THE RELATION OF DIETARY GLYCINE TO GLYCOGENESIS IN THE RAT

by

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INTRODUCTION

Earlier investigations of the ability of the animal body to convert amino acids to carbohydrate were pursued by administering the amino acid in question, by itself, either to starving normal animals, to phlorizinized animals, or to diabetic animals. If this treatment resulted in a diminution of the ketonemia and ketonuria of starving animals or in an increase in the glucose excretion of phlorizinized or diabetic animals, the amino acid given was considered to have been converted into carbohydrate. This interpretation was undoubtedly correct for the conditions under which such experiments were conducted. Several years ago, however, investigations were undertaken in this laboratory which approached this problem from a somewhat different standpoint. In these experiments, the effect of individual amino acids, fed as part of an otherwise complete diet, upon the carbohydrate stores of rats following a fast, was studied. By eating the amino acid as part of his diet, not only was the animal in a different physiological state when he received the amino acid, but he also ingested a considerably larger dose than that given in the other type of studies. It was found[1] that rats fed for two days on a diet containing 10 to 15 per cent glycine showed liver glycogen levels exceeding one per cent after a 24 hour fast while the livers of rats pair-fed on a diet similar in composition except for the replacement of glycine by an equal weight of carbohydrate had glycogen contents of about 0.3 per cent after the fast. A few other amino acids were studied in a similar manner and it was found that l-leucine and l-glutamic acid failed to produce such a picture,

while dl-alanine exerted only a slight effect on carbohydrate reserves.

Mirski and associates[2], whose work has been confirmed by Guest[3] and by Newburgher and Brown[4], had demonstrated some years earlier that a very high protein diet, as compared to a high carbohydrate diet, had a similar effect upon glycogen stores after a fast. They demonstrated further, that animals prefed high protein diets, when subjected to stresses other than fasting, maintained and regained glycogen stores more efficiently than did animals prefed a high carbohydrate diet. These workers designated this phenomenon as the "protein effect", a term which has been adopted to indicate a somewhat similar action of glycine[1]. It had also been demonstrated by Mirski et al.[2] that the "protein effect" could not be elicited in adrenalectomized animals. Likewise, Todd, Barnes, and Cunningham[1] found the "protein effect" of glycine to be abolished by adrenalectomy, indicating that the enhancement of carbohydrate stores observed as a result of glycine feeding may be mediated by the adrenal glands.

Investigations of the effects of other amino acids fed in this way were then abandoned in favor of further inquiry into the mechanism of the "protein effect" of glycine. Observations such as those made up to this point could be explained on the basis of increased glycogenesis, decreased glycogenolysis, or a combination of the two, but the data then available offered no clue as to the mechanism involved. In an attempt to clarify this picture, the reaction of animals prefed diets containing added glycine to a different type of stress was studied.

A large dose of insulin (12 units per kilogram) was the stress chosen[5]. The animals were fasted for 8 hours prior to insulin administration and after another 5 hours they were sacrificed. Glycine-fed animals were found to have 2.5 times as much muscle glycogen, more than 10 times as much liver glycogen, and a considerably smaller decline in blood sugar than did control-fed animals. The notably higher blood sugar levels of glycine-fed animals after insulin ruled out decreased glycogenolysis as an explanation for the improved carbohydrate reserves previously noted. Furthermore, this observation, coupled with the increased glycogen stores noted in glycine-fed animals, supported the theory of increased glycogenesis. The latter concept also gained strength from the observation that glycine-fed animals after an 8 hour fast and before the administration of insulin, exhibited noticeably higher blood sugar levels than did control-fed animals even though the latter had received a diet richer in carbohydrate.

The fact that the "protein effect" cannot be elicited in adrenalectomized animals, combined with the well-recognized effects of the adrenal cortex upon carbohydrate metabolism, led to the hypothesis that the increased glycogenesis observed following glycine feeding resulted from a stimulation of adrenal cortical activity. Supposing for the moment that adrenal cortical activity were enhanced, did this increased supply of hormones act only upon glycine stored in the body during the feeding period or was some of the extra carbohydrate found derived from sources other than glycine? To answer this question, investigations into the extent of body storage of glycine were carried

out with a view to determining whether or not rats could retain enough glycine, free or combined, during the feeding period to account for the extra carbohydrate found after the action of insulin or after a 24 hour fast. It was found(6) that glycine was not stored to any great extent in any of the tissues studied. Calculations were made which indicated that glycine-fed rats had in their bodies, after an 8 hour fast and 5 hours insulin action, about 6 times the amount of extra carbohydrate that could be accounted for by the carbon to carbon conversion to carbohydrate of the excess glycine present before the administration of insulin. Such results lent further support to the theory of increased glycogenesis as a result of glycine feeding, but offered no further clue as to the mechanism involved.

Investigations of other metabolic effects of glycine feeding for the purpose of comparing them with the metabolic effects of adrenal cortical extract or adrenocorticotrophic hormone were delayed by the lack of suitable metabolism cages. A cage was eventually designed which fulfilled the requirements. Its use prevents the contamination of excreta with food, allows for the collection of food spilled during the day, and affords a good separation of urine and feces. With such cages available it was possible to initiate studies of the urinary excretions of various substances by animals fed the experimental diets.

The literature of the past ten or fifteen years abounds in reports on the effects of adrenal cortical extract, adrenal cortical steroids, and adrenocorticotrophic hormone (ACTH) upon the excretion

of various urinary constituents. In 1938, Long and Dohan[7] reported that adrenal cortical extract increased the urinary nitrogen and glucose outputs of adrenalectomized-depancreatized cats and dogs and of hypophysectomized-depancreatized cats. Long, Katzin, and Fry[8] obtained similar results using hypophysectomized-depancreatized rats and extended the work to include normal rats, in which increases in urinary nitrogen and liver glycogen following the administration of adrenal cortical extract were demonstrated. In addition to adrenal cortical extract, the effects of several steroid compounds were studied by this group. It was discovered that cortical steroids having an oxygen atom attached to carbon 11 of the steroid nucleus had the property of increasing the glycosuria of depancreatized and adrenalectomized-depancreatized animals, while desoxycorticosterone (having no oxygen atom on carbon 11), although potent in maintaining the life of adrenalectomized animals, had essentially no effect on the glycosuria of such animals. This was the first observation of such a difference in the physiological actions of the two types of compound. Shortly afterward, Lewis et al.[9] demonstrated increased glucose and nitrogen excretions in both normal and adrenalectomized phlorizinized rats following treatment with adrenal cortical extract. The steroid compound 17-hydroxy-11-dehydrocorticosterone (now known as cortisone) produced a similar picture in such animals while desoxycorticosterone failed to induce such changes. Soon after this Ingle and Thorn[10] observed the complete failure of 11-desoxycorticosterone to induce glycosuria and to increase nitrogen excretion in partially

depancreatized and adrenalectomized-partially depancreatized rats while 17-hydroxy-11-dehydrocorticosterone produced marked rises in both. The effects of these two steroids upon phosphorus excretion were also investigated. Both increased the excretion of inorganic phosphate in the urine, but the increase resulting from 17-hydroxy-11-dehydrocorticosterone was far more striking. Thus, Ingle and Thorn confirmed the previous observations of Long et al.(8) and Lewis et al.(9) and emphasized the striking differences in the physiological actions of the two types of compound. It is now well established that corticoids having an oxygen atom or oxygen containing substituent attached to carbon number 11 of the steroid nucleus act primarily upon carbohydrate metabolism, whereas corticoids lacking an oxygen function in that position are concerned primarily with electrolyte balance. Since the problem to be discussed here is directly concerned with carbohydrate metabolism, the effects of the hormones involved in electrolyte balance have largely been omitted from this brief review.

Ingle (11) extended such studies to the normal force-fed rat and found 17-hydroxy-11-dehydrocorticosterone to be capable of producing a negative nitrogen balance in such animals. A few years later it was established that 17-hydroxycorticosterone also exerted such an action(12). Glycosuria accompanied the negative nitrogen balance, producing a diabetic type of picture, and the condition so induced has been termed "adrenal steroid diabetes"(12). A similar picture might be expected to appear following the administration of pure

adrenocorticotrophic hormone and this proved to be the case when Ingle, Li, and Evans[13] treated normal force-fed rats with this material. In confirmation of this, Bennett and Li[14] have since reported an intensification of the glycosuria and nitrogen loss of alloxan diabetic rats as a result of the administration of adrenocorticotrophic hormone.

The effects of adrenal cortical extract, adrenal cortical steroids, and adrenocorticotrophic hormone upon human metabolism have been shown to resemble closely those demonstrated in animals. From at least one point of view, studies in man may have more significance with respect to the problem under discussion here than do the animal studies. Dosages employed in human studies have been very much smaller than those used in animal work and the effects of a mild stimulus such as glycine feeding may mimic more closely the results of such a small dose than the effects of a rather large one. In 1943, Browne[15] reported that a normal male treated with adrenocorticotrophic hormone exhibited a definite impairment of carbohydrate tolerance, indicating a tendency toward the "adrenal steroid" type of diabetes demonstrated in animals by Ingle and associates[11, 12, 13]. The subject failed to go into negative nitrogen balance in the course of the experiment, but Browne felt that his failure to do so could be attributed to the fact that he was maintained on a high caloric carbohydrate diet. Thorn, Forsham, and coworkers[16, 17, 18, 19, 20] have studied thoroughly the metabolic changes produced in man by both adrenocorticotrophic hormone and various cortical steroids. They have found that the effects of adrenocorticotrophic hormone upon

carbohydrate metabolism, in the presence of functional adrenal glands, are comparable with those produced by the 11-oxysteroids. An increased excretion of uric acid was a constant finding, total urinary nitrogen increased in some cases although not at all consistently, while creatinine excretion remained essentially constant. Rabad[21, 22] had reported a number of years before that adrenal cortical extract increased the uric acid excretion of rats but failed to lead to any change in the urinary output of creatinine. The consistent rise in urinary uric acid in the presence of an unchanged excretion of creatinine has led the group headed by Thorn and Forsham to propose the use of an increase in the uric acid : creatinine ratio after treatment with adrenocorticotrophic hormone as one index of adrenal cortical response to stimulation. It is also noteworthy that this same group has reported a rise in the excretion of inorganic phosphorus in several cases, although the finding was not consistent. As mentioned earlier, Ingle and Thorn[10] had observed rises in the phosphorus excretions of rats treated with adrenal steroids.

Other groups have been active in this field and have reported similar results with regard to nitrogen and uric acid excretion consequent to the administration of adrenocorticotrophic hormone or 17-hydroxy-11-dehydrocorticosterone. Among these groups are Mason et al.[23], Sayers et al.[24], Conn et al.[25, 26] and Sprague et al.[27]. The last-mentioned group also made the interesting observation that urinary creatine nitrogen was increased during the administration of adrenocorticotrophic hormone and 17-hydroxy-11-dehydro-

corticosterone, and that this increase often persisted for several days after hormone treatment had been discontinued. This finding correlated with the observation of Bebad[22] that treatment of normal fasted rats with adrenal cortical extract intensified their creatinuria.

Studies of the effects of adrenocorticotrophic hormone and 17-hydroxy-11-dehydrocorticosterone have been pursued so intensively in the past few years that it is well nigh impossible to cite all the original references. Some excellent compilations and reviews of the subject have appeared recently, however. Among them are the _Proceedings of the First Clinical ACTH Conference_[28] and _Pituitary-Adrenal Function_[29].

