THE METABOLISM OF THE SUGAR ALCOHOLS AND THEIR ANHYDRIDES

By

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ACKNOWLEDGEMENT

It is the desire of the author to recognize here the assistance of his colleagues in the Department of Pharmacology in these experiments and especially the guiding influence of Dr. John C. Krantz, Jr. in directing the research to a successful consummation.

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L I S T O F T A B L E S

THE METABOLISM OF THE SUGAR ALCOHOLS

Introduction - Historically it has been assumed that all metabolizable monosaccharides are of equal value in serving as precursors of the active intermediary of carbohydrate metabolism. In recent years investigation has demonstrated that not all the simple sugars have the same physiological behavior. Not only has it been shown that enamtiomorphously related sugars have not the same fate in the body but also many structurally different sugar compounds have been found to behave as physiological entities. A study of the metabolism of the rare sugars is developing as a new field of exploration for biochemical and pharmacological study with the synthetic production in large quantities of many rare sugars.

The investigational study herein reported was undertaken on a group of compounds intimately related to the sugars with the expectation that some useful generalization may be drawn from their related chemical, physical and physiological properties. A study of the metabolism in the animal body of certain sugar alcohols and their anhydrides, and a review of the knowledge of the metabolism of previously studied, related compounds is included in this discussion.

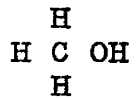
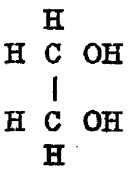
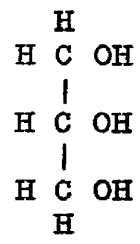
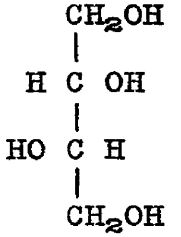
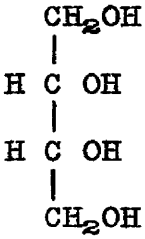
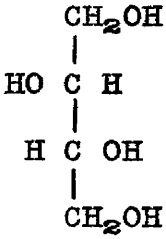
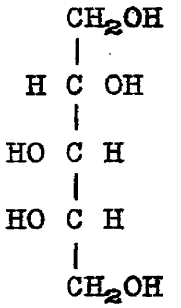
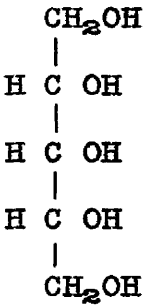
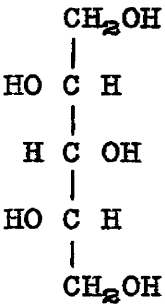
Classification of the Sugar Alcohols - The carbohydrate alcohols or sugar alcohols may be classified as a chemical group representing reduction products of sugar ketoses or aldoses. Since it is generally agreed that two hydroxyl groups cannot ordinarily exist in combination with a single carbon atom the sugar alcohols may be defined as compounds of carbon, hydrogen and oxygen containing an OH group for every carbon atom in the molecule. Boeseken (1923) suggests the name saccharol for this class of substances. The compounds comprising this series with their isomers

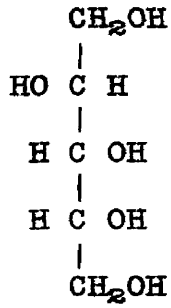
are given in Table I.

Physiological Importance - The saccharols as a class have never been systematically studied in regard to their metabolism notwithstanding their intimate relationship to the extensively studied aldoses and ketoses. Not any of these sugar alcohols, with the exception of inositol and glycerin,occurs as normal physiological entities in the metabolism of the vertebrates. In this connection it is interesting to point out the wide distribution of the saccharols in plants and the ease with which they are attacked by various bacteria and moulds. Ruhland and Wolf (1934) in a recent review state that the sugar alcohols have become known as typical metabolic products of certain plants, particularly the lower plants, Phaeophyceae. Another example of the remarkable distribution of these isomeric substances has been pointed out by Armstrong (1934). The isomeric form of inositol, called scyllitol, has been discovered in such widely distributed forms of life as the spear dog-fish, the cocoanut palm and the oak. The polyhydroxy alcohols have been studied as sources of carbohydrate for the bee, the rhizobium bacteria of legumes, and the blow-fly. This latter organism possesses the rather remarkable ability of utilizing sorbitol for food better than glucose or cane sugar. As a carbohydrate for man quebrachitol and sorbitol have been recommended as sugar substitutes in diabetes.

The physiological importance of certain of the anhydrides of the sugar alcohols to the plant is demonstrated by the discovery of such compounds as styracitol and polygalitol. The details of our knowledge of this class of substances will be considered in a later section.

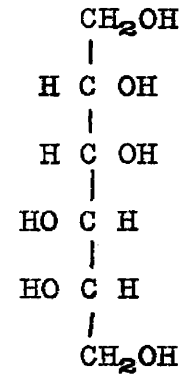
TABLE I

			Optically inactive No asymmetric C atom Liquids
Methyl alcohol	Ethylene glycol	Glycerol	
 M.P. 88 $[a]_D$ -4.4 l-Threitol	 M.P. 120 inactive meso Erythritol	 M.P. 89 $[a]_D$ +4.4 d-Threitol	Tetrahydroxy Alcohols (3) Two meso varieties inactive and identical d-l Erythritol M.P. 72 inactive
 M.P. 102 $[a]_D$ -5.4 l-Arabitol	 M.P. 102 inactive Adonitol	 inactive Xylitol	Pentahydroxy Alcohols (4) d-l Arabitol M.P. 105 inactive

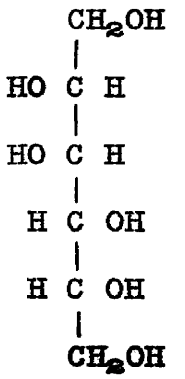


M.P. 102
$[a]_D$ +6.5
d-Arabitol

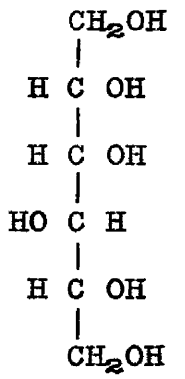
TABLE I (Continued)



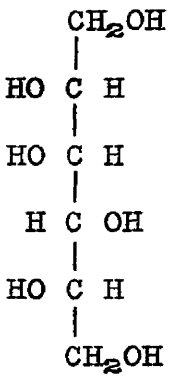
M.P. 163
$[a]_D$ -20 B
l-Mannitol



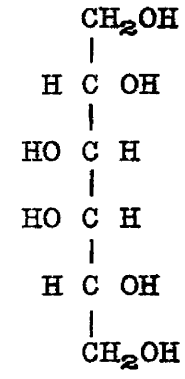
M.P. 166
$[a]_D$ +20 B
d-Mannitol



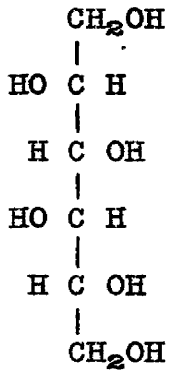
M.P. 89-93
$[a]_D$ -1.75
d-Sorbitol



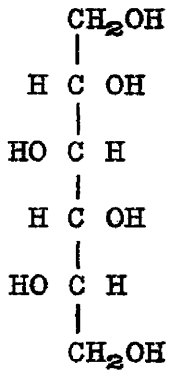
M.P. 77
$[a]_D$ -1.4 B
l-Sorbitol



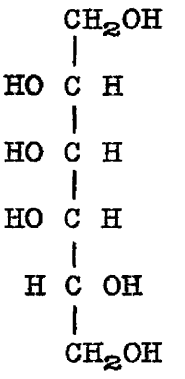
M.P. 188
inactive
Dulcitol



M.P. 73.5
$[a]_D$ -3.5
d-Iditol
d-Sorbierite



M.P. 73.5
$[a]_D$ +3.5
l-Iditol



M.P. 86
$[a]_D$ +3.1
d-Talitol

d-l Mannitol (α-Acritol) M.P. 168 inactive

d-l Talitol M.P. 67 inactive

l-Talitol unknown

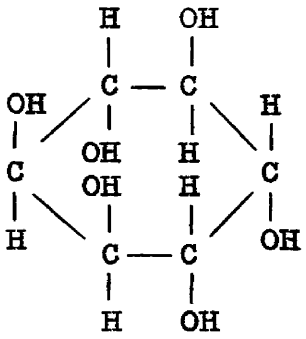
d and l Allitol unknown

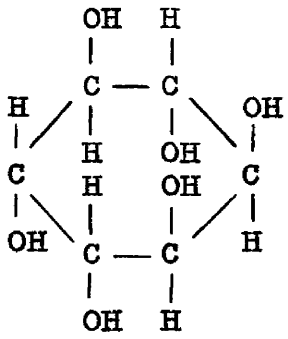
Hexahydroxy Alcohols (10)

B= In Borax solution

TABLE I (Continued)

Inositols (9)



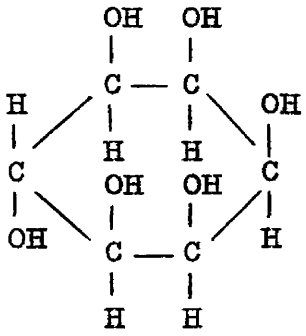


d and l Inositol

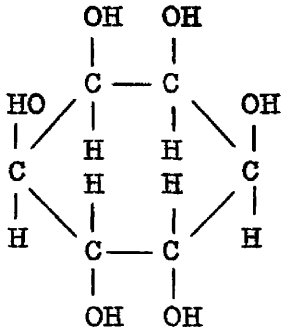
d- M.P. .248 C. $[\alpha]_D$ +68°

l- M.P. .247 C. $[\alpha]_D$ -65°

Note - Absolute configuration of enentiomorphs is not known.



M.P. 225
inactive
meso Inositol

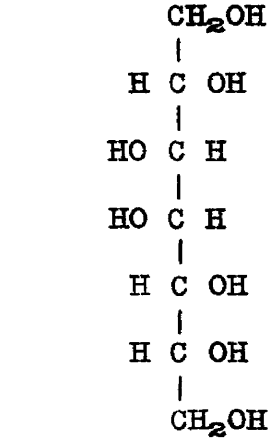


M.P. 350
inactive
Scyllitol

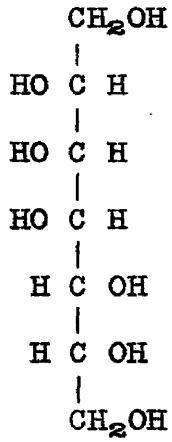
d-l Inositol M.P. 253 inactive.

TABLE I (Continued)

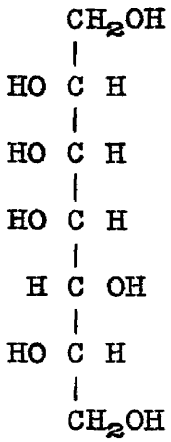
Heptahydroxy Alcohols (16)



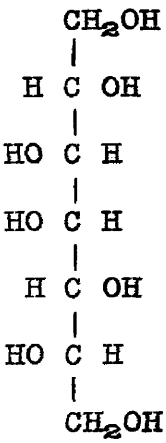
M.P. 188
$[\alpha]_D$+4.8 B*
Persitol
α -d-Mannoheptitol



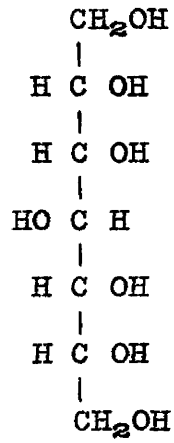
M.P. 153 (217)
$[\alpha]_D$+2.27
Volemitol
β -d-Mannoheptitol
α -Sedoheptitol
Primulitol



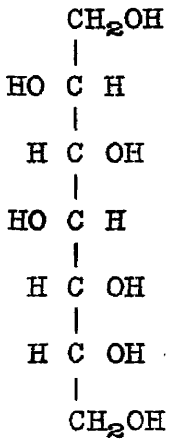
M.P. 128
inactive
β -Sedoheptitol



M.P. 141
Perseulitol
α -l-guloheptitol
β -l-galaheptitol



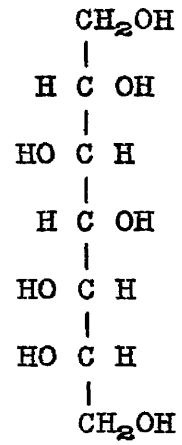
M.P. 128
inactive
α -d-Glucoheptitol



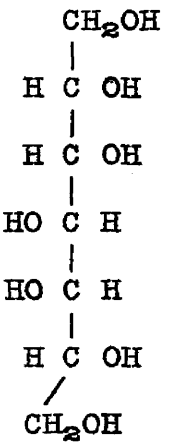
M.P. 131
$[\alpha]_D$ +0.8
β -d-Glucoheptitol

