A STUDY OF THE EFFECT OF FEEDING GLYCINE AND ALANINE ON THE CARBOHYDRATE STORES OF RATS BEFORE AND AFTER ADMINISTRATION OF INSULIN

by

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INTRODUCTION

Students of the effects of feeding individual amino acids on carbohydrate metabolism were originally concerned chiefly with the extent to which these metabolites are converted into glycogen or glucose. Unfortunately, since the first work of Ringer and Lusk[1], published in 1910, reports on this subject have become more and more contradictory. Recent studies with isotopic tracers have challenged the accepted views of the gluconeogenetic capacities of glycine[2] and alanine[3], the two amino acids which have been most frequently employed in such studies.

Meanwhile, knowledge has been gained which suggests the impracticability of considering the metabolism of any one amino acid separately from that of other substances. Interactions of amino acids in metabolism are exemplified by the role of glutamic acid in transamination, and by that of arginine in the formation of urea. Certain of the amino acids have even been reported to cause striking physiological effects of the kind commonly associated with the action of drugs. Examples are the growth inhibition produced by feeding glycine[4], and the improved learning ability repeatedly claimed to follow feeding of glutamic acid[5] [6]. Contradictions in the evidence for gluoneogenesis from alanine and glycine have prompted a search for unknown complicating factors in their relations to carbohydrate metabolism.

It was established by McKay, Wick, and Carne[7], in 1940, that administration of glycine by stomach tube to fasted rats has a delayed effect on liver glycogen. This began to increase after ten hours and reached a maximum concentration about six hours later. Racemic alanine, in doses equivalent in carbon content, caused a similar, but immediate and shorter-lasting increase; added glycogen appeared almost

as rapidly as after administration of a corresponding amount of glucose. However, Olsen, Hemingway and Nier[2], using a similar technique in mice but feeding glycine tagged with C^{13} in the carboxyl group, found a concentration of the isotope in the liver glycogen, after sixteen hours, corresponding to only one glycine unit in every four or five glucose units. (However, elimination of CO_2 increased and half the isotopic carbon was recovered in this form.) There is some evidence, also, that the period of increased gluconeogenesis following administration of dl-alanine, indicated by the work of McKay et al, involves the transformation into carbohydrate of other amino acids than that fed. Gurin and Wilson[3], after injecting alanine containing C^{13} into phlorhizinized dogs, found only small amounts of the isotope in the extra excreted glucose.

It should be pointed out that McCay *et al*, Olsen *et al*, and others using similar techniques, departed widely from natural conditions in that they fed glycine separate from other amino acids. Obviously, only artificial conditions could permit its absorption and entry into tissues without the simultaneous absorption and entry into tissues of other amino acids liberated by hydrolysis of protein. There is no proof that the presence of other recently absorbed amino acids might not alter the physiological effect of glycine. Nor can it be assumed that the effect of feeding it after a prolonged fast is the same as that of administering it to recently-fed animals.

Recently, Todd, Barnes and Cunningham[8] have reported studies of the effects of amino acids on liver glycogen, using a technique which approaches more nearly to normal conditions. Glycine was fed to rats as part of a satisfactory synthetic diet, at levels of 10 per cent

and 15 per cent of the whole ration. Controls were fed an identical ration, except that the free amino acid was replaced by an equal amount of dextrin. Racemic alanine was studied similarly at levels equimolar with 10 and 15 per cent glycine. All these diets had a normal carbohydrate content, and contained the essential foodstuffs in adequate amounts, with an especially rich supplementation of "B" vitamins. Animals fasted 24 hours after feeding 10 per cent or 15 per cent glycine diets showed a reduction in the rate of loss of liver glycogen, as compared to the levels found in animals fed control rations. Alanine, at the highest level studied, gave similar but less striking effect.

Maintenance of relatively large glycogen stores during fasting has also been observed in rats after feeding diets made up predominantly of protein[9] [10] or of fat[11]. The similarity of these effects to those of glycine-supplemented diets indicated that such effects might have a common cause, namely, accelerated gluconeogenesis. A study of the literature suggested that, if these three types of diets did influence carbohydrate stores through the same mechanism, glycine feeding should increase the resistance to the hypoglucemic action of insulin.

Several workers have reported a marked decrease in the incidence of coma, convulsions, and other signs of insulin hypoglucemia in animals fed high-fat diets, compared to those fed normal diets. Roberts and Samuels[12], using rats fed the two contrasting diets (85 per cent of calories as carbohydrate or as fat), showed, after injection of fairly heavy doses of insulin, that the blood-sugar curves were similar in the two groups until three hours after

injection, when the values began to rise from minimum levels. The high-fat-fed group then recovered more rapidly, reaching essentially normal levels by the fifth hour while the others had not. Using delicate technique with human subjects, Himsworth[13] [14] demonstrated increased resistance to the hypoglucemic action of very small intravenous doses of insulin, after a high-fat diet. His work was later confirmed by Lundbaeck and Magnussen[15], using larger insulin doses given to schizophrenics.

That feeding high-protein (casein) diets to rats resulted in changes in resistance to the hypoglucemic action of insulin similar to those seen after feeding high-fat diets is indicated by two early reports. One of these[16] is unfortunately marred by use of a small number of animals, the other[17] by use of rectal temperatures, as the only means of determining the degree of hypoglucemia. However, this effect of a high-protein diet (70 per cent casein) has been recently confirmed in this laboratory.

Previous reports on the effect of glycine on the response to insulin have been contradictory and inconclusive, and in no case have the workers used a technique for administration of the glycine comparable to that of Todd et al. Shortly after the discovery of insulin, Voegtlin, Dunn, and Thomson[18] observed the effect of various substances, fed by stomach tube to fasted rats, on the mortality following simultaneous injection of a very heavy dose of insulin. No determinations of blood sugar are reported. It is not surprising that under these conditions glycine afforded no protection. This is scarcely surprising in view of the delayed effect on liver glycogen reported by McKay[7]. Alanine, however, gave protection equal to that of glucose, given in amounts

equivalent in carbon content. (Contrary to the hypothesis of these workers, the difference between the two amino acids must have been independent of their rates of absorption from the intestine, which have been shown subsequently to be practically the same.[19] [20].) Costa[21] [22] has recently reported essentially opposite results, using rabbits given insulin shortly after the administration of glycine; he claims to have obtained a hyperglucemic effect of insulin under such circumstances, together with a marked increase of liver glycogen. He postulates that insulin accelerates gluconeogenesis from glycine, contrary to the generally accepted belief that insulin depresses gluconeogenesis.

Because of the difficulty in interpreting such evidence as this, it was decided to investigate the effect of diets containing 15 per cent glycine and 18 per cent alanine on resistance to the hypoglucemic action of insulin, using a technique comparable, as nearly as possible, to that of Todd et al[8]. At first, studies were limited to effects on blood sugar, but the results obtained prompted extension of the work to cover liver and muscle glycogen in animals fed the control and 15 per cent glycine diets. Other experiments were performed in attempts to determine the mechanism of the effects observed.

PROBLEM

The following investigations were carried out:

1. Blood sugar of rats fed various diets was determined before and after administration of insulin. The following diets were fed:

A. A diet containing 15 per cent glycine as the free amino acid.

B. A diet containing 18 per cent dl-alanine as the free amino acid.

C. A control diet containing 16 per cent casein.

II. The following determinations were performed on animals fed the control diet, and on animals fed the glycine diet, before and after administration of insulin:

A. Blood sugar.

B. Liver glycogen.

C. Muscle glycogen.

III. On other rats fed the control and glycine diets, the following determinations were performed, before and after administration of insulin:

A. Liver glycogen.

B. Muscle glycogen.

C. Total carbohydrate of the contents of the stomach and small intestine.

D. Total free glycine of the contents of the stomach and small intestine (in rats fed the glycine diet, only).

EXPERIMENTAL

Animals. Healthy albino rats of the Sprague-Dawley strain, raised in our own colony, were used exclusively. Because the supply of animals was limited, and because rats once used in these experiments could be employed later only for breeding#, two types of rats had to be used: young, virgin females, two to three months old weighing approximately 150 to 200 grams, and adult or nearly-adult males weighing approximately 275 to 400 grams. For individual experiments, rats of the same sex and approximately the same age were used throughout. The weight-range for individual experiments seldom exceeded 10 per cent above or below the average weight, and the different experimental groups were matched as closely as possible according to weights of individual rats. For the experiments in which glycogen was measured, only young females were employed.