In this previous work, particularly that in which adrenocorticotrophic hormone was used, alterations in the urinary output of substances other than those mentioned here in some detail have been noted. For example, changes in the output of electrolytes and of 17-ketosteroids and 11-oxysteroids have been shown to occur. Inasmuch as the theory has been advanced that the "protein effect" of glycine reflects enhanced adrenal cortical activity and adrenal cortical activity in the intact animal presupposes the secretion of adrenocorticotrophic hormone, alterations in the electrolyte and steroid excretions of animals fed this amino acid might be expected to occur if this theory is correct. The technical difficulties encountered in trying to collect urine samples free of external contamination with sodium and potassium, however, made it seem unprofitable to attempt such determinations. Furthermore, if one were successful

in making satisfactory urine collections, investigations of electrolyte excretion during feeding would have little meaning unless the amounts of sodium and potassium ingested were known. In short, balance studies would be required. As for investigation of the steroid excretion of these animals, determination of 17-ketosteroids and 11-oxysteroids both require such large volumes of urine as to make it impractical to attempt such analyses on rat urine. Since it was not practical, from a technical standpoint, to study electrolyte and steroid excretions, previous findings with respect to these substances have been omitted from the review of the literature for the sake of brevity.

The phenomenon investigated in this project relates mainly to carbohydrate metabolism, however, so that inability to study electrolyte output was not a serious disadvantage, although a study of steroid excretion, especially of 11-oxysteroids would have been very informative. There remained, nevertheless, a number of other metabolic effects identified with the action of the adrenal cortical secretions upon carbohydrate and protein metabolism which could be studied adequately. Accordingly, the urinary excretions of such things as nitrogen, uric acid, creatine, and glucose appeared likely to offer valuable information regarding the problem at hand. Glycosuria, as a result of treatment with adrenal cortical hormones or adrenocorticotrophic hormone, occurred in normal animals only as a result of rather large doses administered over a relatively long period of time. Normal animals subjected to as mild a stimulus, and one of as short duration, as 48 hours of glycine feeding would not be expected to

exhibit glycosuria and qualitative tests have shown that this does not occur. In addition to total nitrogen, uric acid, and creatine, the excretion of inorganic phosphate had been shown to increase, on occasion, after cortical hormone treatment. Furthermore, in order to answer a number of questions, it was desirable to study in more detail the constituents contributing to the figure for total nitrogen. Does glycine, excreted as such, make a significant contribution to total urinary nitrogen or is any increase primarily due to urea? What effect does glycine feeding have upon urinary ammonia and creatinine? Accordingly, investigations were initiated in which rats were fed known amounts of food of known nitrogen contents and their excretions of total nitrogen, urea, ammonia, uric acid, creatinine, creatine, glycine, and inorganic phosphate were studied.

EXPERIMENTAL

Animals: Adult male rats (200 - 300 grams) of the Sprague-Dawley strain were used. A pair of animals of nearly the same weight was removed from the colony about a week before the experiment was to begin, placed in a cage together, and allowed as much Purina Laboratory Chow and water as desired during this period. This was done to make sure that the animals were well nourished at the beginning of the experiment. At the completion of an experiment, the animals were returned to a diet of Purina Laboratory Chow for a week or ten days, at which time a new experiment was begun in which there was a reversal of diet for a given animal, i.e. the animal receiving the control diet in the first experiment received the glycine diet in the second experiment and vice versa. In this way, each animal was, in effect, serving as his own control. When each one of a pair of animals had received both diets, this pair was discarded and a new set used for the next experiments.

Rations: Colony rats were maintained on Purina Laboratory Chow.

In the preceding investigations on the "protein effect" of glycine[1, 5, 6], dry rations made up of dextrin, glucose, casein, Brewer's yeast, salt mixture, salad oil, and cod liver oil, with glycine replacing an equal weight of dextrin in the experimental ration, had been employed. It was desirable, however, to use liquid rations in studying the urinary excretion of various substances. The use of this form of diet made it easy to feed accurately measured meals, to collect and analyze any ration spilled by the animal, and to

prevent contamination of the excreta with food. Therefore, liquid rations, in which evaporated milk was used as a suspension medium, were developed for this work.

Evaporated milk contains enough fat, vitamins A and D, and mineral salts to fulfill the requirements of the diet. The proteins of the milk were supplemented by Essenamine (a protein preparation derived from lactalbumin which has been shown to be capable of replacing casein in the dry ration), while the carbohydrate of the milk was supplemented with white corn syrup. As in the dry rations, Brewer's yeast was added as a source of B vitamins. These rations have been demonstrated in this laboratory to have the same effect on liver glycogen as the dry rations. The compositions of the control ration and the glycine ration are shown below.

	Control Ration	10% Glycine Ration
Evaporated milk (Fortified with vitamin D)	100 g.	100 g.
Brewer's yeast (Squibb)	10 g.	10 g.
Essenamine (Winthrop-Stearns)	9 g.	9 g.
White corn syrup	61 g.	50 g.
Glycine	0 g.	10 g.

It can be seen that glycine is substituted for an equivalent amount of carbohydrate (corn syrup) in the experimental ration.

For the control ration, all ingredients were stirred together and warmed in a water bath for 5 to 10 minutes. The mixture was then

transferred to a Waring blender and beaten, at intervals, for five to ten minutes. This procedure was necessary to break up lumps of Essenamine and permit its uniform dispersion throughout the liquid. The mixture was then transferred to a 200 ml. volumetric flask and diluted to that volume with distilled water. For the glycine ration, all the ingredients except glycine were mixed together and treated in the same manner as the control ration. Before transferring the mixture to the volumetric flask, however, the glycine was weighed out accurately and placed in a funnel sitting in the neck of the volumetric flask. The glycine was then washed into the flask with the mixture from the Waring blender. When the glycine had all dissolved in the milk mixture, the volume was made up to 200 ml with distilled water. By weighing the glycine accurately and diluting to volume in a volumetric flask, it was possible to estimate accurately the amount of glycine ingested from the total amount of food consumed. This ration was worked out so that 1 ml. is equal to 0.5 grams of dry ration.

Cages: Metabolism cages of the type illustrated in Figure 1 were used. The cages are made of 1/4 inch mesh galvanized screen throughout except for the bottom which is 1/2 inch mesh screen to allow feces to drop through freely. Below the cage bottom is a removable piece of 1/4 inch mesh screen to separate feces from urine. This screen is soldered to a loop of wire of about the same diameter as the cage to facilitate its removal. The urine receptacle, into which the cage fits and to which it is fastened by means of heavy

Figure 1

Metabolism Cage

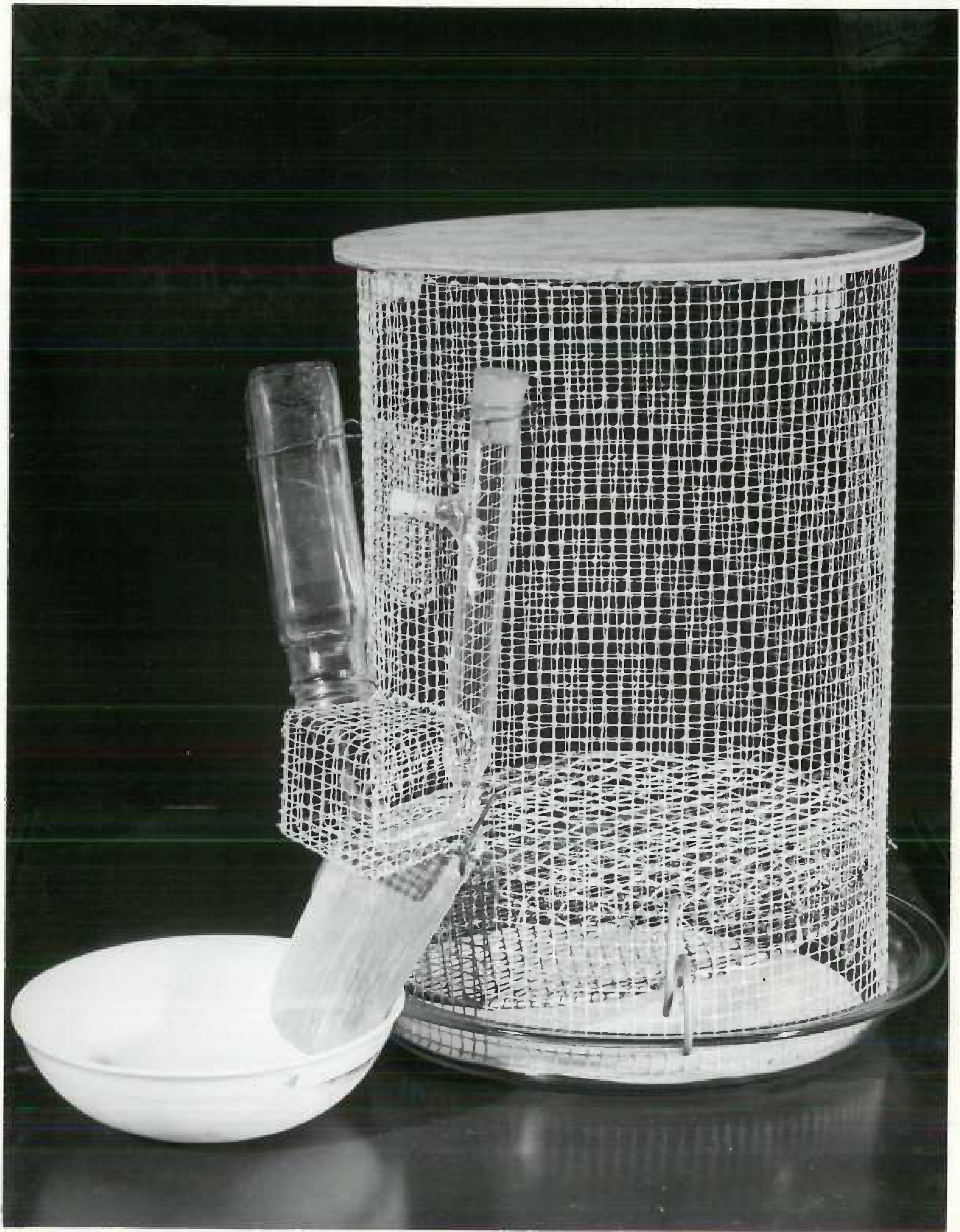

Figure 1

wire clips, is simply a Pyrex pie plate with an outside diameter of 10-1/2 inches. As may be seen from the illustration, food and water containers are inserted into a small addition built onto the side of the cage in such a way that the animal acutally eats outside the cage proper. A detachable metal plate fastened beneath this feeding compartment and extending into a dish permits the collection of any ration spilled during the day.

Methods: The nigrogen contents of the two rations were determined by the micro-Kjeldahl procedure to be described below. Each meal given was pipetted accurately so that the amount of food offered was known. At the end of each twenty-four hour period, the spillage, any uneaten ration, and washings from the feeding tube were combined and diluted to a known volume in a volumetric flask. Nitrogen determinations were carried out on suitable aliquots of this dilution. Knowing the amount of ration of a known nitrogen content offered to an animal and the fraction of this nitrogen found in the spillage, one could calculate the food consumption in the following manner:

$$\frac{\text{Total amount of N spilled}}{\text{N content per ml. ration}} = \text{ml. ration spilled}$$

$$\text{ml. ration offered} - \text{ml. ration spilled} = \text{ml. ration eaten}$$

Such a calculation was made for each twenty-four hour period.

In the case of the glycine ration, which contained 10.0 grams of glycine (weighed accurately) in 200 ml. of ration or 0.05 grams in 1.0 ml., glycine consumption was determined as follows:

ml. ration consumed x 0.05 x 1000 = mgs. glycine consumed

Collection and preservation of urine: As indicated in the description of the cages, urine was collected in a 10-1/2 inch Pyrex pie plate. The urine was preserved by pipetting 10.0 ml. of 1.0 N sulfuric acid into the pie plate at the beginning of each collection period. A large piece of coarse filter paper, which just covered the bottom of the pie plate, was used to insure uniform distribution of the acid over the bottom of the pie plate. At the end of the collection period, the cage was loosened from the pie plate, the separator screen was removed with the aid of a wire hook, the feces were discarded and the screen was placed with the pie plate. A clean separator screen was inserted and the cage attached to a fresh pie plate.