*B= In Borax solution

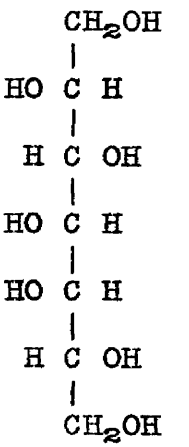
TABLE I (Continued)



M.P. 144
$[\alpha]_D$-2.24
α-Glucoheptulitol

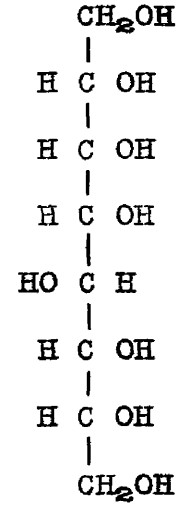


M.P. 188
$[\alpha]_D$-4.35 B
α-d-Galaheptitol
α-l-Mannoheptitol

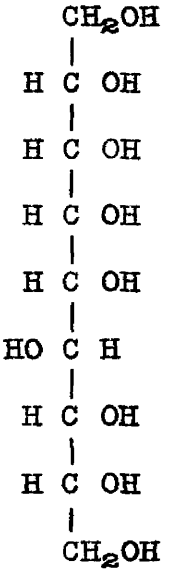


M.P. 144
Rotation unknown
β-d-Galaheptitol

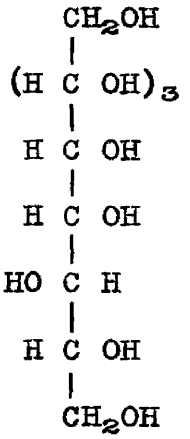
d-l Mannoheptitol M.P. 203 inactive



M.P. 158
$[\alpha]_D$+1.9°
d-gluco-$\alpha\alpha$octitol



M.P. 198
$[\alpha]_D$+3.6° B
d-gluco-$\alpha\alpha\alpha$Nonitol



M.P. 222
$[\alpha]_D$+1.2°
d-gluco-$\alpha\alpha\alpha\alpha$Decitol

d-Manno-Octitol M.P. 258

B= In Borax solution

THE METABOLISM OF METHYL ALCOHOL

Methyl alcohol, chemically the simplest member of the series, cannot be said to possess any of the characteristic properties of the sugar alcohols. For the sake of completeness and because of its interesting metabolism this compound has been included in the survey of the literature of this subject.

While slightly less poisonous than ethyl alcohol in large doses, methyl alcohol is not utilized in the body as a food (Hunt 1907)(Hufferd 1932). Oral administration is followed by serious gastric irritation and blindness owing to a specific action on the second cranial nerve (Buller and Wood 1904). Methyl alcohol is oxidized very slowly to formic acid, most of which is excreted in the urine (Pohl 1899, 1920). This alcohol is not converted into glucose in the body and is incapable of causing a deposition of glycogen in the liver.

THE METABOLISM OF ETHYLENE GLYCOL

Dakin in 1907 in his studies on oxidation in the animal organism reported ethylene glycol as probably having the following fate in the body:

$$\begin{array}{l} CH_2OH \\ | \\ CH_2OH \end{array} \rightarrow \begin{array}{l} CHO \\ | \\ CH_2OH \end{array} \rightarrow \begin{array}{l} COOH \\ | \\ CH_2OH \end{array} \rightarrow \begin{array}{l} COOH \\ | \\ C=(OH)_2 \\ H \end{array} \rightarrow \begin{array}{l} COOH \\ | \\ C=O \\ H \end{array} \rightarrow \begin{array}{l} COOH \\ | \\ COOH \end{array} \rightarrow 2CO_2 + H_2O$$

Dakin was unable to demonstrate oxalic acid excretion in his animals and assumed complete combustion. Later work challenged the view of this investigator.

Von Oettingen and Jirouch,(1931) reporting on the pharmacology of ethylene glycol and related compounds, state that given orally in large doses this material produces severe gastric irritation. The M.F.D. reported was 2.5 cc. per Kg. of mouse subcutaneously while Page and Cryllos (1926) administered 9 cc. per Kg. to dogs without apparent harm and Page took orally himself 15cc. without harmful results. Hanzlik (1931) reports continued drinking of glycol solutions by rats during one third of their life span caused stunting of growth and pathological renal changes. The daily dose was about 0.7 Gm. per Kg. Animals receiving daily doses of 2.2 Gm. per Kg. showed oxaluria and renal calculi variably. He concludes, "Used in doses coming within the range of therapeutic possibilities (as a pharmaceutical solvent) ethylene glycol is comparatively innocuous." Macht and Ting (1922) found 0.120 Gm. per 100 Gm. of rat injected intraperitoneally produced a narcotic effect when measured on the behavior of rats in the circular maize. Hunt (1932) points out that ethylene glycol, while perhaps suitable as a pharmaceutical solvent cannot be considered as a foodstuff since he finds this substance to be approximately three times as toxic as methanol. These conflicting data do not permit an interpretation of whether or not glycol is metabolized as a carbohydrate food.

The most complete quantitative study on the metabolism of ethylene glycol was made by Shapiro (1935) working in Deuel's laboratory. This worker compared the glycogenic and ketolytic action of various carbohydrate substances in the rat. The results are given in Table II.

TABLE II

GLYCOGEN FORMATION FROM GLUCOSE, ETHYLENE GLYCOL AND GLYCEROL

	6 hours after 1 mgm. glucose per sq. cm. or equivalent		4 hours after 3 mgm. glucose per sq. cm. or equivalent	
12 experiments for each substance	Mean per cent. Liver Glycogen	Muscle Glycogen	Mean per cent. Liver Glycogen	Muscle Glycogen
Control	0.17	0.26	0.17	0.26
Glucose	0.63	0.38	1.88	0.37
Ethylene glycol	0.22	0.26	0.27	0.29
Glycerol			1.91	0.35

Av. of 4 experiments each, 3,4,6 hours after administration of ethylene glycol isodynamically equivalent to 1 mgm. of glucose. (Shapiro)

Ethylene glycol did not show any appreciable glycogen storage, neither did it decrease the ketonuria produced in rats by the administration of diacetic acid. Shapiro points out the striking similarity in the metabolism of ethyl alcohol and ethylene glycol. Unfortunately, respiratory studies along with determinations of actual amounts metabolized were not carried out.

Glycol is not converted to sugar in dogs made totally "diabetic" with phlorhizin (Page 1927). Külz found no glycogen storage in hens after feeding glycol in his early work (1905).

It is apparent that the animal body is incapable of converting this two carbon atom sugar alcohol to glucose. There is some indication that

ethylene glycol is itself burned for energy but is incapable of appreciable glycogen storage or utilization for growth and development.

The addition of a $H-\overset{H}{\overset{|}{C}}-OH$ group to methyl alcohol destroys the acute oral toxicity of this compound and converts it into a partially utilizable carbohydrate substance. With this compound sweet taste first appears in this series. The relative sweetness of the sugar alcohols as compared with pure crystallin glucose has been studied in this laboratory and is shown in Table III (Carr 1936).

THE METABOLISM OF GLYCEROL

In the test tube glycerol is completely oxidized to dihydroxy acetone and glyceric aldehyde by hydrogen peroxide, and Dakin (1922) has stated that this oxidation closely resembles biological oxidation. When administered to the phloridzinized dog it is excreted as glucose (Lusk 1928), (Chambers 1925), (Luthje 1904). Cremer showed that when glycerol was taken by the diabetic there resulted an increase in urine sugar (1902). Noble and Macleod (1923) have reported glycerol as valueless in the relief of insulin hypoglycemia while Voegtlin et al. (1924-1925) demonstrated both a curative and preventive action when the alcohol was administered in twenty per cent. solution either intraperitoneally or intravenously. The relief of insulin shock by glycerol is in conformity with the hyperglycemia noted in rabbits by these same workers (1925 A) after oral or intraperitoneal introduction. This last effect has also been noted by Ferber and Rabinowitch (1929).

In Shaffer's <u>in vitro</u> ketolytic reaction with aceto-acetic acid and hydrogen peroxide in alkaline medium, glycerol permits the complete oxida-

TABLE III

RELATIVE SWEETNESS OF THE SUGAR ALCOHOLS AND THEIR ANHYDRIDES
SUCROSE TAKEN AS 100

Product	Carbon Atoms Number	OH Groups Number	Mol. Wt.	Ratio
Sucrose				100
Ethylene glycol	2	2	62	130
Glycerol	3	3	92	108
i-Erythritol	4	4	122	238
Pentaerythritol	5	4	136	110
l-Arabitol	5	5	152	100
d-Mannitol	6	6	182	57
d,l-Sorbitol	6	6	182	54
i-Dulcitol	6	6	182	74
Inositol	6	6	180	50
Ethylene Oxide	2	0	44	+
Epihydrin Alcohol	3	1	74	+
Mannitan	6	4	164	+
Mannide	6	2	146	+
Isomannide	6	2	146	+
Dulcitan	6	4	164	+
Erythritan	4	2	104	50
Polygalitol	6	4	164	75

+ Compounds possessing a bitter taste or tasteless.

tion of the acid as does glucose or fructose (1921).

In Carlson's laboratory (Johnson 1933) glycerol was shown to be non-toxic when consumed in large quantities as a food. Recently Catron and Lewis (1929) using the Cori technique have shown that glycerol is capable of forming glycogen in the liver of the rat (3 per cent.) when administered by stomach tube. Table II shows similar results obtained by Shapiro (1.4 per cent.). In addition this author reports glycerol as being ketolytic in rats fed diacetic acid.

In a study of the relative nutritive value of certain of the sugar alcohols Ariyama and Takahashi found glycerol to be of high nutritive value as measured by the growth rate of rats. (1929). Kulz (1905) also had noticed some storage of glycogen by glycerol in his studies.

The addition of a $H-\overset{H}{\underset{|}{C}}-OH$ group to ethylene glycol converts this compound into a utilizable carbohydrate that the body is able to convert into glucose and glycogen. With this three carbon member of the series practically all toxicity is lost and the compound resembles more closely a true sugar both physically and physiologically. At once the interesting question arises, will the four carbon member be still more readily utilized or will six carbon atoms be necessary for complete utilization?

THE METABOLISM OF ERYTHRITOL

The literature on the metabolism of erythritol is very limited. Bacteria with few exceptions do not metabolize erythritol. The sorbose bacteria, which attack secondary alcohol groups, change the alcohol to a reducing sugar. The earliest reported work on the metabolism of erythritol in higher forms of life is that of Kulz (1905) who found this substance incapable of storage as glycogen in the livers or tissues of hens.

Von mering (1877) in three experiments on rabbits was unable to demonstrate any glycogen in the livers of these animals after the administration of erythritol over a one to twelve hour period, although he was able to show the presence of erythritol in the urine of his animals.

In a complete analytical study of the metabolism of erythritol carried out in this laboratory by Beck (1936) erythritol was not utilized by mice, rats or rabbits. In a series of 41 experiments where erythritol comprised one-third of the diet of rats,the average liver glycogen content was 0.20 per cent. with a minimal value of 0.05 per cent. and a maximum of 0.70 per cent. Control animals varied from 0.09 per cent to 0.15 per cent. with a mean of 0.12 per cent. Tissue glycogen was not influenced. In 33 experiments on the respiratory metabolism of the rat,using the apparatus as described under the section on 'Methods', erythritol by stomach tube in 4 cc. quantities of 10 and 50 per cent. concentrations respectively gave an average R.Q. of 0.734 (low 0.690, high 0.815). Toxicity experiments carried out in connection with this work and reported elsewhere are of considerable pharmacologic interest. Orally amounts as high as 1.8 grams per 100 grams of rat showed no toxic effects. Intraperitoneal injection into mice in doses of 0.8 to 0.9 gram per 100 grams of animal produced a definite hypersensitivity, changing into convulsions and ending in death in two to three hours. The value of 0.8 gram per 100 grams was established as the M.L.D. Macht (1922) reports narcotic depression in rats with quantities of 0.290 gram per 100 grams of rat as measured by their behavior in a circular maze.

The addition of a $H\text{-}\overset{H}{\overset{|}{C}}\text{-}OH$ group to glycerin converts this compound into an alcohol which is not metabolized in the body and there is a partial

return of toxicity that was lost in the series between ethylene glycol and glycerin. Apparently the animal body is incapable of converting a four carbon atom sugar alcohol to a triose or synthesizing to a hexose, although the metabolism of erythrose is unknown.