Rations. Before use in experiments, rats received Purina Laboratory Chow exclusively. The composition of the synthetic experimental rations conformed to the diets employed in earlier work in this laboratory. All rations contained Squibb's Brewer's Yeast, 10 per cent; cod liver oil, 2 per cent; Wesson oil, 5 per cent; casein, 16 per cent; and glucose, 8 per cent. Other ingredients, varying with the diet, are shown in Table I:

#In preliminary experiments, the desirability of using each animal as its own control was investigated. A cross-over technique, like that used in assaying insulin in rabbits (23), was applied. However, the variations in response of individual animals after injection of insulin proved to be as troublesome as variations between different animals of the same group, and the results were not as significant, statistically, as they would have been if each value had been obtained from a different rat.

Table I

Percentage of Dextrin and of Amino Acids in Rations

Ration	Dextrin	Added Amino Acid
"Control"	54	0
"Glycine"	39	15
"Alanine"	36	18

It will be noted that all these rations contain more carbohydrate than fat or protein. Even in the alanine ration, total carbohydrate is 44 per cent, total protein and free amino acid, 38 per cent (counting the protein of the yeast).

Food consumption on the glycine ration tended to be lower than on the other rations. This difficulty was handled by paired feeding as described under Plan of Experiments.

To permit stomach-tubing, the rations described above were divided into two parts, one to be eaten ad libitum by the rats, one to be dissolved in water and administered by stomach tube, each to be fed separately. Solutions of the amino acid (for the glycine and alanine diets) and a solution of glucose, were prepared, mixed in proper proportions after cooling# and diluted to a volume such that the volume of fluid to be given by tube (4-8ml, depending on the size of the rat) contained an amount of amino acid and of carbohydrate corresponding to the amount of ration intended to be given by the two methods combined. Thus, glucose replaced an equal weight of dextrin, which was not used for stomach tubing because of its low solubility. Other constituents of the ration, mixed in proper proportions, were fed separately.

#Glycine and glucose react, above room temperature, to give colored products, as described by Maillard (24). Similar reactions are also observed on standing at room temperature over a period of weeks.

Determinations. Blood for analysis was obtained by a technique similar to methods previously described[25]. The animal was immobilized by rolling snugly in a towel. Escape was prevented by fastening together free edges of the towel with hemostats, leaving the tail outside the pack. The tail was soaked in xylol to promote vasodilatation and increase blood flow, then dried, and severed about one centimeter from the tip. This operation appears to cause the animal temporary pain, but usually no marked agitation.# The tail was passed between the thumb and forefinger with very gentle pressure to reverse the flow of blood in the veins, and enough blood for sampling thus obtained in a few minutes. The color and manner of flowing of such blood is characteristic of a venous, rather than a capillary, origin.

Drops of blood were collected in a depression of a spot plate containing dried potassium oxalate. A 0.1 ml. sample was obtained with a micropipette for glucose determination by Somogyi's modification of the Shaffer-Hartman method, as further modified by Nelson[26] for colorimetric use. The blood was laked in fifteen parts of water. Protein was precipitated according to the method of Somogyi, by the addition of barium hydroxide and zinc sulfate, and the sample was centrifuged. One ml. of the clear, supernatant fluid was mixed in a Folin-Wu sugar tube with one ml. of Nelson's reagent, which is identical with Somogyi's modification of the original Shaffer-Hartman reagent, except that it

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That excitement attendant on obtaining blood samples by this technique has no significant effect on blood sugar is indicated by results of preliminary experiments. Fasting blood sugars of unanesthetized rats, and of rats anesthetized with 56 mg/kg Nembutal, given intra-peritoneally one-half hour before sampling, were wholly comparable.

contains no iodide or iodate. After this mixture was heated for 20-minutes on a boiling water bath, it was cooled, and one ml. of Nelson's chromogenic reagent, an arsenomolybdate complex, was added, producing a stable color. The solutions were made up to a given volume and read in a Klett-Summerson photoelectric colorimeter. This method has been found to have many advantages: it is quite rapid; readings are directly proportional to the quantity of glucose present; standard readings are highly reproducible over long periods of time and blood sugar values agree well with those obtained by other methods. Duplicate readings, obtained with known quantities of sugar, seldom differ by more than one part in seventy. For good agreement of duplicate blood samples, thorough stirring is necessary before drawing the samples, a fact which seems to confirm Himsworth's opinion[13] that sedimentation of erythrocytes is an important source of error in such work.

Liver and muscle glycogen were determined by the method of Good, Kramer and Somogyi[27], which has been widely used for this purpose. Animals were given large doses of Nembutal intraperitoneally (0.5 ml.). After onset of anesthesia, but before death, the abdomen was opened and about one gram of the left lobe of the liver removed, minced with scissors, and dropped into a weighed centrifuge tube containing 2 or 3 ml. of 30 per cent KOH. Less than 30 seconds elapsed between excision of the liver and its emersion in the alkali. Then a sample of the gastrocnemius muscle was obtained similarly, using almost the whole muscle. Tubes were reweighed and contents digested on a boiling water bath. Glycogen was precipitated with 1.1 volumes of 95 per cent ethyl alcohol. Tubes were centrifuged, decanted, and allowed to

drain and the excess alcohol was evaporated on the water bath. An excess of .6N HCl was added and the glycogen hydrolyzed for two hours on the boiling water bath. Glucose found was determined by the Shaffer-Hartman method.

For determination of total carbohydrate of the contents of the stomach and small intestine, these organs were removed after liver samples were obtained, and preserved on ice. After a few hours, they were slit lengthwise and the contents thoroughly removed (using the fingers) and washed into a beaker. Twenty-five ml of concentrated HCl were added and the suspension made up to 500 ml, bringing the concentration of HCl to 0.6N. A 10-cc aliquot was then hydrolyzed on a boiling water bath for two hours, made up to known volume, filtered, producing a clear solution for determination of glucose by the Shaffer-Hartman method.

Plan of Experiments. On the first day of each experiment, rats were weighed and earpunched, and divided into two, three, or in one instance, four, groups, usually of four rats, each group to be fed different diets. Because rats if fed ad libitum would usually eat more of the control and alanine diets than of the glycine diet, pair-feeding was used in all experiments. Rats to be fed the glycine diet were started on the experimental regimen immediately, while those to be fed other diets were given Purina Laboratory Chow for one more day. It will be convenient to describe the treatment of the rats fed the glycine diet first. They were placed in individual cages which were provided with water fountains, screen floors to prevent coprophagy, and large food cups so designed as to reduce spilling and contamination of food to a minimum.

On the afternoon of the first day rats were given weighed amounts of control ration (the amount varied with the size of the rats, but usually was 6 or 8 grams). The purpose of the initial feeding of control ration was to accustom the animals to powdered synthetic rations, to promote more uniform consumption of the glycine ration by limiting and controlling previous food intake, and to avoid feeding of the glycine ration during the period of adjustment to changed conditions in the new cage when the animals tend to increase their activity and lose weight.