To extract the urine from the filter paper, the separator screen was first washed down thoroughly with distilled water from a wash bottle, the washings being allowed to run into the pie plate, and about 100 ml. of distilled water were added to the washings. After the paper had become thoroughly soaked, the dilute urine was filtered into a 500 ml. volumetric flask through a small plug of Pyrex glass wool placed in the stem of an ordinary funnel. A small smear of stopcock grease placed under the lip of the pie plate prevented any loss of urine in the process of pouring it from the pie plate. The pie plate and funnel were washed thoroughly with distilled water and the washings combined with the urine. Before diluting to the mark, 10.0 ml. of 1.0 N sodium hydroxide were pipetted into each sample to

neutralize the 10.0 ml. of 1.0 N sulfuric acid used in the preservation of the urine. From this point on, the urines were preserved with toluene and kept under refrigeration until all analyses were completed. By choosing suitable aliquots, no further dilution of the urine was necessary for the various analyses with the exception of creatine and creatinine.

Plan of a typical experiment: Two animals which had been separated from the colony a week or so earlier as mentioned above were weighed and placed in metabolism cages prepared as described. It was usually found convenient to begin an experiment at about 10:00 A.M., the animals being given a meal of 10.0 ml. at this time. Another meal (10.0 ml.) was given at about 4:30 P.M., and another at about 9:00 A.M. In this way, the animals were well-fed during the entire twenty-four hour period. Since the animals were very fond of these rations, these small meals given at rather wide intervals were eaten almost immediately so that settling out of any of the ingredients did not present any great problem so long as the rations were shaken well before the meal was measured out. Furthermore, giving the day's nourishment in this way allowed one to correct for any obvious differences in the amount of food consumed by the two animals at the next meal. Ordinarily, however, no difficulty was encountered in getting the animals to eat. Animals were allowed water ad libitum.

At the end of twenty-four hours, pie plates, separator screens, and dishes for the collection of spillage were changed. It is estimated that the urine collection period did not vary from twenty-four hours

by more than ten minutes. During the first twenty-four hour period, designated as the control day, both animals received the control ration. This procedure was instituted by Todd, Barnes, and Cunningham[1] at the beginning of this series of investigations to accustom the animals to synthetic rations. It also, in this work, provides a base line for comparison of the results of the experimental period. During the second and third twenty-four hour periods, designated as the first and second experimental days respectively, one animal continued to receive the control ration while the other animal was given the glycine ration. Thus, the experimental animal was fed on the glycine ration for a period of forty-eight hours. The last meal on the second experimental day was given about one-half hour before the urine collection period ended. This was sufficient time to allow the animals to consume the food offered. During the last twenty-four hour period, designated as the fast day, neither animal received any food. At the end of this period, the animals were weighed and returned to a diet of Purina Laboratory Chow if they were to be used again or discarded if they were not to be used again.

Methods: (Analytical)

Total nitrogen: For the determination of the nitrogen contents of the rations, 2.0 ml. aliquots of each ration were diluted to 200 ml. Analyses were carried out on 10.0 ml. aliquots of this dilution of the control ration and on 5.0 ml. aliquots of the glycine ration. Total urinary nitrogen was determined on 2.0 ml. aliquots of the diluted urine obtained as previously described.

Aliquots for analysis were pipetted into 100 ml. Kjeldahl flasks, two or three selenized Hengar granules were dropped in followed by 3 ml. of sulfuric acid digestion mixture. This digestion mixture consisted of concentrated sulfuric acid diluted with an equal volume of distilled water and saturated with K_2SO_4. Digestion was carried out with the aid of a small manifold connected to an aspirator. Boiling was continued for at least one-half hour after the digest had cleared.

Distillation of the digests was carried out under vacuum using the apparatus described by Rinehart, Grendahl, and West(30). When the digests had cooled, about 25 ml. of distilled water were added to each and they were again allowed to cool. The flask was then attached to the appropriate arm of the distillation apparatus; a receiving flask, consisting of a 125 ml. Erlenmeyer flask, containing 25.0 ml. of N/70 sulfuric acid being attached to the other arm. The apparatus was then connected to an aspirator and evacuated for three to five minutes. After evacuation, 10 ml. of 40 per cent sodium hydroxide were added cautiously with shaking, taking care not to lose the vacuum in the process. For the actual distillation, the digestion flask was immersed in a boiling water bath while the receiving flask was cooled by running a stream of tap water over the outside of the flask. It was found necessary to continue the distillation for twenty minutes. When the distillation was completed, the excess standard acid was back titrated with N/70 sodium hydroxide, using

Tashiro's indicator. Each milliliter of titration difference is equal to 0.200 mgs. of nitrogen.

Urea nitrogen: Urine urea nitrogen was determined by an adaptation of the method for blood urea nitrogen described by Rinehart, Grondahl, and West[30]. A 2.0 ml. aliquot of the diluted urine was transferred to a 100 ml. Kjeldahl flask and 1.5 ml. of 2.5 per cent potassium dihydrogen phosphate and 4 drops of urease in glycerol were added. The flasks were immersed in beakers of warm water and placed in an incubator set at 50° - 55° C for an hour and a half to two hours. This period of incubation gave the best recoveries. At the end of the incubation, one or two Hengar granules and about 15 ml. of distilled water were added and the ammonia was distilled out with the use of the distillation apparatus used for the total nitrogen determinations. Again a 25.0 ml. aliquot of N/70 sulfuric acid was placed in the receiving flask. After evacuation at the pump for three to five minutes, 6 ml. of potassium carbonate-oxalate reagent were added cautiously, taking care not to admit any air to the system. The distillation was allowed to proceed for five minutes in the manner described for the total nitrogen estimations. Excess standard acid was back titrated with N/70 sodium hydroxide as before. Calculations were again based upon the factor of 0.200 mgs. of nitrogen per ml. of titration difference.

Ammonia nitrogen: Urinary ammonia nitrogen was determined as described by Rinehart, Grondahl, and West[30]. A 25.0 ml. aliquot of the diluted urine was transferred to a 100 ml. Kjeldahl flask and

one or two Hengar granules were dropped in. The distillation was carried out exactly as described for urea nitrogen, except that 10 ml. of potassium carbonate-oxalate reagent were used to liberate the ammonia. Titration of the excess standard acid and calculations were carried out as previously indicated.

Uric acid: The method of Buchanan, Block, and Christman(31) was used for this estimation. A 10.0 ml. aliquot of the diluted urine was placed in a 50 ml. volumetric flask and 2.5 ml. of urea-cyanide solution were added. Immediately after the addition of 1.0 ml. of arsenophosphotungstic acid, the sample was diluted to the 50 ml. mark and mixed. Exactly thirty minutes after dilution, the sample was read in a Coleman Junior Spectrophotometer at 690 millimicrons. A reagent blank was run with the samples and the instrument was set at zero against this blank. As originally described by Buchanan and associates(31), this method involved a determination of residual color after incubation of the urine with uricase, the difference in the two determinations giving the true uric acid excretion. However, Buchanan et al.(32) have also demonstrated that the amount of non-uric acid chromogenic substances in urine is quite constant so long as methylxanthines such as caffeine or theophylline are excluded from the diet so it was not deemed necessary to include the uricase incubation for the purposes of this investigation.

Inorganic phosphate: This analysis was made by the method of Fiske and SubbaRow(33) as described in Hawk, Oser, and Summerson(34), except that amidol (2,4-diaminophenoldihydrochloride) was used as

the reducing agent rather than 1,2,4,-aminonaphtholsulfonic acid. This estimation was made on 5.0 ml. aliquots of the diluted urine which were placed in 100 ml. volumetric flasks, after which the total volume was brought to about 70 ml. After the addition of 10 ml. of ammonium molybdate, 3 ml. of amidol (1 per cent in 20 per cent sodium bisulfite) were added to each and mixed, followed by dilution to the mark with distilled water and mixing again. The color was read in a Coleman Junior Spectrophotometer at 690 millimicrons after 5 minutes. The instrument was set at zero against the reagent blank.

Creatinine: This determination was done by the method of Hare and Hare as outlined to Haskins[35] in a personal communication. Hare has since published the method[36], the technique described differing from the one to be outlined here only in minor details. For this analysis, a further dilution of the urine was required. Dilution of 10.0 ml. to 50 ml. proved satisfactory. Estimations were made on 5.0 ml. aliquots of this second dilution. This amount was transferred to 15 ml. centrifuge tubes and 0.5 ml. of saturated oxalic acid was added for each 5 ml. of solution. About 40 mgs. of Lloyd's reagent (a small scoop holding about this amount was made) were added regardless of the volume. The tubes were tightly stoppered and shaken by hand for two minutes after which they were centrifuged in an angle centrifuge for about five minutes. The supernatant was then aspirated off through a small tipped glass tube and discarded. Creatinine was eluted from the packed sediment with 10.0 ml. of alkaline picrate made just before use as follows:

5 parts 0.04 N picric acid

1 part 10% sodium hydroxide

12 parts water

After the alkaline picrate was pipetted onto the packed Lloyd's reagent, the tube was again stoppered with the same stopper used in the previous shaking and allowed to stand, with occasional agitation, for ten minutes. After centrifugation, the supernatant was transferred to a cuvette and read in a Coleman Junior Spectrophotometer at 500 millimicrons. Standard creatinine solutions must be run with each set of determinations. Aliquots of standard creatinine containing 20 and 30 micrograms were satisfactory for this work. A reagent blank was also run and the instrument was set at zero with this solution.

Creatine: The methods in use for many years for the determination of creatine depend upon its conversion to creatinine by heating it in the presence of acid, usually in an autoclave. Since the method used for the estimation of creatinine in these studies requires the addition of saturated oxalic acid in proportion to the volume of the aliquot taken for analysis, the idea presented itself that this acid might serve to convert creatine to creatinine as well as to produce the proper conditions for the adsorption of creatinine on Lloyd's reagent. Accordingly, a series of standard creatine solutions were analysed using saturated oxalic acid in the proportion of 0.5 ml. to each 5 ml. of creatine solution, plugging the mouths of the tubes with a wad of Pyrex glass wool, and heating in an autoclave at

120° - 125° centigrade for one-half hour. After the solutions had cooled to room temperature, the analysis was completed exactly as described above for creatinine. No additional oxalic acid was required for this process. The creatine standards were prepared as follows: A solution of 1.318 grams of creatine hydrate in 1000 ml. contains the equivalent of 1.00 mg. per ml. of creatinine. By making suitable dilutions of this stock standard, solutions were obtained from which aliquots containing the equivalent 10, 20, 30, and 40 micrograms of creatinine could be pipetted. Recoveries from such aliquots are tabulated below.

Creatine Std. Equivalent to:	Creatinine Recovered	Per cent Recovery
10 mcg.	8.40 mcg.	84.0
20 mcg.	18.0 mcg.	90.0
40 mcg.	37.2 mcg.	93.0
10 mcg.	9.17 mcg.	91.7
20 mcg.	20.2 mcg.	101.0
30 mcg.	30.1 mcg.	100.0
40 mcg.	39.6 mcg.	99.0
10 mcg.	11.0 mcg.	110.0
20 mcg.	20.1 mcg.	100.0
30 mcg.	30.6 mcg.	100.2
40 mcg.	39.6 mcg.	99.0

Average recovery 97.1%

Since saturated oxalic acid in this proportion was efficient in converting creatine to creatinine, urinary creatine was determined as just described. Analyses were carried out on 5.0 ml. aliquots of the dilution made for the creatinine determination. Creatine was calculated in the usual way:

Creatine = Total creatinine - Preformed creatinine x 1.16.

