IN VIVO EFFECTS OF VARYING BLOOD GLUCOSE LEVELS ON ASCORBIC ACID SYNTHESIS BY DEPANCREATIZED AND PHLORIDZINIZED DIABETIC RATS

by

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INTRODUCTION

From the discovery of ascorbic acid as an antiscorbutic drug to the present day, the role of this vitamin in animal and plant metabolism has been the center of intense and arborizing research. Yet, at present, few specific biochemical actions have been elucidated. Relative to animal metabolism three rather specific activities are worthy of mention. Ascorbic acid appears to promote the absorption of iron in the ferrous form via the gastrointestinal tract. The apparent mechanism involved here is one of reducing ferric ions (commonly found in foods) to ferrous ions and concomitantly oxidizing ascorbic acid to dehydroascorbic acid. In addition vitamin C probably acts to maintain the iron in the reduced ionic state until active absorption is begun. The action of vitamin C in this case is a direct one, taking place in the gastrointestinal tract. It does not influence the absorption of iron if it is given parenterally.(1) Another and perhaps more intriguing action of ascorbic acid is its role in maintaining the function of the enzyme aconitase in the tricarboxylic acid cycle. The compound α,α'-dipyridyl inhibits aconitase which is immediately concerned in the metabolism of citric acid in the tricarboxylic acid cycle. It has been shown that guinea pigs given α,α'-dipyridyl excrete increased amounts of citrate after administration of citrate or malate (which forms citrate in the body) while the control animals do not. This indicates deficient aconitase activity in the α,α'-dipyridyl treated animals. Similarly in ascorbic acid deficiency, guinea pigs will excrete increased amounts of citric acid if treated with citrate or butyrate. In mitochondrial preparations from livers of α,α'-dipyridyl treated and scorbutic guinea pigs, there was a marked decrease in

citric acid oxidation. The addition of ferrous ion and/or ascorbic acid to these preparations restored depressed citric acid oxidation[2]. Though the role of ascorbic acid in aconitase maintainance has not been entirely elucidated, it seems possible that it acts to maintain the iron ion in the reduced state which in turn is necessary for aconitase action. Lastly, ascorbic acid has been closely linked to the oxidation of tyrosine, or more specifically to the synthesis of homogentisic acid from p-hydroxyphenylpyruvic acid. At present the bulk of research seems to indicate that ascorbic acid acts independently to maintain enzyme function rather than acting as a coenzyme or chemical part of the enzyme[3-6]. In addition there is doubt that any free intermediate compounds exist between p-hydroxyphenylpyruvic and homogentisic acids[3]. It would appear that in this conversion there is only one enzyme mechanism and that any intermediate compounds existing are bound to this enzyme. The role of ascorbic acid appears to be nonspecific in maintaining enzymatic activity and can be replaced by numerous other reducing compounds.[8] In all likelihood the two or three enzymes which have been proposed in the past to mediate this reaction are partial breakdown products of only one enzyme.

Contrasting with the paucity of material on specific chemical actions, there is a wealth of material relating ascorbic acid to various physiological processes. Unfortunately, most of this material has been obtained from clinically scorbutic humans or laboratory animals with in vivo experimental setups. As a result of these rather crude preparations, only the grossest of chemical reactions have been observed, and as a result, multiple theories and hypotheses, often contradictory, have evolved. Such data does serve the purpose of

directing future, more specific chemical exploration. It also has markedly broadened the field of ascorbic acid research and, as will be mentioned below, has linked ascorbic acid to the even less well understood branch of hormone metabolism.

A paramount problem in ascorbic acid research has been the mechanism of its biological synthesis in both plants and animals, exclusive of primates and the guinea pig. Considerable effort has been directed to this end by C.G. King and co-workers involving animal biosynthesis, and by F.A. Isherwood and L.W. Mapson and co-workers explaining both animal and plant biosynthesis. It is largely through the efforts of King and associates, employing isotopic tracer techniques, that the mechanism of rat ascorbic acid biosynthesis has been almost entirely explained[9-13]. The mammalian biosynthetic process is at present believed to proceed through D-glucose as a precursor in the following manner: D-glucose $\xrightarrow{\text{oxid.-H}_2\text{O}}$ D-glucuronic acid $\xrightarrow{\text{inversion}}$ 2-keto L-gulonic acid $\xrightarrow{\text{enolisation}}$ L-ascorbic acid. The experimental technique used to demonstrate this was basically to administer first uniformly labelled glucose C-14 to the intact chloretone* stimulated rats and recover ascorbic acid C-14 uniformly labelled from the urine. Partial degradation of the recovered ascorbic acid indicated that there apparently had been no splitting of the glucose molecule which acted as a direct precursor. Further studies utilized D-glucose 6C-14 and 1C-14 with similar recovery techniques in the urine revealing, again through the degradation of the ascorbic acid, that there had been

* Chloretone (trichlorotertairy butyl alcohol) has been shown to maximally stimulate rat ascorbic acid synthesis without becoming incorporated into the structure of vitamin C. References to this as well as other stimulating compounds are listed in the bibliography[14-16].

inversion of the glucose molecule producing respectively L-ascorbic acid 1C-14 and 6C-14. Previous studies having suggested that glucuronic acid might be a more direct precursor of ascorbic acid than is glucose, prompted similar experiments utilizing this substance. Results proved that glucuronic acid not only acts similarly to glucose but gives greater quantities of labelled ascorbic acid establishing a more direct relationship. 2-keto L-gulonic acid was postulated as the final intermediate. Isherwood has confirmed much of King's work by testing numerous possible precursors of ascorbic acid in the rat by infecting these substances and observing urinary ascorbic acid excretion changes(17). He has also observed plant synthesis and finds that certain plants may form ascorbic acid via D-galactose as a precursor in the following scheme: D-galactose ——→D-galacturonic acid——→L-galactonic acid——→L-ascorbic acid(17). Isherwood suggests that either mechanism is possible in rats but that enzyme specificity in that animal strongly favors the oxidation through L-gulonic acid-like compounds. Both such mechanisms may be found to work in various types of plant material, but in these cases enzyme specificity favors L-galactonic acid-like compounds. A significant fact about ascorbic acid synthesis in rats is that it proceeds as a part of carbohydrate breakdown. Such a link to glycogen-glucose metabolism immediately suggests possible hormonal influence on this process exerted by pancreatic, adrenocortical, and pituitary secretions. To date little has been elucidated along this line.

Numerous attempts to relate the metabolism of ascorbic acid to the adrenal steroids and vice versa have yet to contribute more than theory to this field. Certain observations, however, are worthy of mention if only to indicate that chemical relations do seem to exist between these

compounds. Ascorbic acid appears in high concentration in the adrenal cortex and is rapidly depleted under conditions of stress to the animal or by the administration of ACTH. This suggests an important role for this vitamin in hormone production. In addition stress causes hypertrophy of the adrenal cortex, depletion of vitamin C, depletion of cholesterol, and an increase in the oxygen consumption. The common denominator in such reactions appears to be the effect of ACTH. The difference between stress reactions and effects of exogenously administered ACTH appears to be only quantitative and not qualitative. As partial confirmation of this statement it is noted that hypophysectomized animals subjected to stress show no acute drop in adrenal cholesterol or vitamin C. (These substances show a drop chronically as the gland atrophies.) It has been demonstrated by Long, Oesterling, and Stepto that the adrenal cortical ascorbic acid level drops more rapidly in scurvy than does the adrenal cholesterol level[18-20]. Their observation indicates that the drop in cholesterol is secondary to the nonspecific stress of scurvy. Nadel and Clayton and many others have shown that there is a marked rise in urinary corticoids in scorbutic guinea pigs[21,22]. This occurs despite adrenal ascorbic acid levels being reduced to 4% of normal. The conclusions drawn from such experimental data would seem to be: ACTH activates the adrenal cortex and in doing so markedly decreases the adrenal level of both ascorbic acid and a steroid precursor, cholesterol. The more rapid decrease in ascorbic acid levels, as compared to those of cholesterol, in scorbutic animals suggests that ascorbic acid is not acting as a precursor of steroids but does affect their synthesis. It appears that large concentrations

of vitamin C are not required for the synthesis of cortical hormones. Lastly, since increased quantities of adrenal corticoids are excreted in the urine of scorbutic animals, it would seem that the deficiency of ascorbic acid either stimulates increased corticoid synthesis or decreases peripheral corticoid utilization or both. Ascorbic acid prevents adrenal hypertrophy induced by cold stress.