On the afternoon of the next day, rats were again weighed. The uneaten ration, if any, was weighed and discarded. Eight to twelve grams of the glycine ration were then offered, depending on the size of the rat. About noon of the next day, animals were again weighed, and their food consumption noted. They were offered a smaller amount of the same ration (usually four grams, but sometimes two, or even three, for smaller rats). This was usually all eaten by the evening of the same day, when food cups were removed and consumption again noted.

In order to measure the fasting period accurately, it was necessary to prevent, as far as possible, individual differences in the speed with which the last meal was eaten. Fortunately, the carbohydrate and amino acid of the diets, which are the most soluble portions, and therefore the most easily given by stomach tube, are theoretically he most important constituents from the standpoint of response to the hypoglucemic action of insulin. Besides, they would appear to constitute the principal limiting factors for the consumption of the synthetic diets; the carbohydrate because of its bulk, and the amino acid because of its taste. Therefore, the following technique was adopted:

About midnight, after the second day on the glycine ration, the rats were offered a mixture of the protein, salts, yeast and fats of the ration, the amount being in proportion to the desired weight of dry ration in the last meal (4-8 grams). A majority of the rats ate all of their portion promptly, within the allowed time (1-1/2 to 2 hours). (Failure to do so could not be correlated with the results of the experiments.) At about 1:30 AM, the rest of the ration, prepared in water solution as described above, was given by stomach tube, all rats being fed in this way within fifteen minutes. Rats were then replaced in individual cages, without food. Rarely, rats showed distress or diarrhea following this procedure, and therefore were not used for the rest of the experiment.

Next morning, rats were again weighed. After 8-9 hours (depending on the individual experiment) of fasting following tubing,# blood samples were obtained and insulin immediately administered. The time of insulin injection for each rat having been recorded, further blood samples were obtained at desired intervals.

Animals to be fed other diets were paired with glycine-fed rats according to weight. Each manipulation was performed 24 hours later than the corresponding procedure with glycine-fed rats. Their particular diet was substituted for the glycine ration which was eaten by the corresponding glycine-fed rats in the corresponding period.

Absorption of carbohydrate and glycine at this time has been shown to be practically complete.

Almost invariably they ate all, or practically all, the ration allowed them. Utmost care was exercised to insure that time intervals between stomach-tubing and injecting insulin, and between injection of insulin and further blood samplings, were, as nearly as possible, identical in pairs of rats fed different diets.

In two experiments, only glycine and control diets were studied, and animals were sacrificed for liver and muscle glycogen determinations. The plan of the experiments was the same as that described above, with these exceptions: Among both glycine-fed and control-fed animals, four rats, in one group, were killed after the first blood sample was obtained; without receiving insulin; four rats in a second group were killed immediately after a second blood sample was obtained, five hours after receiving insulin.

In one experiment, rats fed glycine and control rations were killed before, and five hours after, receiving insulin, and determinations made of total carbohydrate of the contents of the stomach and small intestine. Liver and muscle glycogen determinations were also carried out.

Insulin. For each experiment, an insulin dose was chosen for all the animals, which, previous experience indicated, would permit partial, but not complete, recovery from minimum blood sugar levels within five hours after injection in the control animals. The doses employed were 7-1/2, 10, and 12 units per kilogram, given subcutaneously. The cause for the variation in general resistance to the hypoglucemic action of insulin, which made such adjustments necessary from time to time, remains unknown. In general, however, young female rats appeared to be more resistant than older males.

The choice of intervals for sampling of blood after insulin injection is discussed in the section on results.

RESULTS

Listing of results will follow the outline of the investigation given under PROBLEM.

1. Blood sugar of rats fed various diets was determined before and after administration of insulin. The diets studied were: 15 per cent glycine, 18 per cent alanine and control.

Times for obtaining blood samples after injection of insulin were determined according to two different plans. In some experiments three or four samples were obtained at intervals so that blood sugar curves could be drawn for each rat. These experiments will be considered first. Composite curves for animals fed control and glycine diets are shown in Figure I. The general shape of these curves is essentially independent of the pre-fed diet, although maximum depression is greater in control-fed animals, and the rate of recovery is greater in animals fed glycine, (Curves obtained on animals fed alanine follow, in general, an intermediate course.) It will be noted that the greatest divergence between glycine-fed and control-fed animals occurs in the period of recovery from hypoglucemic levels (Period II, Fig. I).

In other experiments blood sugar determinations were made just before and at 5 or 6 hours after insulin administration, thus curves for these have not been plotted. The curves in Fig. I show the relationship of these time-intervals and the typical blood sugar curves. Such experiments were conducted on about 60 rats to compare effects of different diets on the resistance to the hypoglucemic action of insulin. Table II gives, for a typical experiment, values for blood sugar before and after insulin. Table III gives average post-insulin blood sugar values for a number of such experiments.

FIGURE I

Typical blood sugar curves of animals fed the control and glycine diets, showing the effect of insulin.

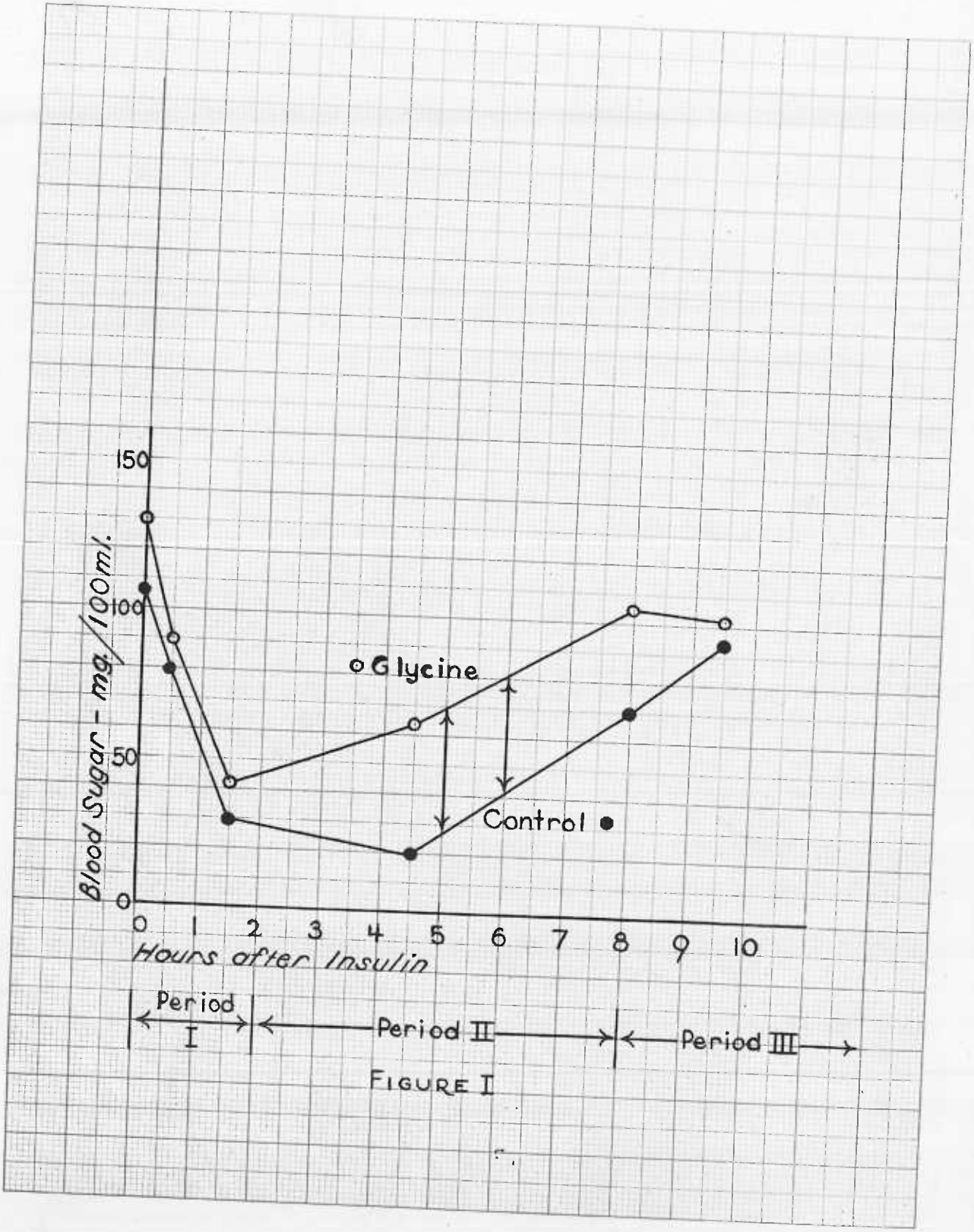
150
100
50
0
Blood Sugar - mg./100ml.
o Glycine
Control •
0 1 2 3 4 5 6 7 8 9 10
Hours after Insulin
Period I
Period II
Period III

