good evidence of continuance of the catabolic phase induced by starvation into the first day of feeding. (Figure 3)

Lower values of muscle glycogen in galactose-fed animals may reflect lowered glucose levels in the blood of these animals[14]. It also may be due to a specific tendency for galactose to inhibit glycogen deposition in muscle. In any case, by the fourth day total muscle glycogen values in growing galactose-fed animals were well below levels found in the 32-hour fasted controls.

Though the galactose diet contains 46 per cent galactose as fed, only one-fourth of this was retained. Therefore, both sets of animals were utilizing a high protein, high fat, relatively low carbohydrate diet. Fat is a poor source of carbohydrate, but protein is converted to glycogen by deaminization of amino acids and their conversion to glucose percursors. The dietary source of both liver and muscle glycogen is therefore uncertain.

Liver glycogen levels are influenced by the composition of the diet, glycogen deposition being favored by high carbohydrate diets and decreased by high protein

or high fat diets[15]. This may in part account for the low levels of liver glycogen found on days two, three, and four. It is also possible that the animals would have consumed somewhat more calories if permitted to feed ad libitum, but it was necessary to keep them slightly hungry in order that they would promptly consume the diet at each feeding period.

Much of the controversy regarding the efficiency of galactose as a glycogen procursor is a reflection of the differences in absorption, rate of removal from the blood, urinary loss, and sites and rates of metabolism.

In most quantitative experiments the investigators used one dose of concentrated sugar solution given by stomach tube. Galactose is somewhat more rapidly absorbed than glucose. Cori[16] gives the relative rates as 110 to 100. Thus galactose reaches the blood more quickly than glucose. However, presumably all body tissues can remove and utilize glucose from the blood while the major and, probably for practical purposes, the only active site of galactose metabolism is the liver. Even the liver has been shown to have rather limited ability to take up galactose. Hence blood galactose soon rises to high levels with consequent losses in the urine in animals eating the

galactose ration. Under conditions of the present experiments a 200 g rat is able to utilize only 1.8 g of galactose per 24 hours. This may well be close to the maximum possible rate for this strain of rat since the method of feeding is believed to present the liver with high and relatively constant galactose levels. Total blood sugar was found to be 160 mgs per cent 10 hours after the last feeding at 9 am, which indicates fair amounts of galactose were still present in the body at this time. If blood galactose is estimated at 100 mgs per cent at this time, and galactose at this concentration is distributed throughout body water (taken as 70 per cent of body weight) calculations show that 140 mgs of galactose remain unutilized.*

Galactose quickly diffuses into extracellular water and more slowly into intracellular water. Consequently considerable galactose can be "stored" intracellularly, gradually passing back into the blood as blood levels fall.

In single dose experiments a wide range of relations between the two sugars as glycogen formers could be achieved by varying the dose and the time of sampling. If low doses of galactose are given, so

* At 70 per cent of weight as body water, a 200 g rat would have 140 ml. of body water. This would contain 100 mgs galactose per 100 ml., or a total of 140 mgs of galactose in body water.

that blood levels do not exceed the ability of the liver to clear it from the portal vein, liver glycogen deposition would begin and end early. There would be little or no galactose lost in the urine. Thus by giving small amounts and measuring liver glycogen soon after sugar administration the relative position of galactose as a glycogen former would be improved.

At higher sugar dosages the liver would be flooded with galactose bringing about high urinary losses and diffusion into intracellular water. Liver metabolism of galactose would continue at a low but maximal rate for some time due to the back diffusion of intracellular galactose. At 12 or even 24 hours the liver might still be converting this stored galactose to glycogen. Glucose, on the other hand, would have been much more quickly taken up, coverted to glycogen, and as time progressed be diffused back into the blood from the breakdown of this newly formed glycogen.

Measurements taken early would show a high efficiency for glucose and a lower efficiency for galactose as glycogen formers. Even if corrected for the amount retained, as Deuel and associates did, galactose would still be less efficient as not all galactose retained (fed minus that in gut and urine) would have been metabolized.