THE METABOLISM OF THE PENTAHYDROXY SUGAR ALCOHOLS

The five carbon atom sugar alcohols have never been studied as physiological carbohydrates possibly owing to the fact that all these compounds are extremely difficult to obtain in large quantities necessary for metabolic studies. Their importance to the animal body is still a completely unexplored problem.

THE METABOLISM OF MANNITOL

The sugar alcohol, mannitol comprises approximately 75 per cent. of Manna obtained from Fraxinus Ornus official in the U.S.P. X. In 1883 Jaffe observed that mannitol when fed to dogs was recovered unchanged in large quantities from their urine. Rosenfeld (1900) was unable to observe any significant increase in liver glycogen after feeding mannitol to dogs. Kulz found no storage in the liver of hens. The accuracy of this early work is questionable as Pflüger has pointed out (1905). Field studied the effect of the ingestion of 100 grams of mannitol on the blood sugar level of five normal, colored males and observed an average rise of 10 mg. per 100 cc. of blood (1919). This work may be questionable, for glucose under similar conditions gave only an average rise of 40 mg. per 100 cc. of blood. No mention is made of the blood-sugar method used nor was the effect of administration of an equal volume of water over the same time period with the same cases studied. The experimental error was

not determined. Mannitol failed to relieve the hypoglycemia of insulin shock in rats as shown by Voegtlin et al. (1925). Ariyama found mannitol to be far inferior to glycerol or ethyl alcohol as a carbohydrate in the diet of rats (1929). Lecoq (1934) reports, however, that mannitol was utilized by pigeons when this alcohol comprised 35 per cent. of their diet. Silberman and Lewis (1933) in fourteen experiments were unable to demonstrate any glycogen storage in rats' livers after administration of 2 to 4 cc. of a 15 per cent. mannitol solution by stomach tube, allowing from 2 to 6 hours for absorption. The fasting values observed for nine control animals were an average of 0.05 per cent (low 0.02, high 0.06). A serious doubt may be raised regarding the values obtained by these workers. The method of glycogen determination used involved the use of chloroform in the terminal procedure in spite of the repeated observations of numerous investigators showing the mobilization of hepatic glycogen by chloroform. In addition, no determinations were made on the animals demonstrating absorption of the mannitol in the time periods used.

The apparent resistance of mannitol to oxidation in the body is extraordinary when contrasted with the behavior of sorbitol which readily undergoes oxidation. In an attempt to clarify our understanding of the metabolism of mannitol the following experiments were carried out.

1. The capacity of this substance to form glycogen in the liver and tissues of white rats has been reinvestigated.
2. The ability to increase the oxygen consumption and raise the R.Q. of the fasting rat.
3. The relief of hypoglycemia in mice.
4. The influence on blood sugar of rabbits.

5. The acute toxicity of mannitol has been thoroughly studied. A discussion of the methods follows.

METHODS OF STUDY

1. Glycogen Storage in the Livers and Tissues of White Rats. Essentially the well developed method of the Coris (1928) was used in these experiments. This type of experiment which assumes that if a sugar substance is capable of storing glycogen in the liver the sugar will follow the normal metabolism of glucose, is finding wide adoption in many laboratories.

Male white rats weighing between 125 and 150 grams were fasted over a period of forty-eight hours and then placed in small individual cages. Water was allowed at all times. The control rats were given a weighed liberal supply of cacao butter and allowed to continue on this diet for a period of eighty-four hours. The experimental rats were fed mixtures of one-third sugar alcohol with two-thirds cacao butter. After the feeding period the amount of the food ingested was determined. In some experiments the rats were killed by exanguination, im others they were anesthetized with 0.5 cc. of 10 per cent. sodium amytal intraperitoneally. The livers were immediately removed and the glycogen estimated by Pfluger's method as described by Cole (1926). This method involves the dissolving of the accurately weighed liver in 60 per cent. KOH and the precipitation of the glycogen with alcohol in the cold. The glycogen is collected on a filter, dissolved in hot water and hydrolyzed with 5 per cent HCl for one hour on a boiling water bath. In later experiments the more recently developed modification of Pfluger's method by Good (1933) was employed. This method has been used

successfully in this laboratory by Reindollar (1935). By precipitation of the glycogen from 30 per cent. KOH in the hot with alcohol and subsequent centrifugilization and hydrolysis the time period is shortened.

The glucose formed by hydrolysis has been estimated by the Munson, Walker method (1906) if present in large quantities (20 mgm. or above) or by the Shaffer and Hartmann (1920-21) modification of the Folin-Wu method or the idometric-copper method of Shaffer and Somogyi (1933).

Table IV gives an illustration of the accuracy that can be obtained by using these methods.

Tissue glycogen determinations were carried out in the following manner. The carcass was rapidly weighed and cut into ten fragments by means of a cleaver. Each portion was transferred immediately to 100 cc. of a 60 per cent. KOH solution heated to 100°C. The entire operation consumed not longer than 3 minutes. The method of the Coris involving freezing of the carcass in CO_2 snow was not found suitable, in fact, these workers have recently shown that freezing apparently produces a measurable breakdown in muscle glycogen (Steiner 1935). After a heating period of 3 hours, the bones were removed by straining and the solution was made up to 250 cc. The glycogen was determined on a 50 cc. aliquot by the methods previously described.

After the liver had been removed from the animal the alimentary canal was removed from the posterior portion of the esophagus to the anus. This part of the animal was combined with the feces in order to determine the exact amount of food which remained unabsorbed. The urine of certain animals was collected for analysis of excreted sugar alcohol or anhydride.

TABLE IV

ACCURACY OF GLYCOGEN DETERMINATIONS

Analysis of Eight Rats' Livers Pooled. Aliquots Determined by Pflüger's Method Using Munson-Walker Method for Glucose.

No.	Glycogen Per cent.	Deviation from Mean Per cent.
1.	0.250	+ 0.004
2.	0.254	+ 0.008
3.	0.245	- 0.001
4.	0.244	- 0.002
5.	0.242	- 0.004
6.	0.244	- 0.002
7.	0.248	+ 0.002
8.	0.239	- 0.007
Mean	0.246	

Analysis of Five Rats' Livers Pooled. Aliquots Determined by Good's Method Using Shaffer and Somogyi Method for Glucose.

No.	Glycogen Per cent.	Deviation from Mean Per cent.
1.	0.032	- 0.001
2.	0.038	+ 0.005
3.	0.030	- 0.003
4.	0.032	- 0.001
5.	0.032	- 0.001
Mean	0.033	

2. Oxygen Consumption and Respiratory Quotient. In an effort to establish the fate of these compounds in the body a series of experiments was conducted to measure the gaseous exchange of the rat after administration of each substance by stomach tube. During the course of this investigation and certain related studies, to date over 600 respiratory quotients have been determined (Krantz 1935-1936)(Carr 1934-1937). Similar studies have been conducted by Benedict and Macleod (1928), Wesson (1930), Cori and Cori (1926) and Sherwood (1936).

With certain modifications Haldane's (1892) open circuit apparatus was employed to measure the respiratory quotient. The animals used in these experiments were fed a balanced ration and were kept in a room of constant temperature of 28°C. in the dark. They were fasted forty-eight hours prior to the experiment. The animal was placed in the metabolism chamber and air was blown at the rate of two liters a minute through two scrubbing bottles of moist soda lime No. 4 mesh and one bottle of calcium chloride, and then through pumice, which had been thrust into concentrated sulfuric acid at bright red heat according to the Haldane technique. This air passed over the animal for twenty or thirty minutes. After this period the metabolism chamber was closed, the air within having been adjusted to atmospheric pressure. The metabolism chamber and animal were weighed on a balance with a load capacity of 2,000 grams and a sensitivity of 1 mgm. Third decimal place weighings were employed. The metabolism chamber was then inserted in the air line. The exit tube was connected with a series of absorption bottles containing sulfuric acid and pumice for water absorption, and soda lime for absorption of carbon dioxide. Check absorption bottles for water and carbon dioxide were kept in the chain and weighed

with each determination. The intake air was checked by blank runs before each series of determinations. In this set-up the efficiency of the water absorption apparatus is perfect. The soda lime requires moistening or replenishing after every ten or twelve experiments. Five hundred cc. Woulff bottles were employed and later 500 cc. pyrex flasks with ground glass joints and stoppers were used. All permanent connections were sealed with sealing wax.

No correction was made for the nitrogen content of the urine of the animals. This was deemed unnecessary for the purpose of the experiment, as Cori and Cori (1926) have shown that the metabolism of a fasting rat is 90 per cent. fat oxidation. Most of the experiments were conducted between 10 A.M. and 4 P.M., according to the suggestion of Horst et al. (1934). Occasionally an animal selected for an experiment was found to have an exceptionally high or low respiratory quotient; such animals were discarded. The values obtained in this study are for apparently normal rats under very carefully controlled conditions.

Accuracy of the Determination. A series of determinations of oxygen consumption, heat production, respiratory quotients of 92 fasting white rats have been reported elsewhere. An examination of the frequency distribution of the respiratory quotients of the rats of this series shows the median value practically identical with the mean of 0.725. The modal series falls within 0.720 and 0.729. Oxygen consumption, on the other hand seems to fluctuate within very wide limits. Twenty-two per cent. of the determinations fell outside of the range 150 to 250 mgm. O_2 per 100 Gm. of animal per hour, with a mean of 185. In later experiments the extreme variations in oxygen consumption have been eliminated by more careful control of temper-

ature and body weight of the animals and an average value established with the standard deviation. The results are given in Table V.

Administration of Glucose. As a control series glucose was administered to fasting rats in the following manner. The fasting metabolism of the animal was determined over a two and one half hour period. The animal was taken from the metabolism chamber, 4 cc. of a 60 per cent. glucose solution warmed to 37°C. was administered by stomach tube and the rat immediately replaced in the apparatus for a second two and one half hours measurement. The results of these experiments are outlined in Tables VI and VII along with blank determinations using water in place of sugar solution.

Having mastered the technique of the experimental method and having established the reliability of the apparatus in the measurement of the gaseous exchange for fasting and carbohydrate fed rats this method was used in a study of the metabolism of the sugar alcohols and their anhydrides.

3. Ability of Sugar Alcohols to Relieve Insulin Shock. In an effort to understand the physiological effect of these alcohols their influence on insulin hypoglycemia was studied. Mice were used in these experiments. Five units of insulin per 100 Gm. of mouse were injected intraperitoneally according to the standard method. The animals were kept in an incubator at 30°C. At the beginning of the convulsive period the solution of sugar substance was injected intraperitoneally. Recovery, partial recovery or death of the animal was recorded within ten minutes, one hour and twelve hours. Animals receiving glucose recover within five to ten minutes. Suitable control experiments were carried out in all cases.

