STUDIES ON THE RELATION OF AMINO ACID FEEDING TO LIVER GLYCOGEN LEVELS IN RATS

by

JOAN M. BARNES

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. . . [redacted] . .

(Professor in Charge of Thesis)

. . . [redacted] . . . . . .

~~(Dean, Graduate Division)~~

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INTRODUCTION

There are numerous environmental factors which may influence the accumulation of liver glycogen in unfasted rats and the maintenance of liver glycogen in fasted rats. The amount of glycogen deposited in the liver of a well-nourished animal depends on the composition of the diet consumed, the season of the year and assorted other conditions, some of which are unknown. The level of liver glycogen found in animals fasted for 24 hours was found to be uniformly low by such workers as Barbour, Chaikoff, et al.(1) who maintained their experimental animals on high carbohydrate diets. Other early experiments indicated that liver glycogen could also be reduced to very low levels by exposure of animals to cold, exercising them, etc.

Hynd and Rotter(2) performed one of the first experiments dealing with the influence of diet, per se, on the carbohydrate metabolism of fasting rats. These authors found that on a carbohydrate-free diet rats showed lower liver glycogen levels than did the carbohydrate-fed controls both before and after fasting. Similar results were obtained by Mackay and Bergman(3) who maintained rats on low and high protein diets. Before and after a 24-hour fast the animals which had received a low protein-high carbohydrate diet showed the highest liver glycogen values. This was also demonstrated by Bollman and Mann(4) in dogs. It should be mentioned, however, that in all of these experiments the animals were maintained for considerable periods of time on the restricted diets and that some of the high protein diets did not contain an adequate supply of B vitamins. It has been shown that a high protein intake necessitates a high B vitamin intake if the most efficient utilization of the diet and the optimum food consumption are to be obtained(5).

Until 1938 it was generally assumed that a high carbohydrate diet would produce the optimum accumulation and maintenance of carbohydrate reserves. The first suggestion that a high protein diet might be superior to a high carbohydrate one in the maintenance of carbohydrate reserves during fasting was made by Mirski, Rosenbaum, Stein and Wertheimer[6]. They stated that ". . . the carbohydrate reserves which are laid down on a protein diet behave differently from those deposited on a diet of high carbohydrate."

These authors found that in animals maintained for from 5 to 12 days on diets containing from 20 to 90 per cent casein there was little difference in the blood sugar or muscle glycogen levels immediately after the feeding period, but that the liver glycogen content of the carbohydrate-fed animals might be four times as high as that of the protein-fed rats. At the end of a 24-hour fast, on the other hand, animals maintained on a high carbohydrate diet (either wheat or a 20 per cent casein synthetic ration) showed liver glycogen levels of less than 0.10 per cent compared to a level of one per cent in animals fed meat or 70 per cent casein. Insignificant differences in blood sugar or muscle glycogen were observed after fasting. This capacity of a high protein diet to maintain liver glycogen reserves during a 24- or 48-hour fasting period was termed the "protein effect." This effect could be detected as early as 12 to 15 hours after the last food intake and was still evident at the end of a 48-hour fast. At the latter time, however, the control animals showed liver glycogen values of around 0.30 per cent.

Further experiments by Mirski et al.[6] showed that under other conditions which ordinarily deplete the carbohydrate reserves of the

liver, the "protein effect" was evident. Animals were exposed to cold, phloridzinized, injected with paratyphus B etc. In each case the animals which had received high protein diets were better able to maintain and regain liver glycogen reserves.

Work experiments were conducted and it was found that while immediately following the work period the carbohydrate reserves of all animals were very low, there was a more rapid rate of recovery of both muscle and liver glycogen in the protein-fed rats.

A three-day fasting experiment was performed. Significantly larger amounts of nitrogen were excreted during the first two days of the fast by the animals which had consumed a high protein ration. By the third day the difference had decreased but was still significant. The total acetone excretion of the protein-fed animals was less than that of the carbohydrate controls. At the end of the experiment the liver glycogen levels of both groups of animals averaged around one per cent.