Later on in a fast, the advantage passes to galactose

as intracellular galactose moves into the blood and is converted to glucose and glycogen by the liver, while at the same time glycogen levels in glucose-fed animals would be falling progressively.

In the present experiments an attempt was made to avoid these pitfalls by providing a fairly constant rate of metabolism of galactose, glucose, fat, and protein.

Though there are no significant differences seen between the two diets as liver glycogen formers, it is possible that a relatively large amount of glycogen came from protein sources in both cases and that this obscured real differences in levels of glycogen derived from the two sugars (Figure 4). Isotope studies would help to discern the sources and relative origins of liver and muscle glycogen on these diets.

It is worth noting that though the total muscle glycogen in glucose-fed animals is higher on days two, three, and four, the residual glycogens are significantly lower on the first, second, and third days than in the corresponding galactose-fed animals (Table 2). Residual glycogen is determined by subtracting TCA soluble glycogen from total or KOH soluble glycogen. Therefore, comparatively much larger fractions of muscle glycogen must have been soluble in TCA in the glucose-fed animals. There are two possible explanations for this:

1) Glycogen from glucose animals is actually more soluble in TCA, and 2) There was a difference in grinding technique and therefore, in tissue particle size. The latter explanation is supported by the fact that it was necessary to begin using a new, very tight, homogenizer at the time the glucose experiments were begun, due to the breakage of the old and quite loose homogenizer. A few muscle glycogens were done on glucose animals with the old homogenizer prior to its breaking, and residual glycogens here (Table 3) were quite a bit higher than those done a few days later with the new instrument.

Similarily the homogenizer used for liver samples was also new, and quite tight at first. It became somewhat looser with continued use. This may account for the rather low values for residual glycogen found throughout the experiment. Three of these values are not statistically different from zero. However, in view of their small size, and because they are determined by the difference of two relatively large numbers, the numerical values for liver residual glycogens are probably more inaccurately determined than the standard deviations in Table 1 would indicate. It is safe to say only that the quantity of residual glycogen in liver is indeed small.

SUMMARY AND CONCLUSIONS

1. The metabolism of galactose and glucose, as reflected by liver and muscle glycogen levels in rats fed diets containing these sugars, was studied. To compensate for urinary losses of galactose, diets were prepared containing either 46 per cent galactose or an appropriate amount of glucose, along with equal quantities of protein, fat, salts, and yeast. The diets were given four times per day at the level of 15 g of galactose diet (or an equivalent level of glucose diet) per 200 g fasted weight per 24 hours to male Sprague-Dawley rats previously fasted 32 hours. Composition of the two diets was so calculated that rats utilized sugar, fat, protein, salts, and yeast from each of them at constant and equal rates. TCA and KOH soluble liver and muscle glycogens were determined after one, two, three, and four days of feeding.

2. At the dietary levels used no significant differences were found in liver glycogen values at one, two, three, or four days feeding in rats utilizing equal amounts of galactose or glucose along with the same amounts of protein and fat when metabolism of these substances was spread evenly throughout the day.

3. Following a 32 hour fast, muscle and liver glycogen levels were highest after one day of feeding

and lower and relatively constant thereafter, with the exception of muscle glycogen in galactose-fed rats, which tended to decline with time to less than the fasting level at four days.

4. No evidence was found for a difference of liver glucose derived from dietary galactose, as against that derived from dietary glucose, as a glycogen former.

5. Low values of residual glycogen found in liver and in glucose-fed muscle might have been due to finer tissue particle size in TCA samples produced by grinding in a tight homogenizer. The relation of residual glycogen and particle size in grinds produced by different homogenizers should be further investigated immediately.