FIGURE I

TABLE II
Blood Sugar Values of a Typical Experiment

GLYCINE DIET	Before Insulin	After Insulin
Rat No.		
1	135	99
2	123	101
3	117	85
4	120	81
AVERAGE	124	91

ALANINE DIET		
Rat No.		
1	104	88
2	116	90
3	93	87
4	121	47
AVERAGE	108	78

CONTROL DIET		
Rat No.		
1	104	78
2	117	55
3	104	46
AVERAGE	108	60

TABLE III

Blood Sugar Values After Insulin

Experiment No.	GLYCINE		ALANINE		CONTROL	
	No. Rats	Average Blood Sugar	No. Rats	Average Blood Sugar	No. Rats	Average Blood Sugar
1	3	95	4	60	1[X]	30[X]
2	4	91	4	78	3	60
3	3	80	4	49	4	38
4	5	97	-	---	2[XX]	46
5	7	104	-	---	8	73

[X] 2 rats died of hypoglucemia before samples were obtained.

[XX] 3 rats died of hypglycemia before samples were obtained.

It will be seen from Table III that the post-insulin blood sugar values for glycine-fed animals are always markedly higher than for animals fed the control ration, although values for the latter, especially, vary greatly in different experiments. This relation is consistent, in spite of differences in age and sex of animals, and differences in details of experimental technique. The differences between the glycine ration and the alanine ration, and between the alanine ration and the control ration, are small, but the t-test of Fisher[28] shows that they are significant. The value of P for the difference between alanine and control is .02, which is well within the limits of statistical significance. The value of P for the difference between glycine and alanine is .003, which may be termed highly significant.

A comparison of the blood sugar values before insulin also brings out significant difference between different diets. In general, the values in all experiments are similar to those in Table II, and there is much less variation from experiment to experiment than is shown among the post-insulin values, in Table III. The average blood sugar levels of 28 rats fed the glycine diet is 131, with a standard deviation of 19.6. The average for 28 rats fed the control diet is 110, with a standard deviation of 17.8. The difference between these averages has a very high order of significance. The average sugar values of rats fed the alanine diet is not significantly different from that of those fed control, but the difference between the averages for alanine-fed rats and the glycine-fed rats is highly significant. (P equals .006.)

There appears to be little correlation between pre-insulin and post-insulin blood sugar values in individual rats, except for the fact,

as pointed out previously, that the glycine-fed rats have a markedly higher blood sugar, both before and after insulin. This lack of correlation is illustrated by Table II. It is possible that data on larger numbers of animals would demonstrate such a relation does exist.

II. Blood sugar, liver glycogen, and muscle glycogen of rats fed the glycine and control diets were determined, before and after administration of insulin. Average values are shown graphically in Figure II, and data from typical animals appear in Table IV. The blood sugar values are quite comparable to those obtained in experiments in which the animals were not sacrificed. It will be seen that a total carbohydrate stores, as shown by liver and muscle glycogen stores, are greater in the glycine-fed animals, before insulin, and that this difference is markedly accentuated after giving insulin.

III. Absorption of glycine and carbohydrate was studied, together with liver and muscle glycogen determinations. The amounts of carbohydrate and of glycine found in the contents of the stomach and small intestine, were negligible. Total carbohydrate ranged from 40-milligrams to amounts too small to detect, and was not significantly altered by diet or by insulin. Free glycine was present in amounts of three milligrams or less, in the animals fed the glycine diets. It was not determined in controls. Corresponding values for liver and muscle glycogen for these animals were quite similar to those found, using comparable technique, in the experiments described under II.

FIGURE II

Liver glycogen, muscle glycogen, and blood sugar of rats fed the control and glycine diets, before and after administration of insulin.

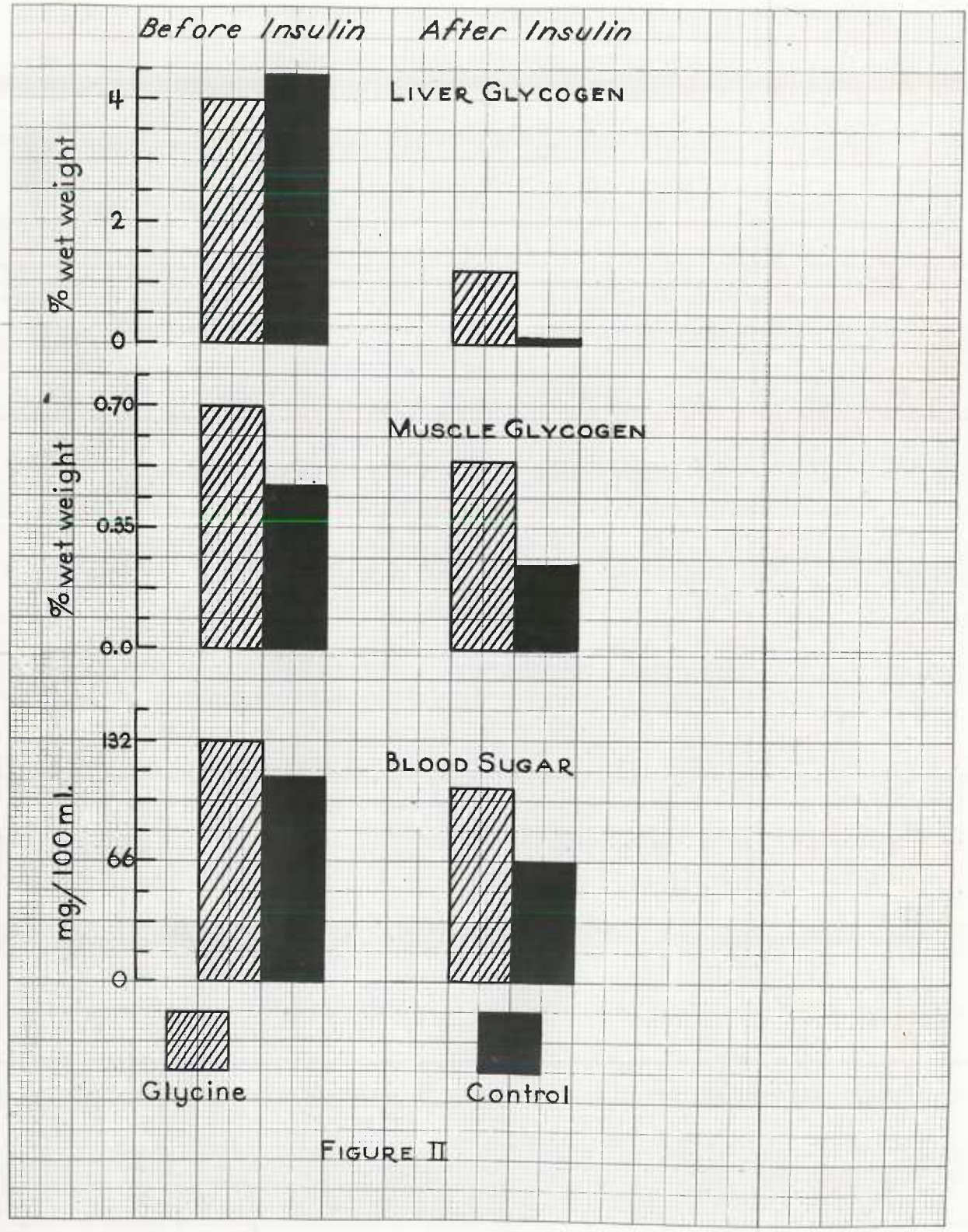
Before Insulin
After Insulin
LIVER GLYCOGEN
% wet weight
4
2
0
MUSCLE GLYCOGEN
% wet weight
0.70
0.35
0.0
BLOOD SUGAR
mg./100 ml.
132
66
0
Glycine
Control