It was shown by Mirski and co-workers[6] that the "protein effect" is abolished by the removal of the adrenal glands. This is scarcely surprising since there is much evidence that the adrenal cortex is intimately associated with carbohydrate metabolism, particularly during fasting. Long, Katzin, and Fry[7] have shown that although adrenalectomized animals are capable of normal carbohydrate metabolism while fed, there is a rapid disappearance of carbohydrate reserves and diminished nitrogen excretion in these animals during fasting. The significant decrease in nitrogen excretion has also been pointed out by Evans[8]. Furthermore, it has been demonstrated by Wells[9] that adrenalectomized animals are able to metabolize dietary protein but

unable to metabolize tissue protein during fasting.

According to Miraki[6] the minimum level of protein in the diet capable of eliciting a "protein effect" is 50 per cent, and the optimum level is 70 per cent. These workers found that as little as 24 hours of high protein feeding sufficed to produce the effect, and that high carbohydrate feeding for this length of time eliminated it. They were unable to explain the mechanism of the "protein effect" but concluded that "an intensified glyconeogenesis is the most important cause of the phenomena described."

Their findings in regard to the effect of high protein diets on the 24-hour fasting level of liver glycogen in rats have been confirmed by Newburger and Brown[10], and Mason Guest[11]. The latter used a more careful technique for controlling the length of the fasting period and found that within the inevitable limits of individual variations the liver glycogen levels of rats could be controlled by varying the composition of the diet.

Since there were no reports in the literature regarding the possible "protein effect" of individual amino acids the work reported below was undertaken.

PROBLEM

Individual amino acids were included in an otherwise satisfactory synthetic ration. The liver glycogen levels obtained after prefeeding these diets were determined after various fasting periods. In short, the capacity of these rations to elicit a "protein effect" was investigated.

EXPERIMENTAL

Animals. Albino rats of the Sprague-Dawley strain were used exclusively. Since Deuel[12] and others have shown that sex influences the rate and amount of glycogen deposited in rats, only male animals were employed. Heyman and Modis[13] found significant differences in the liver and muscle glycogen levels in rats from different age groups. For this reason an attempt was made to use animals between 2 and 3 months of age. The weights of the animals varied from approximately 200 to 300 grams. It is well known that the liver glycogen levels reported for fed and fasted rats show a significant lack of consistency even when the experimental conditions employed are similar. Hence a group of control animals was included in each experiment reported here.

Rations. Colony rats were maintained on Purina Laboratory Chow. The compositions of the control ration used in this work, and of two typical experimental rations are indicated in Table I. It will be noticed that in the experimental rations the amino acid (or casein) was substituted for an equal weight of dextrin in the control diet, but that all other constituents of the rations were identical. Thus a 10 per cent glycine diet refers to one containing 10 per cent glycine and only 44 per cent dextrin.

Table I
Composition of Typical Rations in Percentage

	Control	70% Protein	10% Glycine
Casein	16	66	16
Yeast (Squibb)*	10	10	10
Salt Mixture[14]	5	5	5
Cod Liver Oil	2	2	2
Wesson Oil	5	5	5
Dextrose	8	8	8
Dextrin	54	4	44
Glycine	0	0	10

*4.3 per cent protein was supplied by 10 per cent Squibb's Brewer's Yeast

Food intake was equalised among experimental and control animals. The amount usually eaten was 12 to 16 grams per rat per day, and only data from animals which consumed the designated amount of ration are included here.

Methods. Liver glycogen determinations were made by the method of Good, Kramer and Somogyi[15]. Animals were anesthetised with nembutal. When surgical anesthesia had been obtained a longitudinal abdominal incision was made and the liver excised. Liver samples of from 0.5 to 1 gram were minced into previously weighed tubes containing 30 per cent KOH. Thirty to 60 seconds elapsed between the time that the liver was excised and the samples emersed in the alkali. The tubes were again weighed to determine the size of the samples. After digestion on a boiling water bath, glycogen was precipitated by the addition of 1.1 volumes of 95 per cent ethyl alcohol. After appropriate centrifuging and draining, dilute HCl was added to each tube and the samples hydrolyzed at 100° C. for two hours. Glycogen was determined as glucose by the Shaffer Hartmann method.