Glycine: The method of Alexander, Landwehr, and Seligman[37] was used for this estimation. This method requires the use of a Stotz all-glass still[38]. To 2.0 ml. of phosphate buffer (pH 5.5) and 1.0 ml. of 1 per cent ninhydrin (triketohydrindene hydrate) solution in the flask of the still were added 5.0 ml. of the diluted urine. The condenser was attached and the contents of the flask were distilled rapidly into a test tube calibrated at 10.0 ml. When about 7 ml. of distillate had been collected, the flask was allowed to cool. As soon as it was cool enough to prevent cracking, it was immersed in a beaker of cold water to bring it to room temperature more quickly. The condenser was disengaged, 2.0 ml. of distilled water were added, the condenser replaced, and the distillation was continued to dryness. At the end of the distillation, the neck of the still was heated gently to drive over the few drops of moisture remaining there. The entire distillation was completed in 15 to 20 minutes. Care must be taken not to heat the flask too strongly. Such treatment may drive a red reaction product of ninhydrin over with the distillate and this interferes with the accuracy of the determination. The distillate in the receiving tube was then diluted to the 10.0 ml. mark with

distilled water, mixed thoroughly, and a 5.0 ml. aliquot was pipetted into a test tube (6 inch). To this aliquot was added 4.0 ml. of concentrated H_2SO_4 slowly and with agitation while the tube was immersed in ice water. When the resulting solution had cooled to room temperature, 3 drops of 5 per cent chromotropic acid (1,8-dihydroxynaphthalene-3,6-disulfonic acid) were added and mixed. The mouth of the tube was covered with a marble and the tube was heated in a boiling water bath for 30 minutes. After the solution had cooled, the color intensity was read in a Coleman Junior Spectrophotometer at 575 millimicrons. A reagent blank was run with the analyses and the instrument was set at zero against the blank.

RESULTS

All results are summarized in Table I. Figures 2, 3, 4, 5, 6, and 7 are graphic presentations of data included in this table. All values have been expressed as milliliters or milligrams per 100 grams of body weight so that equal amounts of body tissue were dealt with in making interpretations.

It can be seen from Table I that the volumes of food ingested per 100 grams of body weight were very nearly the same for the two diets. Reducing the liquid rations to the dry basis (1 ml. = 0.5 g.), the greatest difference in average intake was 0.3 gram per 100 grams of body weight. Although the diets were isocaloric, the substitution of glycine for carbohydrate to make the experimental ration resulted in a considerably augmented nitrogen content in that ration so that animals receiving the glycine diet ingested more nitrogen in a given volume of food than did animals receiving the control diet. This difference in nitrogen intake is reflected, of course, in a greater 24 hour excretion of nitrogen by glycine-fed animals. Figure 2 illustrates graphically the nitrogen excretions in the two diets. It can be seen there that the animals eliminated almost exactly the same amount of nitrogen during the day they were eating the control ration and that control-fed animals continued to excrete nitrogen at a nearly constant rate during the experimental period. On the other hand, the urinary nitrogen of the glycine-fed animals increased sharply during the first day of glycine feeding and continued to increase, although much more slowly, during the second day of glycine feeding. The amount of nitrogen excreted during the fasting day

Table I

Food Consumption and Nitrogen Intake with Corresponding Excretions of Various Urinary Constituents

	Control Day			1st Experimental Day			2nd Experimental Day			Fast Day		
	Control Diet	Control Diet	t	Control Diet	Glycine Diet	t	Control Diet	Glycine Diet	t	Control Diet	Glycine Diet	t
Ration Consumed Per 24 hours (Ml./100 g. Body Wt.)	11.7 (11)	11.5 (11)		11.3 (11)	11.0 (11)		11.2 (11)	10.6 (11)		0 (11)	0 (11)	
Nitrogen Ingested Per 24 hours (Mgs./100 g. Body Wt.)	163 (11)	161 (11)		156 (11)	245 (11)		156 (11)	237 (11)		0 (11)	0 (11)	
Total Nitrogen Excreted Per 24 hours (Mgs./100 g. Body Wt.)	97.4 (11)	96.7 (11)		96.8 (11)	160 (11)		96.8 (11)	190 (11)		80.4 (11)	108 (11)	
Nitrogen Retention Per 24 hours (Mgs./100 g. Body Wt.)	65.6 (11)	64.3 (11)	-0.283	59.2 (11)	85 (11)	3.68	59.2 (11)	47 (11)	-0.495	-80.4 (11)	-108 (11)	-8.45

Values are averages for the numbers of animals shown in parentheses.

Table I (Cont.)

Food Consumption and Nitrogen Intake with Corresponding Excretions of Various Urinary Constituents

	Control Day			1st Experimental Day			2nd Experimental Day			Fast Day		
	Control Diet	Control Diet	t	Control Diet	Glycine Diet	t	Control Diet	Glycine Diet	t	Control Diet	Glycine Diet	t
Urea Nitrogen Per 24 hours (Mgs./100 g. Body Wt.)	85.1 (11)	80.6 (11)		83.2 (11)	155 (11)		82.7 (11)	175 (11)		66.5 (11)	96.0 (11)	
Ammonia Nitrogen Per 24 hours (Mgs./100 g. Body Wt.)	5.81 (11)	6.16 (11)	1.38	7.21 (11)	9.09 (11)	5.84	7.14 (11)	10.53 (11)	8.21	7.45 (11)	7.80 (11)	0.842
Uric Acid Per 24 hours (Mgs./100 g. Body Wt.)	1.30 (10)	1.33 (10)	0.344	0.92 (10)	1.52 (10)	3.10	1.12 (10)	1.55 (10)	2.75	1.34 (10)	1.64 (10)	2.60
Creatinine Per 24 hours (Mgs./100 g. Body Wt.)	3.71 (11)	3.66 (11)	-0.579	3.80 (11)	3.71 (11)	-0.094	3.92 (11)	3.86 (11)	-0.649	3.85 (11)	3.81 (11)	-0.243

Values are averages for the numbers of animals shown in parentheses.

Table I (Cont.)

Food Consumption and Nitrogen Intake with Corresponding Excretions of Various Urinary Constituents

	Control Day			1st Experimental Day			2nd Experimental Day			Fast Day		
	Control Diet	Control Diet	t	Control Diet	Glycine Diet	t	Control Diet	Glycine Diet	t	Control Diet	Glycine Diet	t
Uric Acid : Creatinine Ratio*	0.35	0.34		0.24	0.41		0.29	0.40		0.35	0.44	
Creatine Per 24 hours (Mgs./100 g. Body Wt.)	0.28 (9)	0.09 (9)	-1.46	0.15 (9)	1.28 (9)	4.22	0.14 (9)	2.95 (9)	6.20	0.73 (9)	3.37 (9)	4.43
Inorganic Phosphate Per 24 hours (Mgs./100 g. Body Wt.)	7.23 (11)	7.71 (11)	0.927	7.86 (11)	7.33 (11)	0.801	7.02 (11)	7.95 (11)	1.84	8.85 (11)	9.51 (11)	1.15
Free Glycine Per 24 hours (Mgs./100 g. Body Wt.)	0.22 (10)	0.23 (10)	0.326	0.28 (10)	0.36 (10)	2.15	0.26 (10)	0.39 (10)	4.01	0.22 (10)	0.32 (10)	4.50

Values are averages for the numbers of animals shown in parentheses.
*Calculated as suggested by Forsham, Thorn, Prunty, and Hills (19)

Figure 2

A Comparison of Total Urinary Nitrogen Excretion, per 100 g. Body Weight, with Urinary Urea Nitrogen Excretion per 100 g. Body Weight.

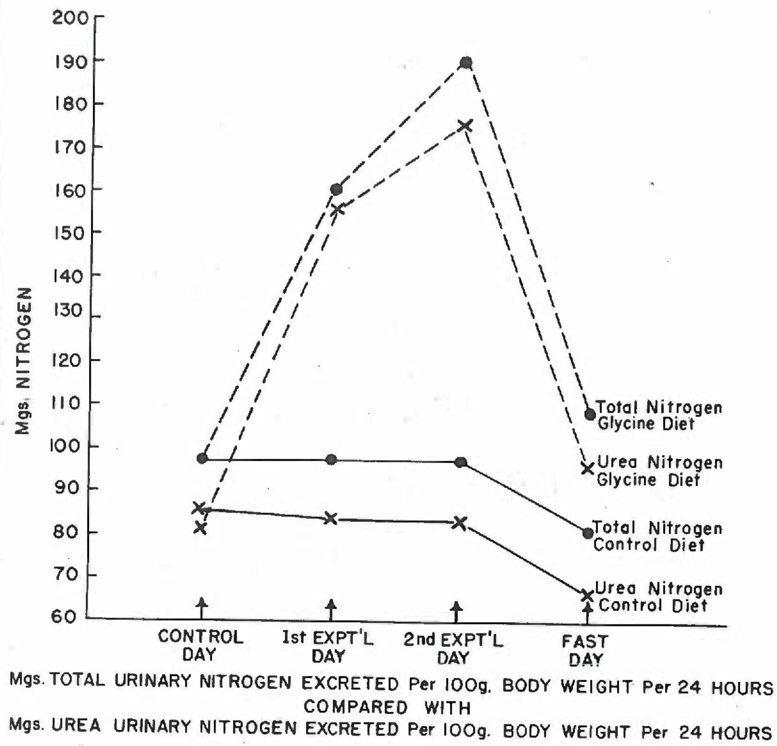

Figure 2

was lower in both control-fed and glycine-fed animals than that observed during feeding, the decrease being much more marked, however, in the glycine-fed animal. Even so, the nitrogen output of glycine-fed animals remained about 28 mgs. per 100 grams of body weight above that of control-fed animals.

A clearer picture of the relationship between nitrogen intake and nitrogen excretion in these animals may be gained by reference to Figure 3. The figures plotted represent differences between nitrogen ingestion and total urinary nitrogen excretion. It may, perhaps, be poor terminology to refer to these figures as nitrogen balances since the feces were not analyzed for this element, but such usage of the term is not without precedent. Ingle[11, 12] has referred to an increased urinary nitrogen output in the presence of a constant food intake as a negative nitrogen balance. This same investigator, in his many investigations of adrenal cortical function, has seldom, if ever, referred to fecal nitrogen. In previous studies in this series[5], it was demonstrated that all glycine and carbohydrate had been absorbed from the gastrointestinal tract at the end of an 8 hour fast. This demonstration of complete absorption of the diet coupled with the short duration of these experiments made it seem unlikely that any great changes in fecal nitrogen might occur during the experimental period. In any case, a comparison of urinary nitrogen excretion with nitrogen consumption was of interest. Figure 3 shows that glycine-fed animals retained significantly greater amounts of nitrogen than did control-fed animals during the first experimental day, then began to exhibit evidence of adaptation to

Figure 3

Nitrogen Balances per 100 g. Body Weight per 24 Hours Based on Total Urinary Nitrogen Excretions.