In so far as the effects of steroids on ascorbic acid metabolism are concerned, a few facts are available. The administration of ACTH, in humans, produces increased blood and urinary ascorbic acid levels as well as increased urinary corticoids. A partial explanation for this lies in the fact that ACTH has been shown by Ingbar et. al. to increase glomerular filtration rate and renal plasma flow, and to affect tubular function[23]. ACTH also seems to produce tissue ascorbic acid mobilization (and/or utilization?) as evidenced by the rapid drop in adrenocortical vitamin C. On the other hand, administration of exogenous corticoids (cortisone) reduces urinary vitamin C excretion, increases storage of vitamin C in the adrenal gland, and prevents its fall as a result of stress[24,25]. Booker demonstrated that the effects of ACTH on plasma and urine levels is antagonized by the administration of adrenocortical extracts. This evidence indicates that the effect of ACTH on ascorbic acid is essentially an adrenocortical phenomenon, and that the other tissues are only secondarily affected. However, there appears to be little doubt that ACTH does exert a marked metabolic effect on ascorbic acid, as is evidenced by Halley's report in 1951[26] in which patients on minimal ascorbic acid intake were treated with ACTH (9-110 days) and developed scorbutic hemorrhagic symptoms immediately relieved by ascorbic acid therapy[27].

Attempts to link insulin and ascorbic acid metabolism are equally intriguing and obscure. To date, no information has ever been presented demonstrating a direct effect of insulin upon ascorbic acid or vice versa. Ralli et. al. have reported one experiment in vitro in which exogenous insulin was added to plasma and produced no drop in the plasma ascorbic acid level. On the other hand, numerous experiments have been performed demonstrating an indirect effect of insulin upon ascorbic acid in some tissues and in the blood and plasma. Ralli has demonstrated that insulin decreases blood and urine ascorbic acid and increases white blood cell and platelet ascorbic levels in vivo[28,29]. Vogt et. al. and de Gandarias have shown that insulin produces a rapid fall in adrenal ascorbic acid levels[30,31]. There are reports that both diabetic humans and dogs have low blood ascorbic acid levels when they are on exogenous insulin[23,26]. In dogs these levels are normal or elevated if no insulin is administered[29]. As an interesting sidelight, Basu claims that insulin dosage in controlling diabetics may be halved and carbohydrate intake may be increased if twenty-five milligrams of ascorbic acid are simultaneously administered with the insulin[32]. Though some relationship exists between insulin and the adrenal steroids, no clarification has yet been forthcoming. It is becoming abundantly clear that no true understanding of the effect of insulin on ascorbic acid metabolism may be obtained until the role of the adrenal steroids has been elucidated.

Prior to the discovery of alloxan as a diabetogenic agent, the glucoside, phloridzin, had been widely utilized to produce diabetic-like states in experimental animals. Though its mode of action was

not fully understood, it was empirically found to stimulate marked glucosuria. With the discovery of insulin, the development of the modern concept of the diabetic state, and the experimental use of alloxan to produce diabetes, phloridzin has found little experimental use until recently. In the past few years phloridzin has been utilized for its ability to prevent renal tubular glucose absorption, thereby lowering blood glucose levels. This particular action has made phloridzin useful in studying gluconeogenesis (glucose synthesis from proteins) as well as other aspects of carbohydrate metabolism. The renewed interest in phloridzin has brought some rather interesting data on its mode of action. Shapiro has demonstrated that though phloridzin prevents glucose phosphorylation in the proximal tubules of the kidneys, the mechanism is not one of inhibiting glucose phosphorylation directly, but rather a block in the production of ATP (adenosine triphosphate) which is required for glucose phosphorylation. Evidence suggests that inhibition occurs most markedly in the dehydrogenases of pyruvic and citric acids. The action of phloridzin requires aerobic conditions indicating that ATP production under anerobic conditions is not affected[33]. More recently, Lotspeich has carried out studies on the effects of phloridzin on citric acid oxidation by guinea pig kidney cortex. Phloridzin inhibits this oxidation. Succinic and fumaric acids tend to augment the action of phloridzin. He concludes that phloridzin primarily inhibits the enzyme aconitase blocking the TCA cycle between citric and cis-aconitic and isocitric acids[34]. Such information as the above suggests a basic action as an enzyme poison for phloridzin worthy of

study in other tissues as well as the kidney. Because phloridzin prevents phosphorylation of glucose, it prevents glucose resorption in the kidney tubules. It thus becomes a convienent agent to produce lowered blood glucose levels via renal wastage and an agent which will block the resorption of other substances in the kidney by similar mechanisms. The effects of phloridzin upon ascorbic acid metabolism are not understood at present, but it would appear that some relation must exist at the level of aconitase in the TCA cycle. That phloridzin will inhibit the resorption of ascorbic acid in the kidney is known(35).

Three review articles covering much of the extensive research theory on ascorbic acid are included in the bibliography. They are intended to supplement the introductory material already presented(36-38).

Because of the intimate relation between glucose metabolism and ascorbic acid synthesis in rats, the possibility of hormonal influence via insulin has been suggested. Insulin is known to govern the phosphorylation of glucose to glucose-6-phosphate by the enzyme hexokinase. It was believed that perhaps glucose phosphorylation might be a prerequisite for the biosynthesis of ascorbic acid. The possibility that insulin might exert some other specific action in this synthesis also exists. It has been the intent of this thesis to provide such experimental data as would prove or disprove the hypothesis that ascorbic acid biosynthesis is controlled by insulin. In addition to this, certain other possible influences upon ascorbic acid synthesis have been studied: i.e., the mass action effect of elevating blood sugar levels, the possible effects of producing glucose wastage via

renal losses, and as will be reviewed from other workers data, the effects of glucose and of hypertonic salt infusions upon ascorbic acid biosynthesis, and possible renal tubular competative mechanisms between ascorbic acid and glucose. Some preliminary studies on ascorbic acid excretion and synthesis under the influence of phloridzin will be discussed.

This thesis specifically has included studies of urinary ascorbic acid excretion by depancreatized rats and by phloridzinized rats.

EXPERIMENTAL WORK

General Plan of Experiments.

Twenty-six male rats of the Sprague-Dawley strain were utilized in these experiments. The weights were generally maintained between 250-300 grams since at this weight range, growth changes were rather slight. Thirteen of the twenty rats depancreatized in the diabetic group of animals died apparently from surgical trauma in addition to dietary inadequacy. Most deaths occurred within one month post-operatively. There was marked gastric dilitation in all cases.