TABLE V

METABOLISM OF NORMAL MALE RATS, FASTING 48 HOURS

SERIES A, 45 ANIMALS

Rat No.	Wt. Gm.	Gm. O_2 per 100 Gm. per hr.	R.Q.	Calories per sq.M. per 24 hrs.
1	117	0.216	0.720	1198
2	120	0.197	0.711	1111
3	121	0.204	0.718	1162
4	125	0.230	0.715	1341
5	110	0.165	0.733	892
6	151	0.224	0.717	1077
7	139	0.155	0.735	921
8	118	0.191	0.704	1069
9	140	0.199	0.725	1190
10	127	0.204	0.733	1218
11	149	0.178	0.695	1043
12	121	0.169	0.721	967
13	126	0.159	0.712	942
14	149	0.172	0.704	1023
15	146	0.218	0.700	1281
16	138	0.163	0.721	974
17	135	0.186	0.708	1072
18	133	0.168	0.727	966
19	163	0.162	0.733	996
20	130	0.174	0.712	988
21	131	0.162	0.710	916
22	143	0.208	0.709	1222
23	130	0.181	0.726	1030
24	143	0.207	0.718	1222
25	128	0.185	0.713	1039
26	133	0.193	0.716	1101
27	148	0.196	0.735	1171
28	133	0.218	0.728	1254
29	128	0.188	0.696	1053
30	133	0.203	0.725	1156
31	119	0.229	0.693	1258
32	134	0.221	0.706	1264
33	114	0.178	0.719	968
34	108	0.233	0.719	1250
35	130	0.194	0.700	1100
36	133	0.196	0.688	1107
37	123	0.193	0.700	1067
38	141	0.159	0.729	934
39	143	0.160	0.706	940
40	122	0.171	0.725	958
41	126	0.198	0.707	1135
42	132	0.223	0.722	1273
43	128	0.172	0.714	977
44	119	0.227	0.705	1270
45	132	0.182	0.692	1038
Mean				1096
Sigma				118

TABLE V (Continued)

METABOLISM OF NORMAL MALE NON-FASTING RATS

SERIES B

Rat No.	Wt. Gm.	Body Temp.	Gm. O_2 per 100 Gm. per hr.	R.Q.	Calories per sq.M. per 24 hrs.
1	156		0.199	0.825	1263
1	158		0.184	0.892	1189
1	153	100.0	0.206	0.843	1301
1	158	101.0	0.194	0.933	1264
1	151	101.8	0.192	0.800	1194
1	152	101.0	0.197	0.885	1253
2	146		0.195	0.882	1229
2	145		0.175	0.830	1142
2	145		0.184	0.874	1096
2	144	98.8	0.180	0.775	1091
2	140	98.3	0.178	0.830	1093
2	142	100.8	0.182	0.878	1135
2	142	101.0	0.177	0.885	1103
2	148	99.3	0.198	1.022	1287
2	167	99.0	0.195	1.072	1318
2	172	100.4	0.205	0.915	1373
2	171	98.8	0.192	0.963	1296
2	178	98.4	0.187	0.855	1243
2	187	101.0	0.166	0.754	1096
3	138		0.206	1.060	1296
4	120		0.181	0.996	1100
4	128		0.210	1.070	1304
5	128		0.189	0.955	1163
5	136		0.198	0.893	1217
6	137		0.179	0.950	1124
6	138		0.161	0.925	1006
6	137		0.187	0.867	1151
6	138	98.8	0.201	0.832	1228
6	135	97.2	0.179	0.880	1096
6	136	98.8	0.176	0.802	1065
6	140	98.4	0.170	0.844	1046
6	144	99.0	0.164	1.000	1060
6	156	97.8	0.197	0.770	1237
6	163	98.5	0.164	0.872	1067
6	160	98.6	0.169	0.880	1096
6	167	98.2	0.201	0.796	1302
6	188	98.6	0.164	0.810	1108
7	159		0.172	0.805	1091
7	160		0.157	0.867	1016
7	154	98.6	0.151	0.974	993
7	164	101.8	0.165	0.905	1063
7	155	98.2	0.158	0.915	1021
7	164	101.2	0.191	1.061	1287
7	184	100.4	0.195	1.010	1362
7	197	99.0	0.183	0.820	1249
7	193	100.0	0.159	0.838	1086

TABLE V (CONTINUED)

METABOLISM OF NORMAL MALE NON-FASTING RATS

SERIES C. Metabolism by Closed Chamber Method

Rat No.	Wt. Gm.	Body Temp.	Gm. O_2 per 100 Gm. per hr.	Calories per sq. M. per 24 hrs.
8	164		0.197	1261
8	168	98.3	0.190	1226
8	172	98.3	0.187	1216
8	176	100.5	0.159	1062
8	183	99.6	0.185	1227
9	155		0.167	1018
9	167	99.5	0.196	1275
9	170	98.5	0.181	1189
9	174	98.3	0.171	1130
10	165	99.4	0.197	1232
10	198	99.4	0.172	1184
10	199	99.0	0.150	1034
10	199	98.7	0.211	1450
10	199	101.0	0.157	1081
10	200	98.7	0.195	1350
10	201	98.6	0.150	1037
10	194	99.1	0.173	1170
10	201	98.9	0.152	1054
11	148		0.172	1076
11	158	98.8	0.210	1342
11	161	101.1	0.182	1168
11	167	98.0	0.173	1123

Mean	=	1169
Sigma	=	104
Number of Determinations	=	68
Number of Animals	=	11

SUMMARY OF TABLE V

GRAND AVERAGE, 113 DETERMINATIONS ON 51 ANIMALS

Body Wt. Gm.	Body Temp. ° F	Gm. O_2 per 100 Gm. per hr.	R.Q.		Calories per sq. M. per 24 hrs.
			Fasting	Non-Fasting	
149	99.3	0.185	0.714	0.894	1132
108-201	97.2-101.8	0.150-0.233	0.688-0.735	0.754-1.072	892-1450

TABLE VI

RESPIRATORY QUOTIENTS DURING FASTING AND AFTER WATER

Rat No.	Wt. Gm.	Gm. CO_2 per 100 Gm. per hr.	Gm. O_2 per 100 Gm. per hr.	Material Administered	R.Q.	Run in hrs.
1	142	0.181	0.175	Fasting	0.749	2.5
		0.191	0.187	4 cc. H_2O	0.740	2.5
2	130	0.168	0.172	Fasting	0.710	2.5
		0.156	0.155	4 cc. H_2O	0.735	2.5
3	155	0.160	0.162	Fasting	0.715	2.5
		0.196	0.204	4 cc. H_2O	0.699	2.5
4	176	0.155	0.154	Fasting	0.730	2.5
		0.171	0.172	4 cc. H_2O	0.720	2.5
5	142	0.159	0.164	Fasting	0.707	2.5
		0.206	0.208	4 cc. H_2O	0.722	2.5
6	166	0.150	0.155	Fasting	0.704	2.5
		0.157	0.159	4 cc. H_2O	0.720	2.5
7	148	0.164	0.164	Fasting	0.726	3.0
		0.218	0.218	4 cc. H_2O	0.726	2.5
8	161	0.137	0.142	Fasting	0.705	2.5
		0.181	0.183	4 cc. H_2O	0.720	2.5
			Average	Fasting	0.718	
			Average	4 cc. H_2O	0.722	

TABLE VII

RESPIRATORY QUOTIENTS DURING FASTING AND AFTER GLUCOSE

Rat No.	Wt. Gm.	Gm. CO_2 per 100 Gm. per hr.	Gm. O_2 per 100 Gm. per hr.	60 per cent Glucose by Stomach Tube	R.Q.	Run in hrs.
1	220	0.137	0.136	Fasting	0.729	2.5
		0.173	0.145	4 cc.	0.854	2.5
2	116	0.217	0.220	Fasting	0.715	1.0
		0.215	0.205	2.5 cc.	0.765	1.0
3	101	0.190	0.194	Fasting	0.713	1.0
		0.224	0.198	2.5 cc.	0.817	2.0
4.	109	0.208	0.212	Fasting	0.712	1.0
		0.214	0.208	1.5 cc.	0.747	2.0
5	158	0.149	0.157	Fasting	0.693	1.0
		0.196	0.177	1.5 cc.	0.804	2.0
6	106	0.200	0.206	Fasting	0.708	2.5
		0.170	0.161	4 cc.	0.768	2.5
7	108	0.230	0.224	Fasting	0.747	2.5
		0.244	0.220	4 cc.	0.805	2.5
8	133	0.198	0.201	Fasting	0.714	2.5
		0.144	0.125	4 cc.	0.839	3.0
	Average	Fasting	0.193	Fasting	0.716	
		Glucose	0.179	Glucose	0.799	
		Difference	0.014	Difference	0.083	

4. Blood Sugar After Sugar Alcohol. Rabbits weighing approximately 2 kilograms were fasted for a period of 48 hours. Their fasting blood-sugar levels were determined by the Shaffer-Hartmann (1920) or the Folin (1928) methods. The sugar alcohol in solution in water was administered by stomach tube and at one half hour, one hour, two hour and three hour periods after administration blood samples were withdrawn from the ear vein. In order to determine the effect of the administration of the stomach tube to the animal in two cases water alone was given. Glucose solution served as a control in most experiments.

THE METABOLISM OF MANNITOL

Materials Employed. The mannitol used in this investigation was of C.P. quality, soluble 16 parts in 100 parts of cold water and very soluble in hot water, odorless and possessing a sweet taste, M.P. 166-167°C., neutral in aqueous solution.

Glycogen Storage After Mannitol Feeding. The results given in Table VIII demonstrate the capacity of mannitol to store glycogen in the livers of rats when fed according to the method described.

Glycogen Storage After Mannitol by Stomach Tube. The results given in Table IX demonstrate the inability of the rat to store glycogen in the livers from mannitol when administered in 15 per cent. solution by stomach tube. These data support the findings of Silberman and Lewis. Analyses of the intestinal contents indicate absorption of most of the mannitol and the amount recovered in the urine was approximately one tenth of the total amount administered.

TABLE VIII

GLYCOGEN STORAGE IN LIVERS OF RATS ON MANNITOL DIET

Control Rats

Rat No.	Wt. of Rats Gm.	Food Ingested Gm.	Livers Gm.	Liver Glycogen Gm.	Liver Glycogen per cent.
1	100 + 122	20.0	9.0	0.005	0.06
2	70 + 75	15.0	5.6	0.005	0.09
3	105	0.5	3.2	0.013	0.41
4	75 + 80	10.0	5.2	0.017	0.32
5	98 + 95	5.5	6.5	0.004	0.07
6	99	4.0	3.3	0.004	0.12
7	107	4.0	3.1	0.005	0.16
8	91	5.0	3.2	0.006	0.19
				Mean	0.16

Mannitol Feeding

1	141 + 105	20.5	8.7	0.155	1.80
2	144 + 98	23.2	8.2	0.083	1.00
3	127 + 113	23.5	8.6	0.135	1.60
4	122 + 127	19.7	8.8	0.055	0.62
5	113 + 102	22.4	8.7	0.138	1.60
6	60 + 70	14.5	5.6	0.038	0.69
7	85 + 70	17.5	6.1	0.046	0.74
8	80 + 70	16.5	5.3	0.064	1.22
9	122 + 116	13.4	6.9	0.122	1.77
10	124	10.0	4.1	0.062	1.52
11	122	10.0	4.3	0.029	0.68
12	129	9.0	5.0	0.026	0.53
13	91	6.5	3.5	0.035	1.00
14	117	10.0	4.1	0.029	0.70
				Mean	0.94

TABLE IX

GLYCOGEN STORAGE IN THE LIVERS OF RATS AFTER MANNITOL BY STOMACH TUBE

Rat No.	Wt. Gm.	Mannitol by Stomach Tube Gm.	Livers Gm.	Liver Glycogen Gm.	Liver Glycogen Per cent.	Mannitol Unabsorbed Gm.
1	125	0.6*	3.92	0.003	0.08	0.031
2	137	0.6	4.17	0.004	0.09	0.041
3	148	0.6	4.34	0.007	0.16	0.058
4	150	0.6	5.29	0.003	0.06	0.052
5	167	0.6	4.44	0.002	0.05	0.033
6	140	0.6	4.23	0.002	0.05	0.063
7	156	0.6	4.24	0.004	0.09	0.054
8	179	0.6	4.86	0.003	0.07	0.035
			Control Animals			
9	146	Water	4.62	0.003	0.07	
10	156	Water	4.88	0.006	0.12	
11	150	Water	4.46	0.002	0.05	
12	142	Water	5.24	0.005	0.10	

*4 cc. 15 per cent solution

0.782 Gm. mannitol recovered from combined excreted urine.

Influence of Mannitol on Respiratory Quotient. Table X sets forth the results of a series of experiments which demonstrate the inability of the white rat to metabolize mannitol directly.

Influence of Mannitol on Blood-Sugar Level of Rabbits. Table XI gives the results of this series of experiments as compared with glucose. An appreciable rise in blood sugar may be noted within one half hour.

Acute Toxicity of Mannitol. Table XII sets forth the results of experiments upon the acute toxicity of mannitol by stomach tube to rats. The M.L.D. of mannitol by stomach tube for white rats is approximately 1.3 grams per 100 grams of body weight.