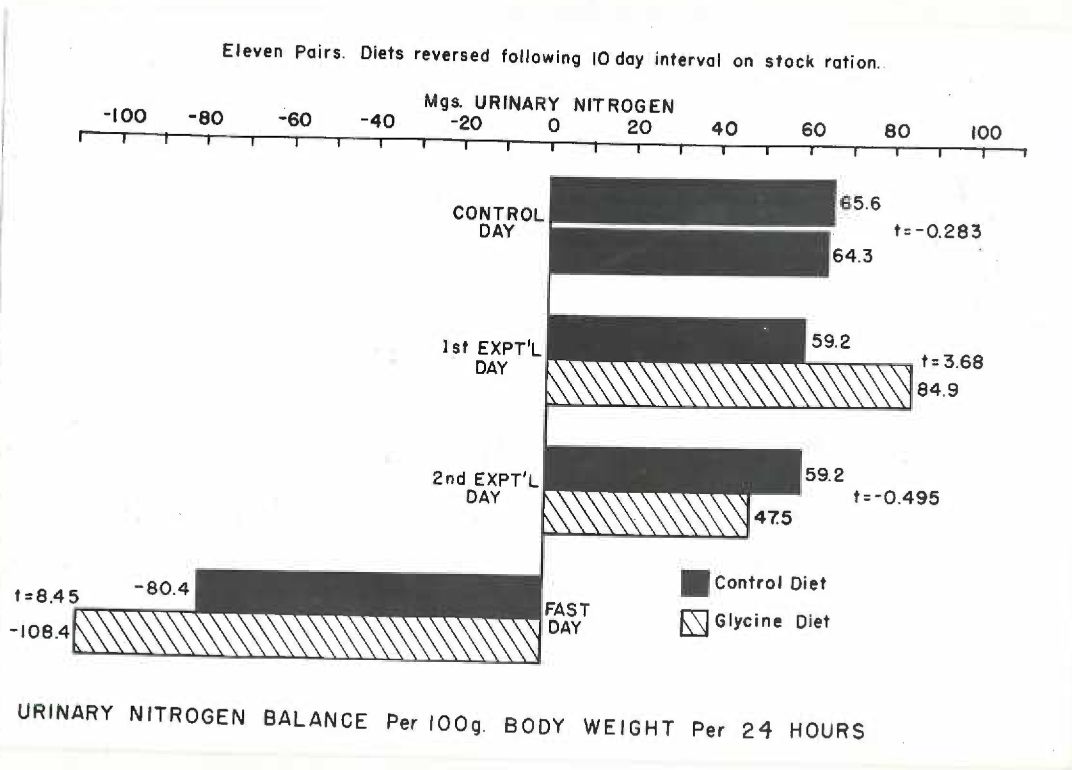

Figure 3

the higher nitrogen intake during the second experimental day. During the latter day, they retained a somewhat smaller amount of nitrogen than did control-fed animals, although the difference was not statistically significant. As previously noted, animals prefed the glycine diet excreted more nitrogen during the fasting day than did animals prefed the control diet and this difference was statistically highly significant.

Inasmuch as urea is the chief nitrogenous constituent of urine, one would expect the excretion of urea by normal animals to parallel their total nitrogen output. This actually proved to be the case as Figure 2 shows clearly. The parallelism is excellent in control-fed animals but not quite so good in glycine-fed animals. In the case of the latter, the total nitrogen output rose slightly faster than the urea output, reflecting the increased excretion of other nitrogenous compounds by glycine-fed animals, but still it can be seen that the bulk of the rise in total urinary nitrogen was due to urea.

Glycine feeding was found to augment ammonia excretion very significantly during the first and second experimental days, but the difference disappeared during the fasting day. Figure 4 illustrates these results. The cause of the increase in urinary ammonia exhibited by control-fed animals beginning on the first experimental day is not clear. It must be kept in mind, however, that excretions of the various substances during the control day reflected to a considerable extent the effects of the previous diet. In some way, assimilation even of the control diet resulted in the excretion of more ammonia

Figure 4

Urinary Excretions of Ammonia Nitrogen per 100 g.

Body Weight per 24 Hours.

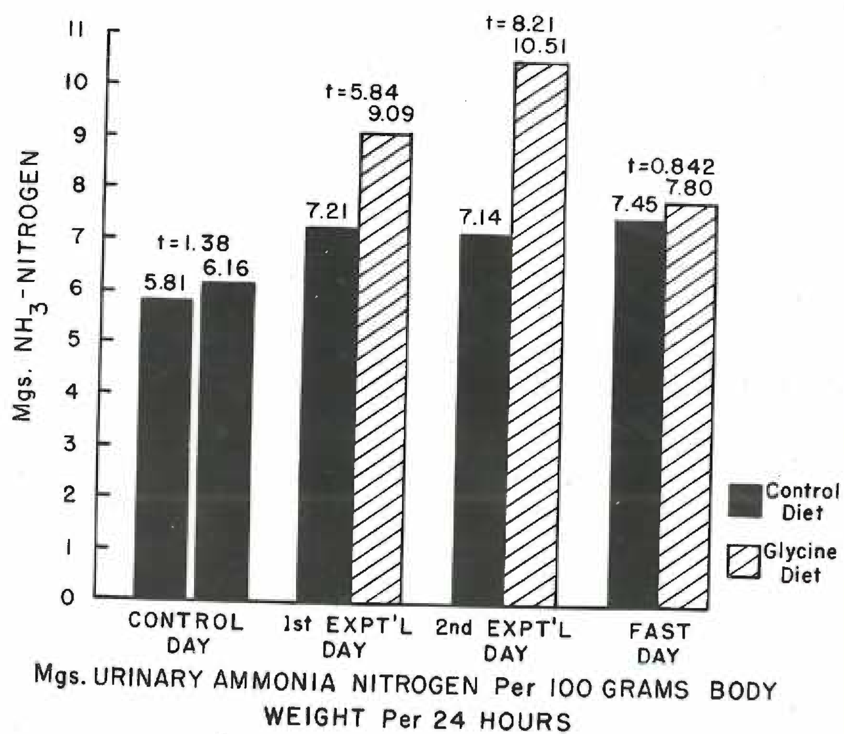

Figure 4

than assimilation of the Purina Laboratory Chow on which stock animals were maintained. Nevertheless the ammonia excretion of animals treated in exactly the same way except for the substitution of glycine for carbohydrate in their diet rose even higher.

Significantly larger amounts of uric acid appeared in the urine of animals receiving the glycine diet beginning on the first experimental day and continuing through the fasting day. Figure 5 compares the uric acid outputs of animals on the two diets. Again, it is not known why control-fed animals exhibited a reduction in uric acid excretion during the first and second experimental days as compared to the control day, but it is likely that this also is a result of the transition from Purina Laboratory Chow to the control ration. As for animals receiving the glycine ration, not only was their uric acid excretion greater than that of control-fed animals, it was also greater than the uric acid excretions of the control day.

Although the creatinine output of glycine-fed animals appears to be consistently less than that of control-fed animals (Table I), this difference is extremely slight and not at all significant when subjected to statistical analysis. Forsham, Thorn and associates (17, 18, 19, 20) have utilized the increased uric acid and nearly constant creatinine excretions observed in human patients given adrenocorticotrophic hormone to calculate the uric acid : creatinine ratio which they use as one index of adrenal cortical response to adrenocorticotrophic hormone. Their purpose in calculating this ratio rather than determining the daily uric acid output of their

Figure 5

Urinary Uric Acid Excretions per 100 g. Body Weight per 24 Hours.

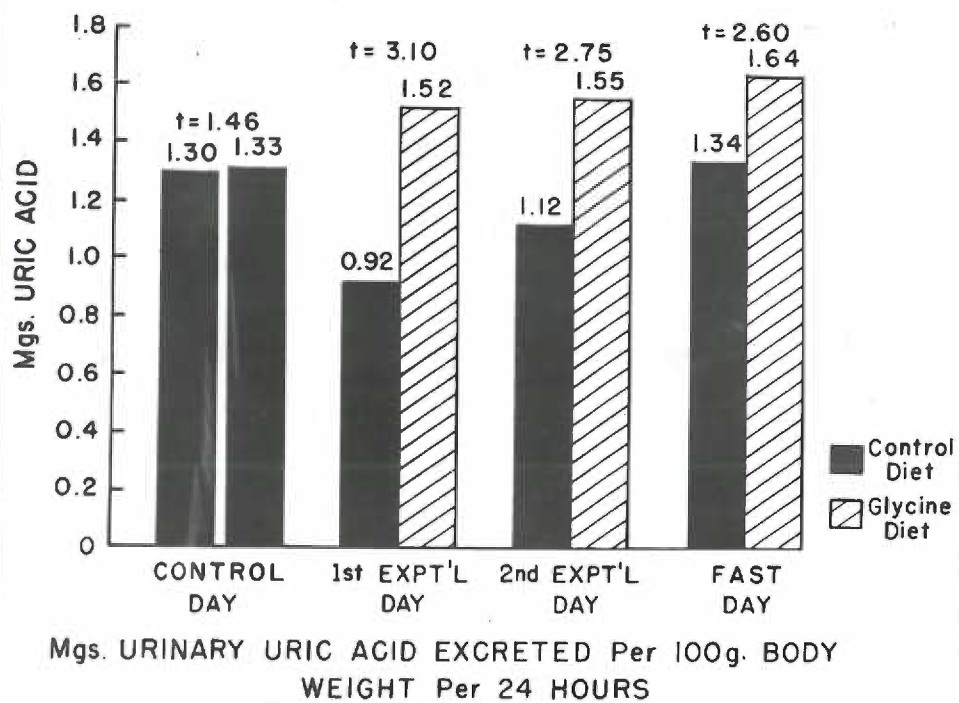

Figure 5

patients was to avoid the necessity for accurate 24 hour urine collections. Although accurate urine collections were made in the experiments reported here, it was still of interest to calculate this ratio for each day and it has been included in Table I and is illustrated graphically in Figure 6. Glycine-fed animals showed a uric acid : creatinine ratio on the first experimental day that was 71 per cent higher than that of control-fed animals. On the second experimental day, the ratio was still 38 per cent higher for glycine-fed than for control-fed animals and on the fast day it was 26 per cent higher.

Creatine excretion is markedly elevated in glycine-fed animals during the first experimental day and continues to increase through the fasting day. Figure 7 shows the striking differences in the outputs of this compound by animals on the two diets. Creatinuria always appears very promptly after the initiation of a fast and the animals fed on the control ration in these experiments were no exception. There was some augmentation of their creatine excretion during the fasting day, but still their output failed even to approach that of the animals prefed the glycine ration.

The excretion of inorganic phosphorus appeared to be somewhat lower in glycine-fed animals than in control-fed animals during the first experimental day and somewhat higher during the second experimental day and the fasting day, but none of these differences proved to be statistically significant.