Two groups of animals were tested. The first group of rats was divided into two subdivisions differing only in that they were run at two separate times. Since experimental conditions were nearly identical in both, they may be grouped together. In this first group, the rats were placed in metabolism cages (discussed later) and fed an evaporated milk (Darigold) diet, to be taken ad libidum. They were maintained on this diet for one week. Following this period, several 24 hour urine samples were collected from each rat, and the output of ascorbic acid was determined as mg./gm. rat/24 hours. Having determined the control values for each animal, the rats were depancreatized (99%) and their fasting blood levels determined. All those reaching fasting blood levels of 350 mg.% and up were considered diabetic. Again, several determinations were made upon the urine samples of each rat, but this time for both glucose and ascorbic acid. The amount of each was listed in the units as given above. Following a suitable number of determinations, 20 mg. of chloretone, disolved in 1 ml. of

50% alcohol was added daily to the milk diet. Roughly 35 mg. of milk was consumed per rat per day, therefore by limiting the amount of milk provided to 35 ml. the amount of chloretone ingested was practically constant. Daily urine glucose and ascorbic acid excretions were again determined. The time required for the above experiments varied between one and one half to two months.

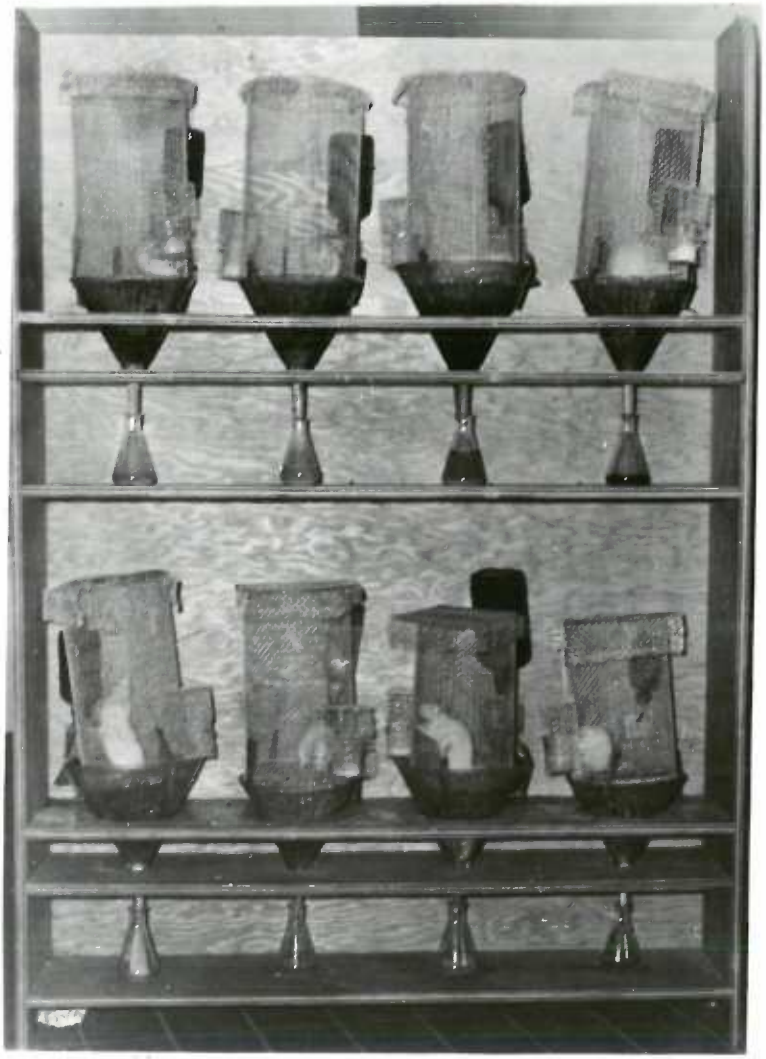
In the second group of rats the experimental design was similar except that diabetes was produced by phloridzin instead of by pancreatectomy. The six animals were placed on similar evaporated milk diets and urinary ascorbic acid control values determined. There was difficulty in obtaining phloridzin which produced the desired biological effects. No pure phloridzin was obtained, but that from the Nutritional Biochemicals Corporation was found to produce a glucosuria which was one half to two thirds as great as that of the depancreatized animals. This phloridzin was prepared as a 40% solution in propylene glycol and 50 mg./100 gm rat/day was administered subcutaneously. As with the chloretone, the rats were maintained on this dosage a few days before determinations on the urine were attempted. Several urine determinations were run for glucose and ascorbic acid content as in the depancreatized rats. Blood sugars were determined. In the last phase of this experiment, chloretone was added to the diet as in the first group and urine ascorbic acid and glucose values obtained. Hematocrit values were done both during the phloridzin administration and during phloridzin plus chloretone. The analysis for this second group required approximately two months.

The type metabolism cage used in both experimental groups was constructed of quarter inch galvanized wire screen and set upon eight or

FIGURE I

TYPE OF METABOLISM CAGES

AND THE SUPPORTING RACK



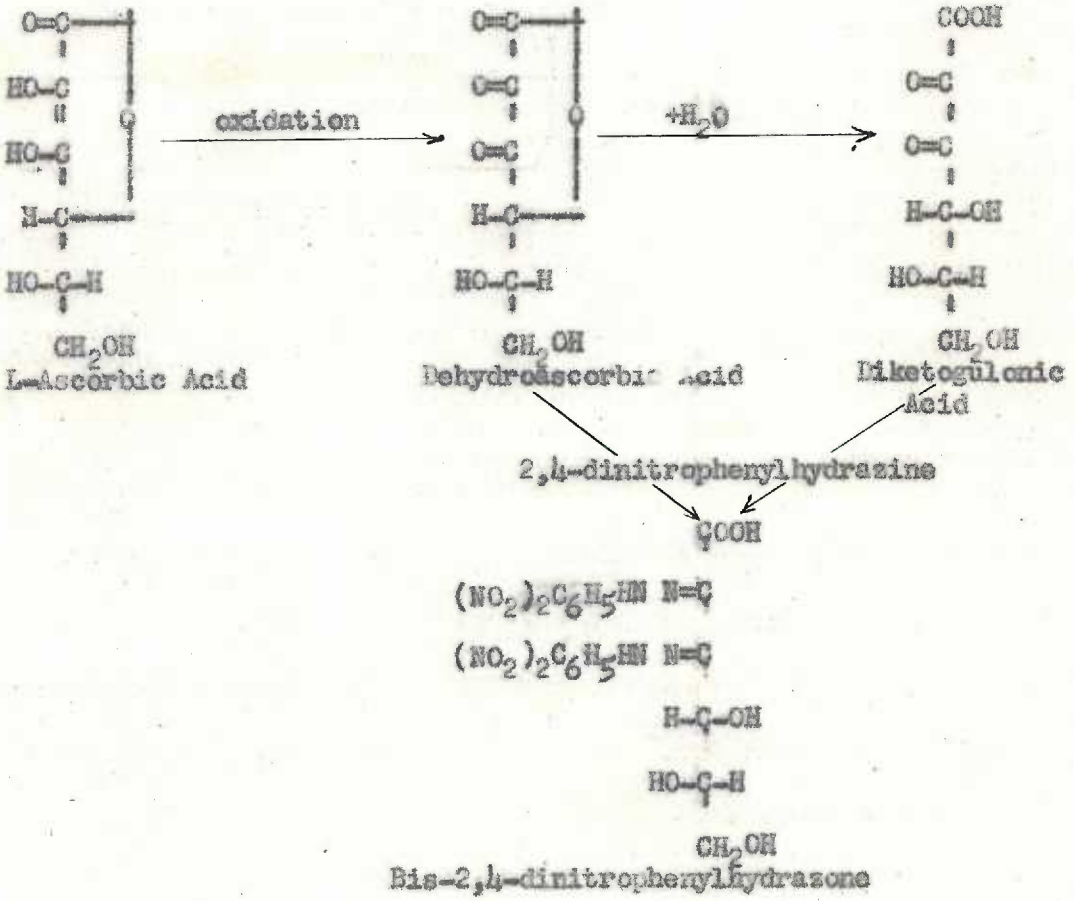
ten inch glass collecting funnels which contained a wire gauze to remove the feces. (See picture on page 13.) The urine was collected in 250 ml. Erlenmeyr flasks. The urine collected from each rat over a twenty-four hour period was preserved in 20 ml. of a solution containing 5% metaphosphoric acid and 10% glacial acetic acid. It was necessary to clean the metabolism cages at least once every week and the collecting funnels more often.