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TABLE 1

MEAN LIVER GLYCOGEN

IN

GALACTOSE AND GLUCOSE FED RATS[a]

GALACTOSE-FED

Days fed	No.[b]	KOH	SD mean[c]	TCA	SD mean	RES	SD mean
0[d]	4	0.022	0.004	0.006	0.003	0.014	0.005
1	7	2.500	0.193	2.360	0.198	0.140	0.027
2	5	1.138	0.174	1.047	0.201	0.091	0.030
3	5	0.988	0.069	0.967	0.079	0.021	0.035
4	6	0.944	0.193	0.917	0.219	0.027	0.037

GLUCOSE-FED

Days fed	No.	KOH	SD mean	TCA	SD mean	RES	SD mean
1	11	2.450	0.261	2.380	0.248	0.070	0.021
2	13	1.182	0.178	1.055	0.167	0.127	0.029
3	12	0.997	0.131	0.965	0.141	0.033	0.022
4	8	1.083	0.231	0.988	0.235	0.094	0.026

a all values expressed in per cent glucose wet weight

b number of animals

c standard deviation of the mean

d fasted 32 hours

TABLE 2

MEAN MUSCLE GLYCOGEN

IN

GALACTOSE AND GLUCOSE FED RATS[a]

GALACTOSE-FED[h]

Days fed	Ng[b]	KOH	SD mean[c]	TCA	SD mean	KES	SD mean
0[d]	4	555	12.6	338	15.2	217	17.5
1	7	666	15.2	408	16.8	257	13.0
2	5	527	11.7	268	8.6	258	6.8
3	5	512[e]	22.3	276	21.3	234[e]	8.8
4	6	468	29.1	274	14.0	204	12.9

GLUCOSE-FED[i]

Days fed	Ng	KOH	SD mean	TCA	SD mean	KES	SD mean
1	11	644	13.6	438	11.5	206	11.0
2	13	576	11.4	354[f]	13.9	222[g]	7.3
3	12	965	15.0	349	14.3	205[g]	7.5
4	8	595	14.5	366	13.9	229	13.8

a all values expressed in mgs per cent wet weight

b number of animals

c standard deviation of the mean

d fasted 32 hours

e five animals, excluding probable outlier

f eight animals, excluding those done with loose homogenizer

g seven animals, excluding probable outlier

h TCA glycogens done with loose homogenizer

i TCA glycogens done with new, tight homogenizer

TABLE 3

MEAN RESIDUAL MUSCLE GLYCOGEN

IN SAMPLES TREATED WITH LOOSE AND TIGHT HOMOGENIZERS

RELATED TO KOH MUSCLE GLYCOGEN[a]

LOOSE HOMOGENIZER[d]					TIGHT HOMOGENIZER				
No.[b]	KOH	SD mean[c]	HBS	SD mean	No.	KOH	SD mean	HBS	SD mean
5	468	7.7	212	6.1	-				
5	516	2.8	252	6.3	5	536	3.2	215	14.2
5	535	2.8	245[e]	7.4	2	544	5.0	197	22.5
6	570	1.4	252	12.7	10	566	1.2	212	10.4
4	588	4.7	263	10.1	6	592	3.3	208	13.0
3	610	3.1	275	17.4	3	611	7.2	231	24.3
2	650	3.5	257	30.7	8	649	5.2	217	10.4
5	697	10.0	270	20.0	3	689	10.3	242	6.2

