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The chemistry of alloxan and the history of alloxan diabetes are reviewed. A comprehensive review of the literature concerning the etiology, pathogenesis, symptomatology, and clinical course of alloxan diabetes is presented.

The purpose of this study was to ascertain the possibility of utilizing alloxan diabetes as a means of studying intermediary metabolism in much the same way that the condition of phlorizin diabetes has been used in the past.

Rats and rabbits were used as experimental animals in this study. The rats were rendered diabetic by the administration of varying doses of alloxan monohydrate by the intravenous or subcutaneous routes. Intravenous injection of 40 mg./Kg of alloxan monohydrate to rats receiving food ad lib produced the most consistent results. The rabbits were rendered diabetic by the intravenous injection of alloxan monohydrate in doses of 125 mg./Kg.

The first part of this study consisted of daily collections of urine from alloxan diabetic rats and the analysis of the urine for glucose and nitrogen. The results of the urine studies on alloxan

diabetic rats were not found to be too satisfactory.

The second part of this study consisted of blood-sugar determinations on fasting alloxan diabetic rats and rabbits following the administration of various amino acids by stomach tube.

Using the latter technique, the carbohydrate-like actions of Glycine, DL-Alanine, Dl-Valine, DL-Phenylalanine, L-Tyrosine, L-Cystine, and DL-Methionine were investigated. It was found that Glycine, DL-Alanine, DL-Valine, DL-Phenylalanine, L-Tyrosine, and L-Cystine caused a definite increase in blood sugar when administered to alloxan diabetic rats. DL-Methionine, however, did not have a carbohydrate-like action when administered to alloxan diabetic rats. On the contrary, it caused a decrease in blood sugar.

It is concluded that alloxan diabetes in rats is a good qualitative technique for the study of gluconeogenesis and that rats are much better test animals then rabbits when this technique is used to study gluconeogenesis.

ALLOXAN DIABETES AS A POSSIBLE MEANS OF STUDYING INTERMEDIARY METABOLISM

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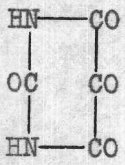
CONTENTS

ALLOXAN DIABETES AS A POSSIBLE MEANS OF STUDYING INTERMEDIARY METABOLISM

INTRODUCTION

CHEMISTRY. Alloxan is a ureide of mesoxalic acid and has the formula



It has been known for more than 100 years, since Wöhler and Liebig (1) produced it by oxidation of uric acid. It is a white powder melting at 256 degrees Centigrade, and it is freely soluble in water and alcohol. When stored in tightly stoppered bottles, it is liable to explode, especially in hot weather (2, 3, 4, 5). It decomposes on hydrolysis into urea and mesoxalic acid, and it is rapidly changed to alloxanic acid by alkalis. Alloxan is an oxidizing agent, having a special affinity for sulfhydryl groups (6, 7). It reduces to dialuric acid with which it acts as an oxidation-reduction system (8), and its structure is found as a constituent part of the flavine molecule and hence of the yellow enzyme. It might thus be expected to influence cellular enzyme systems, and it has been found to enhance the endogenous metabolism of liver suspensions (9), and to be a capillary poison (10). It also causes reversible inactivation of papain and cathepsin due to conversion of sulfhydryl groups into -SS- groups (11), inactivates succinic dehydrogenase (12), and, in suitable concentration, inhibits the formation of Robison and Cori esters (13).

ALLOXAN DIABETES. Alloxan diabetes is a new type of experimental diabetes. The way to its production was shown by the great discovery of Dunn, Sheehan and McLetchie (14) that alloxan has a selective necrosing effect on the pancreatic islet cells. Alloxan diabetes is a chemical diabetes in contradistinction to the surgical diabetes produced by pancreatectomy, which was first described by von Mering and Minkowski (15), and the endocrine diabetes produced by repeated injections of anterior pituitary extract, which was first reported by Young in 1937 (16). In the experimental diabetes occurring after pancreatectomy or after injections of anterior pituitary extract, other tissues than the insulin-producing islands are destroyed or affected. But when alloxan is injected intravenously, there occurs a selective necrosis of the islands of Langerhans of the pancreas, while the acinar tissues remain histologically normal, and the other endocrine organs are apparently unaffected.

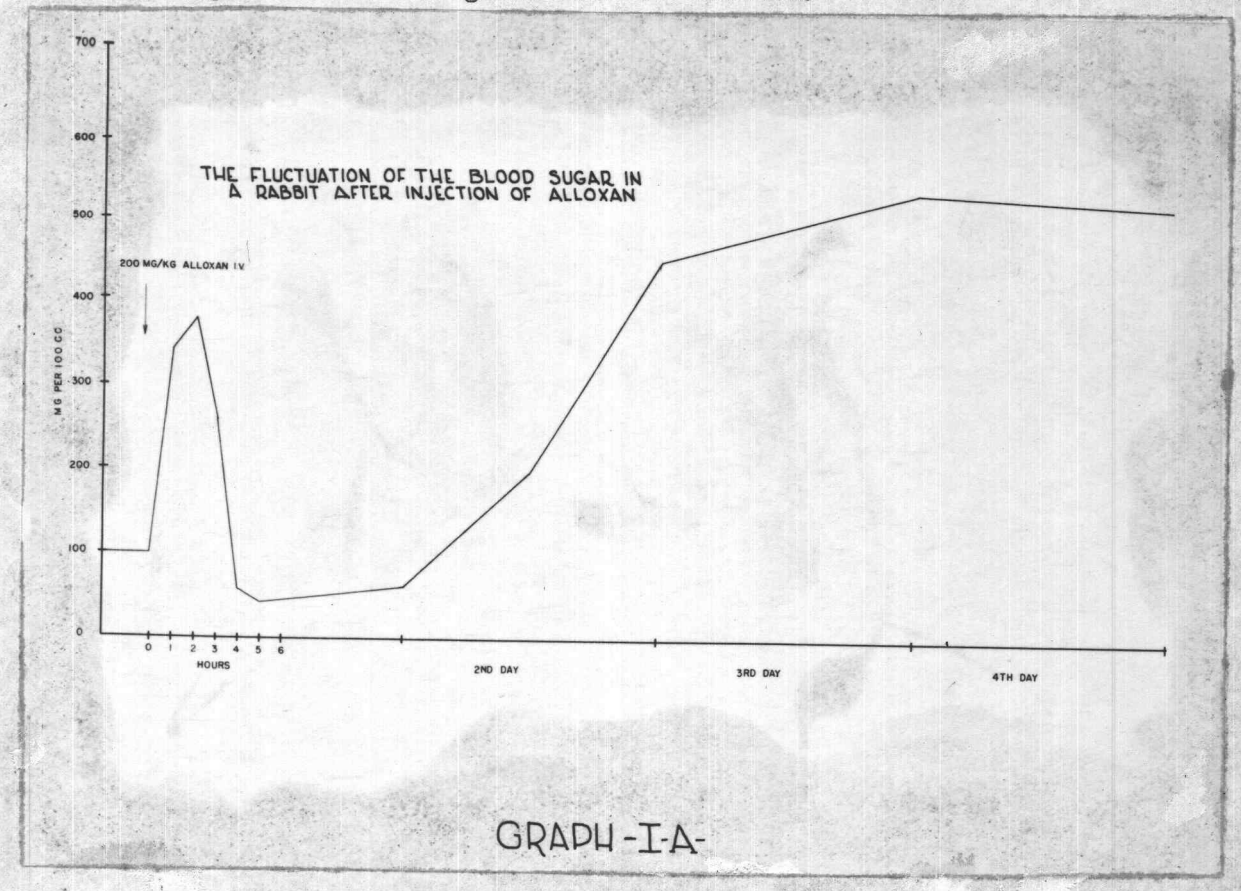
The history of alloxan diabetes begins with the observations of Jacobs (17) in 1937. He found that the injection of alloxan into normal fasting rabbits resulted in an initial hyperglycemia which was soon succeeded by a severe progressive hypoglycemia, lasting up to eight hours or longer, and terminating with death of the animals unless glucose was administered. Jacobs attributed this effect to an insulin-like action of alloxan. However, he missed the full effect of alloxan because he did not follow the blood-sugar in his animals beyond 24 hours. He also failed to make any histological studies.

Six years later, Dunn, Sheehan and McLetchie (14, 18) working on the effect of ureides on the kidneys, found this fluctuation of blood sugar

in alloxan treated rabbits and reported their pioneer observations of selective necrosis of pancreatic islet cells in these animals. They recognized that the early hypoglycemia represented a true insulin effect, and, though their rabbits had all died in a few days, they suggested that a sustained diabetes should be the final result of alloxan poisoning.

The report of Dunn et al. stimulated work by others, and within a short time alloxan diabetes was demonstrated in various animal species -- dogs, rabbits, rats, monkeys, pigeons, cats, hamsters and sheep (19-26). On the other hand, it has not been found possible to produce alloxan diabetes in guinea pigs, ducks, owls and chickens (23, 27, 28).

In the typical experiment, there is a triphasic reaction of the blood sugar to a diabetogenic dose of alloxan.



GRAPH -I-A-

The three stages are: 1) an initial hyperglycemia reaching a maximum in 2 or 3 hours; 2) a transitory hypoglycemia, lasting about 24 hours, often accompanied in rabbits by convulsions and frequently fatal unless relieved by glucose; 3) a final diabetic hyperglycemia accompanied by the classical signs and symptoms of diabetes, viz., glycosuria, polydipsia, polyuria, polyphagia and loss of weight. The response of the individual animal to a single dose of alloxan varies considerably, however, so that the duration and severity of the three phases is not a predictable constant, and the diabetic phase may vary from a mild transitory type to a progressive fatal condition. The minimal dose of alloxan which will produce diabetes in experimental animals varies from species to species. The margin between the fatal and the diabetogenic dose is widest in the rabbit, rat and dog, which makes these animals the animals of choice.

The course of alloxan diabetes in a group of injected animals may take two entirely different forms. Some animals lose weight rapidly, become seriously emaciated and die within a week. Their blood sugar is well over 400 mg. per cent, and there is a severe glycosuria. Other animals respond with similar levels of blood and urine glucose, but after an initial slight weight loss seem to recuperate and regain their weight, appearing in excellent health. Such animals survive without insulin treatment for months, despite the severe diabetes and are apparently capable of compensating for the loss of glucose in the urine by increasing their food intake. A few animals may give the impression of clinical cure of the diabetic condition, the blood sugar returns to normal, the urine is sugar free, but a persistently decreased glucose

tolerance test remains to indicate the diabetes.

In the discussion of the pathogenesis of alloxan diabetes the three phases should be considered separately. The initial hyperglycemic phase could conceivably be due either to an inhibition of insulin action or to a mobilization of extra glucose. The first possibility was ruled out when it was shown that alloxan does not inactivate insulin in vitro and that if insulin is injected simultaneously with alloxan, the initial hyperglycemia will not develop (29). It seems likely therefore that this phase of alloxan action is due to a mobilization of extra glucose. Such glycogenolysis may occur in the liver under the influence of epinephrine, in which case the initial hyperglycemia should not occur if the adrenals are removed. This was demonstrated by Goldner and Gomori (29), and it is supported by the observations of Hard and Carr (30) who found evidence of histological changes in the adrenal medulla and recorded typical epinephrine-type blood pressure curves immediately following an injection of alloxan. Houssay, Orias and Sara (31), however, found that the initial hyperglycemia appears after adrenalectomy or adrenal denervation, but not after hepatectomy or evisceration. Houssay et al. therefore conclude that the hyperglycemia is due to a direct action of alloxan on the liver.