FIGURE II

TABLE IV

A typical experiment in which muscle and living glycogen and blood sugar were determined.#

BEFORE INSULIN

	GLYCINE			CONTROL		
	Liver Glycogen %	Muscle Glycogen %	Blood Sugar %	Liver Glycogen %	Muscle Glycogen %	Blood Sugar %
	4.7	.75	133	4.1	0.50	164
	3.2	.41	130	4.9	0.45	101
	4.0	.78	138	4.2	0.46	86
	3.9	.72	135	3.7	0.41	103
Average	4.0	.66	134	4.2	0.45	113

AFTER INSULIN

	GLYCINE			CONTROL		
	Liver Glycogen	Muscle Glycogen	Blood Sugar	Liver Glycogen	Muscle Glycogen	Blood Sugar
	1.3	0.57	108	0.06	0.20	64
	1.0	0.72	108	0.04	0.26	44
	1.1	0.42	122	0.03	0.15	47
	1.4	0.58	86	0.13	0.27	40
Average	1.2	0.57	106	0.07	0.22	49

All data represent individual determinations.

DISCUSSIONS AND CONCLUSIONS

The effects of the glycine-supplemented diet will be analyzed before those of the alanine-supplemented diet.

Carbohydrate content of the fasted rats before administration of insulin. In these experiments, the last meal was force-fed, and followed by a relatively short fasting period. Nevertheless, absorption of carbohydrate and glycine was practically complete. It does not seem surprising, therefore, that the values for liver glycogen under these conditions are comparable to those reported by many authors for unfasted rats[9]. However, in the animals fed the control diet, blood sugar values were not significantly higher than those found in this laboratory for 48-hour fasted animals. In contrast, the glycine-fed animals showed an elevated fasting blood sugar level. Associated with this hyperglucemia in the glycine-fed rats was a definite increase in muscle glycogen.

The total carbohydrate content of the glycine-fed rats, estimated as shown in Table V, is greater than that of rats fed the control diet, because of the difference in muscle glycogen. Carbohydrate storage in tissues other than muscle or liver may be assumed to be negligible[29]. (Adipose tissue has been found to contain considerable quantities of glycogen under special conditions, different from those studied here[29].)

The liver glycogen values for glycine-fed animals do not appear to be significantly different from those of the animals fed the control diet. This may appear to be inconsistent with the elevation of blood sugar and muscle glycogen in the same animals. In rats fasted 24 hours, Guest[9] has shown that elevation of blood sugar is

TABLE V.

Calculated carbohydrate content, in milligrams, of various tissues, before and after administration of insulin, with the quantities of carbohydrate lost after insulin. #

GLYCINE

Tissue	Before Insulin	After Insulin	Lost
Blood	0.16	0.12	0.4
Liver	350	136	214
Muscle	504	415	89
Total Glycogen	854	551	303

CONTROL

Tissue	Before Insulin	After Insulin	Lost
Blood	0.13	0.10	0.6
Liver	389	13	376
Muscle	344	168	176
Total Glycogen	733	181	552

Calculations are made on the basis of a 200-g. rat having blood, muscle, and liver weights as given by Donaldson (30).

accompanied by increased glycogen storage in liver as well as muscle. Under these conditions, however, the liver reservoir was not as full as in the animals considered here. In unfasted animals studied by the same author, the level of liver glycogen was very high, and it was not related to the level of blood sugar. On the other hand, muscle glycogen was increased in the presence of elevated blood sugar in both unfasted and fasted rats. It appears that when the liver is well supplied with glycogen, carbohydrate added to the blood is deposited in the muscles preferentially. It is not surprising, therefore, that muscle glycogen, and not liver glycogen, contained the added carbohydrate store of the glycine-fed rats.

The glycine-supplemented diet contained less carbohydrate than the control diet. Therefore, only a relative increase in the rate of glyconeogenesis (compared to the rate of carbohydrate utilization) could provide the added carbohydrate stored by the rats fed the glycine diet. Such a relative increase in glyconeogenesis might be brought about by a reduction in the rate of carbohydrate utilization, or by an absolute increase in glyconeogenesis, or by both processes.

The effect of insulin. The greater resistance of glycine-fed rats to the hypoglucemic action of insulin does not appear to be dependent upon their initially elevated blood sugar level. Rather, it appears that the fall in blood sugar is more rapid, at first, if the starting level is higher. (See Figure 2.) The same phenomenon has been reported by other investigators[30a].

It is not known whether the glycogen stores suffered an initial fall, followed by a recovery period, like that seen in blood sugar. This seems probable, in view of the rapid rate of inactivation of

injected insulin[31]. In any event, Table V and Figure II show that, five hours after injection of insulin, the carbohydrate content of the rats fed the control diet was reduced to a much greater extent than that of the glycine-fed rats.# Thus, the net effect of previous glycine feeding was the same after injection of insulin as before, namely, a relative increase in glyconeogenesis. The action of insulin, directly or indirectly, accentuated this effect.

Speculation as to the point of action of insulin under the conditions studied appears irrelevant. There is no evidence that the mechanism responsible for the relative increase in glyconeogenesis after injection of insulin is essentially different from that operative before its administration. At present, only a tentative hypothesis may be advanced as to the nature of this mechanism. Certain clues, however, are to be found in the literature concerning the role of glycine in general metabolism. Other clues are afforded by investigations of various other factors which increase resistance to the hypoglucemic action of insulin.

Relation of glycine to carbohydrate metabolism.

Glycine is the simplest and most readily available of the amino acids, and one of the first to be discovered (Braconnot, 1820)[33]. Its physiological properties have been widely studied, and its unique role

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Small amounts of glycogen are deposited in the adipose tissue of the rat, especially in the interscapular fat, after injection of large doses of insulin (32), but the quantity of carbohydrate involved is negligible, compared to the content of the liver and muscle stores.

in detoxication and other processes has been established. Many of the products of its utilization are known. The carboxyl carbon readily gives rise to carbon dioxide, and it may also appear in glycogen[2]. Glycine nitrogen may give rise to urea[34]. Tissue oxidation-reduction may convert glycine to glyoxylic acid[35], or ethanolamine[36]. In general, however, surprisingly little is known about the intermediary metabolism of glycine--less than is known about the intermediary metabolism of most of the other amino acids.