Results are expressed as per cent of wet weight of liver tissue, since it has been found that the water content of the livers of rats on the various diets studied, does not vary significantly. Fuhrman[16] has also reported that the water content of rat liver is independent of the glycogen content.

Muscle glycogen determinations on the gastrocnemius muscle were made in the manner outlined above for liver glycogen.

Plan of a Typical Experiment. Rats were removed from the colony, weighed, earpunched and placed in individual cages. The cages were equipped with wire screen floors which limited coprophagy and water

was available at all times. For the first day all animals received a given amount of the control ration. This accustoms them to a synthetic diet and leads to improved food consumption on the experimental rations.

Twenty-four hours later all animals were reweighed and some put on experimental, and others left on the control ration. The experimental feeding period was 48 hours. If the animals were to be fasted, one-third of the second day's ration was withheld until the last two hours of the feeding period in order to limit the fasting period as closely as possible. Thus a 24-hour fast might in reality be a 24- to 26-hour fast, but not longer. Animals which were to be killed at 0-hours fast were removed from the cages at the end of the feeding period, weighed and sacrificed for liver glycogen determination as described above. In general animals were sacrificed between 8 and 11 a.m. A diurnal variation in the liver glycogen level of rats has been reported(17) but it is minimized if the animals are not fed in such a manner that all food consumption occurs during the night.

The typical experiment outlined above should not be confused with the type of experiment performed when the glycogenetic property of an amino acid, or other substance, is to be determined. The work reported here is primarily concerned with fasting levels of liver glycogen after prefeeding various diets. If the conversion of a particular compound to carbohydrate is to be determined, on the other hand, the experimental animals are fasted previous to the administration of the substance in question. In this manner liver glycogen levels are first reduced to a low level, then the capacity of the substance to form liver glycogen measured at various time intervals. The experiments reported here are intended to indicate the ability of various diets to maintain carbohydrate

reserves during fasting. They do not necessarily measure the conversion of an amino acid or a protein to carbohydrate.

Amino Acids Studied. Glycine, dl-alanine, l-leucine and l-glutamic acid were investigated and the capacity of each to cause liver glycogen accumulation during the feeding period, and maintenance of liver glycogen during fasting was determined. The "protein effect" of a high casein diet was also studied. It was felt that the effect of a 70 per cent protein diet on the liver glycogen level of 24-hour fasted rats, under the conditions described above, should be established. This value could then be used as a reference standard for the values obtained after prefeeding various amino acid diets.

RESULTS

I. Experimental diets containing 35, 50 or 70 per cent protein were studied. Data presented in Table II illustrate the type of results obtained after feeding diets high in casein. It is evident that while 35 per cent protein exerts no significant "protein effect," 50 per cent protein does elicit a "protein effect" and 70 per cent an even greater effect. As mentioned previously others have found that prefeeding a high protein diet allows maintenance of the carbohydrate reserves of the liver during a 24-hour fast.

Table II
Liver Glycogen Levels of Animals Prefed Protein Diets, and then Fasted 24 hours

Diet	No. of Rats	Average Liver Glycogen (Per cent wet weight)
Control	8	0.34
35% Protein	8	0.48
50% Protein	4	0.89
70% Protein	6	1.05

II. Glycine, or aminoacetic acid, was the first amino acid studied. Tentatively, it was included in the diet at a level of 50 per cent. This proved to be highly unsatisfactory as the food intake was low and the animals lost weight.

It was found that if 5, 10, or 15 per cent glycine were included in the ration the animals would eat an adequate amount and gain or maintain weight during the 48-hour feeding period. These levels were investigated with respect to their capacity to maintain liver glycogen during a subsequent 24-hour fasting period. Typical data are given in Table III.

Table III
Liver Glycogen Levels of Animals Prefed Various Glycine Diets, and then Fasted 24 hours

Diet	No. of Rats	Average Liver Glycogen (Per cent wet weight)
Control	4	0.40
5% Glycine	4	0.40
10% Glycine	4	1.20
15% Glycine	4	1.19

While 5 per cent glycine in the diet does not result in fasting liver glycogen levels which differ significantly from the control values, the effect of feeding 10 or 15 per cent glycine is striking. In both cases the liver glycogen levels, after a 24-hour fast, are comparable to those obtained in animals prefed a 70 per cent protein diet. (See Table II)

Since casein contains less than one per cent glycine[16] it was felt that one or more other amino acids might be capable of eliciting a "protein effect."