Determination of Ascorbic Acid in the Urine.

Ascorbic acid was determined in the earlier part of the diabetic experiment by the method of Roe and Kuether[39] as modified by Roe and Oesterling[40], Bolomey and Kemmerer[41], and Bolin and Book[42]. The urine was collected as described by Schwartz and Williams[43]. This modified technique was suggested by Straumfjord[44] and was found to be completely satisfactory though the results for duplicate determinations showed variations up to 0.3 mg.%. In so far as the experiment was concerned, this was of no significance since experimental conditions produced much greater variations in the urinary ascorbic acid output.

The urine was collected over twenty-four hour periods. Determinations were in most cases carried out immediately. It was found possible to preserve these urine samples, after filtration through crude filter paper to remove extraneous material, by freezing in corked containers. No ascorbic acid was lost by this technique. Following filtration, the urine volume was recorded along with the weight of the rat. Dilution of the urine was found necessary to produce ascorbic acid concentrations in a suitable range to be read

colorimetrically. In the normal and diabetic animal, dilutions of one to five were adequate, (while on a diet of evaporated milk). The diabetic animals with twenty milligrams chloretone added to the diet required urine dilutions of one to twenty. The phloridzinized animals required urine dilutions of one to fifty, and the phloridzinized chloretone stimulated animals needed a maximal urine dilution of one to one hundred. This method of ascorbic acid determination is based upon the oxidation of ascorbic acid to dehydroascorbic acid and diketogulonic acid, both of which react with 2,4-dinitrophenylhydrazine to form the same colored hydrazone, which is estimated colorimetrically.



Reagents used in Ascorbic Acid Analysis

1. 2,4-dinitrophenylhydrazine reagent. Dissolve 2 grams of 2,4-dinitrophenylhydrazine in 100 ml. of approximately 9N sulfuric acid. Let this stand for equilibration and filter using glass wool or high grade filter paper. This reagent may be filtered at once if necessary. It will normally show some precipitation at room temperature and may be refiltered as is necessary. A new solution should be prepared once a month.

2. Glacial acetic acid.

3. Metaphosphoric-thiourea solution. A 20% solution of metaphosphoric acid is prepared in cool distilled water (Bakers Metaphosphoric Acid was found most satisfactory). Fifty milliliters of this solution and 2 grams of thiourea are added to a 100 milliliter volumetric flask and sufficient distilled water is added to make 100 milliliters of solution. This solution should be kept under refrigeration and seems to be most satisfactory if freshly prepared every two weeks.

4. Sodium 2,6-dichlorobenzeneone-indophenol dye solution. Dissolve 100 mg. of the dye in 50 ml. of hot water (70°C roughly) and filter while hot. The solution should be kept under refrigeration. This solution should be prepared fresh every two or three weeks.

Technique for Ascorbic Acid Determination.

This technique was utilized for most of the diabetic animal experiments. A second technique will be described later in the paper.

Two milliliters of the diluted urine are transferred to each of

three test tubes (150 by 18 mm.). One drop of the indophenol dye (two drops if necessary) is added to each tube. This dye oxidizes ascorbic acid to the dehydroascorbic acid form. A pink color should persist, if not, the ascorbic acid concentration is too great and greater dilution of the urine is required. Two milliliters of the thiourea-metaphosphoric acid are added to all three tubes to reduce the excess dye and maintain any heavy metal contaminants in the reduced state. Such contaminants interfere with the determination. One of the three tubes is separated from the rest to be used as a control. To the other two tubes, one milliliter of the phenylhydrazine reagent is added. This is to form the bis-2,4-dinitrophenylhydrazone, and to give an acid medium necessary to maintain the dehydroascorbic and diketogulonic acids in a stable acid form. All three tubes are mixed well and placed in a controlled temperature bath and 37°C for exactly three hours. This develops the hydrazone derivative in the two phenylhydrazine tubes. At the end of this period the tubes are removed and placed in a cold water bath for five minutes to bring them to room temperature and to stop the reaction. Five milliliters of the glacial acetic acid are then added to all three tubes. Lastly, one milliliter of the indophenol dye is added to the blank tube. The tubes are allowed to stand for thirty minutes and then the solutions are transferred to pyrex tubes (125 by 15 mm.) which are calibrated and show equal transmission of light in the color range used. The solutions are read in a Klett-Summerson photo-electric colorimeter (model 800-3) using a green filter number 54 which has a spectral range of 500-570 millimicrons. The colorimeter is set at zero with

each blank, and the readings of the two unknown tubes averaged and read against the blank. A standard curve, which has been previously constructed with Klett readings on the ordinate, and ascorbic acid as milligrams percent on the abscissa, is used to determine ascorbic acid concentration in the tubes. The milligrams percent reading is multiplied by the dilution factor for the urine and by the volume of urine in milliliters over one hundred and this value divided by the weight of the rat in grams. This gives the milligrams ascorbic acid per gram of rat per twenty-four hours.

During the latter part of the diabetic experiment, technical difficulties developed in the use of the Klett-Summerson colorimeter, and it was deemed advisable to swith to a Coleman Junior model 6A spectrophotometer which was available. A new transmittance curve was established and a maximal transmission of 510 millimicrons was determined as most satisfactory for the color reaction utilised in this experiment. This instrument was employed for the remainder of the experiments. A new standard curve was made. A standard solution of 100 mg.% ascorbic acid was utilised to prepare solutions varying from 0.5 mg.% to 2.5 mg.%. This standard was freshly made whenever curve points were to be checked, however, it was found that a standard solution could be kept for several days (and maybe longer) without deteriorating if made up in the urine preservative and kept under refrigeration.

Because of limited time for experimental procedure in the phloridzin experiment, a shorter method for ascorbic acid determination was sought. Following the suggestion of Schaffert and Kingsley[45] the incubation temperature was elevated to 100°C on the assumption that the

incubation time could be correspondingly shortened. By elevating the temperature the diketogulonic acid apparently combines most rapidly with the hydrazine reagent to form the bis-2,4-dinitrophenylhydrazone. Since this experiment sought only to determine the total ascorbic acid content of the urine (ascorbic acid, dehydroascorbic acid, and diketogulonic acid), the difference in rapidity of combination of each of these substances with the hydrazine reagent was of little significance. Varying times for incubation at 100°C were tested for giving results comparable to those of the older technique. Twenty minutes was found quite satisfactory. The results showed the same variability as in the older technique but the determination time was reduced by two hours and forty minutes. This shorter incubation was used for the phloridzinized animals. The total determination for ascorbic acid remained identical with the method first used except for the shorter incubation and the use of the Coleman type B colorimetric tubes for colorimetric readings.