The knowledge that carbohydrate may be formed from glycine immediately suggests an explanation of the relative increase in glyconeogenesis following glycine feeding. This theory deserves consideration, primarily, because of the limited period of time during which the various determinations were performed in the work reported here. There might have been increased relative glyconeogenesis during the period of study as a compensation for the reduced supply of carbohydrate from all sources previously. For instance, a rat might ingest 2.5 g. of glycine during the experiment, of which 1.5 g. was converted to glycogen, during the feeding period, the other one gram stored as glycine, representing potential carbohydrate. A comparable rat fed the control diet, in which glycine is replaced by dextrin or glucose, would therefore be supplied with one gram more of carbohydrate than the glycine-fed rat, during the feeding period. (For simplicity, it is assumed that one gram of glycine could be converted into one gram of glucose or glycogen. This is an overestimation of about 25 per cent.) The control-fed rat, however, would have one gram less of potential carbohydrate, and would not store all of its added gram of

actual carbohydrate, but would use a large part of it for fuel. The sum of actual and potential carbohydrate stored by the glycine-fed rat would, accordingly, be greater than that stored by the rat fed the control diet. The stored glycine could be used to great advantage for glyconeogenesis by the glycine-fed rat during the fasting period, especially to replace the glycogen depleted by the action of insulin.

The observed facts may be explained similarly without postulating conversion of glycine to carbohydrate. Glycogen stores might be spared by oxidation of stored glycine. If the absolute rate of glyconeogenesis remained the same, glycogen stores would be increased by this process as well as by glyconeogenesis from glycine.

Tracer studies have shown that glycine may undergo both glyconeogenesis and oxidation. The other process demanded by the thoery--namely, fluctuation of the glycine content of tissues after glycine ingestion--has also been demonstrated. However, it remains to be demonstrated that the hypothesis is capable of explaining the observed results. This appears to involve assumptions which, at best, are not supported by the available evidence.

Obviously, the effectiveness of the type of mechanism which is postulated depends on the efficiency of utilization of stored glycine for oxidation and glyconeogenesis. Unfortunately, there is not enough evidence concerning tissue glycine to make possible such an evaluation. Such data have been obtained only for exogenous glycine. Different investigators have administered glycine by various routes (usually in fasted animals), and observed subsequent changes in blood sugar or liver glycogen levels. The results so obtained tend to minimize the

importance of glycine as a precursor or sparer of carbohydrate.

It has been pointed out that the absorption of glycine without the simultaneous absorption of other amino acids is an imphysiological condition. Obviously, one, at least, of the normal pathways for ingested glycine is blocked under these circumstances, since, without a supply of essential amino acids, glycine, or other amino acids derived from glycine, could not be incorporated in newly-formed protein. In the presence of a large surfeit of unusable glycine, one would expect that the amino acid would be oxidized as rapidly as possible, with the mechanisms available for this process. Olsen, Hemingway, and Nier[2], have studied the rate of production of carbon dioxide from the carboxyl group of glycine, labeled with C13, in 48-hour fasted mice. Only about half of the isotopic carbon which was ingested was recovered in exhaled CO_2 in sixteen hours. There appears to be no evidence, therefore, that energy may be derived readily enough from glycine to permit significant carbohydrate-sparing, except under highly artificial conditions.

For an attempt to estimate the efficiency of glyconeogenesis from glycine, the most pertinent data are those obtained in studies of liver glycogen after glycine administration. Several workers[37] [38] [19] [39] have reported that glycine gives rise to very little liver glycogen, as compared to glucose, or to certain other amino acids. Results from studies of liver slices in vitro have been given a similar interpretation[40]. On the other hand, some investigators[7] [22] have reported higher levels of liver glycogen after administration of glycine than those found after administration of a comparable quantity of glucose. MacKay[22] and his associates appear to have resolved

these contradictions, on the basis of time relations. Formation of liver glycogen after feeding glycine is markedly delayed. This delay is not due to slow absorption[20] [19]. However, the tracer studies of Olsen and coworkers[2] demonstrated that, during the period when liver glycogen storage was greatest following glycine administration, only a small portion of this glycogen had arisen from the carboxyl carbon of glycine. It is theoretically possible that the other carbon atom of glycine after separation from the carboxyl carbon might be used for glyconeogenesis to a greater extent, but there is no evidence for such a series of reactions. Olsen and his coworkers suggest that amino acids other than glycine are the chief precursors of the glycogen formed after glycine feeding[2].

Several studies of the effect of glycine administration on blood sugar, similar to those in which liver glycogen was determined, may be mentioned. After parenteral administration of glycine and other amino acids and fatty acids, in rather massive doses, prompt hyperglucemia is produced[41-44]. Ingested glycine has been claimed to cause both hyperglucemia[45] [21] and hypoglucemia[46] by different authors, the evidence as presented being rather weak on both sides. Descriptions which have been given of the methods used for the parenteral administration of the amino acids[41-44], arouse the suspicion that the observed effects were toxicological, rather than physiological. There is good evidence that the effect is due to release of epinephrin[41-43]. It is said that in rabbits there is an associated suppression of the hypoglucemic action of insulin[42] [21]. However, this is not true in rats immediately after ingestion of glycine, as mentioned in the Introduction.

Glycine, like alanine and glucose, reduces ketonemia when fed to fasting rats[46a]. This may indicate increased glyconeogenesis from protein, but not necessarily from glycine, to any great extent.

After studying the excretion of glucose and nitrogen in fasted, phlorizinized dogs after administration of glycine, Ringer and Lusk[1] concluded that all the ingested glycine was converted to glucose, both the carbon atoms of glycine entering into the glucose molecule. Although this technique is still used[46b], the assumptions made in the required calculations are open to serious criticism, and the conclusions which are drawn from such work are in disagreement with the results of studies using other techniques.

It will be seen from Table V that a typical glycine-fed rat gained an added margin of 0.25 g. of carbohydrate over the typical rat previously fed the control diet, during the five hours after insulin injection. The smallest quantity of stored glycine which would have to be utilized, according to the theory under consideration, to produce this difference, would also be about 0.25 g. However, the foregoing discussion has shown that even uncombined glycine probably could be used for this purpose (either by glyconeogenesis or by sparing carbohydrate oxidation) only rather slowly. Therefore, the quantity of glycine which would have to be liberated from tissue stores would probably be much larger than 0.25 g. This, in turn, leads to further difficulties, which constitute the principal objection to the glycine-storage theory.

The requisite quantity of glycine could hardly be stored in any form except as a protein constituent. The only other alternatives are, that it might be deaminated before storing, or that it might enter the non-protein-nitrogen fraction. The only known method by which

residues of deaminated amino acids may be stored in large quantities is their conversion to glycogen, a possibility which is expressly excluded by the glycine-storage theory. Retention of the necessary quantities of glycine in the non-protein fraction of tissue would be expected to give rise to amino-nitrogen levels far higher than those previously reported[47].

Furthermore, the protein containing the stored glycine would necessarily be laid down during the feeding of glycine-supplemented ration since, as is well known, the amino acid composition of pre-existing protein is definite and fixed.

Although excess dietary nitrogen may be retained for short periods of time and then released[48] [49], it is generally admitted that the protein store of the body is much less flexible than those of carbohydrate and fat[50]. Since most tissue proteins contain relatively small quantities of glycine[51], it appears that the nitrogen retention of rats storing dietary glycine would be much greater than that due to glycine alone. The suggestion that glycine might be converted to other amino acids before storage would tend to reduce this difficulty. However, tracer studies indicate that ingested glycine is stored largely or entirely as glycine, and there is no conclusive evidence that it can give rise to any other amino acids[51a].

Even if the requisite glycine could be stored in protein during the feeding period, it appears doubtful that more than a small fraction of it could be made available, as required by the glycine-storage theory, during the period of recovery from insulin hypoglucemia. It is evident that if a large portion of the glycine (or other glyconeogenetic amino acids) of tissue, in general, were liberated after

injection of insulin, the accompanying widespread protein hydrolysis would make tissue destruction the most prominent effect of overdoses of this hormone. The effect would be even more striking, perhaps, if some proteins, and not others, suffered loss of their glycine. No protein is known which can be destroyed by insulin, but it appears that the existence of one must be postulated, to satisfy the demands of the glycine-storage theory.