III. Casein contains approximately 22 per cent glutamic acid[18]. This amino acid was studied next. It was fed at 10 and 20 per cent levels, or in amounts approximately equimolecular with 5 and 10 per cent glycine respectively. The liver glycogen values found are illustrated in Table IV. It is apparent that at the levels studied this amino acid does not stimulate the maintenance of liver glycogen during a 24-hour fast.

Table IV
Liver Glycogen Levels of Animals Prefed Glutamic Acid Diets, and then Fasted 24 hours

Diet	No. of Rats	Average Liver Glycogen (Per cent wet weight)
Control	4	0.40
10% l-Glutamic	4	0.42
20% l-Glutamic	6	0.27

IV. Alanine is available in casein in considerably larger amounts than glycine, and resembles it in structure. It was investigated at levels of 6, 12, 18 and 24 per cent. The first three are equivalent to 5, 10, and 15 per cent glycine. Table V presents the type of data regularly obtained when 6 or 12 per cent dl-alanine were fed.

Table V
Liver Glycogen Levels of Animals Prefed dl-Alanine Diets, and then Fasted 24 hours

Diet	No. of Rats	Average Liver Glycogen (Per cent wet weight)
Control	4	0.24
6% dl-Alanine	4	0.27
12% dl-Alanine	4	0.10

It is clear from the above data that neither 6 nor 12 per cent dl-alanine in the diet elicits any "protein effect." In Table V_a are reported the results obtained in two experiments in which 18 per cent dl-alanine was fed, and one in which 24 per cent dl-alanine was used.

Table V_a
Liver Glycogen Levels of Animals Prefed dl-Alanine, and then Fasted 24 hours

Diet	No. of Rats	Average Liver Glycogen (Per cent wet weight)
Control	4	0.35
18% dl-Alanine	4	0.63
Control	4	0.46
18% dl-Alanine	4	0.35
Control	4	0.33
24% dl-Alanine	4	0.50

The above data show that 18 per cent dl-alanine elicits an inconsistent effect. In the first experiment above, all experimental animals had liver glycogen values above the control level. In the second experiment all of the experimental animals showed lower liver glycogen levels than the controls. In still other experiments with

this level of dl-alanine the values obtained have ranged from below the control value to one per cent, in a particular group of experimental animals. The effect of prefeeding 24 per cent dl-alanine seems to be somewhat similar.

V. A limited investigation was made of the effect of prefeeding l-leucine. It was found that a 17 per cent l-leucine diet (approximately equimolecular with 9 per cent glycine) showed no "protein effect." No data are available on the possible "protein effect" of this amino acid at other levels.

VI. It was thought that the liver glycogen levels of animals immediately after feeding various diets might relate to the liver glycogen levels obtained after a 24-hour fast. Figure 1 illustrates the total lack of correlation found.

VII. In Table VI the total nitrogen contents of some of the diets studied are tabulated and the "protein effect" of the diets indicated. It is evident that no correlation exists (except in the case of the casein diets) between the total nitrogen contents of the diets and their capacity to maintain liver glycogen during a 24-hour fast.

Table VI
Total Nitrogen of Diets Studied and "Protein Effect"

Diet	Total N (Per cent)	"Protein Effect"
Control	3.20	--
10% Glycine	5.07	++
12% dl-Alanine	5.08	--
20% l-Glutamic	5.10	--
17% l-Leucine	5.03	--
35% Protein	5.46	--
15% Glycine	6.00	++
18% dl-Alanine	6.03	±
50% Protein	7.77	+
70% Protein	10.85	++

FIGURE 1

Liver glycogen levels of

animals prefed various diets

for 48 hours, then fasted

0 or 24 hours.