Several comparisons between the two techniques were made as to the accuracy of results. In both the short and long determinations two factors seemed to account for variation. One of these seemed to be the urine itself. For an as yet unaccounted for reason, duplicate urine ascorbic acid values tend to show some variation, probably from some undiscovered substance present in the urine. The second and more important reason appeared to be the use of the weak acid (glacial acetic) to dissolve the bis-hydrazone formed in the reaction. Periodic readings in color density (every fifteen minutes) following the addition of five milliliters of acetic acid showed a progressive rise in color density over a two and one half hour period. The color fades in

twenty-four hours. Though this produced considerable variation in readings, it did not interfere with the gross variations demonstrated in the experiment. Therefore, the use of the weak acid was continued. Preliminary tests were undertaken to see if the use of a stronger acid would eliminate this fallacy in accurate determinations. The use of sulfuric acid as in the original Roe technique has a tendency to char various preparations to be tested for ascorbic acid. It is also rather time consuming to add drop-wise to a solution in an ice bath. Because of these reasons, the use of glacial acetic acid had been proposed. Schwartz and Williams recently have suggested the use of a concentrated hydrochloric-85% phosphoric acid combination in parts of three to two respectively[46]. This must be prepared within one half hour before use, but has the advantages of a strong acid without the heat production and charring effect. The few tests run utilizing this combination showed practically no variation in colorimeter readings over a forty-five minute period. In addition, five millilitors of this acid combination could be added rapidly. Though this change in technique has not been fully tested or used in any of these experiments, it would seem to fill a gap in present methods of ascorbic acid determination.

Method of Determining Blood and Urine Glucose.

Somogyi's technique was utilized in determining blood and urine glucose levels in depancreatized and phloridzinized rats[47,48]. When blood glucose levels were to be determined, the rats were fasted for a twenty-four hour period prior to obtaining blood samples. The samples were obtained in the afternoon around one to two o'clock. The

urine samples were collected and filtered as mentioned earlier. Collection time was usually at ten to eleven AM.

Reagents.

1. 0.3 N Barium Hydroxide.

2. 5.0% solution of Zinc Sulfate Hepta-hydrate. It is most important that these first two solutions neutralise each other exactly: their exact concentrations are less important. Phenolphthalein is used as the indicator and titration is carried to a definite pink color which will last one minute.

3. Somogyi Sugar Reagent (making one liter)

Anhydrous Disodium Phosphate	28 gm.
0.1 N Sodium Hydroxide	100 ml.
Rochelle Salt	40 gm.
Cupric Sulfate (crystaline)	8 gm.
Anhydrous Sodium Sulfate	180 gm.
Potassium Iodide	8 gm.
N Potassium Iodate	25 ml.

Initially the Rochelle salt and the phosphate are added to about seven hundred milliliters of distilled water and dissolved. The sodium hydroxide is added and followed by the slow addition of the cupric sulfate which has been previously dissolved in about eighty milliliters of distilled water. The solution must be stirred while adding the copper salt. Next, the sodium sulfate is added and allowed to dissolve. Lastly the iodide and the iodate are added and the entire solution is diluted to one liter. It is allowed to sit for one to two days and then filtered through good grade filter paper. This

solution will usually keep for a year or more but should be checked against good thiosulfate every month or so.

5. 2 N Sulfuric Acid.

6. 0.005 N Thiosulfate. This is preserved by the addition of two milliliters of a ten percent sodium hydroxide.

7. 2% Sodium Oxalate solution. To prepare an oxalated spot dish use two drops of the oxalate in each cup of the dish and dry. This produces sufficient anti-coagulant for determinations of 0.1 ml. of blood.

Procedure for Blood Glucose Determination.

The rat is wrapped in a cloth with the tail exposed. The tail is then washed in warm water both to stimulate blood flow and to clean. The tip of the tail is cut off with scissors and a few drops of blood deposited in one cup of the oxalated spot dish. The blood is stirred with the tip of the cut tail to mix the oxalate and blood well. Using a one tenth milliliter blow pipette, on tenth milliliter of the blood is laked in 3.5 milliliters of distilled water. To this is added 0.2 ml. of the barium hydroxide follwed by 0.2 ml. of the 5% zinc sulfate. The solutions are mixed well and centrifuged rapidly for approximately ten minutes in the usual laboratory centrifuge. Two milliliters of the clear supernatent fluid are accurately pipetted into a large (25 by 200 mm.) tube. It is well to have the tip of the pipette covered with a small amount of cotton to prevent collecting any of the precipitate in the supernatant. Two milliliters of the sugar reagent is added to the supernatant via an accurate blow pipette which is utilized for all sugar determinations. The tube is

covered with a large glass marble and placed in a boiling water bath for twelve minutes. After heating, the tube is cooled in cold water for a few minutes (actually one minute is enough) and one millilicter of 2 N sulfuric acid is added rapidly via a free flowing one milliliter pipette. The mixture is swirled and should be titrated with the tiosulfate solution within a few mimutes. The end point of the titration is determined by adding one or two drops of a starch solution in half saturated sodium chloride. The starch solution gives a blue color which at the end point will change to a faint green color lasting one minute. The titration is best carried out using a five milliliter burette which is calibrated in hundredths and has a needle tip. The thiosulfate is added at a constant rate with constant swirling of the tube. With every group of blood or urine determinations, two blanks are run. These are prepared by using distilled water. Otherwise, they are run as above. The difference between the blanks and the unknown is obtained and value of glucose concentration in the unknown read from a previously constructed curve having an ordinate of thiosulfate difference values and an abscissa of milligrams percent glucose. The blood glucose levels are reported in milligrams percent.

Urine Glucose Determination.

One tenth milliliter of the filtered diabetic urine (two tenths if glucose is less concentrated) is pipetted into 6.9 (or 6.8) milliliters of distilled water. Four tenths milliliter of the barium hydroxide and four tenths milliliter of the zinc sulfate are added and the mixture stirred well. The mixture is centrifuged, and two tenths

milliliter of the supernatant are added to the large incubation tube. To this are added 1.8 ml. of distilled water and 2 ml. of the sugar reagent. The determination is run as above. The value in milligrams percent is multiplied by the dilution factor of ten or twenty and by the volume of urine in milliliters over one hundred, and the result by the weight of the rat in grams. This gives the final value of mg./gram rat/ 24 hours, as in the ascorbic acid results.

Determination of Rat Hematocrit.

Hematocrit values are obtained by the method of Van Allen using Van Allen hematocrit tubes and 0.01 ml. of blood[49]. The blood for this determination is obtained as in the manner described above, only potassium-ammonium oxalate (two drops of a 2% solution) is used instead of the sodium oxalate to prevent cell distortion. The blood is drawn into the hematocrit tubes and two wide rubber bands are applied lengthwise to hold the blood in the tubes. The tubes are centrifuged in a right angle centrifuge for thirty minutes to obtain constant volume readings.

RESULTS

The results on the three animals surviving the first experiment in 1953 are recorded on Table I. In this group, the urinary ascorbic acid of the controls, the urinary ascorbic acid and glucose of the depancreatized rats, and of the depancreatized plus chloretone stimulated rats are listed. The results of this experiment are given separately from those for 1954 because they were run at different periods and because the daily determinations extend over a slightly different period of time. In the concluding analysis, both groups are considered as one.

By the termination of this first experiment, rat number three was in extremus. The urine sugars are depressed from lack of food though the urinary ascorbic acid values appear to remain at least doubled even on a starvation diet.