The temporary hypoglycemic phase was at first interpreted as due to an insulin-like effect of alloxan itself. This hypothesis was discarded, however, when it was shown that alloxan does not have a hypoglycemic effect in animals previously treated with alloxan or depancreatized (29, 32) or eviscerated (33). The presence of a normal

pancreas seems necessary, therefore, for the occurrence of the hypoglycemic phase. If the hypoglycemia is due to the effect of alloxan upon the pancreas, two mechanisms for its production are possible. They are that alloxan may stimulate an overproduction of insulin or that preformed insulin may be suddenly released by the destruction of the islet cells. The first hypothesis was suggested by Dunn et al. (14, 18, 21); the second hypothesis was suggested by several investigators (29, 34, 35). These latter workers showed that the initial hyperglycemia, which, according to the theory of insulin over-production is its physiological stimulus, can be prevented by the injection of insulin with alloxan without modifying the subsequent hypoglycemic reaction. Also, beta cell degeneration precedes the hypoglycemia (33), and whenever the animal survives, it is impossible to separate the hypoglycemic effect from the diabetogenic action of alloxan, which always develops. Banerjee (36) also brought evidence against the theory of over-stimulation of the islet cells. He removed part of the pancreas of rabbits leaving just enough tissue to maintain a normal fasting blood sugar. If alloxan stimulates the islet cells to the extent of producing subsequent necrosis due to overwork, the remaining pancreatic tissue should secrete enough insulin to produce marked hypoglycemia in the partially depancreatized rabbits. This did not occur, and the slight hypoglycemia observed could be better explained by the liberation of small amounts of insulin from the relatively few necrosed islet cells still present in the animals. Both of these explanations for the hypoglycemic action were refuted by Houssay,

Orias and Sara (31), who demonstrated the secondary hypoglycemia in recently pancreatectomized but not in hepatectomized dogs. Accordingly, these authors concluded that this hypoglycemic phase must be extrapancreatic in origin, and probably due to a lack of glucose production by the liver.

There seems to be general agreement that the final and permanent rise in blood sugar is due to necrosis of the islet cells and therefore to the permanent lack of insulin production. However, the extreme hyperglycemia of alloxan diabetes suggests that the drug may also affect the liver directly (29, 31).

The clinical syndrome of alloxan diabetes is associated with a pathological picture which is characteristic. The pancreatic changes, described first by Dunn, Sheehan and McLetchie in the rabbit (14) consist of acute necrosis of almost all the islet cells, starting at the center of the islet in proximity to the blood supply (18). Although the alpha cells of the islets of Langerhans may suffer from alloxan, the most characteristic lesion is the disappearance of the beta cells, following granulation and shrinkage of the entire cell body (37). There is no hydropic degeneration which is so prominent in pituitary diabetes, although it can be induced by repeated small doses of alloxan (33). The lesions of the beta cells are apparent five minutes after an intravenous injection of alloxan (33, 35) without any sign of inflammatory reaction. In dogs the pancreatic ducts usually show a characteristic vacuolization (38).

By the use of proper doses of alloxan, the pathologic lesions can

be limited to the beta cells of the insular tissue, except for a mild and temporary degeneration of the tubular epithelium of the kidney, mild fatty metamorphosis of the liver, and some congestion of the adrenal glands. If amounts of alloxan greater than the diabetogenic dose are given, necrosis of the renal convoluted tubules, severe rise in N.P.N. and albuminuria can be observed, as well as fatty degeneration and necrosis of the liver, congestion, necrosis, and leucocytic infiltration of the adrenal cortex and hydropic degeneration and necrosis of the anterior pituitary. (14, 19, 21, 24, 30, 39).

Associated with the severe glycosuria occurring in alloxan diabetes, there is a loss of glycogen from the liver and the skeletal muscle, but not from the heart, and this fact reveals a fundamental metabolic difference between the two types of muscle (40). Hyperlipemia is frequently marked (20) and is associated with hypercholesterolemia (39) and fatty infiltration of the liver (38). Studies with deuterium revealed that the synthesis of fatty acids from glucose is decreased to about 5% of normal in the alloxan diabetic animal; it is probable, therefore, that the excess fat found in the plasma and in the liver is derived from the fat depots (41). Other metabolic disturbances consist of a rise of the inorganic phosphate which, in coma, can reach the value of 26 mg. per cent and is associated with a decrease in the organic phosphorus compounds of the liver (42); and, in pigeons, a rise in the blood uric acid level to 100 times the normal value, associated with large deposits of sodium urate crystals on the serous membranes and in the kidneys -- a condition which has been known for a

long time as visceral gout (38).

It seems likely, therefore, that alloxan has a specific necrotizing action on the insulin-producing cells of the pancreas resulting in the liberation of preformed insulin and terminating in a true permanent pancreatic diabetes. A confirmation of this view is found in the fact that the alloxan-treated pancreas contains only about one-fourth the amount of insulin found in the normal organ (29).

The reason for the specific necrotic effect of alloxan on the cells of the pancreatic islets is unknown. Alloxan does not inactivate insulin either in vitro or in vivo; although alloxan is known to inactivate many enzymes by oxidation of their sulfhydryl groups, rather high concentrations (about 0.01 M.) are required for this action; and since the administration of other oxidizing agents of the same or greater power does not produce diabetes, this property of alloxan would not seem to be the basis of its action on the pancreas (31). Brückmann and Wertheimer (43) found that alloxan and some nondiabetogenic compounds cause a rapid fall in blood glutathione but fail to affect the glutathione content of pancreas, liver and kidney. This work is presented as further evidence against the view that alloxan diabetes depends on SH oxidation. Alloxan in low concentration may act as hydrogen acceptor for certain dehydrogenases (29, 44); it seems likely, therefore, that some specific enzyme in the beta cells may have a high affinity for alloxan and be inactivated by union with it.

Some differences have been observed between alloxan diabetic animals and depancreatized animals. Houssay, Orias and Sara (31)

noted that the final rise in blood sugar was sometimes higher in alloxan diabetic dogs than in depancreatized dogs. Thorogood and Zimmermann (45) also found that the glycosuria was more severe and the insulin requirement higher in alloxan diabetic dogs than in pancreatectomized dogs; also, the former were able to live longer without insulin treatment and failed to develop ketosis and diabetic coma. Removal of the pancreas from alloxan diabetic dogs reduced glycosuria and the insulin requirement, but these animals passed quickly into coma when insulin was withheld. The finding that the insulin requirement of the alloxan-diabetic dog is much greater than that of the depancreatized animal would seem to indicate that the amount of exogenous insulin necessary to prevent glycosuria in depancreatized animals does not represent the actual output of insulin by the pancreas under normal circumstances. Thorogood and Zimmermann proposed the hypothesis that there is secreted by the alpha cells of the pancreas a second endocrine factor which acts to increase blood sugar but prevent ketosis in insulin deficient animals. Pathological evidence would support this theory, for in those diabetics who show histological pancreatic changes, there is hyalinization involving all cells of the islets. This theory could explain the different tendencies towards ketosis in different diabetic patients and also the small insulin requirements of completely depancreatized men.

Since alloxan is able to induce experimental diabetes in animals, it has naturally been surmised that it may be connected with the cause of

human diabetes mellitus. In 1943, the theory was projected by many men, including Dunn et al. (18, 21), that an abnormality or derangement of purine metabolism giving rise to alloxan might conceivably cause damage to the pancreatic islets over a long period of time. It therefore was of interest to examine the tissues and fluids of normal animals for the possible presence of alloxan. Prior to the discovery of alloxan's diabetogenic effect, the only such observations, made many years ago, appeared to be its detection in 1862 in the gelatinous mucus from a patient with intestinal catarrh (46) and (as murexide) in the urine of a patient suffering from heart disease (47, 48). Tipson and Ruben (49) developed techniques intended for the detection of alloxan in small amounts, and applied them to the analysis of a number of animal tissues and fluids. They reported what they assumed to be alloxan or its reduction products in several animal tissues: brain, liver, spleen and thymus of beef; liver of fowl, duck, guinea pig, lamb, pig, rabbit and rat; liver, ovaries, thyroid and uterine muscle of the human being; and liver and pancreas of diabetic persons.

Of particular interest is the finding that alloxan or its reduction products may be present in the livers of normal animals. It was shown many years ago, by Ascoli and Izar (50, 51) and Preti (52), that an enzyme system which decomposes uric acid in the presence of oxygen, and which synthesizes uric acid from dialuric acid plus urea in the absence of oxygen, is present in dog and calf livers. This is of significance, because dialuric acid is closely related to alloxan; indeed dialuric acid, as well as other compounds closely related to alloxan, viz., methylalloxan, alloxantin, dimethylalloxantin and

methyl dialuric acid have been shown to produce diabetes in animals, just as does alloxan (53). It is further known that, allowing for variation in individual response, alloxan is tolerated by normal animals, producing no observable adverse effects when administered below certain critical concentrations. It exerts a rather general cytotoxic effect only when present in amount above this threshold. As a tentative hypothesis, it was suggested by Tipson and Ruben (49) that overproduction of alloxan or inability to destroy it, may result in its reaching the pancreas in sufficient concentration to destroy the beta cells in the islets of Langerhans.

The views of those who postulate that alloxan plays some significant role in the causation of human diabetes were recently echoed by Joslin, one of the foremost medical specialists in diabetes. He stated his belief that the discovery of the specific action of alloxan in destroying the beta cells of the islands of Langerhans has brought medical scientists nearer to the explanation of diabetes than any step made hitherto (54).

Other men, however, do not seem as enthusiastic. Duff (55) offers the objection that the early changes in the islets produced by alloxan do not resemble any lesion commonly observed in human diabetes, in spite of Dunn's uncovering in the literature of four or five cases in which necrosis of the islets was described (14). Further, the speed with which alloxan is destroyed when injected into the animal body (37, 56) and the high resistance that human subjects have to the diabetogenic

effects of alloxan (57, 58, 59, 60, 61) argue against the possible importance of the drug in the etiology of the disease in humans.

The purpose of this study was to ascertain the possibility of using alloxan diabetes as a means of studying intermediary metabolism in much the same way that the condition of phlorhizin diabetes has been used in the past.

EXPERIMENTAL PART

The animals used in the experiments described in this thesis were young adult male and female rats and young adult male rabbits. The rats were, for the most part, of the Evan-Long-Wistar strain (116 animals) with some Sprague-Dawley strain (11 animals) and a few hooded rats (2 animals). All rats previous to injections were maintained on our standard stock diet, the composition of which is as follows:

	Per cent
Ground corn	38.0
Ground wheat	32.0
Dried skimmed milk powder	20.0
Ground alfalfa leaves	6.0
Cod liver oil	2.0
Irradiated brewer's yeast	1.0
Sodium chloride	0.5
Calcium carbonate	0.5

To render the animals diabetic alloxan monohydrate (Eastman Kodak Co.) was administered to the rats by the subcutaneous or intravenous routes. Freshly prepared aqueous solutions were used in all injections -- a 2% solution of alloxan monohydrate being used for the subcutaneous injections and a 5% solution for the intravenous injections. Twenty-two rats were injected by the subcutaneous route in dosages of 200 mg./kg. of alloxan monohydrate (or 175 mg./kg. of alloxan after correction for the molecule of water in alloxan monohydrate). These

animals were fasted for 48 hours prior to injection in accordance with the method recommended by Kass and Waisbren (62). All the remaining rats were injected by the intraveous route in dosages ranging from 20 mg./kg. to 40 mg./kg. of alloxan monohydrate. Most satisfactory results were obtained using 40 mg./kg. of alloxan monohydrate (or 35 mg./kg. of alloxan) as previously reported by Lazarow and Palay (63). All injections were made into the tail vein; the animals were not starved prior to injection.

URINE STUDIES ON RATS

The animals were placed in individual metabolism cages set over large glass funnels and were fed a high fat diet free from reducing substances. The composition of the high fat diet was as follows:

	Per cent
Sucrose	33.0
Vegetable shortening	30.0
Casein	20.0
Cellu-Flour	10.0
Salt mixture	5.0
Yeast	2.0

Urine collections were made every 24 hours. A small crystal of thymol was placed in the receiving flask as a preservative. Sugar was quantitatively determined according to the method of Shaffer-Hartmann (64), and nitrogen by the Kjeldahl procedure. These collections were started 24 hours post injections and were continued for about 10 to 14 days or

until the animals showed sufficient urinary sugar to establish beyond any question that they were truly diabetic. After this was determined, various amino acids were administered to the animals in the form of aqueous solutions, or, when dealing with an acid which was sparingly soluble, suspensions were made with the amino acids in a concentration of 10%. Gum tragacanth was used as a suspending medium. All animals were starved for at least 24 hours before administration of any amino acid.