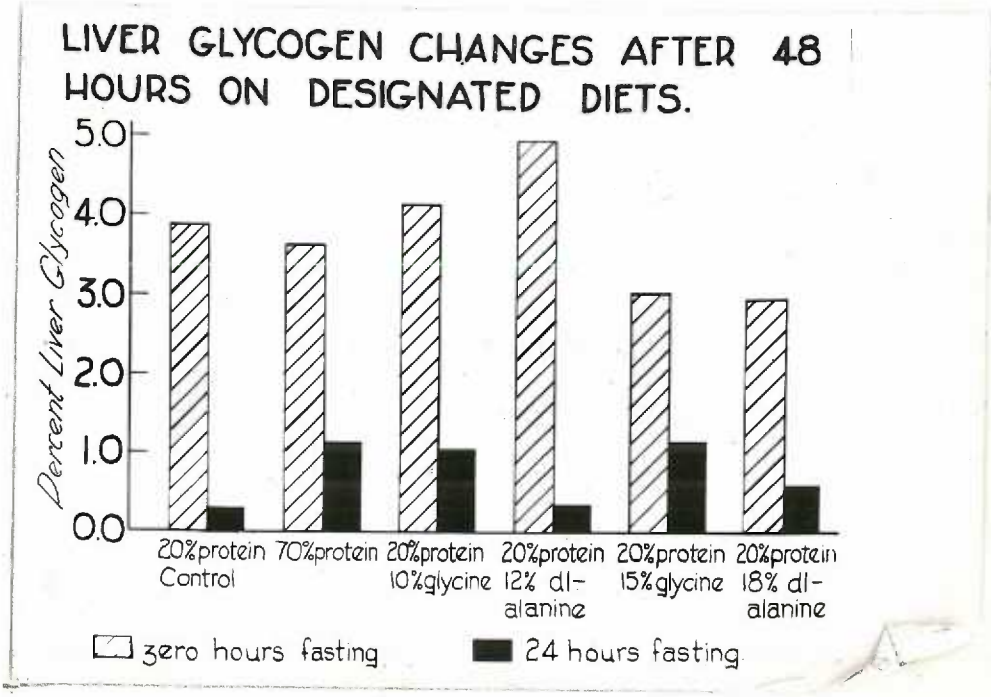
LIVER GLYCOGEN CHANGES AFTER 48 HOURS ON DESIGNATED DIETS.
Percent Liver Glycogen
5.0
4.0
3.0
2.0
1.0
0.0
20% protein Control
70% protein
20% protein 10% glycine
20% protein 12% dl-alanine
20% protein 15% glycine
20% protein 18% dl-alanine
zero hours fasting
24 hours fasting

FIG. 1

FIGURE 2

Liver glycogen levels of

animals fed 10% glycine or control diet

for 48 hours, then fasted

16, 24, 32 or 48 hours.