Table II records the results from the four animals utilized in the second part of this first experimental group. Since only three animals were used in the first part, it was necessary to conduct a second similar experiment to increase the sample size of depancreatized animals to six or seven.

Table III presents the crude data for the second major group of experimental animals. The determinations are recorded as in the previous charts with the addition of hematocrit values. Because of space limitation these values are placed on the chart during the general period in which they were run but not on the exact day.

Table IV summarizes the results of each experimental group by listing the means of each major determination, i.e., urinary ascorbic

Table I

RESULTS OF PANCREATECTOMY AND PANCREATECTOMY PLUS CHLORETONE ON URINARY ASCORBIC ACID AND GLUCOSE PRODUCTION IN THE ADULT MALE RAT

(all values in mg./ gm. rat/ 24 hrs. unless listed otherwise) (BG= Blood Glucose)

Date 1953	Rat 1 Vit. C	Rat 1 Urine G, Blood G	Rat 2 Vit. C	Rat 2 Urine G, Blood G	Rat 3 Vit. C	Rat 3 Urine G, Blood G
7/21	0.009				0.004	
7/22	0.010				0.007	
7/23	0.003				0.013	
7/24	0.006				0.008	
7/25						
7/26						
7/27	0.009					
7/28	Depancreatised				0.011	
7/29					Depancreatized	
7/30						
7/31			0.008			
8/1						
8/2						
8/3	0.007	0.398	0.009		0.010	0.52
8/4	0.023	5.23			0.014	9.11
8/5	0.017	7.50	0.013		0.023	6.76
8/6	0.016	8.84	0.013		0.024	13.35
8/7	0.021	12.65			0.022	13.36
8/8			Depancreatized			
8/9						
8/10	0.024	BG 354 mg.%			0.022	BG 354 mg.%
8/11	0.021	15.82				
8/12					0.023	14.52
8/13					0.023	17.81
8/14	0.025	22.00				
8/15						
8/16						

Table I (contd.)

Date 1953	Rat 1 Vit. C	Rat 1 Urine G, Blood G	Rat 2 Vit. C	Rat 2 Urine G, Blood G	Rat 3 Vit. C	Rat 3 Urine G, Blood G
8/17	0.028	BG 390 mg.%	0.022	BG 402 mg.%	0.026	BG 420 mg.%
8/18	0.027	20.51	0.023	13.64		
8/19			0.025	15.12	0.030	13.99
8/20						
8/21		BG 394 mg.%	0.036	BG 358 mg.%		BG 400 mg.%
8/22						
8/23	Chloretone 20 mg.				Chloretone 20 mg.	
8/24						
8/25			0.034	19.34		
8/26						
8/27			0.036	BG 400 mg.%		
8/28	0.099	14.23	0.035	20.51	0.088	18.30
8/29			Chloretone 20 mg.			
8/30	0.101	12.08	0.133	19.49	0.086	6.37
8/31	0.100	13.83	0.124	17.73	0.088	13.44
9/1			0.159	24.47	0.103	5.98
9/2	0.113	10.17	0.130	21.62	0.098	1.58
9/3	0.112	11.58	0.129	17.23	0.067	2.63

Table II

RESULTS OF PANCREATECTOMY AND PANCREATECTOMY PLUS CHLORETONE ON URINARY ASCORBIC ACID AND GLUCOSE PRODUCTION IN THE ADULT MALE ALBINO RAT

(all values in mg./ gram rat/ 24 hrs. unless listed otherwise) (BG= Blood Glucose)

Date 1954	Rat 4 Vit. C	Rat 4 Urine G Blood G	Rat 5 Vit. C	Rat 5 Urine G Blood G	Rat 6 Vit. C	Rat 6 Urine G Blood G	Rat 7 Vit. C	Rat 7 Urine G Blood G
7/20	0.006						0.017	
7/21	0.014				0.014		0.017	
7/22	0.017				0.014		0.011	
7/23	0.011				0.010		0.010	
7/24								
7/25								
7/26	0.011				0.011			
7/27	0.006				0.012		0.017	
7/28	0.009							
7/29	0.021						0.020	
7/30			0.021		Depancreatised			
7/31								
8/1								
8/2	Depancreatised						Depancreatised	
8/3			0.017					
8/4								
8/5								
8/6			0.017					
8/8								
8/9			0.015					
8/10			0.014					
8/11								
8/12			0.011					
8/13			0.015					

Table II (contd.)

Date 1954	Rat 4 Vit. C	Rat 4 Urine G / Blood G	Rat 5 Vit. C	Rat 5 Urine G / Blood G	Rat 6 Vit. C	Rat 6 Urine G / Blood G	Rat 7 Vit. C	Rat 7 Urine G / Blood G
8/14								
8/15								
8/16								
8/17								
8/18		Depancreatized						
8/19								
8/20								
8/21								
8/22								
8/23								
8/24								
8/25								
8/26		BG 380 mg.%		BG 404 mg.%		BG 375 mg.%		BG 417 mg.%
8/27								
8/28								
8/29								
8/30								
8/31	0.046	28.54	0.028	21.74	0.033	18.62	0.053	20.38
9/1	0.036	26.36	0.054	21.66	0.037	20.83	0.047	24.14
9/2	0.050	26.47	0.036	15.41	0.032	19.79	0.047	23.51
9/3	0.052	BG 315 mg.%	0.056	20.38	0.041	BG 385 mg.%	0.065	BG 376 mg.%
9/4								
9/5								
9/6								
9/7	0.035	20.11	0.044	18.94	0.041	22.52	0.036	18.03
9/8	0.046	22.70	0.045	28.98	0.047	22.84	0.050	21.36
9/9	0.040	25.22	0.040	21.80	0.032	15.88	0.044	18.53
9/10	Chloretone 20 mg./D		Chloretone 20 mg./D		Chloretone 20 mg./D		Chloretone 20 mg./D	
9/11								

Table II (contd.)

Date 1954	Rat 4 Vit. C	Rat 4 Urine G / Blood G	Rat 5 Vit. C	Rat 5 Urine G / Blood G	Rat 6 Vit. C	Rat 6 Urine G / Blood G	Rat 7 Vit. C	Rat 7 Urine G / Blood G
9/12								
9/13								
9/14								
9/15	0.095	17.65	0.146	17.16	0.078	15.61	0.088	14.84
9/16	0.144	23.44	0.184	20.21	0.134	18.81	0.140	17.55
9/17	0.127	18.71	0.162	18.94	0.144	16.69	0.122	15.32
9/18		BG 360 mg.%		BG 427 mg.%		BG 354 mg.%		BG 465 mg.%
9/19								
9/20	0.166	17.52	0.143	23.25	0.132	17.70	0.112	18.71
9/21	0.122	14.80	0.156	14.95	0.117	15.90	0.108	15.20
9/22	0.142	17.33	0.126	14.40	0.106	15.11	0.097	14.24

Table III

RESULTS OF PHLORIDZIN AND PHLORIDZIN PLUS CHLORETONE ON URINARY ASCORBIC ACID AND GLUCOSE PRODUCTION IN THE ADULT MALE ALBINO RAT
(Values in mg./ gram rat/ 24 hours) (Blood Glucose listed as mg.%)