BLOOD SUGAR STUDIES ON RATS

A second phase of this research was to study the blood-sugar response of animals, which were known to be diabetic, when varying amounts of amino acids were fed either in aqueous solution or in gum tragacanth suspension.

The animals were starved a minimum of 24 hours before administration of the amino acids. A control sample of blood was taken to determine the fasting level of blood sugar. The amino acid was then administered by stomach tube. At intervals, depending upon the condition prevailing in the individual experiment, blood sugar determinations were made at either one, two, three, four, five, etc. hours. In some cases the studies were carried out for as long as 12 hours.

Blood sugar was determined by the Jeghers-Meyers modification of the Folin-Malmros Micro Blood Sugar Method (65) utilizing 25 cu. mm. of blood. The Klett-Summerson photoelectric colorimeter with a number 54 green filter was used for reading the determinations.

EXPERIMENTS WITH RABBITS

The 10 rabbits used were male New Zealand whites about twelve weeks old. These animals were maintained on a diet of rabbit pellets (General Mills) with fresh greens twice a week. Alloxan monohydrate was administered intravenously by injection into an ear vein in dosages of 125 mg./kg. in 5% solution as recommended by Kendall et al. (39). The animals were not starved prior to the injection.

Various amino acids were administered to the animals by stomach tube in the form of aqueous solutions or suspensions made with gum tragacanth and the resulting blood-sugar levels were determined. In all experiments the animals were starved at least 5 days before the administration of the amino acid. Blood sugar determinations were obtained by puncturing one of the ear veins. The Jeghers-Meyers method (65) was used for this determination. The amino acids used throughout this thesis were either commercial preparations or were prepared in our laboratory, and on analysis gave the following results:

AMINO ACID	N THEORETICAL	N FOUND	% DEVIATION
Glycine	18.67	18.53	0.75
DL-Alanine	15.83	15.77	0.44
DL-Valine	11.96	11.90	0.05
DL-Phenylalanine	8.49	8.55	0.705
L-Tyrosine	7.73	7.75	0.25
L-Cystine	11.66	11.45	1.80
DL-Methionine	9.40	9.49	0.935

RESULTS

EXPERIMENTS ON RATS

URINE STUDIES. Sixty-six animals were used in this part of the study. In all, 403 experimental days were recorded. The urine was analyzed for sugar and nitrogen. The experiments in Tables II, III and IV show typical results. Other experiments are included in the appendix.

BLOOD STUDIES. The results of these experiments are listed in Tables V to XI. The changes from the fasting blood sugar level following administration of varying amounts of amino acids by stomach tube are shown in Graphs I to VII. Table I lists the amino acids fed, and the amount and state in which they were given.

EXPERIMENTS ON RABBITS

The results obtained on the blood sugar studies on alloxan diabetic rabbits following the administration of various amino acids by stomach tube are listed in Table XII.

TABLE I

Amino Acid	Medium	Conc. of Solution or Suspension gms./100 cc.	Amount Fed gms./Kilo
Glycine	Aqueous	10%	2.5-5.0
DL-Alanine	Aqueous	10%	1.0-2.5-5.0
DL-Valine	Aqueous	5%	3.2-4.3
DL-Phenylalanine	1.75% gum Tragacanth	10%	2.5
L-Tyrosine	1.75% gum Tragacanth	10%	2.5
L-Cystine	1.75% gum Tragacanth	10%	2.5
DL-Methionine	1.75% gum Tragacanth	10%	2.5-4.0
DL-Methionine	Aqueous	3%	3.7-4.8-5.2

DISCUSSION

Various techniques have been used in the past to study the gluconeogenic or sugar-forming properties of the amino acids. Among these methods are: 1) phlorizin diabetes; 2) liver glycogen deposition; and 3) the antiketogenic effect.

Phlorizin is a glucoside which, when given subcutaneously, interferes with a number of metabolic processes, one of which is the kidney mechanism for retention of blood glucose. Thus, the renal threshold for sugar is lowered, and glucose is excreted in the urine. In this way the sugar-forming ability of fed or injected amino acids, or similar compounds, may be tested quantitatively by determining the resulting extra urinary glucose excreted following the administration of these amino acids. This involves certain assumptions which may or may not be warranted.

The administration of some, but not all, of the amino acids to fasted animals is followed by an increased deposition of liver glycogen. It generally has been assumed that if glycogen formation occurred, the amino acid has been converted into a substance, presumably glucose, which was incorporated into the newly formed glycogen.

It has long been known that ketogenesis may be related to carbohydrate depletion and that when glucose is administered in such cases, the ketosis disappears. However, animals, with the exception of the primates, do not develop a ketosis on starvation or on a carbohydrate free diet. In such animals, an experimental ketonuria may be caused

in one of two ways, viz., an endogenous ketosis may be created by placing the animals on a high-fat, low-protein diet for 10 to 12 days so that on fasting large amounts of acetone bodies appear in the urine because of enforced fat utilization (66), or an exogenous ketonuria may be evoked by feeding a solution of sodium butyrate to fasting animals (67). The administration of some amino acids, like the administration of glucose, alleviates the experimental ketonuria in rats. Thus the ability of an administered amino acid to reduce the experimental ketonuria in an animal is taken as indication of the sugar-forming tendencies of the amino acid.

A summary of the results of studies reported in the literature on the carbohydrate-like effect of amino acids as ascertained by different methods is shown below (68).

CARBOHYDRATE-LIKE EFFECT OF AMINO ACIDS

Amino Acid	Extra urinary glucose in phlorizinized dog	Hepatic glycogen deposition in fasted rat	Reduction of experimental ketonuria in rat
Alanine	+DL	+L, D, DL	+L, DL
Arginine	+L	+L	+L
Aspartic acid	+DL	+L, DL	+L, DL
Cysteine	+L	-L	
Cystine		-L	-L
Glycine	+	+	+
Glutamic acid	+L	+L, DL	+L
Histidine	+(?) L	+L	+L
Isoleucine	-DL	+(slight) DL	-DL
Leucine	-DL	-DL	-DL
Lysine	-L	-L, DL	
Methionine	+DL	-DL	
Norleucine	+L, D, DL	+DL	+DL
Phenylalanine	-DL	+DL	+DL
Proline	+L		
Serine	+DL	+DL	
Threonine		+DL	+DL
Tryptophane	-L	-L, D, DL	+L, D, DL
Tyrosine	-L	+L	-DL
Valine	+L, D, DL	+DL	+DL

Liver perfusion techniques have also been used to study the intermediary metabolism of the amino acids, but these are in vitro methods and, as far as gluconeogenesis is concerned, are of questionable value.

In our studies, of all the rats rendered diabetic by the subcutaneous injection of 200 mg./kg. of alloxan monohydrate, only 16% survived longer than one week. Among the rats in this group which succumbed during the first week post injection, the males appeared to die earlier than the females. Of the rats rendered diabetic by the intravenous injection of 40 mg./kg. of alloxan monohydrate, 46% survived longer than one week. There did not appear to be any sexual difference in susceptibility to alloxan; however, of the rats that died during the first week after injection, the males appeared to die usually on the third and fourth days post injection, whereas the females usually died on the fourth and fifth days post injection. The significance of this observation, if any, is unknown.

The two different courses of alloxan diabetes described earlier were observed in the animals used in this study, namely - some lost weight rapidly, developed a very high blood sugar and a severe glycosuria and died within a week; others showed similar levels of blood and urine glucose, but after an initial slight weight loss, seemed to recuperate and regain their weight. A few animals gave the impression of a clinical cure of the diabetic condition, the blood sugar returned to normal and the urine was sugar free.

The results of the urine studies on alloxan diabetic rats were

not found to be too satisfactory. The glucose excreted in the urine varied directly with the food intake. Inasmuch as the high-fat diet was not very palatable to the rats, their appetites soon fell off, and their urinary glucose excretion decreased accordingly. On fasting, the urine became sugar-free due to the decrease in blood sugar to normal levels. Thus, sugar formed from the amino acid would have to reach a level above the renal threshold, a variable level, and would not give a true picture of the total transformation.

Qualitatively, the results after amino acid feeding followed the same pattern established by glycogen studies and the anti-ketogenic effect. As Experiment #39 recorded in Table II shows, the removal of food on the 15th. post injection day caused the urine sugar to decrease from 2290 mg. to 448 mg. During the next three days the fast continued and the urine was sugar free. On the 19th. day 1800 mgs. of DL-alamine were fed by stomach tube. The ensuing 48 hours showed a total of 675 mg. of glucose excreted. Experiment #41 reported in Table III showed essentially the same result except that all sugar was excreted within 24 hours. Experiment #56 recorded in Table IV shows similar although less striking results.

TABLE II

TYPICAL EXPERIMENT ON MALE RAT[a] RECEIVING 40 MGS. OF ALLOXAN MONOHYDRATE PER KILOGRAM OF BODY WEIGHT INTRAVENOUSLY. NO PREVIOUS FAST. ANIMAL FED HIGH FAT DIET FOR FIFTEEN DAYS. STARVED FOR NEXT THREE DAYS AND THEN ADMINISTERED 9.0 CC. OF 20% SOLUTION OF DL-ALANINE BY STOMACH TUBE. FAST CONTINUED THROUGH TWENTY-FIRST DAY.

DAYS POST INJECTION	1	2	3	4	5	6	7	8	9	10	11	12	13
URINE OUTPUT (CC.)	-	26	26	26	49	44	57	38	53	46	46	46	25
GLUCOSE (MGS.)	-	299	1526	1798	3692	3782	4910	3610	3830	4250	4210	4000	1895
TOTAL N (MGS.)	-	200	155	163	232	247	286	244	264	271	286	288	190
FOOD INTAKE (GM.)[b]	-	8	4	6½	11	10½	12½	10	12½	11½	12	13	11½
BODY WEIGHT (GM.)	-	-	-	-	-	-	-	-	-	-	-	-	-

DAYS POST INJECTION	14	15	16	17	18	19	20	21
URINE OUTPUT (CC.)	27	20	16	24[c]	14[c]	20	30	18
GLUCOSE (MGS.)	2290	448	0	0	0	326	349	0
TOTAL N (MGS.)	244	157	144	146	150	354	353	-
FOOD INTAKE (GM.)[b]	11½	½[d]	e	e	e	f	e	e
BODY WEIGHT (GM.)	-	225	218	210	200	192	-	-

a - Expt. #39, male rat weighing 246 gms. on date of alloxan injection.
b - High fat diet.
c - Received 5 cc. water by stomach tube.
d - Fast started.
e - Fasted.
f - 9.0 cc. of 20% solution of DL-Alanine administered by stomach tube in two doses of 4.5 cc. each.

TABLE III

TYPICAL EXPERIMENT ON MALE RAT[a] RECEIVING 40 MGS. OF ALLOXAN MONOHYDRATE PER KILOGRAM OF BODY WEIGHT INTRAVENOUSLY. NO PREVIOUS FAST. ANIMAL FED HIGH FAT DIET FOR FIFTEEN DAYS. STARVED FOR NEXT THREE DAYS AND THEN ADMINISTERED 9.0 CC. OF 20% SOLUTION OF DL-ALANINE BY STOMACH TUBE. FAST CONTINUED THROUGH TWENTY-FIRST DAY.