a all values expressed in mgs per cent wet weight

b number of animals

c standard deviation of the mean

d includes 4 control and 10 glucose-fed animals

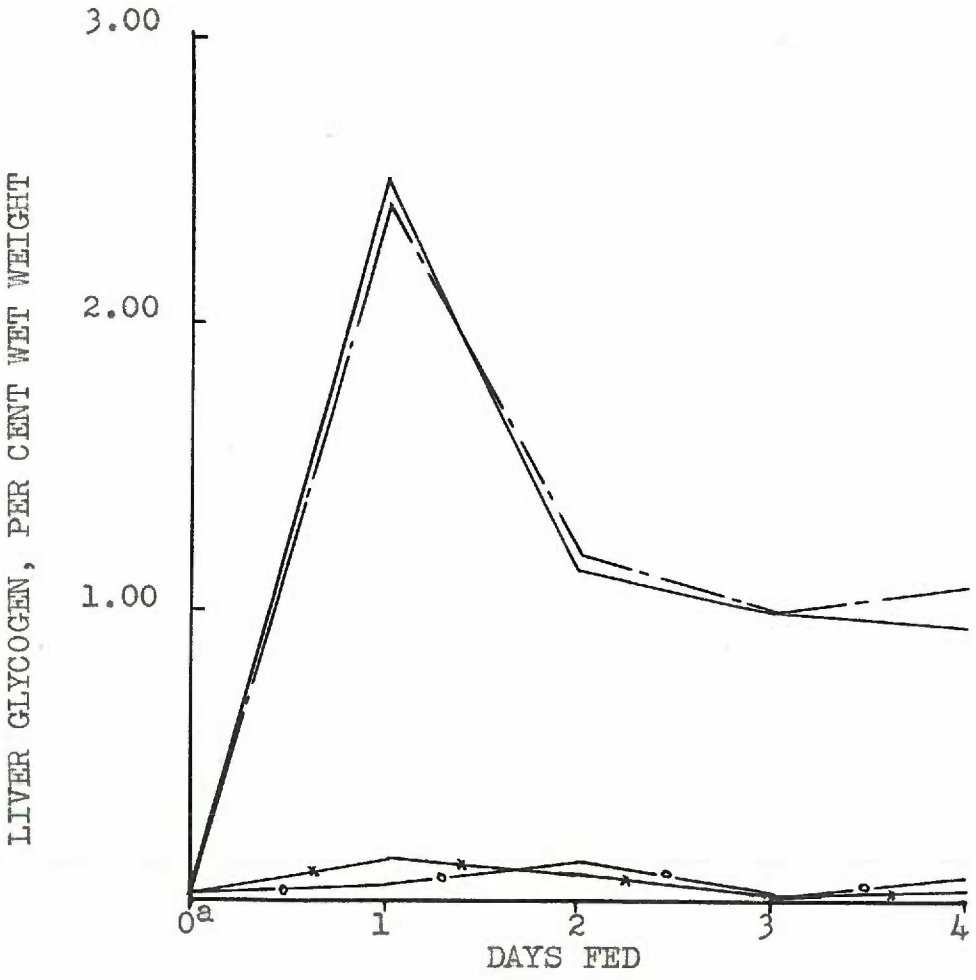
e four animals, excluding probable outlier

FIGURE 1

MEAN LIVER GLYCOGEN

IN

GALACTOSE AND GLUCOSE FED RATS



a 32 hour fasted, 0 time (0.022 per cent)