TABLE X

RESPIRATORY QUOTIENTS DURING FASTING AND AFTER MANNITOL

Rat No.	Wt. Gm.	Gm. CO_2 per 100 Gm. per hr.	Gm. O_2 per 100 Gm. per hr.	25 per cent Mannitol by Stomach Tube	R.Q.	Run in hrs.
1	170	0.254	0.257	Fasting	0.716	2.5
		0.193	0.193	5 cc.	0.727	2.5
2	165	0.196	0.191	Fasting	0.744	2.5
		0.184	0.189	5 cc.	0.705	2.5
3	155	0.226	0.232	Fasting	0.747	2.5
		0.194	0.202	5 cc.	0.755	2.5
4	210	0.194	0.194	Fasting	0.727	2.5
		0.154	0.157	5 cc.	0.713	2.5
5	170	0.216	0.213	Fasting	0.735	2.5
		0.183	0.182	5 cc.	0.731	2.5
671	126	0.176	0.178	Fasting	0.717	3.2
		0.183	0.171	5 cc.	0.778	3.1
672	148	0.154	0.164	Fasting	0.683	2.5
		0.163	0.159	5 cc.	0.740	3.6
673	131	0.167	0.176	Fasting	0.692	2.5
		0.187	0.185	5 cc.	0.734	4.0
674	123	0.150	0.154	Fasting	0.707	2.5
		0.139	0.135	5 cc.	0.751	4.3
675	-	-	-	-	-	-
	123	0.178	0.178	5 cc.	0.730	7.6
676		-	-	-	-	-
	130	0.186	0.186	5 cc.	0.727	7.6
Mean				Fasting	0.719	
				5 cc.	0.735	

TABLE XI

INFLUENCE OF GLUCOSE AND MANNITOL ON BLOOD-SUGAR LEVEL OF RABBITS BY STOMACH TUBE

Rabbit No.	Wt. Kg.	Glucose Gm.	Mgm. per 100 cc. Blood					
			Fasting	1/2 hr.	1 hr.	1.5 hr.	2 hr.	3 hr.
1	2.25	9.0	100	172	265			
2	2.00	8.0	98	149	295	290	279	219
3	2.75	11.0	101	185	202	202	200	177
4	2.95	11.8	90	211	260		250	194
Mean			97	179	255	246	243	197
		Mannitol Gm.						
1	2.25	8.44	122	172	149	146	116	143
6	1.40	5.60	101	146	151	154	146	
7	1.60	6.40	104	175	194	197		
4	3.20	12.80	95	142	127		122	119
Mean			105	159	155	165	129	131

TABLE XII

ACUTE TOXICITY OF MANNITOL

Series A

Rat No.	Wt. of Rat Gm.	Mannitol Gm. per 100 Gm. Rat	Result
1	270	1.0	recovered
2	285	2.0	died after 12 hours
3	295	3.0	died after 5 hours
4	300	3.0	died within an hour

Series B

1	205	1.0	recovered
2	175	1.5	died after 12 hours
3	160	1.7	died after 12 hours
4	210	1.9	died after 12 hours
5	235	2.0	died after 12 hours

Series C

1	195	1.0	recovered
2	200	1.5	died after 15 hours
3	240	1.4	recovered
4	230	1.3	died after 15 hours
5	260	1.2	recovered
6	220	1.1	recovered

THE METABOLISM OF DULCITOL

While mannitol has been the subject of some physiological investigation dulcitol has been practically ignored. Kulz reports no storage in the livers or bodies of hens when fed dulcitol in five experiments. Other physiological experiments were not found in an extensive search of the literature. It seemed of special interest to observe in animal experiments if any changes in metabolism would occur concomitant with changes in the relative position of the hydroxy groups in the isomers mannitol, dulcitol and sorbitol.

Materials Employed. Pfanstiehl's C.P. dulcitol, M.P. 188°C. free from galactose was used throughout this investigation. The compound was white and crystalline, only slightly soluble in cold water or alcohol, extremely soluble in hot water or alcohol.

Glycogen Storage After Dulcitol Feeding. The results of these experiments are set forth in Tables XIII to XV.

Influence of Dulcitol on the Respiratory Quotient. The results of these experiments are given in Table XVI.

Dulcitol and Insulin Shock. Dulcitol in 3 per cent. solution administered to mice in convulsions resulting from insulin injection by the method described previously was ineffective in relieving hypoglycemia. It should be pointed out, however, that owing to the insolubility of this compound it could not be used in the 15 per cent. concentration as employed for glucose.

Acute Toxicity of Dulcitol. Dulcitol administered by stomach tube to rats in quantities of 1.3 grams per 100 gram of rat failed to show toxic properties.

Influence of Dulcitol in Blood-Sugar Level of Rabbits. The results of these experiments are given in Table XVII.

TABLE XIII

GLYCOGEN STORAGE IN LIVERS OF RATS ON DULCITOL DIET

Control Rats

Group No.	No. of Rats	Wt. of Rats Gm.	Food Ingested Gm.*	Livers Gm.	Liver Glycogen Gm.	Liver Glycogen Per cent.
1	3		32.0	14.0	0.025	0.18
2	4		46.0	21.5	0.047	0.22
3	4		49.0	18.4	0.017	0.09
4	4		56.0	13.4	0.004	0.03
5	3		49.0	9.7	0.017	0.17
6	2		12.5	6.9	0.006	0.09
7	2		14.1	8.9	0.032	0.36
8	2		12.4	8.2	0.003	0.03
9	2		10.5	6.8	0.008	0.11
10	2		17.5	8.5	0.010	0.12
11	1		8.2	3.5	0.008	0.24
					Mean	0.16

* Pure cacao butter

Dulcitol Feeding

1	2	128 + 140	4.1	7.5	0.023	0.30
2	2	91 + 153	6.9	7.0	0.029	0.42
3	2	118 + 121	6.4	8.6	0.054	0.63
4	2	115 + 108	7.0	7.6	0.026	0.34
5	2	108 + 140	7.6	8.2	0.042	0.51
6	2	125 + 123	4.7	7.0	0.047	0.68
7	2	133 + 130	5.0	7.2	0.015	0.20
8	1	149	2.0	3.4	0.017	0.50
9	2	144 + 136	5.9	7.8	0.090	1.15
10	2	142 + 135	6.7	6.8	0.011	0.15
11	2	135 + 126	5.9	6.5	0.030	0.46
					Mean	0.49

TABLE XIV

GLYCOGEN STORAGE IN TISSUES OF RATS ON DULCITOL DIET

Control Rats

Group No.	No. of Rats	Tissues Gm.	Liver Glycogen Gm.	Liver Glycogen per cent.
1	1	148	0.161	0.11
2	1	147	0.149	0.10
3	1	142	0.189	0.13
4	1	150	0.247	0.16
5	1	133	0.174	0.13
6	1	130	0.169	0.13
7	1	127	0.218	0.17
8	1	135	0.286	0.21
9	2	161	0.320	0.20
10	2	234	0.258	0.11
11	2	165	0.148	0.09
12	2	171	0.169	0.10
13	2	192	0.288	0.15
			Mean	0.13

Average food consumed- 9 grams cacao butter per rat.

Dulcitol Feeding

1	2	189.5	0.167	0.09
2	2	208.5	0.133	0.06
3	2	146.0	0.118	0.08
4	2	168.6	0.077	0.05
5	2	168.5	0.082	0.05
6	2	174.6	0.131	0.07
7	2	190.1	0.141	0.07
8	1	103.2	0.073	0.07
9	2	175.3	0.201	0.11
10	2	173.2	0.223	0.13
11	2	166.9	0.131	0.08
12	1	97.9	0.058	0.06
13	1	97.3	0.084	0.08
14	1	102.0	0.071	0.07
15	1	94.7	0.059	0.06
16	1	100.7	0.082	0.08
			Mean	0.08

Average food consumed- 7 grams equal to 2.3 grams of Dulcitol per rat.

TABLE XV

GLYCOGEN STORAGE IN THE LIVERS OF RATS AFTER DULCITOL BY STOMACH TUBE

Rat No.	Wt. Gm.	Dulcitol by Stomach Tube Gm.	Livers Gm.	Liver Glycogen Gm.	Liver Glycogen per cent.
1	120	1.25	3.9	0.019	0.37
2	125	1.25	4.1	0.010	0.17
3	145	1.25	4.6	0.014	0.30
4	121	1.25	3.8	0.010	0.26
5	137	1.25	4.7	0.043	0.91
6	119	1.25	4.9	0.042	0.86
7	140	1.25	4.9	0.067	1.35
				Mean	0.60

TABLE XVI

RESPIRATORY QUOTIENTS DURING FASTING AND AFTER DULCITOL

Rat No.	Wt. Gm.	Gm. CO_2 per 100 Gm.	Gm. O_2 per 100 Gm.	Dulcitol by Stomach Tube	R.Q.	Run in hrs.
1	182	0.484	0.482	Fasting	0.728	2.5
		0.428	0.450	1.25 Gm.	0.693	2.5
2	160	0.493	0.513	Fasting	0.697	2.5
		0.523	0.563	1.25 Gm.	0.676	2.5
3	196	0.361	0.401	Fasting	0.718	2.5
		0.435	0.435	1.25 Gm.	0.727	2.5
4	194	0.576	0.558	Fasting	0.751	2.5
		0.400	0.413	1.25 Gm.	0.706	2.5
5	169	0.340	0.323	Fasting	0.765	2.5
		0.554	0.543	1.25 Gm.	0.740	2.5
6	156	0.540	0.556	Fasting	0.706	2.5
		0.490	0.524	1.25 Gm.	0.681	2.5
7	153	0.474	0.459	Fasting	0.748	2.5
		0.556	0.580	1.25 Gm.	0.703	2.5
8	153	0.328	0.354	Fasting	0.676	2.5
		0.364	0.388	1.25 Gm.	0.684	2.5
9	161	0.443	0.439	Fasting	0.734	2.5
		0.702	0.685	1.25 Gm.	0.745	4.0
10	188	0.427	0.430	Fasting	0.723	2.5
		0.805	0.827	1.25 Gm.	0.710	5.0
				Mean Fasting	0.724	
				Mean Dulcitol	0.706	

TABLE XVII

INFLUENCE OF DULCITOL ON BLOOD-SUGAR LEVEL OF RABBITS

Rabbit No.	Dulcitol Gm. per Kg.	Mgm. per 100 cc. Blood Fasting	1/2 hr.	1 hr.	2 hr.	3 hr.
1	4	92	134	132	124	125
2	4	90	86	90	100	104
3	2	95	92	114	119	119
4	2	79	101	87	104	98
5	2	89	75	95	95	104
	Water Gm. per Kg.					
6	10	95	98	90	105	127
7	10	80	104	134	124	124

THE METABOLISM OF SORBITOL

The sugar alcohol, sorbitol, has received more attention as a substitute carbohydrate in the diet of man and experimental animals than any compound in this series. Sorbitol occurs in the free state in nature in mountain ash berries and the fruits and other parts of the plant of the Rosaceae family.

In Europe in recent years a trade product by the name of "Sionin" has been extensively studied. This material is reported to be pure d-sorbitol. (Payne et al. 1933) (Kurez 1931).

Sorbitol has recently been made available as an industrial chemical by reduction of d-glucose. Formerly the expense of this material made experimentation costly but since 1934 it has found a wide variety of commercial uses, principally in the synthetic preparation of vitamin C. Recently sorbitol was reported to be of medicinal value as a diuretic (West et al. 1936).

Drury and Salter (1934) found sorbitol valueless in prolonging the survival time of hepatectomized rabbits, however, many of the compounds studied by these workers admittedly serve as glycogen formers in the intact animal. Salter et al. (1935) report sorbitol as a foodstuff which, in the liver of mice, is capable of being converted into glycogen. Because of the limit imposed by the gastrointestinal tolerance sorbitol could not be fed in amounts as great as the maximum glucose ingestion. In 16 mice sorbitol by stomach tube gave a liver glycogen concentration of 0.44 per cent. After 20 hours glucose in equal concentration gave 0.14 per cent., 48 hour fasting control mice 0.07 per cent. Unfortunately the authors killed their animals by exsanguination and have not determined the average deviation of the in-

dividual experiments.

Lecoq (1934) reports sorbitol to be completely utilized by pigeons when comprising 35 per cent. of their diet and the sole carbohydrate. Kosterlitz (1933) administered d-sorbitol to depancreatized dogs and found significant quantities of glycogen in the livers of these animals. Labbe undertook a study of the metabolism of sorbitol for the Academy of Medicine of Paris in 1932 because of the wide spread use of d-sorbitol by diabetics in France and the divergence of views regarding its value in diabetes. Labbe et al. (1934) present figures to show the utilization of sorbitol by rabbits. The results are summarized in Table XVIII. These workers believe that the inability of sorbitol and mannitol to store glycogen in amounts commensurate with glucose is explained by their non-transfer through the intestinal mucosa. In five diabetics glucose tolerance was compared with sorbitol tolerance and the blood-sugar level determined. These results are summarized also in Table XVIII. Additional studies were undertaken on diabetics receiving a fixed dosage of insulin where sorbitol was used to replace a part of the carbohydrate intake. In these cases good utilization of the sugar alcohol was observed. The authors conclude that a certain amount of sorbitol is relatively better tolerated by the diabetic than glucose. The purgative action so often observed to be produced in animals by all sugar alcohols, imposed a limit upon the amount that could be fed.