DAYS POST INJECTION	1	2	3	4	5	6	7	8	9	10	11	12	13
URINE OUTPUT (CC.)	-	24	23	22	39	43	57	29	55	62	58	70	36
GLUCOSE (MGS.)	-	370	1621	1410	2784	3378	4780	2380	3830	5640	5530	4810	2790
TOTAL N (MGS.)	-	190	176	162	236	233	285	243	278	305	321	348	270
FOOD INTAKE (GM.)[b]	-	7	3½	5	6½	8½	10½	4	14	11½	13	13	6
BODY WEIGHT (GM.)	-	-	-	-	-	-	-	-	-	-	-	-	-

DAYS POST INJECTION	14	15	16	17	18	19	20	21
URINE OUTPUT (CC.)	67[g]	15	6	8[c]	6[c]	20	20	16
GLUCOSE (MGS.)	5900	1002	173	0	0	986	0	0
TOTAL N (MGS.)	335	136	131	94	110	382	90	-
FOOD INTAKE (GM.)[b]	16	½[d]	e	e	e	f	e	e
BODY WEIGHT (GM.)	-	206	200	192	185	176	-	-

a - Expt. #41, Male rat weighing 250 gms. on date of alloxan injection.
b - High fat diet.
c - Received 5 cc. water by stomach tube.
d - Fast started.
e - Fasted.
f - 9.0 cc. of 20% solution of DL-Alanine administered by stomach tube in two doses of 4.5 cc. each.
g - Funnel leaked.

TABLE IV

TYPICAL EXPERIMENT ON FEMALE RAT[a] RECEIVING 40 MGS. ALLOXAN MONOHYDRATE PER KILOGRAM OF BODY WEIGHT INTRAVENOUSLY. NO PREVIOUS FAST. ANIMAL FED HIGH FAT DIET FOR TEN DAYS. STARVED FOR NEXT THREE DAYS AND THEN ADMINISTERED 9.0 CC. OF 20% SOLUTION OF DL-ALANINE BY STOMACH TUBE. FAST CONTINUED THROUGH FIFTEENTH DAY.

DAYS POST INJECTION	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
URINE OUTPUT (CC.)	-	80	59	66	95	86	78	35	55	33	9	19[c]	12[c]	20	22
GLUCOSE (MGS.)	-	2886	2818	4140	6292	6016	5684	2470	3990	2222	0	0	0	296	0
TOTAL N (MGS.)	-	416	377	343	424	439	422	253	305	225	126	91	99	337	113
FOOD INTAKE (GM.)[b]	-	17½	10½	10	18½	18	17½	7½	15	3½[d]	e	e	e	f	e
BODY WEIGHT (GM.)	-	-	-	-	-	-	-	-	-	202	198	192	182	174	-

a - Expt. #56, female rat weighing 210 gms. on date of alloxan injection.
b - High fat diet.
c - Received 5 cc. water by stomach tube.
d - Fast started.
e - Fasted.
f - 9.0 cc. of 20% solution of DL-Alanine administered by stomach tube in two doses of 4.5 cc. each.

Because of the lack of quantitative response, it was felt that more significant results might be obtained through observing blood sugar changes after feeding the amino acids. The necessity of increasing blood sugar above the renal threshold before a response could be obtained would be eliminated.

The technique adopted was to inject the animals intravenously with the standard amount of alloxan (40 mg./kg. as the monohydrate). Blood sugar levels were determined, starting at 24 hours post injection, to insure that the animals were diabetic. At the end of a 24 hour fast a control sample of blood was drawn. The amino acid was then administered and blood sugar changes were noted, usually at 2, 4, 6, 8, and in some cases 10 and 12 hours. In a few experiments samples were taken at 1, 3, 5, and 7 hours.

Using this technique it was found that administration by stomach tube of Glycine, DL-Alanine, DL-Valine, DL-Phenylalanine, L-Tyrosine and L-Cystine resulted in an increase in blood sugar as shown in Tables V to XII and as graphically represented in Graphs I to VI. These findings, with the exception of L-Cystine, are in agreement with those determined by other methods as summarized above. Most significant is the finding that phenylalanine and tyrosine are sugar-formers inasmuch as these amino acids were not considered sugar-formers prior to the work of Butts et al. by liver glycogen and anti-ketogenic techniques (69). Since this is a controversial point, it is important that other techniques be used to study this problem.

TABLE V

BLOOD SUGAR RESPONSE OF ALLOXANIZED RATS TO VARYING AMOUNTS OF GLYCINE ADMINISTERED BY STOMACH TUBE.

GLYCINE FED IN A 10% AQUEOUS SOLUTION.

EXPT. #	SEX	BODY WT. (GMS) AT INJ.	BEG. FAST	END FAST	DAYS AFTER ALLOXAN	FASTING	MATERIAL FED GRAMS/KILO	ON FOOD	BLOOD SUGAR (MGS.%) RESPONSE (HRS. AFTER AMINO ACID ADMINISTRATION) 0	1	2	3	4	5	6	7	8	9	10
72	F.	-	160	132	52	3	Glycine 2.5	307	162		269		202		-		292		393
89	M.	206	158	152	5	1	Glycine 5.0	353	338		584		-		385		-		-
91	M.	192	176	164	5	1	Glycine 5.0	393	92		207		-		88		-		-
93	M.	194	168	148	5	1	Glycine 5.0	457	330		382		-		337		-		-
113	F.	190	172	168	5	1	Glycine 2.5	648	282		365		299		-		-		-
114	F.	180	164	160	5	1	Glycine 2.5	381	224		344		229		-		-		-

TABLE VI

BLOOD SUGAR RESPONSE OF ALLOXANIZED RATS TO VARYING AMOUNTS OF DL-ALANINE ADMINISTERED BY STOMACH TUBE.

DL-ALANINE FED IN A 10% AQUEOUS SOLUTION.

EXPT. #	SEX	BODY WT. (GMS) AT INJ.	BEG. FAST	END FAST	DAYS AFTER ALLOXAN	FASTING	MATERIAL FED GRAMS/KILO	BLOOD SUGAR (MGS.%) ON FOOD	RESPONSE (HRS. AFTER AMINO ACID ADMINISTRATION) 0	1	2	3	4	5	6	7	8	9	10	12
69	F.	210	236	196	65	2½	DL-Alanine 2.5	209	95	183	199		216		280			102	117	
72	F.	-	160	150	50	1	DL-ALANINE 2.5	307	165		328		404				294		277	209
74	F.	170	152	130	57	1	DL-Alanine 5.0	580	213		307		299				289		286	254
91	M.	192	-	160	16	1	DL-Alanine 1.0	393	99	158		128		134						

TABLE VII

BLOOD SUGAR RESPONSE OF ALLOXANIZED MALE RATS TO VARYING AMOUNTS OF DL-VALINE ADMINISTERED BY STOMACH TUBE.

DL-VALINE FED IN A 5% AQUEOUS SOLUTION.

EXPT. #	SEX	BODY WT. (GMS) AT INJ.	BEG. FAST	END FAST	DAYS AFTER ALLOXAN	FASTING	MATERIAL FED GRAMS/KILO	ON FOOD	BLOOD SUGAR(MGS.%) RESPONSE (HRS. AFTER AMINO ACID ADMINISTR'N) 0	1	2	3	4	5	6	7	8
							DL-Valine										
77	M.	160	146	116	15	2	4.3	181	121		180		143			149	
							DL-VALINE										
79	M.	190	190	156	15	2	3.2	178	87		220		201			150	

TABLE VIII

BLOOD SUGAR RESPONSE OF ALLOXANIZED RATS TO 2.5 GRAMS DL-PHENYLALANINE PER KILOGRAM OF BODY WEIGHT FED BY STOMACH TUBE. A 10% SUSPENSION OF THE AMINO ACID WAS MADE USING GUM TRAGACANTH (1.75%) AS THE SUSPENDING MEDIUM.

EXPT. #	SEX	BODY WT. (GMS) AT INJ.	BEG. FAST	END FAST	DAYS AFTER ALLOXAN	FASTING	MATERIAL FED GRAMS/KILO	BLOOD SUGAR (MGS.%) ON FOOD	RESPONSE (HRS. AFTER AMINO ACID ADMIN.) 0	1	2	3	4	5	6	7	8
							DL-Phenylalanine										
113	F.	190	172	156	6	2	2.5	648	254		275		328		-		-
							DL-Phenylalanine										
114	F.	180	164	150	6	2	2.5	381	146		323		228		-		-
							DL-Phenylalanine										
121	M.	200	188	180	8	1	2.5	448	107		114		120		127		-
							DL-Phenylalanine										
121	M.	200	188	166	9	2	2.5	448	95		175		190		146		80
							DL-Phenylalanine										
122	M.	176	170	150	8	1	2.5	496	290		299		386		377		-
							DL-Phenylalanine										
122	M.	176	170	134	9	2	2.5	496	203		366		390		400		397
							DL-Phenylalanine										
128	M.	-	-	166	15	2	2.5	500	96		123		118		120		124
							DL-Phenylalanine										
129	M.	-	-	156	15	2	2.5	452	76		96		80		76		80

TABLE IX

BLOOD SUGAR RESPONSE OF ALLOXANIZED RATS TO 2.5 GRAMS L-TYROSINE PER KILOGRAM OF BODY WEIGHT FED BY STOMACH TUBE. A 10% SUSPENSION OF THE AMINO ACID WAS MADE USING GUM TRAGACANTH (1.75%) AS THE SUSPENDING MEDIUM.

EXPT. #	SEX	BODY WT. (GMS) AT INJ.	BEG. FAST	END FAST	DAYS AFTER ALLOXAN	FASTING	MATERIAL FED GRAMS/KILO	ON FOOD	BLOOD SUGAR (MGS.%) RESPONSE (HRS. AFTER AMINO ACID ADMINISTR'N) 0	1	2	3	4	5	6	7	8
123	M.	196	-	140	19	1	L-Tyrosine 2.5	320	152		177		214		234		262
127	M.	-	218	202	7	1	L-Tyrosine 2.5	431	321		349		376		355		325
128	M.	-	190	180	7	1	L-Tyrosine 2.5	500	81		107		99		106		96
128	M.	-	190	168	8	2	L-Tyrosine 2.5	500	87		133		135		150		132
128	M.	-	-	174	14	1	L-Tyrosine 2.5	500	89		132		122		125		133
129	M.	-	200	188	7	1	L-Tyrosine 2.5	452	236		271		303		303		240
129	M.	-	200	176	8	2	L-Tyrosine 2.5	452	84		175		167		174		188
129	M.	-	-	170	14	1	L-Tyrosine 2.5	452	95		135		127		142		155
133	M.	154	-	124	10	1	L-Tyrosine 2.5	622	123		147		137		100		45

TABLE X

BLOOD SUGAR RESPONSE OF ALLOXANIZED RATS TO 2.5 GRAMS L-CYSTINE PER KILOGRAM OF BODY WEIGHT FED BY STOMACH TUBE. A 10% SUSPENSION OF THE AMINO ACID WAS MADE USING GUM TRAGACANTH (1.75%) AS THE SUSPENDING MEDIUM.

EXPT. #	SEX	BODY WT. (GMS) AT INJ.	BEG. FAST	END FAST	DAYS AFTER ALLOXAN	FASTING	MATERIAL FED GRAMS/KILO	ON FOOD	BLOOD SUGAR (MGS.%) RESPONSE (HRS. AFTER AMINO ACID ADMINISTR'N) 0	1	2	3	4	5	6	7	8
113	F.	190	172	144	7	3	L-Cystine 2.5	648	287		348		325		-		-
113	F.	190	-	124	14	1	L-Cystine 2.5	648	448		583		575		458		471
114	F.	180	164	140	7	3	L-Cystine 2.5	381	93		109		141		-		-
114	F.	180	-	134	14	1	L-Cystine 2.5	381	95		97		102		186		290
121	M.	200	188	158	10	3	L-Cystine 2.5	448	104		197		177		225		149
122	M.	176	170	120	10	3	L-Cystine 2.5	496	205		229		225		244		263
123	M.	196	196	164	10	3	L-Cystine 2.5	320	124		150		134		179		183

TABLE XI

BLOOD SUGAR RESPONSE OF ALLOXANIZED RATS TO VARYING AMOUNTS OF DL-METHIONINE ADMINISTERED BY STOMACH TUBE. DL-METHIONINE FED EITHER IN A 3% AQUEOUS SOLUTION OR IN A 10% SUSPENSION, WHICH WAS MADE USING GUM TRAGACANTH (1.75%) AS THE SUSPENDING MEDIUM.