It can be seen in Table I that the urinary glycine excretions of animals on both diets was very small. Glycine feeding increases

Figure 6

Uric Acid : Creatinine Ratios

Figure 7

Urinary Creatine Excretions per 100 grams Body Weight per 24 Hours

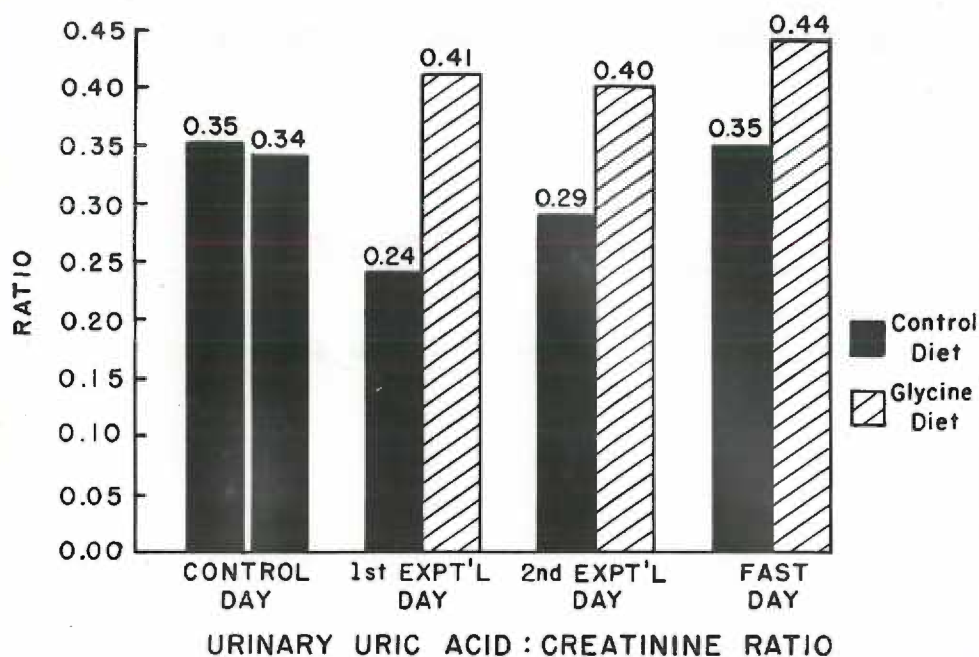

Figure 6

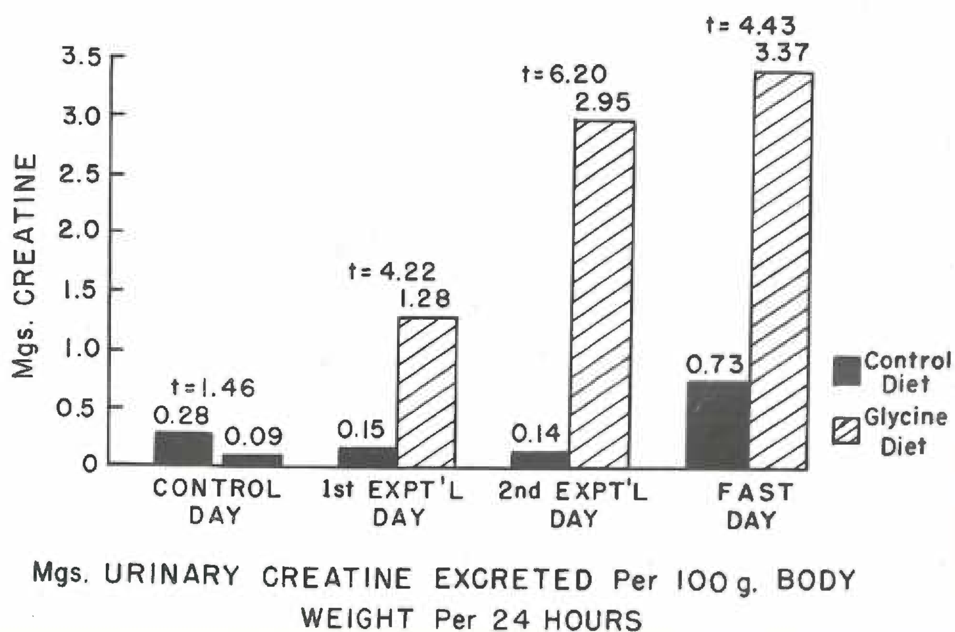

Figure 7

the output of this amino acid somewhat and the differences in its excretion by glycine-fed animals as compared to control-fed animals do become significant, from a statistical standpoint, during and after the second experimental day. However, when one considers that animals on the glycine diet ingested over 500 mgs. of glycine per 100 grams of body weight per day and excreted only about 0.4 mg. per 100 grams of body weight per day, it can be seen that the excretion of uncombined glycine is negligible in comparison with the intake. Since an average of 96 per cent of the total urinary nitrogen as determined by Kjeldahl was accounted for in the various constituents, it is not likely that an appreciable amount of combined glycine was excreted.

Animals on both diets lost weight of course during the experiments since a 24 hour fast was included in the experimental period. Glycine-fed animals lost, on an average, about 4 grams more than the control-fed animals but this difference is not statistically significant.

DISCUSSION

Previous studies on the metabolic effects of adrenal cortical extract, adrenal cortical steroids, and adrenocorticotrophic hormone in animals emphasize an increase in urinary nitrogen in association with alterations in carbohydrate metabolism. Indeed, Long, Katzin, and Fry[8], upon first making such observations, felt that the symptoms of adrenal cortical insufficiency or excess could be accounted for on the basis of increased or decreased glucogenesis from protein. Since that time, however, considerable evidence has been accumulated which indicates that this is not the case. Ingle[10] soon demonstrated that the increase in glucose excretion induced in partially depancreatized or adrenalectomized, partially depancreatized rats upon the administration of 17-hydroxy-11-dehydrocorticosterone could not be accounted for on the basis of increased glucogenesis from protein. Later studies by this same investigator and his associates[12] have established that adrenal steroid diabetes is very resistant to control with insulin and that even if the glycosuria of animals in which this condition had been induced were successfully controlled with insulin, their nitrogen excretions remained high. Furthermore, the initial rise in nitrogen excretion following the initiation of hormone injections preceded the onset of glycosuria. Thus it appeared that the adrenal cortical hormones have a primary protein catabolic action which is not secondary to their effects upon carbohydrate metabolism. In studies of nitrogen excretion following adrenalectomy, Ingle and Oberle[39] found that, after recovery from the operation, force-fed, adrenalectomized animals maintained with 1 per cent sodium chloride

solution for drinking water excreted as much nitrogen as during the pre-operative period and as much as their sham-operated controls. It was concluded from this that rats have mechanisms other than the adrenal cortex for the regulation of nitrogen balance. When Ingle and Prestrud[40] subjected force-fed rats receiving 1 per cent sodium chloride for drinking purposes to adrenalectomy, and treated half of them with cortical extract, the latter animals and the sham-operated controls showed a smaller post-operative increase in urinary nitrogen than did adrenalectomized animals not receiving the extract. Under these circumstances then, adrenal cortical hormones appear to have favored protein anabolism. The clinical studies of Thorn, Forsham, and associates[16, 17, 18, 19, 20] and of Conn and associates[25, 26] have established that impairment of carbohydrate metabolism is often demonstrable in the absence of an increase in urinary nitrogen. Babad[21] had observed a number of years earlier that the administration of adrenal cortical extract to rats maintained on a carbohydrate diet until their nitrogen excretions had reached a constant level resulted in no appreciable increase in total urinary nitrogen although the output of products of purine metabolism rose. Still another factor to be taken into consideration in interpreting the data on urinary nitrogen excretion obtained in the work reported here is the age of the animals used. Although mature male rats were used, they were young enough to be growing slowly so that there was a tendency for them to retain nitrogen during feeding. Considering all these things, then, a negative, or less positive, nitrogen balance in

glycine-fed animals, although good evidence in favor of enhanced adrenal cortical activity, would not be conclusive evidence of its occurrence, nor would the failure of an increase in nitrogen excretion to appear rule out a stimulation of adrenal cortical activity. Reference to Figures 2 and 3 makes it clear, not only that a negative nitrogen balance failed to occur during the period of glycine feeding but also that there was actually considerable nitrogen retention. The fasting period was another matter however. During this time, animals prefed the glycine diet exhibited a highly significant augmentation of urinary nitrogen excretion compared to control-fed animals. The net increase amounted to 28 mgs. of nitrogen per 100 grams of body weight. Since proteins are usually considered, for general purposes, to contain 16 per cent nitrogen, an extra urinary nitrogen excretion of 28 mgs. amounts to approximately 28 x 6.25 or 175 mgs. of dry protein. Most body tissues contain about 80 per cent water, so that quantity of dry protein would represent a loss of about 0.875 grams of body tissue per 100 grams of body weight or more than 2 grams of body tissue in a 250 gram rat. This enhancement of the urinary nitrogen output of glycine-fed animals as compared to that of control-fed animals upon being subjected to the stress of a fast offers evidence in support of the theory of a stimulation of adrenal cortical activity as a result of feeding that amino acid. In this connection, it is interesting to note that the increase in the absolute values for nitrogen output by glycine-fed animals arising from the higher nitrogen content of the glycine diet is attributable for the most part to an increased

urea output (Figure 2) and not to any appreciable extent to the excretion of glycine itself (see Table I). From this it is obvious that the glycine taken in the diet enters into chemical reactions in the body rather than being excreted unchanged. Whether or not this would be the case in the absence of the adrenal glands is not known at this time. It is not impossible that this observation is itself an indication of adrenal cortical activity.

The studies of Thorn, Forsham, and coworkers[16, 17, 18, 19, 20] and of Conn and associates[25, 26] on human beings have established a close correlation between loss of carbohydrate tolerance and increased uric acid excretion during the administration of adrenocorticotrophic hormone. This relationship is much more consistent and predictable than the relationship between loss of carbohydrate tolerance and increased nitrogen excretion. Such observations are compatible with those of Babad[21] in which it was found that the administration of adrenal cortical extract to rats brought to a constant level of nitrogen excretion by feeding on a carbohydrate diet resulted in no significant change in total urinary nitrogen but did increase markedly the elimination of products of purine metabolism. The uric acid excretions of glycine-fed animals proved to be significantly higher than that of control-fed animals, not only during the fasting period but also during the period of feeding on the experimental ration, (see Figure 5). Viewing these results in the light of the clinical studies cited above, the figures for urinary uric acid support the

theory of a stimulation of adrenal cortical activity during feeding as well as during fasting.

Forsham, Thorn, and associates(17, 18, 19, 20) have utilized the increase in uric acid excretion in the presence of an unchanged creatinine output to set up the uric acid : creatinine ratio as an index of adrenal cortical response to adrenocorticotrophic hormone. Since the creatinine excretion of glycine-fed rats proved to be almost the same as for control-fed rats, it seemed permissible to calculate this ratio for the two groups of animals (Figure 6). For the first experimental day, the ratio is 71 per cent higher for glycine-fed animals than for control-fed animals, for the second day, 38 per cent higher, and for the fast day 26 per cent higher. Forsham et al.(19), upon administering 40 mgs. of adrenocorticotrophic hormone per day for four days to a normal human male, noted a 20 per cent increase in his uric acid : creatinine ratio for the entire period of treatment. The increase was much greater in a patient suffering from pituitary insufficiency. Thus, the increase in this ratio for glycine-fed animals serves to emphasize the rise in their uric acid output with its indication of adrenal cortical activation.

The very striking increase in creatine excretion (Figure 7), which appeared promptly after the initiation of glycine feeding and continued through the fasting day, is a most interesting and noteworthy observation. A number of years ago, Babad(22) demonstrated that adrenal cortical extract increased the creatinuria induced by inanition in both intact and castrated male and female rats, although

the effect was somewhat erratic in intact males. Subsequently, Mason et al.[23, 41] reported a decreased excretion of creatine in a young woman treated with adrenocorticotrophic hormone. This subject also developed acne and exhibited only a slight increase in nitrogen excretion even when receiving a large dose of hormone, indicating that androgenic effects were in the ascendancy in this case. Sprague and associates[27] have demonstrated increases in urinary creatine nitrogen after the administration of adrenocorticotrophic hormone and 17-hydroxy-11-dehydrocorticosterone acetate in several cases and the increase sometimes persisted for several days after hormone administration was discontinued. If testosterone propionate were administered simultaneously, there was an initial decrease in urinary creatine nitrogen and the subsequent rise was somewhat reduced in comparison with the rise resulting from adrenocorticotrophic hormone or 17-hydroxy-11-dehydrocorticosterone alone. This suppression of creatine excretion by androgen suggests a possible explanation for the inconsistent increases in creatinuria exhibited by intact male rats after treatment with adrenal cortical extract observed by Babad[22] and the reduced creatinuria of the woman studied by Mason et al.[23, 41] who also presented other evidences of androgenic effects. An increase in creatine excretion as a consequence of adrenal cortical activity is compatible with the tissue breakdown usually associated with an increased supply of these hormones, and the bulk of the evidence just presented supports this idea. The increased output of creatine by animals prefed glycine containing diets appears, then, to offer further

support to the theory of a stimulation of adrenal cortical activity. Although Roth and Allison[42], in studying the effects of adding 4.8 per cent glycine to casein diets, found no increase in urinary creatine or creatinine, neither were Todd, Barnes, and Cunningham[1] able to demonstrate the "protein effect" of glycine when only 5 per cent of that amino acid was included in the ration. Martel, Pagé, and Gingras[43, 44] have also reported the incidence of a severe creatinuria in rats fed diets containing 10 per cent glycine, but differences in materials and techniques prevent a close comparison of their work with the results reported here. The composition of their diet, aside from glycine, was quite different and their experiments covered much longer periods of time. Creatine determinations were done after nine days of glycine feeding at the earliest. They have attributed their findings to deficiencies in certain B vitamins[43, 44, 45] perhaps due in part to a suppression of bacterial synthesis of vitamins in the gastrointestinal tract. Such deficiencies could scarcely account for changes appearing promptly upon the initiation of glycine feeding or even during a 48 hour period of feeding on the glycine ration.