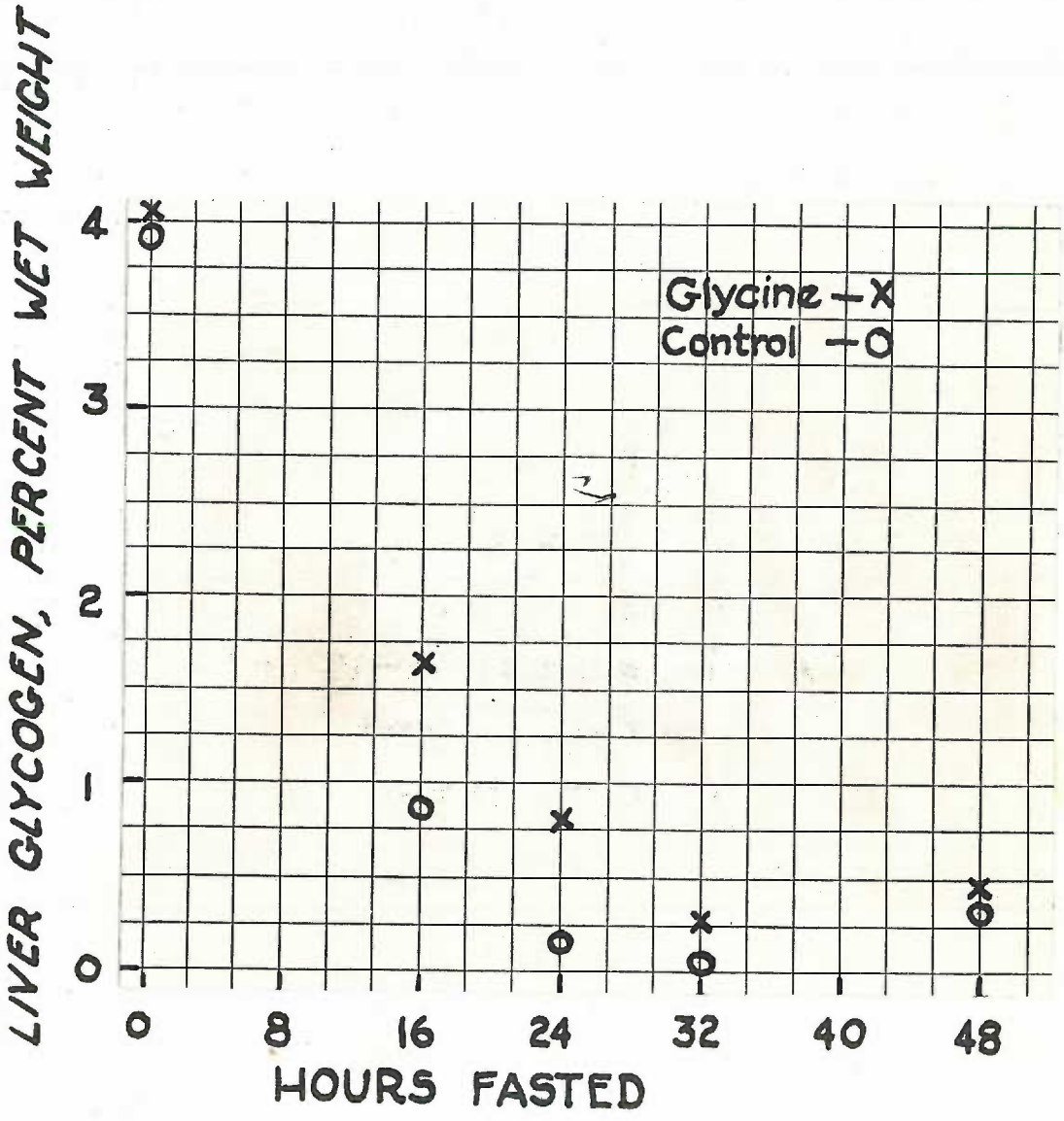
LIVER GLYCOGEN, PERCENT WET WEIGHT
4
3
2
1
0
Glycine – X
Control – O
0
8
16
24
32
40
48
HOURS FASTED

FIG. 2

VIII. A summary of some of the data obtained from animals fed glycine or alanine diets is presented in Table VII. The data show conclusively that in some manner 10 or 15 per cent glycine, when incorporated in the ration for a 48-hour feeding period, causes considerable accumulation of liver glycogen in fed animals and maintenance of liver glycogen in animals which have fasted for 24 hours. On the other hand, 12 per cent dl-alanine causes an extraordinary accumulation of liver glycogen in fed animals but no maintenance during fasting, while 18 per cent dl-alanine behaves in an unpredictable manner.

Table VII
Typical Liver Glycogen Levels of Rats Prefed Diets 48 hours, Fasted 0 or 24 Hours

Diet	Mean Liver Glycogen (Per cent Wet Weight) Hours Fasted	
	0	24
Control	3.9(14)*	0.33(30)
10% Glycine	4.2(8)	1.10(21)
15% Glycine	3.1(13)	1.17(16)
12% dl-Alanine	5.0(8)	0.36(23)
18% dl-Alanine	3.0(14)	0.56(22)

*Figures in parentheses indicate number of animals represented.

IX. An attempt has been made to determine the possible mechanism of the "protein effect" of glycine. Swimming experiments were conducted and the rate of recovery of liver and muscle glycogen after the exhausting exercise was determined. It was not possible to show any increase in rate of recovery in animals which had been prefed glycine nor was it possible to confirm Mirski's[6] results in this particular regard with high protein diets.