TABLE XI

EXPT. #	SEX	BODY WT. (GMS) AT INJ.	BEG. FAST	END FAST	DAYS AFTER ALLOXAN	FASTING	MATERIAL FED GRAMS/KILO	ON FOOD	BLOOD SUGAR (MGS.%) RESPONSE (HRS. AFTER AMINO ACID ADMINISTR'N) 0	1	2	3	4	5	6	7	8
72	F.	-	160	128	53	4	DL-Methionine 4.6	307	97		92		72				79
77	M.	160	156	142	6	1	DL-Methionine 4.2	181	134		109		86				75
77	M.	160	146	110	16	3	DL-Methionine 4.0	181	124		55		56				91
78	M.	186	164	164	6	1	DL-Methionine 3.7	610	124		108		94				88
78	M.	186	164	138	8	3	DL-Methionine 4.3	610	186		281		212				247
78	M.	186	164	132	9	4	DL-Methionine 4.5	610	147				162				124
79	M.	190	184	164	6	1	DL-Methionine 3.7	178	107		100		95				88
79	M.	190	190	156	16	3	DL-Methionine 4.0	178	126		138		101				99
80	M.	160	134	124	6	1	DL-Methionine 4.8	800	290		195		181				321
81	M.	178	148	144	6	1	DL-Methionine 4.2	573	292		147		174				172
81	M.	178	148	116	8	3	DL-Methionine 5.2	573	208		227		158				169
89	M.	206	-	144	8	1	DL-Methionine 2.5	353	127			158		238			
91	M.	192	-	164	8	1	DL-Methionine 2.5	393	96			84		110			
99	F.	174	144	120	7	2	DL-Methionine 2.5	427	193			77		51			
100	F.	162	130	104	7	2	DL-Methionine 2.5	451	370			248		204			
102	F.	150	128	110	7	2	DL-Methionine 2.5	596	100			118		108			

TABLE XII

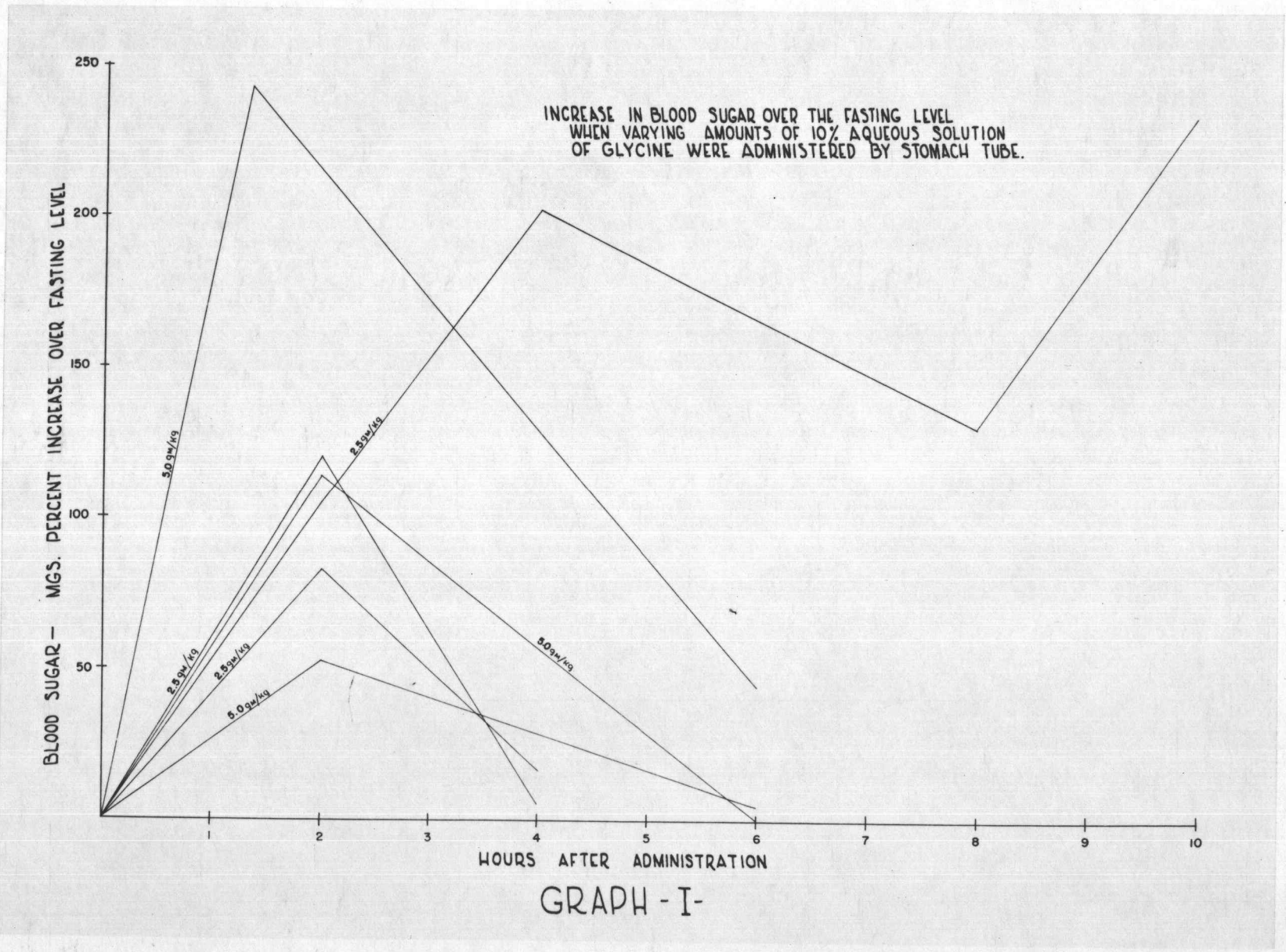
BLOOD SUGAR RESPONSE OF ALLOXANIZED RABBITS TO VARYING AMOUNTS OF GLYCINE, DL-ALANINE, DL-PHENYLALANINE, AND L-CYSTINE FED BY STOMACH TUBE. THESE AMINO ACIDS WERE FED EITHER IN A 10% AQUEOUS SOLUTION OR IN A 10% SUSPENSION, WHICH WAS MADE USING GUM TRAGACANTH (1.75%) AS THE SUSPENDING MEDIUM.

TABLE XII

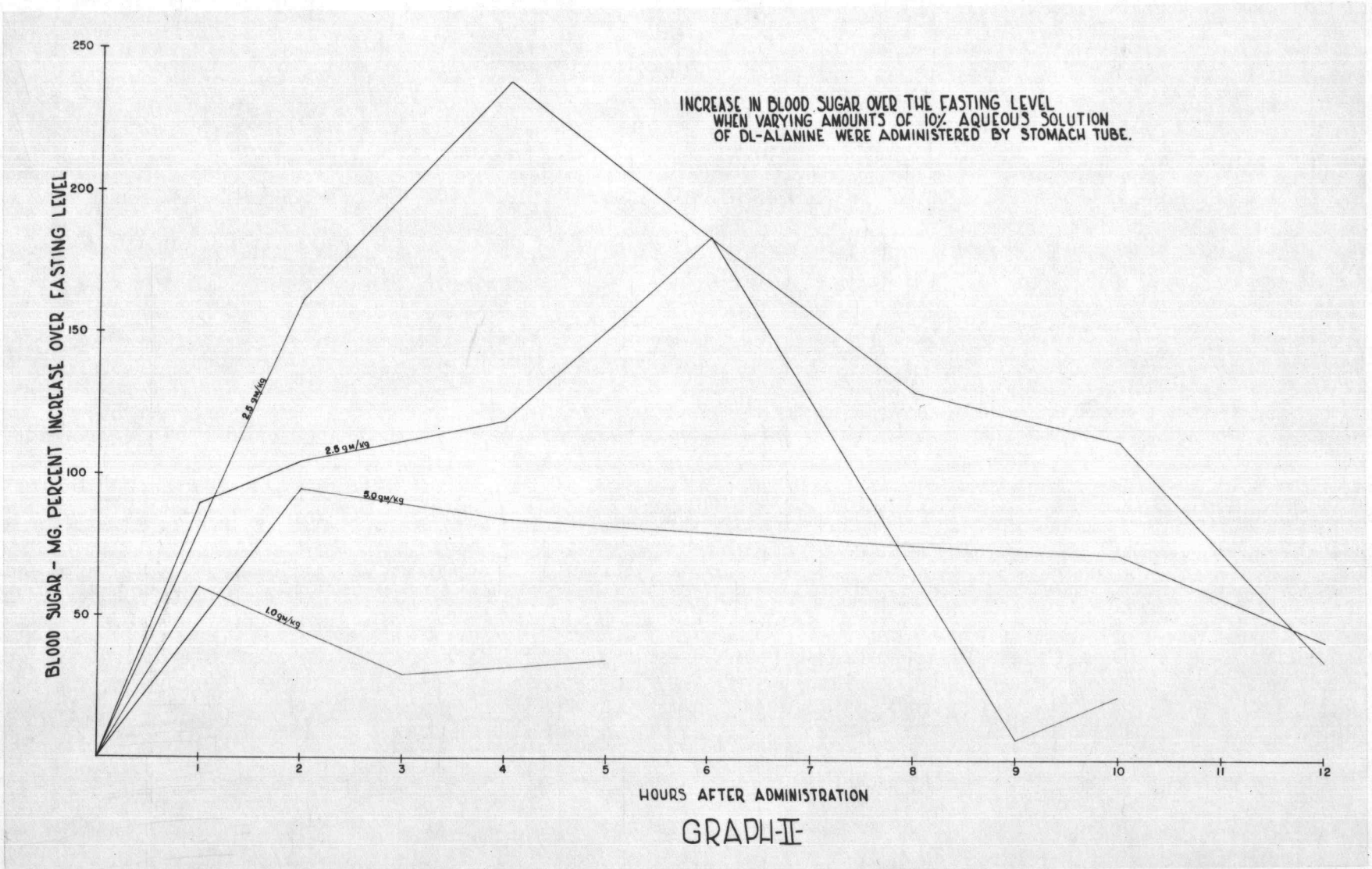
EXPT. #	SEX	BODY WT. (KGS) AT INJ.	BEG. FAST	END FAST	DAYS AFTER ALLOXAN	FASTING	MATERIAL FED GRAMS/KILO	ON FOOD	BLOOD SUGAR (MGS.%) RESPONSE (HRS. AFTER AMINO ACID ADMINISTR'N) 0	1	2	3	4	5	6
105	M.	2.16	2.27	1.85	14	6	DL-Alanine 1.0	520	121		145		132		115
105	M.	2.16	2.27	1.76	15	7	Glycine 2.5	520	121		155		153		116
105	M.	2.16	2.27	1.70	17	9	DL-Alanine 2.5	520	122		161		172		
105	M.	2.16	2.27	1.56	18	10	DL-Phenyl-alanine-2.5	520	124		180		189		
105	M.	2.16	2.27	1.51	19	11	L-Cystine 2.5	520	114		138		143		
107	M.	2.61	2.61	2.36	13	5	DL-Alanine 2.0	537	113		164		172		
107	M.	2.61	2.61	2.27	14	6	DL-Phenyl-alanine-2.5	537	132		169		171		
107	M.	2.61	2.61	2.10	15	7	L-Cystine 2.5	537	138		173		163		
109	M.	2.78	2.64	2.27	13	5	Glycine 2.5	614	289		328		337		282
109	M.	2.78	2.64	2.13	14	6	DL-Phenyl-alanine-2.5	614	180		210		235		226
111	M.	2.41	1.93	1.59	13	5	Glycine 2.5	684	125		153		167		137
111	M.	2.41	1.93	1.36	14	6	DL-Phenyl-alanine-2.5	684	105		119		128		117
112	M.	1.99	1.96	1.70	13	5	Glycine 2.5	663	210		242		249		204
112	M.	1.99	1.96	1.59	14	6	DL-Phenyl-alanine-2.5	663	169		187		202		184

The finding in this study that L-Cystine is a sugar-former is the first direct experimental evidence for this action of L-Cystine. For many years cystine has been considered to be a sugar-forming amino acid, but this idea was merely a deduction from Dakin's experimental work (70), in which he reported an excretion of extra urinary glucose in a phlorizinized dog after the administration of cysteine to the extent which should come from a 100% conversion of this compound to form glucose. The fact that cysteine is a normal breakdown product of cystine was well established. Thus, the results obtained in this study with L-Cystine in alloxan diabetic rats serve as experimental evidence supporting this assumption.