It seems impossible, then, to explain the experimental results on the basis of storage of glycine, or other amino acids derived from glycine, without the assistance of several other purely hypothetical processes. A variant of this theory is suggested by the fact that glycine can be synthesized readily from other amino acids, as it is needed for certain special functions. Glycine is known to enter into the formation of protoporphyrin[52], uric acid[53], ethanolamine[36], (and therefore choline and acetylcholine), creatine and creatinine, glycocholic acid, and glutathione, besides hippuric acid and other products of detoxification[50]. The only amino acid which is known, at present, to be convertible to glycine, is serine[54]. The growth inhibition[55] which has been produced in rats by administration of benzoic acid, which removes glycine as hippuric acid, is evidence of the high priority of glycine formation. Conceivably, a surfeit of glycine might spare a group of other amino acids, which might be more easily stored for later protection of carbohydrate, and might also be better glycogen formers than glycine. This improvement hardly justifies the addition of another imaginary phenomenon.

Clearly, an explanation is to be sought which does not depend on storage of amino acids in large quantities. However, without storage,

it must be postulated that glycine has some indirect effect on carbohydrate utilization or production. Such an effect would probably involve part of the regulating machinery of protein or carbohydrate metabolism.

In confirmation of this line of reasoning, there is good evidence that the ingestion of glycine alters the fate of other amino acids. Part of this evidence has already been given. After ingestion of glycine by a fasted animal, there is a delayed, gradual accumulation of liver glycogen. Most of this added carbohydrate is probably not derived from the administered glycine. The duration and the magnitude of the increase indicate that it could not have been produced merely by oxidation of the ingested glycine, with consequent carbohydrate sparing. Glycine appeqrs to stimulate the oxidation of fat, or protein, or both, probably causing an absolute increase in glyconeogenesis.

From other types of studies, it may be concluded that the action of glycine is specifically concerned with the oxidation of amino acids. Kiech and Lusk[56], studied the rate of urea formation after administration of different amino acids to fasted rats, and compared it to the rate of disappearance of amino nitrogen from the tissues. Whole carcasses of the animals, as well as excreta, were analyzed. With all amino acids studied--glycine, alanine, glutamic acid, and aspartic acid--there was an initial period of about four hours, during which deamination was closely paralled by urea formation. Then, in the animals given alanine and glutamic acid, the rate of urea formation decreased, compared to the rate of disappearance of amino nitrogen. With glycine and aspartic acid, the opposite effect was noted. The rate of disappearance of amino nitrogen decreased, but urea production

was maintained at a disproportionately high level, indicating hydrolysis and deamination of tissue protein. These results were interpreted as showing stimulation of nitrogen metabolism by glycine.

Subsequently, Reid[37] obtained confirmatory results in fasting dogs. The urinary excretion of inorganic sulfur and nitrogen was studied for periods of several days, following ingestion of glycine, or of amounts of glucose or alanine containing equal quantities of carbon. Alanine exerted a sparing action on nitrogen equal to that of glucose. On the other hand, glycine aggravated the negative nitrogen balance of the fasting animals. Changes in excretion of inorganic sulfur were interpreted as being a better indication of the rate of utilization of tissue proteins. The excretion of inorganic sulfur in the glycine-fed animals was almost twice as great as that in the animals given glucose. Alanine fell midway between glycine and glucose in this respect.

In all the experiments which have been cited, so far, as evidence for the belief that glycine-feeding accelerates protein oxidation, the amino acids have been given, alone, to fasted animals. It has already been pointed out that this technique brings about a very artificial situation. Other experiments, however, which are not open to this criticism, support the same theory. Hier[4] has studied the effect on the growth of young rats of the addition of glycine (or of gelatin, which contains 25 per cent glycine) to otherwise satisfactory diets. Significant depression of growth was obtained with the addition of glycine, at a level of 6 per cent of the diet. This may indicate that glycine opposes the nitrogen retention necessary for growth by increasing the rate of utilization of amino acids for glyconeogenesis

or oxidation. Unfortunately, the intake of food on the various diets is not given.

Addition of unnatural forms of amino acids to intravenously-administered amino-acid mixtures causes certain toxic symptoms. Addition of glycine to the racemic mixtures delays the onset of toxic symptoms (vomiting)[58]. This suggests that administration of glycine accelerates the deamination of amino acids, or increases the rate of utilization of the keto acids formed from them.

Using, as a starting point, the fragmentary information which is now available concerning the intermediary metabolism of glycine, it is difficult to explain the effect of feeding this particular amino acid on the metabolism of protein in general. Creatine comes to mind as a possible intermediary. It is well known that feeding glycine appears to increase the formation of creatine in certain creatinuric diseases, and that creatine phosphatehas an important role in anaerobic glycolysis. However, it is not clear in what way increased availability of creatine, resulting from an increased supply of glycine, would affect response to the action of insulin, or the behaviour of carbohydrate stores in general.

Glycine also enters into the formation of glutathione, and, theoretically, glutathione may be concerned with the protection of ascorbic acid[59], and therefore with carbohydrate metabolism. However, the concentration of glutathione in the blood is not affected by oral or intravenous administration of glutathione itself[60].

Like the glycine-storage theory, attempts to explain the results on the basis of increased production of creatine, or of glutathione, involve not one, but a series, of ill-supported assumptions. First,

increased availability of glycine must lead to increased production of a certain substance, "X." "X" must then be stored in unusually large quantities. "X," when liberated from storage, must be able to increase carbohydrate stores, in some way. A large enough proportion of stored "X" must be made available, for this purpose, in a few hours, to cause a marked increase in carbohydrate content during the period of recovery from insulin hypoglucemia, without giving rise to any other physiological effects which would be noted in the glycine-fed animals. Thus, the little-known field of glycine metabolism is bridged by a chain of hypotheses. Until this field is more thoroughly explored, any line of reasoning which involves glycine transformations appears hazardous, although such a scheme, admittedly, would be difficult to disprove. Fortunately, a simpler type of hypothesis remains to be discussed.

The recent development of improved methods for the chemical determination of glycine and alanine has led to studies of the concentrations of these amino acids in human blood[61] [62]. They make up a relatively large fraction of the fasting blood amino acid[61]. Ingestion of glycine caused a marked, sustained elevation in blood glycine, as well as a definite increase in blood alanine and an elevation in total blood amino-acid nitrogen which was sometimes greater than could be explained by the increase in combined glycine and alanine nitrogen[61]. The rise in blood amino-acid nitrogen after giving glycine was more prolonged than that reported to follow administration of other amino acids, possibly because of the low rate of excretion of glycine in the urine[61]. These findings indicate that elevation of glood glycine may constitute one of the most important humoral changes following the ingestion of protein.

It is well known that the rate of nitrogen excretion tends to be adjusted to the nitrogen intake in such a way that a constant balance is maintained. The mechanism of this adjustment does not depend on a special storage form of protein[50]. Rather, ingested amino acids continually enter into formation of tissue protein, which is continuously broken down[34], and variations in protein intake affect different fractions of tissue protein equally[49]. Apparently, then, a delicate mechanism is required for regulation of the rate of deamination and further utilization of amino acids.

It is well known that disturbances in the function of the anterior pituitary, the adrenal cortex, and the islets of Langerhans are reflected in alterations in nitrogen excretion. These endocrine glands are involved in adaptation to fasting and to different levels of carbohydrate intake[50]. Therefore, it does not appear illogical to conjecture that one or more of these glands are directly concerned in adaptation to changes in protein intake, and that a humoral mechanism, sensitive to the level of dietary protein--namely, alteration in blood amino-acid concentration--may be involved in this adjustment. It is known that the secretion of insulin is influenced by the digestion of carbohydrate through an analogous humoral factor, i.e., the level of blood sugar[63]. As the following discussion will show, all the results of glycine-feeding, obtained in these experiments, can be explained by postulating that elevated blood glycine stimulates a single endocrine gland to increase its rate of secretion.