Date	Rat 1		Rat 2		Rat 3		Rat 4		Rat 5		Rat 6	
1955	Vit. C	Urine G / Blood G	Vit. C	Urine G / Blood G	Vit. C	Urine G / Blood G	Vit. C	Urine G / Blood G	Vit. C	Urine G / Blood G	Vit. C	Urine G / Blood G
7/5	0.014		0.018		0.010		0.015		0.016		0.020	
7/6	0.016		0.017		0.011		0.017		0.014		0.016	
7/7	0.014		0.015		0.011		0.019		0.014		0.016	
7/8												
7/9												
7/10												
7/11												
7/12												
7/13	0.017		0.019		0.013		0.021		0.021		0.019	
7/14	0.020		0.027		0.018		0.025		0.022			
7/15	0.022		0.027		0.023		0.033		0.026		0.027	
7/16												
7/17												
7/18												
7/19	0.016		0.019		0.009		0.017		0.012			
7/20			0.022		0.012				0.022		0.021	
7/21	Phloridzin administered 50 mg./ 100 gm. rat/ day											
7/22												
7/23												
7/24												
7/25	Rat 1 showed											
7/26	blood in urine.	0.176	9.55	0.127	9.22	0.151	9.66	0.136	7.84	0.146	6.11	
7/27	Phloridzin	0.178	10.59	0.147	7.34	0.179	10.86	0.132	4.28	0.156	9.13	
7/28	withdrawn for	0.168	11.13	0.194	11.91	0.163	9.44	0.141	7.36	0.154	8.93	
7/29	approx. 28 days.	0.197	12.00	0.136	8.13	0.150	8.10	0.152	7.27	0.130	5.60	

Table III (contd.)

Date 1955	Rat 1 Vit. C	Rat 1 Urine G / Blood G	Rat 2 Vit. C	Rat 2 Urine G / Blood G	Rat 3 Vit. C	Rat 3 Urine G / Blood G	Rat 4 Vit. C	Rat 4 Urine G / Blood G	Rat 5 Vit. C	Rat 5 Urine G / Blood G	Rat 6 Vit. C	Rat 6 Urine G / Blood G
7/30												
7/31												
8/1												
8/2												
8/3			0.130	4.76	0.167	11.62	0.218	12.72	0.106	6.00	0.194	11.60
8/4			0.164	11.27	0.166	11.18	0.216	13.06	0.170	11.39	0.143	8.22
8/5			0.164	10.30	0.152	9.30	0.171	11.21	0.102	6.20	0.180	11.22
8/6												
8/7												
8/8												
8/9												
8/10				BG 136 mg%		BG 111 mg%		BG 133 mg%		BG 141 mg%		BG 133 mg%
8/11												
8/12												
8/13												
8/14				Hematocrit values: Rat 2=32; Rat 3=31; Rat 4=26; Rat 5=33; Rat 6=32								
8/15												
8/16				BG 60 mg%		BG 112 mg%		BG 92 mg%		BG 102 mg%		BG 75 mg%
8/17												
8/18												
8/19	Chloretone plus phloridzin administered to Rats 2-6 --Rat 1 restarted on phloridzin 50mg/ 100gm rat/ day											
8/20												
8/21												
8/22	0.179	8.43	0.315	9.80	0.216	9.76	0.314	10.36	0.279	11.21	0.238	12.40
8/23	0.156	7.62	0.352	11.28	0.268	9.47	0.219	7.31	0.232	8.36	0.293	9.60
8/24	0.124	8.35	0.274	10.36	0.221	8.98	0.271	9.16	0.254	5.08	0.279	10.28
8/25	0.140	10.59	0.336	10.58	0.234	5.12	0.273	10.44	0.186	8.43	0.246	9.11
8/26	0.105	7.70	0.341	13.09	0.228	9.39	0.223	9.22	0.241	10.48	0.280	10.09
8/27			Hematocrit values: Rat 1=30; Rat 2=32; Rat 3=32; Rat 4=32; Rat 5=32; Rat 6=32									
8/28	0.117	8.22	0.362	12.91	0.245	9.62	0.234	8.60	0.252	9.76	0.265	10.11
8/29		BG 100 mg%		BG 140 mg%		BG 105 mg%		BG 111 mg%		BG 111 mg%		BG 130 mg%

Table III (contd.)

Date	Rat 1		Rat 2		Rat 3		Rat 4		Rat 5		Rat 6	
1955	Vit. C	Urine G Blood G	Vit. C	Urine G Blood G	Vit. C	Urine G Blood G	Vit. C	Urine G Blood G	Vit. C	Urine G Blood G	Vit. C	Urine G Blood G
8/30												
8/31	Rats 2-6 back on milk diet with no medication to be tested for normal blood sugar. Rat 1 on phloridzin plus chloretone											
9/1												
9/2	0.275	9.03										
9/3	0.273	9.24										
9/4	0.308	8.36										
9/5	0.245	7.04										
9/6	0.242	8.26										
9/7	0.270	9.86										
9/8		BG 110 mg%										
9/10												
9/11												
9/12				BG 117 mg%		BG 126 mg%		BG 120 mg%		BG 107 mg%		BG 133 mg%

Hematocrit values: Rat 1=32; Rat 2=35; Rat 3=35; Rat 4=34; Rat 5=34; Rat 6=36

Table IV

MEAN VALUES FOR URINARY ASCORBIC ACID AND GLUCOSE TAKEN FROM TABLES I THROUGH III
(Values listed as mg./ gm. rat/ 24 hours)

Group I - Depancreatized Diabetic Rats — 1953

	Rat 1		Rat 2		Rat 3	
	Vit. C	Urine G	Vit. C	Urine G	Vit. C	Urine G
Control	0.007		0.011		0.009	
Depancreatized	0.021	11.62	0.030	17.15	0.022	11.18
Depanc. + Chloretone	0.105	12.38	0.135	20.11	0.088	8.38

Group I - Depancreatized Diabetic Rats — 1954

	Rat 4		Rat 5		Rat 6		Rat 7	
	Vit. C	Urine G	Vit. C	Urine G	Vit. C	Urine G	Vit. C	Urine G
Control	0.009		0.016		0.012		0.015	
Depancreatized	0.043	24.90	0.043	21.27	0.038	20.08	0.057	20.99
Depanc. + Chloretone	0.124	18.24	0.153	18.15	0.118	16.64	0.111	15.96

Group II - Phloridzin Diabetic Rats — 1955

	Rat 1		Rat 2		Rat 3		Rat 4		Rat 5		Rat 6	
	Vit.C	Urine G	Vit.C	Urine G	Vit.C	Urine G	Vit.C	Urine G	Vit.C	Urine G	Vit.C	Urine G
Control	0.017		0.020		0.012		0.021		0.018		0.020	
Phlorid.	0.137	8.48	0.168	9.94	0.156	9.81	0.178	10.72	0.134	7.19	0.158	8.69
Phlorid. + Chlor.	0.269	8.63	0.330	11.34	0.243	8.72	0.256	9.18	0.241	8.89	0.267	10.26

Table V

MEANS AND STANDARD DEVIATIONS OF CONTROLS, AND ALL URINARY GLUCOSE OUTPUTS.
(Values listed in mg./ gm. rat/ 24 hours)

	Mean	N	Standard Deviation
Urinary Ascorbic Acid Control	0.015	84	0.006
Urinary Glucose			
Depancreatised	17.68	45	6.73
Depancreatized + Chloretone	16.89	36	3.23
Phloridzin	9.16	41	2.2
Phloridzin + Chloretone	9.50	36	3.0

acid and urinary glucose. The means are listed for 1953, 1954, and 1955. This table does not list blood glucose levels since these were utilized only as an indication of the degree of diabetes existing in the depancreatized animals and maintainance of normal or depressed blood glucose in the phloridzinized rats.