The results obtained when DL-Methionine was administered to alloxan diabetic rats, as listed in Table XI and graphically depicted in Graph VII, show that, in general, DL-Methionine does not give rise to sugar. This finding is in agreement with that determined by liver glycogen deposit studies in normal fasted rats (71), (72), (73). In the past many have held the view that methionine is a sugar-former. This idea was based on the experimental work of Vars (74) who found extra urinary glucose in a phlorizinized dog injected subcutaneously with DL-Methionine and also on the theory that methionine can be converted in the body to cystine, which is a sugar-former. This latter theory was recently refuted by duVigneaud et al. (75) who, by feeding to rats methionine labeled with S^{34} and with C^{13} in the beta and gamma positions and subsequently isolating the cystine from the hair, found from isotopic analysis of the cystine that the sulfur but none of the carbon had been derived from the methionine. Thus it has been established

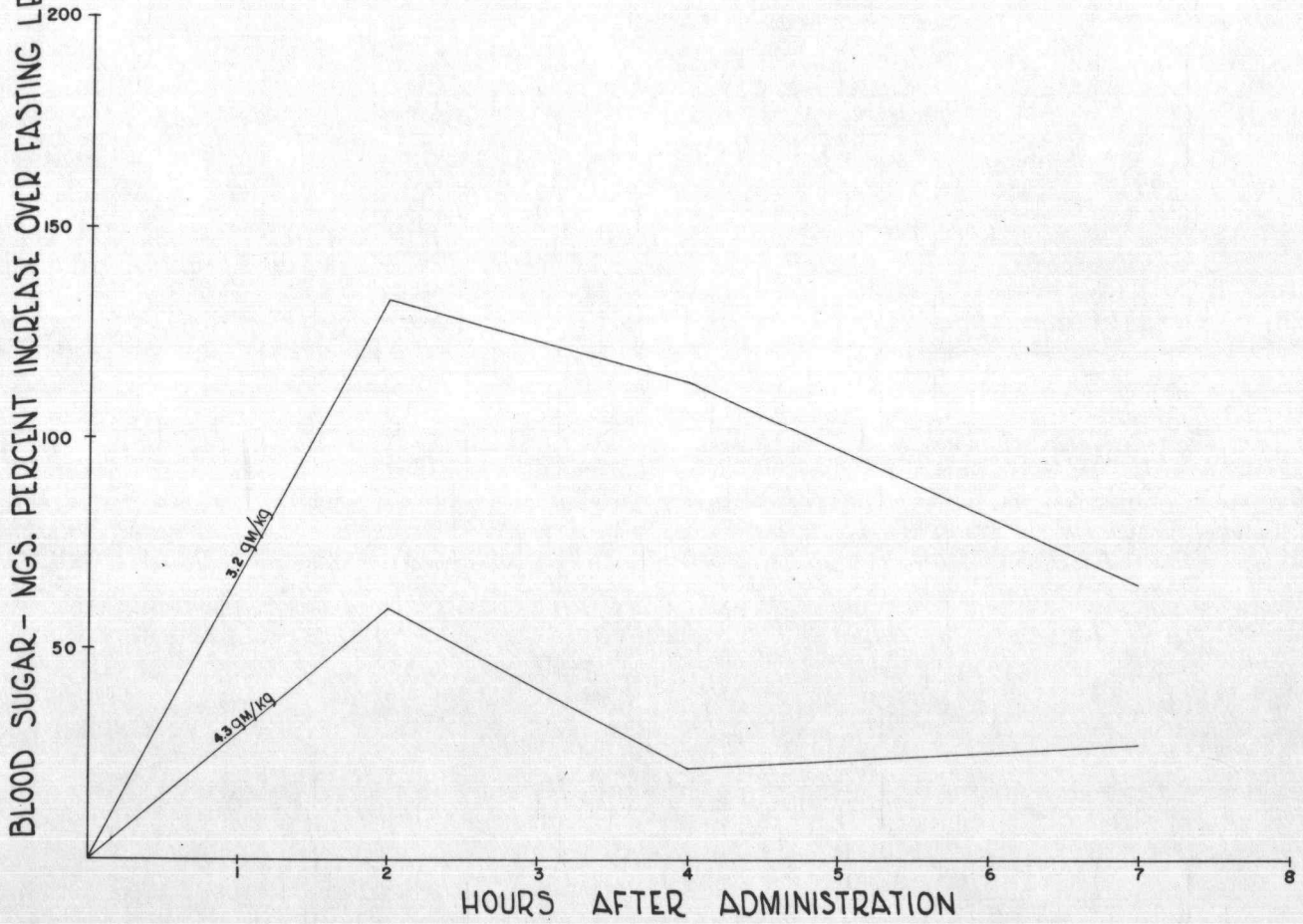

INCREASE IN BLOOD SUGAR OVER THE FASTING LEVEL
WHEN VARYING AMOUNTS OF 10% AQUEOUS SOLUTION
OF GLYCINE WERE ADMINISTERED BY STOMACH TUBE.
BLOOD SUGAR — MGS. PERCENT INCREASE OVER FASTING LEVEL
250
200
150
100
50
5.0 gm/kg
2.5 gm/kg
2.5 gm/kg
2.5 gm/kg
5.0 gm/kg
5.0 gm/kg
1
2
3
4
5
6
7
8
9
10
HOURS AFTER ADMINISTRATION

GRAPH - I-

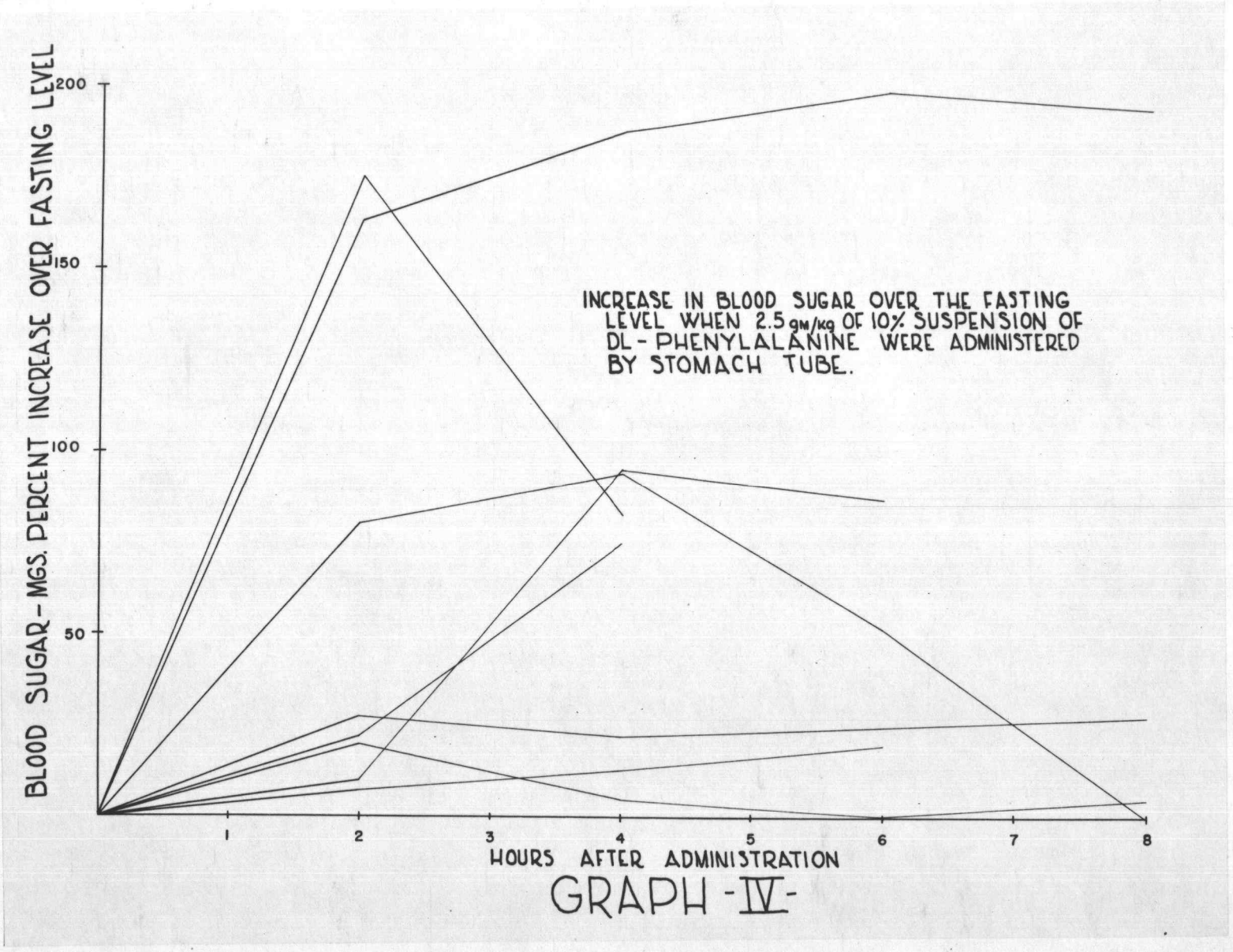

INCREASE IN BLOOD SUGAR OVER THE FASTING LEVEL
WHEN VARYING AMOUNTS OF 10% AQUEOUS SOLUTION
OF DL-ALANINE WERE ADMINISTERED BY STOMACH TUBE.
BLOOD SUGAR - MG. PERCENT INCREASE OVER FASTING LEVEL
250
200
150
100
50
2.5 gm/kg
2.0 gm/kg
5.0 gm/kg
1.0 gm/kg
1
2
3
4
5
6
7
8
9
10
11
12
HOURS AFTER ADMINISTRATION

GRAPH-II-

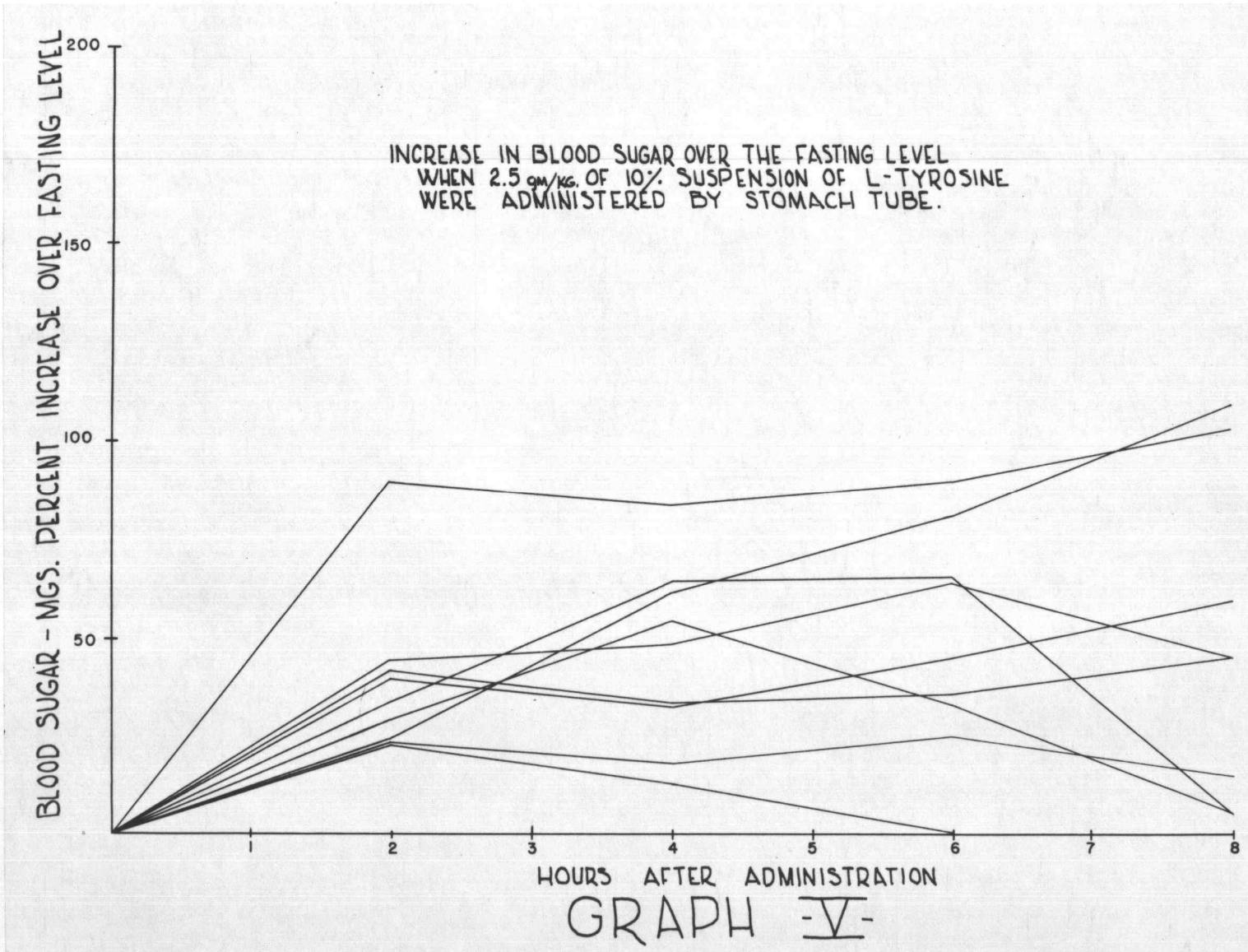
INCREASE IN BLOOD SUGAR OVER THE FASTING LEVEL WHEN VARYING AMOUNTS OF 5% AQUEOUS SOLUTION OF DL-VALINE WERE ADMINISTERED BY STOMACH TUBE.