It was thought that if the liver glycogen level in animals prefed glycine were to drop appreciably during the 24-hour fasting period, this might serve as a stimulus for glyconeogenesis and account

for the high liver glycogen levels found after a 24-hour fast. Figure 2 shows that during the 24-hour fasting period the liver glycogen levels of animals prefed glycine do not appear to drop as rapidly or to as low levels as do those of the controls.

As pointed out previously[6] the "protein effect" cannot be obtained in adrenalectomized animals. Preliminary data obtained in animals adrenalectomized by Mr. Lew Cunningham show that the effect of glycine is also abolished in the absence of the adrenals. Sufficient data are not at hand to indicate whether the adrenal gland must be intact (or cortical extract supplied) during the feeding period as well as the fasting period in order to obtain the "protein effect" of glycine.

DISCUSSION AND CONCLUSIONS

The various points in the following discussion are taken up in the order in which they appear under results.

I. The "protein effect" of a 50 or 70 per cent protein diet (casein and yeast) first reported by Mirski[6] and later confirmed by others[10,11] has been reaffirmed. The mechanism of this effect is not understood. According to Mirski[6] it results from an increased glyconeogenesis. Paschkis and Schwoner[19] have reported that there is a principle in the anterior pituitary, distinct from the growth hormone, which regulates protein metabolism. They say that the stimulus for the secretion of this hormone is protein feeding. It has also been reported that feeding excess protein leads to nitrogen retention during the first days of the feeding period[20] and that animals which have been on a high protein diet excrete significantly larger amounts of nitrogen during the first 24 to 48 hours of a fast than do carbohydrate-fed animals[6,21,22].

The "protein effect" cannot be obtained in bilaterally adrenalectomized animals[6]. This is to be expected since the adrenal glands appear to be essential to the maintenance of carbohydrate reserves in fasting animals regardless of the previous diet[7,9]. The explanation of this lies in the glycostatic capacity of the cortical hormones and their role in glyconeogenesis in fasting animals[9,23,24].

A possible explanation for the "protein effect" involves the assumption that there is increased protein anabolism during the feeding period due to the secretion of the anterior pituitary hormone described by Paschkis and Schwoner[19] and that protein is rapidly mobilized and converted to carbohydrate under the influence of the adrenal cortical

hormones during the fasting period. This explanation, however, lacks proof and does not take into account the fact that a decreased rate of glycolysis may also be instrumental in producing the "protein effect."

II. It has been demonstrated that while 5 per cent glycine included in an otherwise satisfactory diet has no influence on carbohydrate maintenance during fasting, rations containing 10 or 15 per cent glycine elicit a "protein effect" similar to that resulting from a 70 per cent protein diet. The mechanism of the "protein effect" of glycine is also not understood. Discussion of the possible mechanisms will be deferred to paragraph IX.

III. The data show that if l-glutamic acid is fed at levels equimolecular with 5 or 10 per cent glycine no "protein effect" is demonstrable. The possible effect of this acid if fed at higher levels has not been investigated. Why this excellent glycogen former does not elicit a "protein effect" at the levels studied is not clear.

IV. When dl-alanine is fed at levels equimolecular with 5 or 10 per cent glycine, no "protein effect" is obtained. If the level is increased to 18 per cent (approximately equivalent to 15 per cent glycine), however, an inconsistent effect is demonstrable. The great individual differences found in animals prefed 18 per cent dl-alanine are not easily explained.

V. It was found that l-leucine when fed at a level approximately equimolecular with 9 per cent glycine is incapable of maintaining liver glycogen during a subsequent 24-hour fast. No data is available on the possible effect of this acid if fed at higher levels.

VI. It was thought that the level of liver glycogen obtained at 0 hours fast might be related to the level found after a 24-hour fast.

Inspection of the data reveals absolutely no correlation between 0- and 24-hour fasting liver glycogen levels. This indicates that the mechanism of the "protein effect" must be accounted for by more subtle means than a simple, constant glycogen withdrawal during fasting.