Table V demonstrates the standard deviations for certain of the important results in the foregoing charts. This allows for a rapid comparison of important group data with the means listed in Table IV. In this chart, the mean and standard deviation for all ascorbic acid controls are listed. This is to facilitate distinguishing between the normal rat ascorbic acid output and those of the depancreatized, the chloretone stimulated, and the phloridzinized rats. Additionally, the means and standard deviations for all urinary glucose determinations have been determined to demonstrate whether chloretone produces any stimulation of urinary glucose output in the depancreatized rat or the phloridzinized rat.

DISCUSSION

It is evident from the results obtained that the following conclusions may be drawn. Pancreatectomy produces a state of marked insulin insufficiency as is evidenced by both blood glucose levels and urinary glucose output. In this diabetic state, there is a marked increase in urinary ascorbic acid output. With chloretone stimulation of the rat, there is a greater increase in urinary ascorbic acid than in diabetes alone, though there is no detectable change in the diabetic urinary glucose levels, and probably none in blood glucose levels. The decrease in blood glucose levels with phloridzin administration was not sufficient to be detected, though it is probable that some decrease occurred. Phloridzin does produce a marked glucosuria, though not so great as in the depancreatized rats. Phloridzin alone produces a urinary ascorbic acid level roughly comparable to that of the depancreatized-chloretone stimulated rats. The object of this thesis was threefold: First of all to demonstrate whether or not there is any direct effect of insulin upon ascorbic acid production. Secondly, since glucose is a precursor of ascorbic acid in the rat, efforts were made to determine whether or not elevation of blood glucose levels will produce a mass action effect, stimulating the increased production of ascorbic acid. Thirdly, as a corollary to this, does lowering blood glucose decrease the production of ascorbic acid? The answer to this latter point has not yet been obtained since, as mentioned above, blood glucose levels were not noticeably depressed by the administration of phloridzin. Additionally, since the phloridzin is known to inhibit renal resorption

of ascorbic acid, it is entirely possible that this effect was enough to stimulate ascorbic acid production via homeostatic mechanisms despite any fall in blood glucose levels. Finally, the phloridzin utilized in this experiment was sufficiently impure to lead one to suspect that it might have been acting directly as a toxic stimulating substance causing an increase in ascorbic acid production. Even if the phloridzin were pure, one does not know what effects it might have directly upon ascorbic acid production. Nevertheless, the fact that the phloridzin utilized in this experiment did produce both a glucosuria and an ascorbic aciduria seems to confirm other reports that it does block renal tubular resorption.

Important to the theory that elevated blood glucose stimulates ascorbic acid production is the possible competitive reabsorption between glucose and ascorbic acid in the kidney. If there were marked competition between these two substances, one might conclude that the elevated urinary ascorbic acid might be purely the result of blocked tubular resorption. In line with this reasoning, Daughday found that alloxan or depancreatized diabetic rats excrete high levels of inositol in their urine. This does not occur in the normal animal. Continuous intravenous administration of glucose in the normal animal produced both a marked inositoluria and diuresis. Continuous intravenous sodium chloride administration produced the diuresis in the normal animal but did not increase inositol excretion. The conclusion drawn from this experiment was that glucose and inositol compete in renal tubular resorption. That the diuresis itself had no effect is indicated by the saline diuresis without inositoluria[50]. One might assume from these studies that such a competitive mechanism

would also be likely to exist between ascorbic acid and glucose. Selkurt has studied the effect of glucose in blocking ascorbic acid resorption in normal dogs(35). He reports that when glucose is being reabsorbed at a maximal rate, ascorbic acid reabsorption is at first completely blocked, and then gradually recovers to a rate slightly below that of the untreated animal. P-aminohippuric acid excretion interferes with ascorbic acid reabsorption in a manner similar to that of glucose resorption. Although their respective tubular mechanisms do not mutually interfere, glucose and p-aminohippuric acid when simultaneously infused, depress ascorbic acid reabsorption to a greater degree than does each substance singly. Selkurt concludes that because of the non-specific nature of the competition, the interference lies in the system which supplies energy for tubular transfer. A more tenable conclusion might be that both glucose and p-aminohippuric acid do compete with one or more reactions necessary for ascorbic acid resorption. Such a block to ascorbic acid resorption might be partially overcome by production of additional quantities of such substances as are required for resorption of glucose and ascorbic acid and secretion of p-aminohippuric acid. Though this experiment was performed upon dogs one might postulate similar findings in rats. If such were the case the chronic effects of elevated glucose excretion would account at least partially for the elevation in urinary ascorbic acid in diabetic rats. It would not necessarily account for all of the threefold increase observed in diabetic rats. Selkurt and and Houck demonstrated somewhat similar findings upon ascorbic acid resorption utilizing intravenous infusions of hypertonic sodium and

potassium chloride solutions[51]. Again, the experiment utilized dogs and not rats. These two salt solutions produced a significant depression in ascorbic acid resorptive Tm. Production of an osmotic diuresis by a substance not itself resorbed (mannitol) did not influence the resorption of ascorbic acid. The nature of the resorptive block utilizing the salt solutions is not understood.

It is evident that at least four substances do have some blocking effect upon ascorbic acid resorption. However, since diabetic dogs have been shown to have lowered blood ascorbic acid levels as compared to normal when on exogenous insulin control and elevated blood ascorbic acid levels when not given insulin, one might conclude that sustained elevation of blood glucose does stimulate the formation of increased quantities of ascorbic acid. Both decreased renal resorption and increased biosynthesis of ascorbic acid from elevated blood and urinary glucose levels probably account for the increased urine ascorbic acid in diabetic rats.

That insulin is not necessary for the production of ascorbic acid in the rat seems relatively certain. As a corollary to this, it is fairly certain that the hexokinase reaction is not necessary for the production of ascorbic acid. It may be further concluded that insulin does influence ascorbic acid production indirectly through its regulatory effect on blood glucose levels.

Despite the objections to the use of impure phloridzin in this experiment, it is logical to conclude that phloridzin seems to indirectly stimulate the formation of ascorbic acid. This is true despite a possible slight lowering of blood glucose levels that would

tend to depress ascorbic acid synthesis. The basis for this statement lies in the fact that phloridzin very definitely blocks renal tubular resorption of ascorbic acid. With the failure to resorb ascorbic acid, the rat is stimulated to synthesize vitamin C to replenish falling reserves. The reasoning is consonant with the principle of homeostasis. It is interesting to speculate that phloridzin promotes renal ascorbic acid wastage by a mechanism similar to that causing glucose wastage. In utilizing the impure phloridzin in these experiments, one is struck by the marked rise in urinary ascorbic acid. This rise is comparable to the rise stimulated by chlorotone. Though no determinations were made on blood ascorbic acid it seems very possible that the impure phloridzin might have acted as a direct stimulant of biosynthesis as well as a tubular blocking agent. Studies of the action of phloridzin on blood ascorbic acid levels would be of interest.

SUMMARY

1. Insulin does not exert a direct effect upon ascorbic acid biosynthesis in the rat.

2. The elevation of blood glucose, produced by an insulin insufficiency, stimulates ascorbic acid production. This is believed to be the result of mass action.

3. Insulin does exert an indirect effect upon ascorbic acid synthesis by controlling blood glucose levels.

4. Part of the increased urinary ascorbic acid levels can be explained by glucose ascorbic acid competition in renal tubular resorption.

5. Phloridzin produces a marked ascorbic aciduria. This appears to be largely because of blocking renal tubular resorption.

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