GRAPH -III-

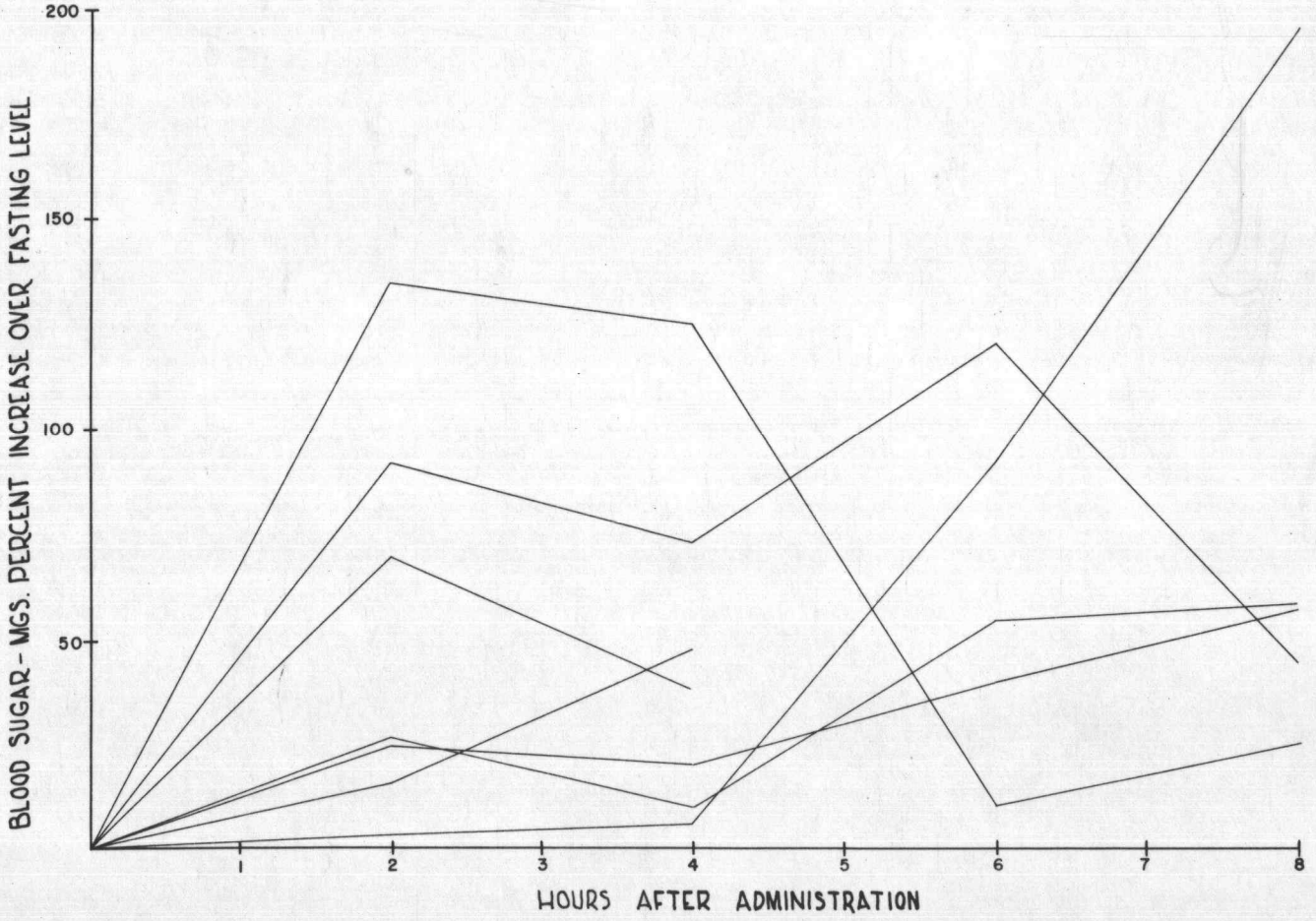

BLOOD SUGAR - MGS. PERCENT INCREASE OVER FASTING LEVEL
200
150
100
50
1
2
3
4
5
6
7
8
HOURS AFTER ADMINISTRATION
INCREASE IN BLOOD SUGAR OVER THE FASTING LEVEL WHEN 2.5 gm/kg OF 10% SUSPENSION OF DL - PHENYLALANINE WERE ADMINISTERED BY STOMACH TUBE.

GRAPH - IV -

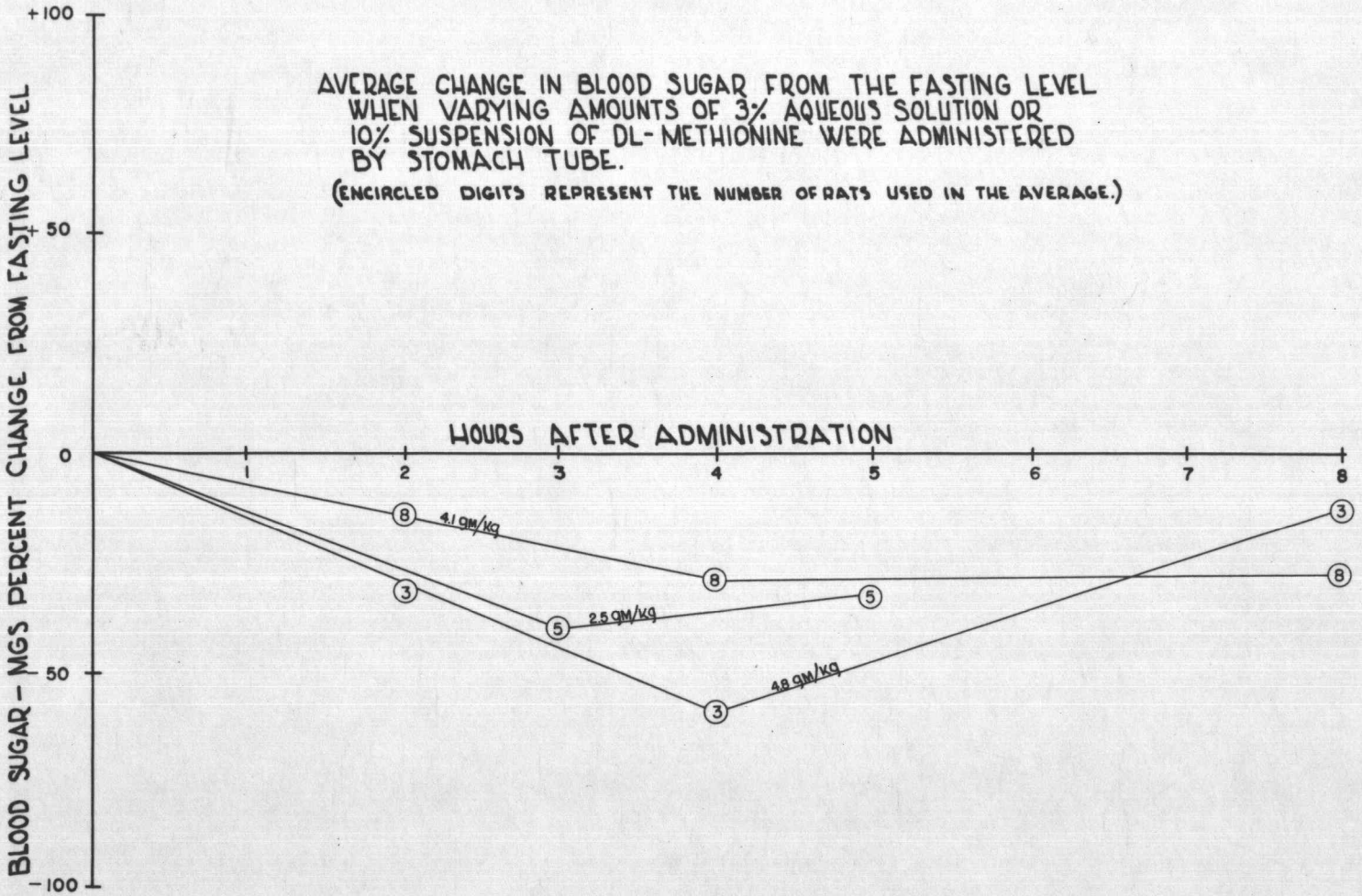

INCREASE IN BLOOD SUGAR OVER THE FASTING LEVEL
WHEN 2.5 gm/kg. OF 10% SUSPENSION OF L-TYROSINE
WERE ADMINISTERED BY STOMACH TUBE.
BLOOD SUGAR - MGS. PERCENT INCREASE OVER FASTING LEVEL
200
150
100
50
1
2
3
4
5
6
7
8
HOURS AFTER ADMINISTRATION

GRAPH -V-

INCREASE IN BLOOD SUGAR OVER THE FASTING LEVEL
WHEN 2.5 gm/kg OF 10% SUSPENSION OF L-CYSTINE
WERE ADMINISTERED BY STOMACH TUBE.



GRAPH -VI-


AVERAGE CHANGE IN BLOOD SUGAR FROM THE FASTING LEVEL WHEN VARYING AMOUNTS OF 3% AQUEOUS SOLUTION OR 10% SUSPENSION OF DL-METHIONINE WERE ADMINISTERED BY STOMACH TUBE.
(ENCIRCLED DIGITS REPRESENT THE NUMBER OF RATS USED IN THE AVERAGE.)
+100
50
0
50
-100
BLOOD SUGAR - MGS PERCENT CHANGE FROM FASTING LEVEL
HOURS AFTER ADMINISTRATION
1
2
3
4
5
6
7
8
4.1 GM/KG
2.5 GM/KG
4.8 GM/KG

GRAPH - VII -

incontrovertibly that the carbon chain of methionine is not utilized in the in vivo conversion of methionine to cystine.

The results obtained on the blood sugar studies on alloxan diabetic rabbits following the administration of various amino acids were not very satisfactory. Although these animals responded to the injection of amino acids by stomach tube as shown in Table XII, the responses were not as sensitive or consistent as those obtained with rats. The reason for this discrepancy between the rabbits and the rats can possibly be explained by the following observations: 1) On the seventh day of fasting, food was still found to be present in the stomach of the rabbits; 2) The observed apathy and marked decrease in bodily activity of the rabbits during prolonged fasting no doubt is accompanied by a similar decrease in absorption from the intestinal tract; 3) The correct dosage of the administered amino acids, using DL-Alanine as a yardstick, was not determined satisfactorily.