Payne et al. (1933) report two cases of juvenile diabetics treated with sionin where a significant rise in blood sugar occurred while the patients were receiving 25 grams per day. Sionin did not relieve the hypoglycemia of increased insulin administration. In rats these work-

TABLE XVIII

INFLUENCE OF SORBITOL ON LIVER AND TISSUE GLYCOGEN OF RABBITS

Material Administered	Liver Glycogen per cent.	Tissue Glycogen per cent
Glucose		
10 Gm. per Kg.	5.43	0.020
6 Gm. per Kg.	3.64	0.029
3 Gm. per Kg.	1.61	0.016
Natural Sorbitol		
3 Gm. per Kg.	0.97	0.016
Synthetic Sorbitol		
6 Gm. per Kg.	2.41	0.045
Mannitol		
3 Gm. per Kg.	0.86	0.031

INFLUENCE OF SORBITOL ON BLOOD AND URINE SUGAR OF DIABETICS

Case No.	Material Administered	Rise in Blood Sugar Above Fasting mgm. per cent.	Glycosuria
1	Glucose	81	+++
	Sorbitol	110	+++
2	Glucose	125	++
	Sorbitol	102	+
3	Glucose	190	+++
	Sorbitol	67	0
4	Glucose	143	+
	Sorbitol	40	0
5	Glucose	135	++
	Sorbitol	85	+

ers found the following average values for liver glycogen after 2 grams of sionin or glucose and 2 cc. of water by mouth: sionin 0.114 per cent.; tap water 0.124 per cent.; glucose 0.980 per cent. They conclude that sorbitol is not a satisfactory carbohydrate for diabetes except as a sweetening agent.

Gottschalk (1929) in reviewing this subject classifies d-sorbitol with dioxyacetone, d-fructose and inulin as substances useful in diabetes of the mild type because of their "insulin enticing" action.

Rayband and Roche (1934) studied the influence of sorbitol on the R.Q. of normal and diabetic patients and failed to observe any rise in quotient. Sorbitol did not give rise to glycogen deposition in animals and in the phloridzinized rabbit it was excreted as glucose. The authors attribute the extra glucose excretion to the abnormality of the animals receiving phloridzin and further point out the inability of sorbitol to relieve insulin convulsions in guinea pigs.

The most complete clinical study carried out on a very large number of diabetic and normal patients is that of Douhoffer (1930). In these patients sorbitol gave rise to an average increase in blood sugar of only 30 mgm. per cent. above normal after 50 grams by mouth. The character of the curve differs in different individuals depending on the absorption of the sorbitol, some cases gave very high blood sugar values. The author permits himself only a statement to the effect that sorbitol may be useful to the diabetic organism. A large number of clinical papers have appeared mostly in France and Germany reporting sorbitol as valuable in the treatment of diabetes, Reinwein (1929); Thannhausee (1929); Bogendorfer (1931); Paolazzi (1931); and finally the master diabetic clinician, Von Noorden (1929) and others.

Fate of Sorbitol in the Animal Body. The paucity of critical data and the divergence of views in the literature regarding the metabolism of sorbitol makes a positive statement impossible. A thorough restudy of the problem is indicated. Judging from the isomeric relationship of sorbitol, dulcitol and mannitol and the data of this study one is inclined to believe that these compounds are partially utilized by the animal body and within the limits of intestinal tolerance follow the same fate in the body as glucose.

THE ANHYDRIDES OF THE SUGAR ALCOHOLS

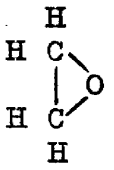
Since the chemical preparation and identification of this group of substances approximately eighty years ago they have lain dormant in scientific literature. Because of the apparent ease of manufacture of most of the anhydrides of the sugar alcohols they were studied in this investigation in the hope that a partially dehydrated compound would oxidize in the animal body. Many of them have been far from easy to prepare and disappointment resulted from studies on their metabolism.

Classification of the Anhydrides of the Sugar Alcohols. The compounds take their names from the parent substance substituting the ending "an" for "ol" in the case of the first anhydrides and "de" for "tol" in the case of the second anhydrides. The anhydride linkage is designated in the usual manner. The compounds comprising this series with their known isomers is given in Table XVIIIa.

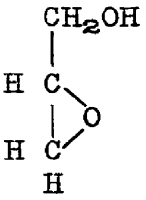
THE METABOLISM OF ERYTHRITAN

The first member of this series that has received any attention from the standpoint of metabolism is erythritan. This compound was com-

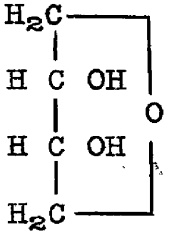
TABLE XVIIIa



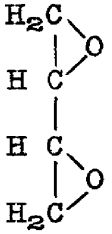
B.P. 10.5°
1-2 Anhydro Glycol
Ethylene Oxide



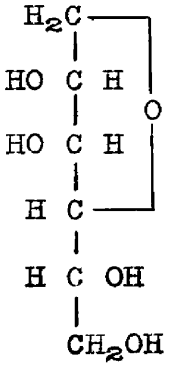
B.P. 70° 10 mm.
1-2 Anhydro glycerol
1- Epihydrin Alcohol
Glycidol
Glycide



B.P. 154° 18 mm.
1-4 Anhydro Erythritol
Erythritan

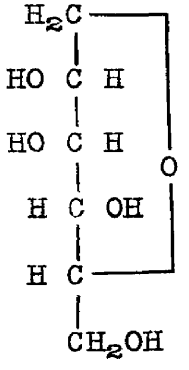


M.P. 15°
B.P. 138° 767 mm.
1-2,3-4 Dianhydro Erythritol



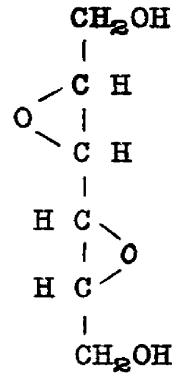
$[\alpha]_D$ + 52.0
1-4 Anhydro d-Mannitol
Mannitan (Syrup)

Crystallin Mannitan
M.P. 145°
$[\alpha]_D$ -23.75

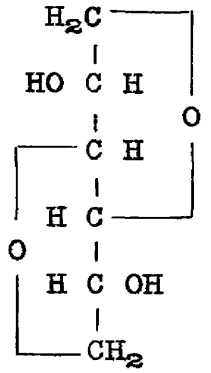


M.P. 142.5°
$[\alpha]_D$ +47.8
1-5 Anhydro d-Mannitol
Polygalitol

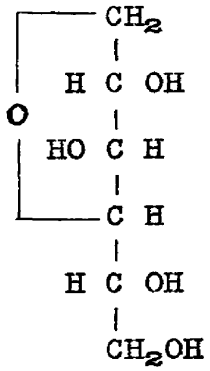
TABLE XVIIIa (CONTINUED)



B.P. 297 - 317°
2-3,4-5 Dianhydro Mannitol
Mannide



M.P. 87°
$[\alpha]_D$ +91.36
1-4,3-6 Dianhydro Mannitol
Isomannide

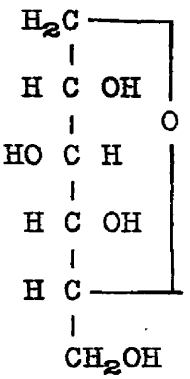


1-4 Anhydro Dulcitol
Dulcitan (?)

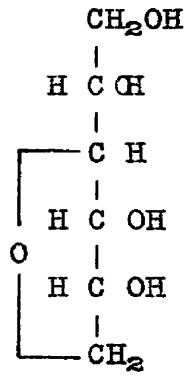
Formula not known

B.P. 189° 18mm.
2-3,4-5 Dianhydro Dulcitol
Dulcide

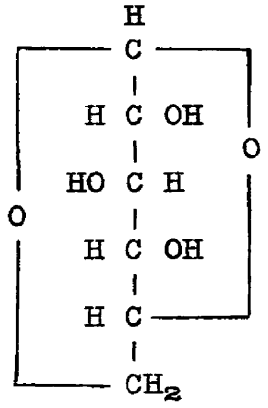
TABLE XVIIIa (CONTINUED)



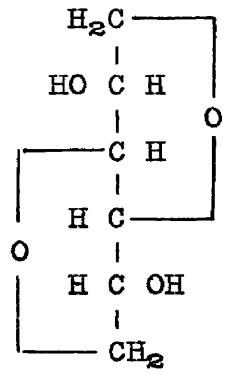
M.P. 157°
$[\alpha]_D$ -50.0
1-5 Anhydro d-Sorbitol
Styracitol



M.P. 113°
$[\alpha]_D$ -7.5
3-6 Anhydro d-Sorbitol



M.P. 179°
$[\alpha]_D$ -66.2
β-Glucosan



M.P. 102°
$[\alpha]_D$ + 95.92
3-6 Anhydro Furano Mannose

pletely studied in this laboratory by Beck (1936). The erythritan used was prepared by a new method which gave 45 per cent. yields; far better than those previously reported. The compound was found to be absorbed from the alimentary tract and excreted unchanged in the urine of rats. The R.Q. was not significantly changed and the compound was neither stored in the liver nor in the tissues. However, the compound was found to be less toxic to white mice by intraperitoneal injection than erythritol. Neither erythritol nor erythritan were toxic to rats by stomach tube in amounts of 1.5 gm. per 100 gm. of rat.

THE METABOLISM OF DULCITAN

Dulcitan is probably the 1-4 anhydride of dulcitol. Its structure has never been accurately determined. The compound used in this investigation was of chemical purity and gave a satisfactory analysis for the proper ratio of C, H_2 and O_2. For details of the method of preparation see section on mannitan. The results have been reported previously (Carr 1934). The inability of the compound to increase the R.Q. of fasting rats is demonstrated in Table XIX. Dulcitan did not store glycogen in the livers or tissues of rats either in feeding experiments or by stomach tube injection, Table XX. The compound was not toxic by oral administration and was incapable of relieving the hypoglycemic shock of insulin in mice. Dulcitan does not raise the fasting blood-sugar level of rabbits.

THE METABOLISM OF MANNITAN

The mannitan used for these and subsequent studies was prepared by a slight modification of the method of Vignon (1874). The residue obtain-

TABLE XIX

RESPIRATORY QUOTIENTS DURING FASTING AND AFTER DULCITAN

Rat No.	Wt. Gm.	Gm. CO_2 per 100 Gm.	Gm. O_2 per 100 Gm.	Dulcitan by Stomach Tube	R.Q	Run in hrs.
1	188	0.575	0.600	Fasting	0.696	2.5
		0.447	0.464	Dulcitan	0.702	2.5
2	188	0.429	0.437	Fasting	0.713	2.5
		0.515	0.535	Dulcitan	0.705	2.5
3	149	0.491	0.491	Fasting	0.727	2.5
		0.468	0.496	Dulcitan	0.686	2.5
4	170	0.402	0.412	Fasting	0.709	2.5
		0.370	0.378	Dulcitan	0.701	2.5
5	135	0.379	0.368	Fasting	0.751	2.5
		0.425	0.418	Dulcitan	0.740	2.5
6	120	0.501	0.500	Fasting	0.729	2.5
		0.535	0.559	Dulcitan	0.696	2.5
7	184	0.382	0.384	Fasting	0.720	2.5
		0.505	0.500	Dulcitan	0.735	2.5
8	125	0.577	0.577	Fasting	0.727	2.5
		0.660	0.701	Dulcitan	0.685	3.5
9	111	0.442	0.425	Fasting	0.765	2.5
		0.750	0.767	Dulcitan	0.708	4.0
10	124	0.421	0.389	Fasting	0.767	2.5
		0.833	0.835	Dulcitan	0.724	4.0
				Mean Fasting	0.729	
				Mean Dulcitan	0.708	

TABLE XX

GLYCOGEN STORAGE IN LIVERS OF RATS ON DULCITAN DIET

Group No.	No. of Rats	Wt. of Rats Gm.	Food Ingested Gm.	Livers Gm.	Liver Glycogen Gm.	Liver Glycogen Per cent.
1	2	125 114	3.4	4.45	0.015	0.33
2	3	105 120 104	5.9	13.50	0.007	0.05
3	1	125	1.5	2.80	0.009	0.32
4	1	125	1.3	2.90	zero	zero
5	1	128	4.0	3.90	zero	zero
6	1	115	3.8	3.50	zero	zero
7	1	128	2.5	3.60	zero	zero
8	1	118	3.6	4.10	zero	zero
9	1	128	2.6	3.60	zero	zero
10	1	112	2.6	4.30	zero	zero
11	1	128	3.3	3.85	zero	zero
					Mean	0.06

GLYCOGEN STORAGE IN TISSUES OF RATS ON DULCITAN DIET

Group No.	No. of Rats	Tissues Gm.	Liver Glycogen Gm.	Liver Glycogen per cent.
1	2	240.0	0.177	0.07
2	3	330.2	0.184	0.05
3	1	83.5	0.039	0.05
4	1	95.5	0.116	0.12
5	1	87.3	0.078	0.09
6	1	91.7	0.099	0.10
7	1	93.5	0.013	0.01
8	1	80.0	0.053	0.07
9	1	74.1	0.033	0.05
10	1	94.8	0.027	0.03
			Mean	0.07

ed after dehydration with sulfuric acid was dissolved in ten times its volume of water and treated with ammonium carbonate to remove barium and filtered with animal charcoal. When dried to constant weight at 110°C. the yield was approximately 50 per cent. of theory. This method yielded a syrupy mass free from barium and sulfuric acid derivatives. The analysis figures have been previously reported (Carr et al. 1933). Later work on the use of mannitan in physico-chemical measurements has established the purity and uniformity of different samples. The feeding of mannitan to rats produced in many animals a complete mobilization of liver glycogen. The results of feeding experiments is shown in Tables XXI and XXII and compared with animals on a levulose diet as controls. The control tissue-glycogen determinations were made on animals as previously described. A series of ten animals in Table XXIII is given to show the apparent mobilization of tissue glycogen concomitant with a decrease in liver glycogen. In later experiments it developed that most animals given a stomach tube after a fasting period have a tissue glycogen content below that of animals fed a cacao butter diet. The M.L.D. for mannitan was found to be approximately the same as for mannitol. Mannitan is incapable of relieving insulin shock in mice. The administration by stomach tube to rabbits does not cause an appreciable rise in the blood-sugar level. The influence on R.Q. is given in Table XXIV.