VII. Since a 35 per cent protein ration produces no "protein effect" while 50 per cent protein elicits a significant effect, and 70 per cent a marked effect, it was thought that the total nitrogen content of the diet might be a determining factor in relation to the "protein effect." With the exception of the casein diets, however, no correlation exists between the nitrogen content of the diets studied and the "protein effect" elicited by them. Ten per cent glycine, for example, elicits a significant "protein effect" while roughly equivalent amounts of dl-alanine, l-glutamic, and l-leucine do not.

VIII. The data obtained from animals prefed various levels of glycine or dl-alanine emphasizes the fact that though dl-alanine is capable of causing normal or excessive accumulation of liver glycogen during the feeding period, it does not elicit a "protein effect" comparable to that exerted by equivalent amounts of glycine in the diet. This data also indicates that the glycogenetic capacity of an amino acid is not a criterion of its ability to elicit a "protein effect" at the levels investigated. There is a growing feeling that glycine is not converted to carbohydrate (see IX) yet glycine elicits a consistent "protein effect." Alanine is generally considered to be an excellent glucose and glycogen former but is of dubious value in maintaining carbohydrate reserves during a 24-hour fast. This last statement also applies to l-glutamic acid.

IX. Some further investigations were made regarding the effect of glycine on the carbohydrate reserves of the liver in exercised, fasted and adrenalectomized animals.

The rapid recovery of liver and muscle glycogen found by Mirski[6] in protein-fed animals after exhausting exercise could not be demonstrated in animals which had received glycine diets.

It was found that during the fasting period following glycine feeding, no rapid decrease in liver glycogen occurred which might have acted as a stimulus for glyconeogenesis.

The removal of the adrenal glands resulted in the elimination of the effect ordinarily exerted by glycine. This is consistent with the well-known functions of adrenal cortical hormones.

The "protein effect" of glycine is not understood. It has been pointed out that the total nitrogen content of the diet is not the determining factor though it may be related to the "protein effect" of the high protein diets. The 0-hour liver glycogen level is not a determining factor. The glycogenetic properties of an amino acid do not determine its capacity to elicit a "protein effect."

It has recently been shown by Olsen, Hemingway and Mier[25] that if glycine containing C^{13} in the carboxyl group is administered to fasted rats, very little of the isotope appears in the considerable liver glycogen deposited. During the 16 hours following the administration of the amino acid, however, 50 per cent of the tagged carbon may be isolated from the respiratory CO_2. These authors conclude that glycine stimulates glyconeogenesis from other molecules. This confirms Dakin's[26] earlier theory that glycine is not converted to carbohydrate but stimulates glyconeogenesis.

The work reported here may be construed as further evidence for the stimulation of glyconeogenesis by glycine. It is apparent that the adrenal cortex is essential to this process, in fasting animals, but the mechanism and site of action are not known. It is clear that, under the conditions employed, glycine is able to cause the maintenance of liver glycogen during a 24-hour fast, that neither l-glutamic acid nor l-leucine behave in this manner, while dl-alanine is only somewhat effective.

SUMMARY

The capacity of a high protein diet to maintain liver glycogen in rats during a 24-hour fast reported by others(6,10,11) has been confirmed. This phenomenon is referred to as the "protein effect."

A marked effect on carbohydrate storage in rats during starvation has been demonstrated as a result of prefeeding glycine.

After consuming a diet containing 10 or 15 per cent glycine (replacing an equal weight of carbohydrate in control ration) for 48 hours, the animals have liver glycogen levels of over one per cent at the end of an ensuing 24-hour fast. Under like conditions rats on the control ration have liver glycogen levels that average around 0.3 per cent.

The amino acids l-leucine and l-glutamic acid do not show this effect when fed at levels equimolecular with 10 per cent glycine. At this level dl-alanine also shows no effect. At a level equimolecular with 15 per cent glycine, however, dl-alanine shows an inconsistent effect.

Adrenalectomized rats exhibit no "protein effect" as the result of prefeeding glycine.

It is felt that sufficient data are not available to state whether the "protein effect" of glycine is due to increased glyconeogenesis, decreased glycolysis, or both. The possibility that glycine stimulates glyconeogenesis from other molecules is discussed.

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