KOH glycogen, galactose-fed

KOH glycogen, glucose-fed

residual glycogen, galactose-fed

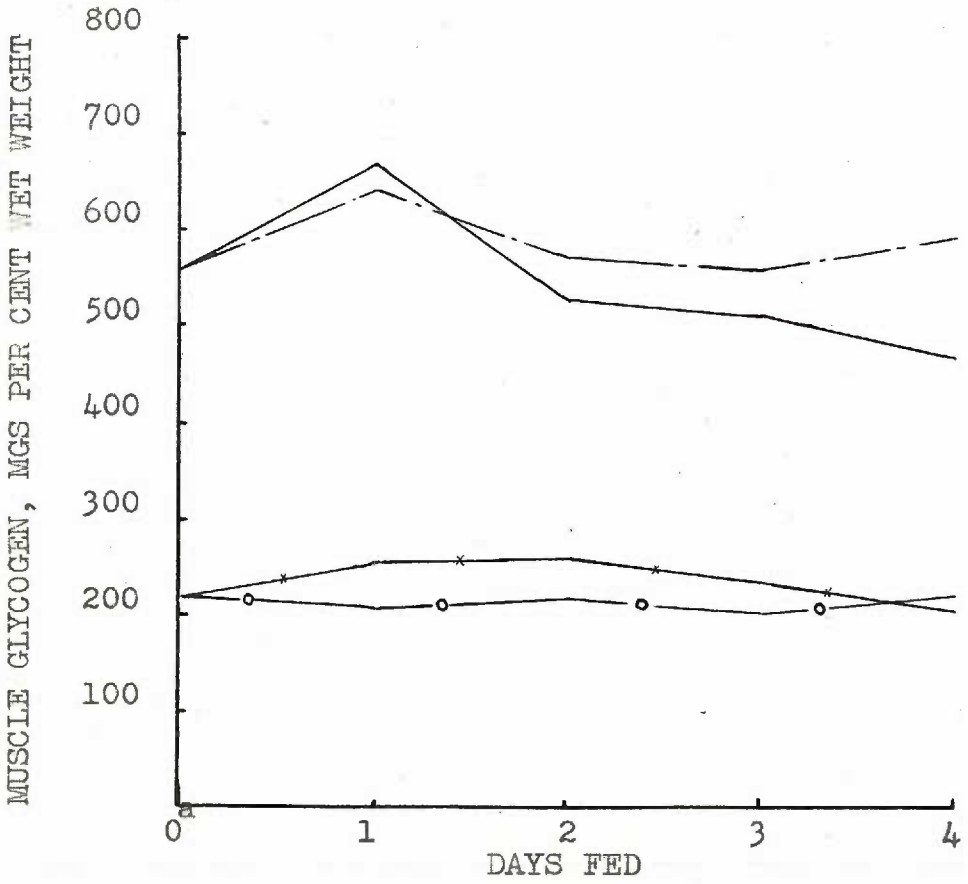
residual glycogen, glucose-fed

FIGURE 2

MEAN MUSCLE GLYCOGEN

IN

GALACTOSE AND GLUCOSE FED RATS



a 32 hour fasted, values at 0 time

KOH glycogen, galactose-fed

KOH glycogen, glucose-fed

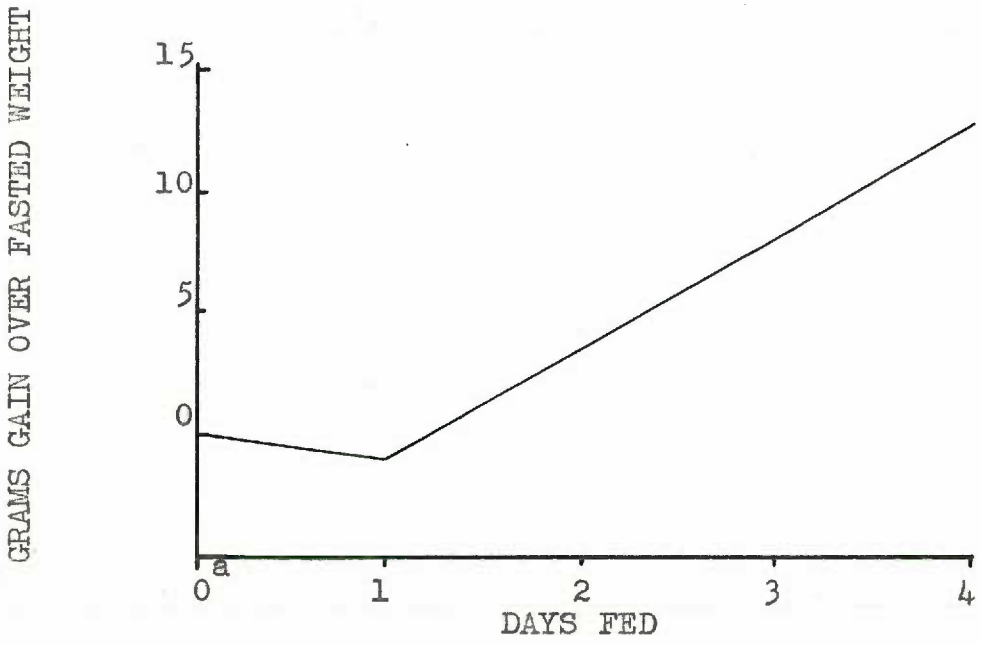
residual glycogen, galactose-fed

residual glycogen, glucose-fed

FIGURE 2

MEAN WEIGHT GAIN IN ALL RATS

IRRESPECTIVE OF DIET



a fasted 32 hours, values at 0 time

FIGURE 4

SCHEME OF GLYCOGEN SYNTHESIS