Since tracer studies have definitely established glycine to be a precursor of both creatine[46] and uric acid[47], an assessment of the increased urinary excretions of these two substances by glycine-fed animals must take into account the possible mass action effects of glycine feeding. However, if the enhancement of creatine excretion were simply a mass action effect, Roth and Allison[42] should have

found increased creatinuria in rats fed diets containing 4.8 per cent glycine and no such increase was noted. When such a large excretion of creatine in the presence of an unchanged output of creatinine is observed, one wonders about the levels of creatine and especially creatine phosphate in muscle. If there were an appreciable increase in the latter, the consequent augmentation of the reservoir of high energy phosphate groups might account for the excellent maintenance of muscle glycogen exhibited by glycine-fed animals during the action of insulin[5]. Investigation of the levels of creatine phosphate in the muscles of rats fed the glycine diet might prove to be of considerable interest. As for uric acid, since glycine is only one compound entering into its synthesis and this amino acid is also capable of entering into the biosynthesis of a number of compounds other than creatine and uric acid[48, 49, 50, 51], it seems unlikely that mass action effects alone could account for increases of the size observed.

Still another question arises with respect to creatine excretion. Pitts[52] has presented evidence, in dogs, of a renal reabsorptive mechanism common to glycine and creatine. As plasma levels of glycine rise, reabsorption of the amino acid takes precedence and creatine is excreted. In this work, glycine was administered by intravenous infusion, so that its plasma concentration was probably greater than that attained by glycine feeding. Such a mechanism might not be demonstrable, then, if the amino acid were administered by mouth. Furthermore, if such a mechanism were operative, Roth and Allison[42]

should have found some augmentation of urinary creatine with the feeding of a diet containing 4.8 per cent glycine.

The urinary output of inorganic phosphorus by animals fed on the glycine diet revealed no significant differences from those of animals receiving the control diet. Ingle and Thorn[16] had observed a large rise in phosphorus elimination when partially depancreatized rats were treated with relatively large doses of 17-hydroxy-11-dehydrocorticosterone, but one need not necessarily expect such a change to occur in normal animals. Although Forsham, Thorn, et al.[17, 18, 19] have reported rises in the inorganic phosphorus excretions of patients suffering from pituitary insufficiency treated with adrenocorticotrophic hormone, they found that the administration of this hormone to a normal human male produced a slight decrease in phosphorus excretion during treatment, followed by a rise after the withdrawal of adrenocorticotrophic hormone. It seems likely that adrenal cortical activity does not have a direct effect upon phosphorus excretion, but rather that alterations in the elimination of this product of metabolism are secondary to the effects of the cortical hormones upon carbohydrate and protein metabolism. If this be true, then a mild stimulation of cortical activity, such as might result from glycine feeding or fasting for a comparatively short time, would not be expected to produce an appreciable change. The data obtained support this view.

In the presence of the greatly increased urea formation exhibited by glycine-fed animals during the period of glycine feeding, one should not be surprised at the increased production of ammonia nitrogen (Figure 4) shown by these animals. The formation of large amounts of

urea is associated with the assimilation of high protein diets which in turn leads to increased urinary acidity and ammonia. Although the isoelectric pH of glycine is around 6 and it would, of course, be dissociated as an acid at body pH, it seems improbable that an amino acid, which is furthermore a very weak acid, would remain in the circulation long enough to produce a significant degree of acidosis. Lotspeich and Pitts[53] have demonstrated that glycine and several other amino acids are capable of increasing the rate of ammonia secretion in the acidotic dog. Furthermore, there was a correlation between the extent of reabsorption of the amino acid and the increase in ammonia secretion associated with it. The observation that animals fed very considerable amounts of glycine excreted very minute amounts of this amino acid along with increased amounts of ammonia suggests that such a mechanism might account for part of the observed increase in urinary ammonia.

The results of these studies, then, give support to the theory of increased secretory activity of the adrenal cortex as a result of glycine feeding. Ingle, Ward, and Kuizenga, however, have obtained evidence that it may not be necessary to postulate increased secretory activity of the adrenals except during periods of acute stress[54], that maintenance of resistance to stress may be quite a different thing requiring only the presence of the adrenal cortical hormones, not an increased supply of them. Such a possibility was indicated by their observation that adrenalectomized, cortin-treated rats, when subjected to fractures of one or both hind legs, although failing to

excrete as much nitrogen during the first 24 hours following fracture as did unoperated animals, excreted amounts of nitrogen after this period that seemed to depend upon the severity of the stress to which they had been subjected rather than the amount of cortin they were receiving. In these experiments, the animals each received 4 cc. of adrenal cortical extract per day, which is rather a large dose for an animal as small as a rat. One wonders if there might not be a rather critical level of cortical hormone activity, above the normal physiological one, required for the successful maintenance of resistance to stress which was exceeded when that amount of exogenous hormone was administered. The stresses used in this work were obviously rather severe, while glycine feeding, if one is to think of it as a stress, is a comparatively mild one. Evidence has been presented that glycine feeding may constitute a stress since it does produce symptoms of toxicity under certain conditions. Martel, Gingras, and Page[43, 44, 45] in reporting the results of feeding glycine-containing diets over long periods of time always refer to the "toxic effects of glycine" or "glycine intoxication". In studying the metabolism of parenterally administered glycine in dogs, Handler, Kamin, and Harris[55] found infusion of this amino acid at rates exceeding 1 mg. of nitrogen per kilogram per minute to be invariably lethal. Nausea and vomiting were the first symptoms of toxicity noted by these workers. If the infusion were discontinued at this stage, the intoxication was reversible and the animals recovered, but if glycine administration were continued until symptoms of respiratory distress appeared, the changes

were irreversible. An animal receiving glycine in his diet would most likely refuse to eat before ingesting enough to carry him to the stage of respiratory distress, but rats receiving the glycine diet have been observed to appear nauseated upon occasion so the possibility of a toxic action of glycine cannot be dismissed. Such a possibility, however, does not rule out adrenal cortical activity as the mediator of the "protein effect" of glycine, but rather lends support to that hypothesis since the stress incident to the ingestion of a toxic substance would be expected to result in an activation of the adrenal cortex. Stresses as severe as those employed by Ingle et al.[54], producing a very acute condition, might result in very rapid depletion of the supply of hormones stored in the gland, leaving the animal dependent upon the daily manufacture of hormones, with no reserve supply, during the period of maintenance of resistance to stress. If these hormones act in or on enzyme systems, or as catalysts in some other way, perhaps the mass action effects of increased physiological demands for various substances produced under the influence of these hormones are sufficient to accelerate the reactions producing them to a considerable degree so long as some, not necessarily more, of the catalyst is present. Ingle does not deny the necessity for increased adrenal cortical secretion during the acute phase of a stress. Coming back to the stimulus of glycine feeding, a stress of such mild degree may not exhaust the supply of hormones stored in the glands rapidly but rather may leave some untouched, so that, as glycine feeding is continued, the gland would be able to augment its daily

output with some of the reserve supply, thus producing an increase in cortical hormone activity in addition to possible mass action effects. It is admitted that such a scheme is speculative, but it is difficult otherwise to correlate Ingle's findings with respect to maintenance of resistance to stress, and this must be considered in a three day experiment, and the results of the experiments reported here which match the effects of increased adrenal cortical activity so well.

The idea that glycine administration promotes carbohydrate formation from substances other than glycine is not a new one. Dakin[56] made such a proposal some thirty years ago. Although glycine had been found by Lusk[57] to augment the glycosuria of a fully diabetic animal by an amount closely corresponding to that theoretically possible if both carbon atoms were completely converted to glucose, Dakin felt that this augmentation could not be due to the direct conversion of glycine to glucose since neither glycollic acid, glyoxylic acid, nor acetic acid exhibited similar properties. Later Reid[58] who had been unsuccessful in demonstrating the conversion of glycine to sugar, established in studies of urinary nitrogen and inorganic sulfur that glycine caused more of an increase in protein catabolism than did alanine. He attributed these findings to the fact that alanine was known to be an excellent sugar former while according to his findings, glycine was not, so that an animal receiving alanine was not forced to draw so heavily upon body proteins for conversion to carbohydrate as was an animal given glycine. Olsen, Hemingway, and

Nier[59], using glycine labeled with C^{13} in the carboxyl group, were able to establish definitely that glycine carbon entered into the formation of liver glycogen to a small degree, according to their calculations to the extent of 1 carboxyl carbon from glycine for every 29.3 normal carbon atoms. They also postulated a promotion of glycogen formation from other body constituents as a result of the ingestion of glycine. The appearance of the carboxyl carbon of glycine in liver glycogen has been confirmed by Sakami[60] who obtained, in addition, good evidence that the entry of glycine into glycogen proceeds by way of serine. He drew no conclusions, however, as to the extent of the entry of glycine carbon into glycogen or to the stimulation of glycogenesis from sources other than glycine. In the work of Sakami, as in that of Olsen et al.[59] glycine was labeled in the carboxyl group with C^{13}. Thus although it was definitely established that the carboxyl carbon of that amino acid was utilized, at least to some extent, in the synthesis of liver glycogen, no direct evidence with respect to the α-carbon was available. This problem was attacked by Barnet and Wick[61] who administered to rats, by gavage, glycine labeled with C^{14} in the α-position as well as glycine labeled in the carboxyl group with that isotope. The results obtained using the carboxyl-labeled acid were in excellent agreement with those of Olsen, Hemingway, and Nier[59] in that they found the carboxyl carbon of glycine to contribute about 1 carbon atom for each 28 carbon atoms incorporated into liver glycogen, whereas Olsen et al. had found 1 carboxyl carbon from glycine to each 29.3 normal carbon

atoms of glycogen. As for glycine labeled in the α-position, Barnet and Wick found that 1 out of each 8.5 carbon atoms of glycogen arose from the α-carbon of glycine. They were able to draw no conclusions from their results, however, relating to the controversy over direct conversion of glycine carbon to glycogen carbon as opposed to the stimulation of glycogenesis from other sources.

Several rather recent studies employing tracers have shed considerable light upon the intermediary metabolism of glycine and point out possible sources of the extra carbon atoms found in carbohydrate following glycine feeding. In the work of Sakami just mentioned(60), not only did he demonstrate the appearance of the carboxyl carbon of glycine in liver glycogen, but he also found that, after feeding C^{14} labeled formate simultaneously with glycine containing C^{13} in the carboxyl group, he could isolate serine from the livers which contained C^{13} in the carboxyl group and C^{14} in the β-position indicating that glycine can be converted to serine by fixation of formate. Since the β-position of serine contained no C^{13} at all, it was concluded that this carbon atom does not arise from formate by CO_2 fixation. A later report by Sakami(49) has presented evidence that the α-carbon of glycine itself, after deamination and decarboxylation, is capable of contributing formate or formate derivative for the conversion of glycine to serine. After administering glycine labeled with C^{14} in the α-position, by stomach tube, to rats, Sakami isolated serine with C^{14} in both the α- and β-positions. Furthermore, he found nearly as much activity in the β-position as in the α-position.