Prior to the undertaking of this investigation no metabolism experiments had been reported on mannitan. Bloor (1912) made some preliminary studies on the absorption of the anhydride esters of mannitol from the gastrointestinal tract of animals and concluded that these esters (mannitan distearate, mannide distearate and isomannide distearate) are about one-

TABLE XXI

GLYCOGEN STORAGE IN LIVERS OF RATS ON MANNITAN DIET

Group No.	No. of Rats	Wt. of Rats Gm.	Food Ingested Gm.	Livers Gm.	Liver Glycogen Gm.	Liver Glycogen Per cent
1	2	90 + 93	30.0	6.30	zero	zero
2	2	80 + 110	17.6	7.90	zero	zero
3	2	95 + 125	20.0	9.50	zero	zero
4	2	95 + 110	20.0	10.80	zero	zero
5	2	80 + 80	15.6	6.60	zero	zero
6	1	125	8.0	3.35	0.010	0.30
7	1	105	8.0	4.05	zero	zero
8	1	115	6.5	4.05	zero	zero
9	1	125	9.0	4.90	0.010	0.21
10	1	105	7.5	3.40	zero	zero
11	1	100	8.0	3.30	0.008	0.23
12	1	125	10.0	4.68	zero	zero
13	2	70 + 75	11.8	6.60	0.006	0.09
14	2	55 + 80	13.0	5.10	zero	zero
15	2	85 + 85	16.5	6.80	zero	zero
16	2	75 + 80	13.5	6.10	zero	zero
17	2	75 + 80	13.5	4.90	zero	zero
18	2	120 + 126	21.0	7.60	0.007	0.09
19	2	94 + 106	15.5	7.00	0.050	0.07
20	1	125	6.5	4.70	zero	zero
21	1	125	7.5	4.45	zero	zero

GLYCOGEN STORAGE IN LIVERS OF RATS ON LEVULOSE DIET

1	2	85 + 80	25.0	6.80	0.144	2.10
2	2	82 + 75	23.0	7.20	0.264	3.67
					Mean	2.89

Feeding experiments No. 20 and 21 were conducted with mannitan prepared by the method of Berthelot which consists essentially of dehydrating mannitol by prolonged boiling under a reflux condenser with several volumes of concentrated hydrochloric acid.

TABLE XXII

GLYCOGEN STORAGE IN TISSUES OF RATS ON MANNITAN DIET

Control Rats

Rat No.	Wt. Gm.	Tissues Gm.	Food Ingested Gm.	Tissue Glycogen Gm.	Tissue Glycogen per cent
1	195	148	7.0	0.161	0.109
2	195	147	0.0	0.149	0.101
3	195	142	7.7	0.189	0.133
4	200	150	10.0	0.247	0.165
5	175	133	10.0	0.174	0.131
6	165	130	10.0	0.169	0.130
7	170	127	10.0	0.218	0.172
8	155	135	4.0	0.286	0.212
				Mean	0.144

Mannitan Feeding

1	160	117	14.5	0.173	0.148
2	165	119	14.6	0.181	0.152
3	170	124	11.0	0.164	0.133
4	165	120	11.0	0.230	0.191
5	155	109	6.0	0.151	0.138
6	175	127	10.8	0.192	0.180
7	170	125	15.0	0.167	0.133
8	155	107	10.8	0.192	0.180
9	170	127	13.0	0.248	0.194
10	175	127	12.5	0.262	0.205
				Mean	0.164

TABLE XXIII

GLYCOGEN STORAGE IN TISSUES AND LIVERS OF RATS AFTER MANNITAN BY STOMACH TUBE

Rat No.	Wt. Gm.	Tissues Gm.	Tissue Glycogen Gm.	Tissue Glycogen per cent	Liver Gm.	Liver Glycogen Gm.	Liver Glycogen per cent.
1	160	125	0.068	0.054	5.55	0.041	0.75
2	200	165	0.183	0.111	6.10	0.000	0.00
3	155	125	0.023	0.018	5.22	lost	lost
4	140	110	0.031	0.028	4.30	0.004	0.09
5	185	150	0.070	0.046	5.75	0.004	0.07
6	150	115	0.029	0.025	5.25	0.007	0.13
7	165	130	0.092	0.071	6.50	lost	lost
8	150	115	0.067	0.058	5.73	0.003	0.05
9	150	118	0.044	0.037	4.80	0.000	0.00
10	145	115	0.040	0.035	5.00	0.000	0.00
			Mean	0.048			

TABLE XXIV

RESPIRATORY QUOTIENTS DURING FASTING AND AFTER MANNITAN

Rat No.	Wt. Gm.	Gm. CO_2 per Hr. per Gm.	Gm. O_2 per Hr. per Gm.	Mannitan by Stomach Tube	R.Q.	Run in hrs.
1	290	0.899	0.835	Mannitan	0.782	2.5
2	290	0.906	0.887	Mannitan	0.746	2.5
3	350	1.163	1.128	Mannitan	0.750	2.5
4	325	1.058	0.966	Mannitan	0.797	2.5
5	200	0.629	0.627	Mannitan	0.729	2.5
6	185	0.653	0.613	Mannitan	0.774	2.5
7	182	0.826	0.834	Fasting	0.720	2.5
		0.717	0.677	Mannitan	0.770	2.5
8	150	0.727	0.750	Fasting	0.705	2.5
		0.547	0.511	Mannitan	0.778	2.5
9	147	0.777	0.783	Fasting	0.720	2.5
		0.624	0.613	Mannitan	0.740	2.5
10	175	0.800	0.814	Fasting	0.714	2.5
		0.621	0.577	Mannitan	0.782	2.5
11	180	0.808	0.792	Fasting	0.743	2.5
		0.641	0.564	Mannitan	0.826	2.5
12	210	1.147	1.144	Fasting	0.728	2.5
		0.944	0.918	Mannitan	0.747	2.5
13	123	0.558	0.601	Fasting	0.675	2.5
		0.600	0.570	Mannitan	0.765	2.5
14	173	0.630	0.643	Fasting	0.713	2.5
		0.602	0.579	Mannitan	0.758	2.5
15	185	0.610	0.638	Fasting	0.695	2.5
		0.775	0.739	Mannitan	0.736	2.5
16	173	0.726	0.716	Fasting	0.737	2.5
		0.625	0.571	Mannitan	0.795	2.5
17	160	0.673	0.675	Fasting	0.725	2.5
		0.673	0.683	Mannitan	0.716	2.5
18	175	0.840	0.829	Fasting	0.735	2.5
		1.314	1.266	Mannitan	0.758	5.0
				Mean Fasting	0.718	
				Mean Mannitan	0.766	

half as "digestible" as ordinary fat. It should be pointed out also that the formulas given by this author for the anhydrides are incorrect.

THE METABOLISM OF MANNIDE AND ISOMANNIDE

Materials Employed. The second anhydride of mannitol, mannide and its isomer were prepared as pure chemical substances for study. The possibility existed, it was argued, that a double anhydride linkage might confer upon the compound the capacity of utilization by the animal body. Isomannide was prepared by Fauconnier's method (1884) in which mannitol is dehydrated by concentrated hydrochloric acid. The isomannide crystallizes from alcohol and is not converted to mannitol by water even at boiling temperature. This anhydride appears to be quite stable and differs in this respect from mannide which is readily converted into mannitol. The chemical configuration of mannide and isomannide has been established by von Romberg and von der Berg (1922) and by observations on the conductivity and combinations with boric acid made in this laboratory. However, Corre' and Manchère in 1931 pointed out that perhaps a better designation of the structure of these compounds would be one in which the OH groups would be considered as migratory and not permanently fixed.

Mannide was prepared by Liebermann's method (1884) which consists essentially of heating mannitol with concentrated butyric acid to 250°C in a sealed tube. The compound is of syrupy consistency and in the presence of water passes slowly into mannitol. The analysis and data on the metabolism of these two substances have been previously reported (Krantz et al. 1935).

Animal Experiments. A summary of the influence of these two anhydrides of mannitol on experimental animals is given in Tables XXV, XXVI, XXVII XXVIII and XXIX. The data indicate that neither isomannide nor mannide is stored in the liver as glycogen. The tissue glycogen of rats has again been found to be reasonably fixed and not subject to change even after a period of prolonged fasting which must have been experienced by these animals since the compounds did not serve as available carbohydrate to an appreciable extent. Isomannide was without effect on the R.Q. An increase occurred after mannide which appears as statistically significant. This increase, especially with certain animals, has been observed a number of times throughout these investigations following administration of a substance that gave no evidence of being metabolised in other types of experiments. The inability to relieve insulin shock or elevate the fasting blood-sugar level was to be expected since the experiments failed to indicate any significant utilization of the anhydrides in general. Neither of these compounds exhibited any poisonous properties in these experiments although the characteristic diarrhoea was present. It is of some interest to note that isomannide given in single doses of 1, 1.5 and 2 grams per 100 grams of rat respectively by stomach tube was without any apparent harmful effect.