The wide individual variations in the response of animals to alloxan injections precludes its use as a quantitative method for the study of intermediary metabolism; however, alloxan diabetes is apparently suitable as a qualitative method for intermediary metabolism studies.

SUMMARY

1. Glycine, DL-Alanine, DL-Valine, DL-Phenylalanine, L-Tyrosine, and L-Cystine have carbohydrate-like actions when administered to alloxan diabetic rats.

2. DL-Methionine does not have a carbohydrate-like action when administered to alloxan diabetic rats. On the contrary, it caused a decrease in blood sugar.

3. Alloxan diabetes in rats is a good qualitative technique for the study of gluconeogenesis.

4. Intravenous injection of 40 mg./kg. of alloxan monohydrate to rats receiving food ad lib produces the most consistent results.

5. Rats are much better test animals than rabbits when this technique is used to study gluceoneogenesis.

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APPENDIX

TABLE XIII

TYPICAL EXPERIMENT ON MALE RAT[a] RECEIVING 40 MGS. ALLOXAN MONOHYDRATE PER KILOGRAM OF BODY WEIGHT INTRAVENOUSLY. NO PREVIOUS FAST. ANIMAL FED HIGH FAT DIET FOR EIGHT DAYS. ON NINTH DAY ANIMAL KILLED ACCIDENTALLY WHILE BEING FED BY STOMACH TUBE.

DAYS POST INJECTION	1	2	3	4	5	6	7	8	9
URINE OUTPUT (CC.)	16	30	33	25	40	35	35	23	c
GLUCOSE (MGS.)	366	2907	2950	1770	3300	2037	1484	1169	c
TOTAL N (MGS.)	145	119	155	135	193	162	157	127	c
FOOD INTAKE (GM.)[b]	11	9	8	2	9	7	2	1½	c

a - Expt. #14, male rat, 59 days old and weighing 112 gms. on date of alloxan injection.
b - High fat diet ad lib.
c - Animal died accidentally.

TABLE XIV

TYPICAL EXPERIMENT ON MALE RAT[a] RECEIVING 200 MGS. ALLOXAN MONOHYDRATE PER KILOGRAM OF BODY WEIGHT SUBCUTANEOUSLY. PRE-INJECTION FAST OF 48 HOURS. ANIMAL FED HIGH FAT DIET FOR SEVEN DAYS. ON EIGHTH DAY ANIMAL KILLED ACCIDENTALLY WHILE BEING FED BY STOMACH TUBE.

DAYS POST INJECTION	1	2	3	4	5	6	7	8
URINE OUTPUT (CC.)	-	22	35	45	50	50	50	c
GLUCOSE (MGS.)	-	2090	2632	3372	3506	3171	3474	c
TOTAL N (MGS.)	-	136	129	148	203	188	213	c
FOOD INTAKE (GM.)[b]	-	6	4	7	11	10	10	c

a - Expt. #16, male rat, 59 days old and weighing 100 gms. on date of alloxan injection.
b - High fat diet ad lib.
c - Animal died accidentally.

TABLE XV

TYPICAL EXPERIMENT ON MALE RAT[a] RECEIVING 200 MGS. ALLOXAN MONOHYDRATE PER KILOGRAM OF BODY WEIGHT SUBCUTANEOUSLY. PRE-INJECTION FAST OF 48 HOURS. ANIMAL FED HIGH FAT DIET FOR SEVENTEEN DAYS AND REMOVED FROM EXPERIMENT ON EIGHTEENTH DAY.

DAYS POST INJECTION	1	2	3	4	5	6	7	8	9	10	11	12
URINE OUTPUT (CC.)	-	24	35	30	25	25	25	18	8	3	15	9
GLUCOSE (MGS.)	-	967	1574	1967	1680	1510	1250	629	202	563	904	202
TOTAL N (MGS.)	-	88	122	124	202	124	170	145	117	138	170	97
FOOD INTAKE (GM.)[b]	-	11	8	10	7	8	6	4	3	4	8	7

DAYS POST INJECTION	13	14	15	16	17	18
URINE OUTPUT (CC.)	7	10	9	9	7	[c]
GLUCOSE (MGS.)	0	0	0	0	0	[c]
TOTAL N (MGS.)	-	-	-	-	-	[c]
FOOD INTAKE (GM.)[b]	6½	6	7½	14⅛	9½	[c]

a - Expt. #19, male rat, 59 days old and weighing 100 gms. on date of alloxan injection.
b - High fat diet ad lib.
c - Animal removed from experiment.

TABLE XVI

TYPICAL EXPERIMENT ON MALE RAT[a] RECEIVING 200 MGS. ALLOXAN MONOHYDRATE PER KILOGRAM OF BODY WEIGHT SUBCUTANEOUSLY. PRE-INJECTION FAST OF 48 HOURS. ANIMAL FED HIGH FAT DIET FOR TEN DAYS AND REMOVED FROM EXPERIMENT ON ELEVENTH DAY.

DAYS POST INJECTION	1	2	3	4	5	6	7	8	9	10	11
URINE OUTPUT (CC.)	-	20	12	8	26	19	8	7	8	8	c
GLUCOSE (MGS.)	-	490	579	318	1069	571	57	70	0	0	c
TOTAL N (MGS.)	-	103	94	79	208	145	90	96	[illegible]	[illegible]	c
FOOD INTAKE (GM.)[b]	-	2	3	2	10	2½	3	6	0	0	c

a - Expt. #24, male rat, 66 days old and weighing 100 gms. on date of alloxan injection.
b - High fat diet ad lib.
c - Animal removed from experiment.

TABLE XVII

TYPICAL EXPERIMENT ON FEMALE RAT[a] RECEIVING 40 MGS. OF ALLOXAN MONOHYDRATE PER KILOGRAM OF BODY WEIGHT INTRAVENOUSLY. NO PREVIOUS FAST. ANIMAL FED HIGH FAT DIET FOR NINE DAYS FOLLOWED BY ELEVEN DAYS ON STOCK DIET AND THEN FED HIGH FAT DIET AGAIN FOR THREE DAYS. ANIMAL REMOVED FROM EXPERIMENT ON TWENTY-FOURTH DAY.

DAYS POST INJECTION	1	2	3	4	5	6	7	8	9	10-20	21	22	23	24
URINE OUTPUT (CC.)	-	16	22	18	18	10	6	9	5	d	70	73	60	c
GLUCOSE (MGS.)	-	304	695	326	438	237	153	41	0	d	6380	5910	2990	c
TOTAL N (MGS.)	-	76	158	153	186	131	108	71	54	d	380	370	181	c
FOOD INTAKE (GM.)[b]	-	½	0	0	0	0	0	0	0	d	16½	14	8	c
BODY WEIGHT (GM.)	-	-	-	-	-	-	-	-	132	d	136	134	134	c

a - Expt. #31, female rat weighing 182 gms. on date of alloxan injection.
b - High fat diet ad lib.
c - Animal removed from experiment.
d - Animal fed stock diet ad lib.

TABLE XVIII

TYPICAL EXPERIMENT ON FEMALE RAT[a] RECEIVING 40 MGS. ALLOXAN MONOHYDRATE PER KILOGRAM OF BODY WEIGHT INTRAVENOUSLY. NO PREVIOUS FAST. ANIMAL FED HIGH FAT DIET FOR TEN DAYS. STARVED FOR NEXT TWO DAYS AND THEN REMOVED FROM EXPERIMENT ON THIRTEENTH DAY.

DAYS POST INJECTION	1	2	3	4	5	6	7	8	9	10	11	12	13
URINE OUTPUT (CC.)	-	30	7	14	17	24	23	9	11	9	12	15c	e
GLUCOSE (MGS.)	-	2494	615	687	1288	1979	1755	71	106	0	0	0	e
TOTAL N (MGS.)	-	338	129	193	142	208	207	124	164	-	-	-	e
FOOD INTAKE (GM.)b	-	15½	1½	4½	8	10	11½	5	11	1½d	f	f	e
BODY WEIGHT (GM.)	-	-	-	-	-	-	-	-	-	128	118	102	e

a - Expt. #49, female rat weighing 140 gms. on date of alloxan injection.
b - High fat diet ad lib.
c - Received 10 cc. water by stomach tube.
d - Fast started.
e - Animal removed from experiment.
f - Fasted.

TABLE XIX

TYPICAL EXPERIMENT ON FEMALE RAT[a] RECEIVING 40 MGS. ALLOXAN MONOHYDRATE PER KILOGRAM OF BODY WEIGHT INTRAVENOUSLY. NO PREVIOUS FAST. ANIMAL FED HIGH FAT DIET FOR NINE DAYS. REMOVED FROM EXPERIMENT ON TENTH DAY.

DAYS POST INJECTION	1	2	3	4	5	6	7	8	9	10
URINE OUTPUT (CC.)	-	25	30	20	18	18	13	14	14	c
GLUCOSE (MGS.)	-	238	2026	668	295	0	0	0	0	c
TOTAL N (MGS.)	-	312	353	249	240	-	-	-	-	c
FOOD INTAKE (GM.)[b]	-	19	10½	11	8	11	14	7½	11	c

a - Expt. #58, female rat weighing 168 gms. on date of alloxan injection.
b - High fat diet ad lib.
c - Animal removed from experiment.

TABLE XX

TYPICAL EXPERIMENT ON FEMALE RAT[a] RECEIVING 40 MGS. ALLOXAN MONOHYDRATE PER KILOGRAM OF BODY WEIGHT INTRAVENOUSLY. NO PREVIOUS FAST. ANIMAL FED HIGH FAT DIET FOR TEN DAYS. STARVED FOR NEXT THREE DAYS. ANIMAL FOUND DEAD ON FOURTEENTH DAY.

DAYS POST INJECTION	1	2	3	4	5	6	7	8	9	10	11	12	13	14
URINE OUTPUT (CC.)	-	11	13	12	32	53	53	29	71	29[c]	12	11	22	e
GLUCOSE (MGS.)	-	104	652	141	1869	4290	4640	3900	5940	1628	0	0	0	e
TOTAL N (MGS.)	-	190	198	177	184	279	313	237	327	207	125	119	110	e
FOOD INTAKE (GM.)[b]	-	10	0	0	7	12	11½	7	16	1½[d]	f	f	f	e
BODY WEIGHT (GM.)	-	-	-	-	-	-	-	-	-	178	170	160	142	e

a - Expt. #60, female rat weighing 214 gms. on date of alloxan injection.
b - High fat diet ad lib.
c - Received 5 cc. water by stomach tube.
d - Fast started.
e - Animal found dead.
f - Fasted.

TABLE XXI

TYPICAL EXPERIMENT ON FEMALE RAT[a] RECEIVING 40 MGS. ALLOXAN MONOHYDRATE PER KILOGRAM OF BODY WEIGHT INTRAVENOUSLY. NO PREVIOUS FAST. ANIMAL FED HIGH FAT DIET FOR TEN DAYS. STARVED FOR NEXT TWO DAYS. ANIMAL REMOVED FROM EXPERIMENT ON THIRTEENTH DAY.

DAYS POST INJECTION	1	2	3	4	5	6	7	8	9	10	11	12	13
URINE OUTPUT (CC.)	-	40	24	40	45	26	13	8	10	10	13	13[c]	e
GLUCOSE (MGS.)	-	3408	2295	2840	2035	217	0	0	0	0	0	0	e
TOTAL N (MGS.)	-	352	202	284	334	266	225	160	166	-	-	-	e
FOOD INTAKE (GM.)[b]	-	13	7	11	15½	11	11½	8½	8	1[d]	f	f	e
BODY WEIGHT (GM.)	-	-	-	-	-	-	-	-	-	154	144	138	e

a -- Expt. #61, female rat weighing 156 gms. on date of alloxan injection.
b -- High fat diet.
c -- Received 10 cc. water by stomach tube.
d -- Fast started.
e -- Animal removed from experiment.
f -- Fasted.