Various endocrine glands, which are known to be related to resistance to the hypoglucemic action of insulin, must be considered.

Endocrine glands which may be related to the effect of glycine feeding on carbohydrate metabolism.

1. The adrenal cortex.

Adrenalectomized, fasting animals are very sensitive to insulin, and develop hypoglucemia spontaneously (even in the absence of the pancreas), with an associated loss of liver glygocen[50]. These disorders are corrected by administration of cortical extracts[50]. Long[64] distinguishes between the "organic" and "inorganic" actions of the adrenal cortex, including the effect on carbohydrate maintenance among the former, and the effect on salt and water metabolism among the latter. Different groups of cortical steroids are responsible for the different types of action, although corticosterone is common to both groups[64] [65]. In the following discussion, reference will be made only to the steroids with a keto or hydroxyl group at carbon eleven, which are known to influence carbohydrate metabolism.

Injection of cortical extract gives protection against the hypoglucemic action of insulin[66] [67] [68], and increases fasting glycogen stores[66] [67] [68] and blood sugar[68], in fasted rodents. Such effects are produced in normal rats[68] and mice[67] [68], as well as in adrenalectomized animals[66]. The reported changes are of such magnitude that it appears that all the observed effects of glycine-feeding on carbohydrate storage could be explained by increased adrenal-cortical activity.

Authorities in this field have not decided whether these actions of the adrenal cortex are dependent on increased glyconeogenesis from protein, or decreased oxidation of carbohydrate, or both.

These explanations do not appear to be mutually exclusive. Either mechanism would involve increased deamination and further oxidation of amino acids. Other lines of evidence, too, indicate that the adrenal cortex is concerned in the utilization of tissue protein for oxidation. Like glycine-feeding, administration of the cortical hormone appears to lead to increased destruction of tissue protein. According to Long[64], the weight loss which is produced by administering cortical extract to fasted animals could only occur at the expense of protein, and the increased potassium excretion effected by the steroids which have an "organic" action is a sign of tissue destruction. Administration of the cortical hormone, or the adrenotrophic principle of the pituitary, may even cause depression of growth[65]. Loss of tissue protein in Cushing's syndrome is well known. Lymphoid tissue appears to be particularly subject to destruction by adrenal cortical action[69]. The action of cortical hormones, like that of glycine, appears to involve increased formation of urea from amino acids[64].

The action of adrenal cortical extracts on carbohydrate metabolism appears to be independent of the presence of the anterior pituitary, since they restore liver glycogen of hypophysectomized animals[70]. As the following discussion will show, however, the reverse is not true.

2. Insulin. Conceivably, the presence of added quantities of glycine in blood or other tissues might accelerate the

inactivation of exogenous insulin,[#] but it is hard to see how a change either in the rate of inactivation or the rate of secretion of endogenous insulin could produce the effects observed after glycine feeding, before insulin injection. Increase in insulin activity may, under some circumstances, increase glycogen stores, and decreased insulin activity may elevate blood sugar. Obviously, then, coexistence of elevated blood sugar and elevated glycogen stores must depend on some other factor. Besides, in preliminary experiments in this laboratory, characteristic effects of glycine on liver and muscle glycogen have been obtained in alloxan-diabetic rats. Thus, glycine feeding increases carbohydrate storage whether the supply of insulin is greatly increased (as in the above experiments), or greatly reduced, as after destruction of islet cells by alloxan. From this there appears to be little likelihood of a glycine-insulin relationship as an explanation of the data presented.

3. The anterior pituitary. While certain hypophyseal extracts appear to have effects on carbohydrate metabolism which are independent of the corticotrophic action(75) (50), the principal action of the pituitary on the response to insulin seems to depend on the presence of the adrenal cortex. Young has named a "glycotrophic" principle of the hypophesis which is capable of suppressing insulin hypoglucemia(76)(77). Jensen and Grattan(78), however, have presented good evidence that this hormone is identical with the adrenotrophic principle.

[#] In vitro, blood can inactivate large quantities of insulin, if the blood-insulin mixture is incubated for several hours (71) (73). The insulin-destroying-power of the blood is altered by the changes in blood chemistry associated with disease (73), including ketonemia (74), or even aging (71). Glutathione is said to inactivate insulin in vitro, but not in vivo (46). It would be interesting to determine whether glycine-feeding increases the insulin-inactivating power of blood.

4. Other endocrine glands. It is well known that removal of the adrenal medulla decreases resistance to insulin hypoglucemia. However, blood sugar is elevated at the expense, first, of liver glycogen, and ultimately of muscle glycogen[50]. Therefore, increased secretion of epinephrin could hardly explain the results obtained.

Much the same situation exists with regard to the thyroid. Thyroidectomy causes a transient reduction in resistance to the hypoglucemic action of insulin[79] [80]. There is evidence that the relation of the thyroid hormone to insulin depends on potentiation of epinephrin[80]. In any case, increased thyroid activity reduces glycogen stores, instead of enrichening them[50].

The evidence for a relationship between androgens[81 - 83] and estrogens[84-86] and insulin activity is contradictory and unconvincing[50], but seems to indicate that these hormones are synergic with insulin, probably because they, like insulin, favor nitrogen retention. The fact that similar results have been obtained with glycine-feeding in both sexes and at various ages seems to argue against the idea that the gonads might play an important role in effects which have been observed on carbohydrate stores.

The role of the posterior pituitary in resistance to the hypoglucemic action of insulin is difficult to evaluate. Apparently the oxytocic fraction, administered simultaneously with insulin, is able to prevent hypoglucemia[87]. However, Russell and her coworkers have shown that removal of the anterior, but not the posterior lobe

of the hypophysis reduces resistance to insulin convulsions[88]. #

At present, increased adrenal cortical activity appears to be the best explanation for the relative increase in glyconeogenesis caused by glycine-feeding.

Alanine. It has been shown that feeding alanine causes increased resistance to the hypoglucemic action of insulin, although this effect is not so marked as with glycine. The difference between the effect of the two amino acids remains to be explained. It may be of importance that the alanine which was fed was a racemic mixture. A special enzyme, d-amino acid oxidase[89], would be involved in the metabolism of the unnatural isomer, which is known to lead to the formation of liver glycogen more slowly than l-alanine, or the racemic mixture, in equal quantities[90]. It is generally agreed that alanine can be readily converted into glycogen[50] [7] [19] [38], although the evidence for this belief is open to some of the same criticisms which apply to that for glyconeogenesis from glycine. Increase in liver glycogen after alanine administration occurs almost as fast as after giving glucose[7]. This might easily antagonise glyconeogenesis from other amino acids. However, ingestion of alanine causes a rise in blood alanine almost as marked as the corresponding increase after glycine administration[62].

In the absence of evidence to the contrary, the most likely assumption appears to be that administration of alanine, like feeding glycine, stimulates the adrenal cortex to greater activity.

In this connection, it may be remarked that various centers in the hypothalamus have been shown to have some effect on blood-sugar regulation, but the evidence is so contradictory that evaluation is impossible (50).

SUMMARY

Pronounced alterations in carbohydrate metabolism may be produced in rats by feeding diets containing 15 per cent glycine (replacing an equal weight of carbohydrate in a generally satisfactory synthetic "control" diet.) These alterations are manifested by elevated blood sugar and increased in carbohydrate stores during fasting, and by increased resistance to the hypoglucemic action of insulin. Prefeeding glycine markedly reduces the loss of carbohydrate content which follows injection of insulin in large doses.

Feeding a diet containing 18 per cent dl-alanine also increases resistance to insulin hypoglucemia, but not to as great an extent as does feeding 15 per cent glycine.

It is tentatively suggested that the effects of feeding both these amino acids depend on stimulation of the adrenal cortex.

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