Serine is an excellent glucose former and this contribution of the α-carbon of glycine to both the α- and β-positions of serine has led Barnet and Wick[61] to advance a pathway via serine for the conversion of glycine to glycogen as an explanation for their observation that the α-carbon of glycine enters into glycogen to a greater extent than does the carboxyl carbon. Siekevitz and Greenberg[51] have confirmed much of Sakami's work in tracer studies carried out using rat liver slices. They found that labeled formate plus unlabeled glycine gave serine labeled in the β-position, that the α-carbon but not the carboxyl carbon of glycine appeared as formate, and that CO_2 was not reduced to formate. Under their experimental conditions, at least, the production of formate from the α-carbon of glycine was not reversible but the formate could condense with glycine to give serine. Although rat liver slices were able to form serine from glycine both anaerobically and aerobically, it was concluded from the percentage of radioactivity in the β-carbon of serine relative to the total radioactivity of the serine that there is another source of formate in the fasted rat liver slice which is more active anaerobically than aerobically. Very soon thereafter, Sakami[62] reported the isolation of serine containing C^{14} in the β-position from rat livers following treatment with C^{14} methyl-labeled choline, indicating that at least one of the methyl groups of that compound may contribute formate for condensation with glycine to give serine. It was also suggested by Sakami that the methyl group of methionine might also serve as a formate donor since it is transferable to choline. Siekevitz and

Greenberg[63] had also been conducting a series of investigations into this problem using liver slices. When either C^{14} methyl-labeled methionine or C^{14} N-methyl-labeled choline were used, both formate containing C^{14} and serine with C^{14} in the β-position were isolated. Their data also suggest that the methyl group of methionine may enter this reaction through some intermediate step or steps, most likely via choline. Formate production from choline was greater anaerobically than aerobically suggesting that this may be the extra-glycine source of the β-carbon of serine which had formerly been shown to be more active under those conditions[51]. Other possible extra-glycine sources of the β-carbon of serine have been suggested. Sakami[64], using C^{14} labeled acetone, has demonstrated that this compound is capable of contributing the β-carbon of serine and the labile methyl groups of methionine and choline. Since C^{14} methyl-labeled acetate failed to yield such results, it was concluded that acetone utilization involves cleavage into acetate and formate or formate derivatives and the formate moiety enters into the biosynthesis of serine, methionine, and choline while the acetate portion follows other metabolic pathways. Meltzer and Sprinson[65] have recently reported the cleavage of threonine *in vivo* to yield acetate and glycine, a process which might serve as an indirect source of glycine to enter into serine formation. That conversion to serine is indeed an important step in the intermediary metabolism of glycine is further emphasized by the recent report of Greenberg and Harris[66] establishing that glycine is not reduced directly to ethanolamine but that this process proceeds instead

instead via the intermediate formation of serine from glycine followed by decarboxylation. Also Sprinson[50] has found that the α-carbon of glycine enters into the formation of acetic and aspartic acids in the intact rat, and it is likely that these reactions also proceed via serine although this has not been proved as yet. MacKay et al.[67] noted some years ago that the peak of liver glycogen formation by fasted rats given glycine was not reached until 14 hours after glycine administration. This delay might well be a reflection of the fact that glycine must proceed through some intermediate compound, such as serine, before being converted to glycogen. It may well be that the increased retention of nitrogen by glycine-fed animals as compared to control-fed animals during the first experimental day, which was found to occur in the experiments reported here, also results from the metabolism of glycine via some roundabout pathway, such as an initial conversion to serine. At any rate, there seems little doubt that the formation of serine represents an important process in the intermediary metabolism of glycine.

While the α-carbon of glycine itself is capable of furnishing the β-carbon to another molecule of glycine to form serine, there are also several other possible sources for this carbon atom such as the labile methyl groups of methionine and choline, acetone, and possibly glycine derived indirectly from the cleavage of threonine in the body. When the animal is presented with a large load of glycine to metabolize, which is the situation with the feeding of a diet containing 10 per cent glycine, all possible sources of β-carbon atoms for the

synthesis of serine may be called upon to a very considerable degree. Although the attachment of another carbon atom to glycine from an extra-glycine source could account for some of the net gain in carbohydrate carbon as compared to glycine carbon previously noted[6], one cannot assume a net gain of one carbon atom per molecule of glycine from such processes. In the presence of such a sizable load of glycine, some serine would surely be formed by the condensation of formate arising from the α-carbon of one molecule of glycine with another molecule of glycine. Since Sickevitz and Greenberg have shown[51] that the carboxyl carbon of glycine is not metabolized to formate but probably to CO_2, for each molecule of serine formed from two molecules of glycine, there would be a loss of one carbon atom. Such a loss would offset, either partly or wholly, the gain of a carbon atom accompanying serine formation from glycine and formate from an extra-glycine source. Thus it seems unlikely that the synthesis of serine from glycine, followed by the conversion of serine to carbohydrate, could account entirely for an enhancement of carbohydrate reserves of the magnitude of that observed after prefeeding of the glycine diet. It is interesting to speculate, however, upon the possible fate of the residue remaining after a compound has supplied formate for serine formation. For example, after utilization of the methyl group of methionine to supply formate, a residue of homocysteine is likely. If this compound were to be converted to carbohydrate, it could make an appreciable contribution to the glycogen stores. If formate for serine formation were derived

from acetone (arising in fat metabolism) or from threonine (via cleavage to give glycine), a residue of acetate would be left. Oxidation of acetate thus produced via the tricarboxylic acid cycle could exert a sparing action upon carbohydrate stores. If methionine and threonine were important participants in the scheme of metabolism of glycine via serine, the assimilation of large amounts of glycine might be expected to require the withdrawal of these amino acids from tissue proteins to some extent. Such a withdrawal would, at the same time, release other amino acids which could not be retained in the tissues in the absence of sufficient methionine and threonine and these, in turn, might also enter into the glycogenic process. It is interesting to note that MacKay and associates(67) found glycine to produce, eventually, higher liver glycogen levels than with glucose or dl-alanine although the doses of the three compounds were adjusted so that their carbon equivalents were the same, and the peaks of glycogen formation from glucose and dl-alanine were nearly the same. Although this fact did not escape their attention, that group felt that the conditions of their experiments did not warrant any conclusions as to the significance of this observation. It is nevertheless very suggestive of a stimulation of glycogen synthesis from materials other than glycine, perhaps through a chain of events such as that just outlined. Such a process is, of course, only theoretical at this time. It is known that the pathway through serine is an important one in the metabolism of glycine, that glycine may condense with formate from several possible sources to yield serine,

and that feeding diets containing 10 per cent glycine results in improved carbohydrate reserves after a fast. It seems probable that serine synthesis from glycine plays a role in this stimulation of glycogenesis, but it has not yet been demonstrated experimentally. If one assumes for the moment that this pathway is important, a question arises as to the relative contributions of serine itself and of compounds arising as by-products in the synthesis of this amino acid to the glycogen stores.

The importance of the adrenal glands to the "protein effect" of glycine was demonstrated early in this series of investigations by the failure of this response to appear in adrenalectomized animals and the theory of an enhancement of adrenal cortical activity has been strengthened by the results of the urine studies reported here. Certainly it is reasonable to suppose these hormones to exert some action upon processes such as those suggested in which several amino acids partisipate. If one assumes an increased supply of cortical hormones to be required in producing the "protein effect" of glycine, a question then arises as to the exact stimulus for the secretion of this extra amount of hormones. It may be that the presentation to the animal body of a large load of amino acid itself serves as a stimulus for cortical activity, with tissue protein mobilization concomitant to such an increase in circulating hormones. The appearance of symptoms of toxicity with the intravenous infusion of glycine[55] coupled with the observation that formate is actually produced in biological systems in the biosynthesis of serine from glycine[51, 63]

lead to an interesting hypothesis. Perhaps the assimilation of a large amount of glycine may result in the production of sufficient formate to reach toxic levels, thus activating the adrenal cortices which in turn bring about the mobilization of tissue proteins.

There is, then, good evidence in support of the theory of enhanced adrenal cortical activity as a result of glycine feeding, but the exact stimulus for it and the site or sites of action of the hormones are not known. Also, little is known concerning the processes of intermediary metabolism involved in producing the "protein effect" of glycine, but the formation of serine by animals receiving the glycine diet appears to offer a fertile field in the further investigation of this problem.

SUMMARY

Earlier investigations had established that rats prefed diets containing 10 or 15 per cent glycine maintained their carbohydrate reserves much more efficiently when subjected to stress than pair-fed control rats prefed diets similar in composition except for the substitution of an equal weight of carbohydrate for glycine. The absence of such a response in adrenalectomized animals was presumptive evidence for the mediation of this effect by the adrenal cortices, possibly through increased secretory activity. Investigations into the extent of tissue storage of glycine during the feeding of that amino acid disclosed insufficient reserves of glycine to account for the net excess of carbohydrate found in the bodies of glycine-fed animals as compared to control-fed animals after insulin, assuming the glycine carbon to be completely transformed to carbohydrate carbon.

More direct evidence in support of increased adrenal cortical activity as a result of glycine feeding was sought from careful studies of the daily urinary excretions of various substances by animals prefed the two diets and comparing the changes noted with the reported results of treatment with adrenal cortical extract, adrenal cortical steroids, and adrenocorticotrophic hormone. Previous work indicated that studies of the total nitrogen, uric acid, creatine, creatinine, and inorganic phosphorus excretions should be of value in such a comparison. In addition, outputs of urea, ammonia, and glycine were investigated. Two animals were given the control diet for a period of 24 hours after which one animal was changed to the

glycine diet while the other continued to receive the control diet for a period of 48 hours. At the end of this time, both animals were subjected to a fast of 24 hours. The urine excreted by each rat during each 24 hour period was analysed for all of the substances mentioned.

Since the substitution of glycine for carbohydrate in the diet increases the nitrogen content of the diet, animals receiving this ration excreted more nitrogen than did their pair-fed controls. However, when nitrogen excretions were compared with nitrogen intakes, it was found that animals receiving the glycine diet retained significantly more nitrogen during the first day of glycine feeding than did control-fed animals. During the second day of glycine feeding, rats ingesting that diet appeared to retain somewhat less nitrogen than animals ingesting the control diet but the difference was not significant. Upon being subjected to a 24 hour fast, rats prefed the glycine diet excreted significantly more nitrogen than rats prefed the control diet, the net excess of nitrogen excretion accounting for an additional loss of more than 2 grams of body tissue in a 250 gram rat.

Urea production by animals receiving both diets paralleled the total nitrogen excretion quite closely. Increased excretions of other nitrogenous compounds by animals fed the glycine diet resulted in a somewhat poorer parallelism in this respect for glycine-fed animals.

Ammonia excretion was found to be significantly increased by glycine feeding during the feeding period, but the differences in ammonia output by animals receiving the two diets disappeared during the 24 hour fast.

A significant rise in uric acid excretion occurred as soon as glycine feeding was initiated and continued through the fasting day. Since there was essentially no change in creatinine output as a result of glycine feeding, the uric acid : creatinine ratio for glycine-fed animals was increased.

The ingestion of glycine resulted in a striking increase in creatine excretion which was well maintained through the fasting day.

No significant changes in the excretion of inorganic phosphorus were found to occur.

The urinary excretion of glycine by animals receiving the glycine diet was somewhat greater than that of animals receiving the control diet and this difference became significant, from a statistical standpoint, during the second experimental day and continued thus through the fasting day. However, the actual amounts excreted were so small as to make the increases of negligible importance.

These results are discussed in the light of previous findings with respect to the effects of adrenal cortical activity and it is concluded that they offer good evidence in support of the theory of increased secretory activity of the adrenal cortices as a result of glycine feeding. Also, suggestions are made with regard to possible pathways of intermediary metabolism, such as the conversion of glycine to serine, which might be involved in the "protein effect" of glycine.

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