THE METABOLISM OF POLYGALITOL

Polygalitol, the 1-5 anhydride of mannitol occurs naturally in the plant Polygala Amara and related polygala species. Chodat in 1888 isolated from polygala amara a crystallin substance which he believed to be an aldose possessing reducing properties and suggested an elaborate hypothesis

TABLE XXV

GLYCOGEN STORAGE IN LIVERS AND TISSUES OF RATS ON ISOMANNIDE DIET

Rat No.	Wt. Gm.	Food Ingested Gm.	Livers Gm.	Liver Glycogen Per cent	Tissues Gm.	Tissue Glycogen Per cent	Isomannide Recovered Urine Gm.	Isomannide Recovered Intestines Gm.
1	130	1.0	2.90	0.56	81.1	0.09		
2	140	1.5	4.05	0.90	77.6	0.13		
3	130	3.4	3.80	0.19	81.0	0.12	6.8	5.2
4	150	3.0	4.20	0.46	93.7	0.16		
5	125	3.0	3.95	0.21	88.8			
6	133	2.0	4.50	0.00	103.5	0.02		
7	111	1.6	4.00	0.00	84.4	0.06		
8	140	2.0	4.50	0.00	107.0	0.02	8.0	0.8
9	120	1.8	3.60	0.40	93.9	0.02		
10	125	1.5	4.20	0.00	96.4	0.05		
11	132	1.3	3.80	1.65	82.7	0.07		
12	144	1.3	4.05	0.96	98.5	0.11		
13	136	1.2	3.65	0.70	87.2	0.09		
14	134	2.9	4.60	0.14	114.2	0.02		
15	130	3.3	4.70	0.33	111.5	0.07		
16	140	2.5	4.55	0.25	125.7	0.04		
17	130	3.7	4.95	0.00	109.5	0.09		
18	121	2.9	3.75	0.54	91.8	0.04		
19-20	2 rats	3.2		0.00				
21-22	2 rats	3.3		0.00				
23-24	2 rats	2.3		0.00				
25-26	2 rats	1.0		0.04				
27-28	2 rats	1.9		0.00				
			Mean	0.19		0.07		

TABLE XXVI

GLYCOGEN STORAGE IN LIVERS AND TISSUES OF RATS AFTER ISOMANNIDE BY STOMACH TUBE

Rat No.	Isomannide Gm.	Livers Gm.	Liver Glycogen per cent.	Tissues Gm.	Tissue Glycogen per cent.
1	0.1	2.7	0.09		
2	0.1	3.4	0.27		
3	0.1	4.0	0.47		
4	0.1	3.9	0.92		
5	0.1	3.9	0.11		
6	0.1	4.0	0.13		
7	0.1	3.8	0.84		
8	0.1	3.6	0.01		
9	0.1	3.6	0.65		
10	0.1	3.9	0.84		
		Mean	0.43		
11	0.2	4.6	0.05	109	0.08
12	0.2	4.6	0.04	106	0.07
13	0.2	4.7	0.74	101	0.07
14	0.2	4.0	0.30	100	0.09
15	0.2	4.6	0.16	87	0.08
16	0.2	3.7	0.42	106	0.07
17	0.2	4.0	0.48	91	0.06
18	0.2	3.5	0.67	91	0.10
		Mean	0.35	Mean	0.08
19-20	0.5	6.8	0.31		
21-22	0.5	7.0	0.03		
23-24	0.5	6.5	0.02		
25-26	0.5	6.2	0.02		
27-28	0.5	6.9	0.02		
29-30	0.5	4.5	1.36		
31	0.5	3.9	0.01		
32	0.5	3.7	0.29		
33	0.5	4.5	0.00		
34	0.5	4.0	0.02		
35	0.5	4.2	0.07		
36	0.5	3.9	0.59		
37	0.5	3.7	0.09		
38	0.5	4.1	0.07		
39	0.5	4.1	0.52		
		Mean	0.22		

TABLE XXVI (CONTINUED)

Rat No.	Isomannide Gm.	Livers Gm.	Liver glycogen per cent.
40	2.0	3.6	0.94
41	2.0	3.3	0.54
42	2.0	3.2	0.92
43	2.0	3.7	0.10
44	2.0	3.9	0.23
45	2.0	3.6	0.90
46	2.0	3.3	0.46
47	2.0	3.4	0.09
48	2.0	4.2	0.50
49	2.0	6.6	0.35
50	2.0	5.1	0.56
		Mean	0.50
	Water-cc.		
51	2.0	3.6	0.36
52	2.0	3.8	0.06
53	2.0	3.8	0.90
54	2.0	3.9	1.18
55	2.0	3.7	0.64
56	2.0	3.5	0.60
57	2.0	3.2	0.52
58	2.0	4.2	0.22
59	2.0	3.9	0.02
		Mean	0.50

TABLE XXVII

GLYCOGEN STORAGE IN LIVER AND TISSUES OF RATS AFTER MANNIDE DIET AND MANNIDE BY STOMACH TUBE

Group No.	No. of Rat	Mannide Consumed Gm.	Glycogen per cent. Liver	Glycogen per cent. Tissue	Rat No.	Mannide by Stomach Tube Gm.	Glycogen per cent. Liver	Glycogen per cent. Tissue
1	2	3.7	0.40		1	2.0	0.09	0.07
2	2	3.7	0.34		2	2.0	0.25	0.09
3	2	3.7	0.60		3	2.0	0.10	0.12
4	2	3.7	0.68		4	2.0	0.17	0.12
5	2	3.7	0.34		5	2.0	0.65	0.13
6	2	2.4	0.07		6	2.0	0.59	0.13
7	2	4.9	0.25		7	2.0	0.05	0.08
8	2	4.2	0.38		8	2.0	0.07	0.10
9	2	3.0	0.03		9	2.0	0.07	0.10
10	2	3.3	0.03			Mean	0.22	0.10
11	1	2.0	0.32	0.11				
12	1	1.9	0.15	0.10		Water by Stomach Tube cc.		
13	1	2.3	0.23	0.08				
14	1	1.2	0.22	0.09				
15	1	2.4	0.37	0.11	10	2.0	0.09	
16	1	2.1	0.69	0.15	11	2.0	0.06	
17	1	2.0	0.45	0.07	12	2.0	0.07	
18	1	3.3	0.19	0.14	13	2.0	0.07	
19	1	3.4	0.20	0.17	14	2.0	0.08	
20	1	2.4	0.39	0.22	15	2.0	0.07	
					16	2.0	0.07	
		Mean	0.21	0.12	17	2.0	0.08	

TABLE XXVIII

RESPIRATORY QUOTIENTS DURING FASTING AND AFTER ISOMANNIDE

Rat No.	Wt. Gm.	Gm. O_2 per 100 Gm. per hr.	Isomannide by Stomach Tube	R.Q.	Run in hrs.
1	125	0.325	Fasting	0.760	2.0
		0.350	Isomannide	0.698	2.5
2	200	0.240	Fasting	0.745	2.5
		0.185	Isomannide	0.723	2.5
3	190	0.191	Fasting	0.739	2.5
		0.176	Isomannide	0.710	2.5
4	149	0.229	Fasting	0.746	2.5
		0.204	Isomannide	0.744	2.5
5	170	0.212	Fasting	0.740	3.0
		0.203	Isomannide	0.745	2.5
6	156	0.266	Fasting	0.708	2.5
		0.230	Isomannide	0.723	2.5
7	157	0.193	Fasting	0.744	2.5
		0.177	Isomannide	0.736	2.5
8	137	0.256	Fasting	0.715	2.5
		0.249	Isomannide	0.701	3.0
9	127	0.189	Fasting	0.753	2.5
		0.196	Isomannide	0.732	5.0
		Mean 0.233	Fasting	0.739	
		Mean 0.219	Isomannide	0.723	

TABLE XXIX

RESPIRATORY QUOTIENTS DURING FASTING AND AFTER MANNIDE

Rat No.	Wt. Gm.	Gm. O_2 per 100 Gm. per hr.	Mannide by Stomach Tube	R.Q.	Run in hrs.
1	110	0.219	Fasting	0.737	2.5
		0.198	Mannide	0.792	2.5
2	115	0.263	Fasting	0.747	2.5
		0.210	Mannide	0.764	2.5
3	186	0.108	Fasting	0.698	2.5
		0.086	Mannide	0.771	2.5
4	110	0.220	Fasting	0.736	2.5
		0.198	Mannide	0.746	2.5
5	109	0.214	Fasting	0.763	2.5
		0.159	Mannide	0.787	2.5
6	138	0.164	Fasting	0.747	2.5
		0.161	Mannide	0.766	2.5
7	108	0.198	Fasting	0.722	2.5
		0.191	Mannide	0.748	2.5
8	137	0.223	Fasting	0.739	2.5
		0.222	Mannide	0.784	2.5
9	129	0.205	Fasting	0.707	2.5
		0.244	Mannide	0.766	2.5
10	87	0.274	Fasting	0.703	2.5
		0.191	Mannide	0.779	2.5
11	124	0.193	Fasting	0.757	2.5
		0.202	Mannide	0.784	2.5
12	144	0.176	Fasting	0.728	2.5
		0.197	Mannide	0.739	2.5
13	151	0.161	Fasting	0.735	5.0
		0.158	Mannide	0.750	2.5
14	110	0.211	Fasting	0.685	2.5
		0.189	Mannide	0.741	2.5
15	130	0.171	Fasting	0.749	2.5
		0.173	Mannide	0.832	2.5
16	128	0.175	Fasting	0.729	2.5
		0.165	Mannide	0.800	2.5
			Fasting	0.730	

to account for the presence of this material in the plant. Considerable confusion existed regarding the nature of the sweet substance in the polygala species and Jankhier (1918) made a study of the sugar substance in polygala amara the subject of a dissertation for the doctor's degree. In 1927 Picard isolated a crystallin sugar alcohol of M.P. 142°from polygala vulgaris and showed this substance to be identical with that of Chodat. Shinoda and Sato in 1932 completely elucidated the chemical nature of polygalitol and reported its isolation from still another variety (P. tennifolia) and demonstrated that the three reported substances are the same compound. These workers also noted the presence in their extracts of a substance formerly called polygarite, which they proved to be styracitol by oxidation with hydrogen peroxide and subsequent treatment with phenylhydrazine. The glucosazone, M.P. 208° is identical with that obtained from polygalitol. The synthesis of styracitol is conclusive evidence of its structure and the similarity of the glucosazone establishes the structure of polygalitol.

Material Employed. Approximately 125 grams , the largest amount ever reported, of pure polygalitol havebeen prepared for these studies from Polygala amara by a modification of the method of Picard. The details of the large scale preparation of all the anhydrides of the sugar alcohols and their chemistry should be recorded in a appropriate chemical publication. Essentially the method consisted of extraction of the air-dry, powdered plant with 50 per cent. hot alcohol until exhausted, concentration in a vacuum and subsequent addition of water and lead subacetate. The lead is precipitated as sulfide and the clear aqueous fluid is concentrated to a syrup from which crystals form upon standing. A 70 per cent. ether-alcohol mix-

ture was found invaluable in removing traces of a reducing sugar-like substance that prevented complete purification. This material undoubtedly was the substance that led Chodat to believe polygalitol to possess a reducing group. The pure crystallin polygalitol may be repeatedly crystallized from hot 95 per cent. alcohol without alteration in M.P. Mixed samples from different batches gave a sharp M.P. 142°C. The specific rotation was $[\alpha]_D^{16}$ +47.5° (Shinoda +47,81°) and $[\alpha]_D^{25}$ +42.1° (Picard +41.1°). After standing thirteen days the solution possessed the same rotation. The pure compound gave no reduction with Fehling's solution before or after hydrolysis. Upon ignition the compound yielded 0.87 per cent. ash. The pH of 4 per cent. aqueous solutions of polygalitol gave a value of 6.7.

Polygalitol and Glycogen Storage. Male rats were fed the mixture of cacao butter and polygalitol as previously described and their liver and tissue glycogen was determined. The inability of this anhydride to increase the liver and tissue glycogen may be observed in Table XXX.

Influence of Polygalitol on Respiratory Quotient. These measurments are presented in Table XXXI. There is some evidence that polygalitol raises the R.Q. especially in certain animals where the determinations were carried out over a period of four or five hours.

Influence of Polygalitol on Blood-Sugar Level of Rabbits. Four rabbits were used in this experiment and the polygalitol was administered by stomach tube. An attempt was made to recover the polygalitol from the urine of one of these animals. Approximately 0.5 Gm. was recovered from rabbit No. 3 which upon purification gave the sharp characteristic M.P. of polygalitol. The details are given in Table XXXII.

TABLE XXX

GLYCOGEN STORAGE IN LIVERS AND TISSUES OF RATS AFTER POLYGALITOL DIET

Rat No.	Wt. Gm.	Polygalitol Consumed Gm.	Livers Gm.	Liver Glycogen Per cent.	Tissues Gm.	Tissue Glycogen Per cent.	Change in Wt. During Experiment Gm.
1	131	2.1	3.86	0.16	110	0.06	-10
2	121	1.2	3.61	0.21	91	0.05	-10
3	125	2.1	3.14	0.16	101	0.06	- 9
4	143	2.7	4.15	lost	114	0.04	- 7
5	137	1.0	4.23	0.05	106	0.06	-12
6	125	2.3	3.73	0.27	77	0.08	-27
7	129	2.3	3.99	0.42	72	0.09	-42
8	128	1.6	3.82	0.14	83	0.10	-26
9	118	2.0	3.92	1.06	71	0.13	-26
10	125	1.0	3.60	0.12	76	0.10	-34
11	112	1.6	3.05	0.14	77	0.08	-23
			Mean	0.18		0.08	
				Controls			
12	135	Cacao Butter	4.09	0.37	109	0.06	- 9
13	121	Cacao Butter	3.90	0.10	99	0.06	- 8
14	133	Cacao Butter	4.63	0.17	111	0.03	- 7
15	101	Cacao Butter	3.27	0.12			-11
16	110	Cacao Butter	3.15	0.16			-17
17	106	Cacao Butter	3.20	0.19			-22
18	122	Glucose	4.87	4.19			-18
19	103	Glucose	2.99	0.06			-19
		Cocao Butter	Mean	0.18		0.05	

TABLE XXXI

RESPIRATORY QUOTIENTS DURING FASTING AND AFTER POLYGALITOL

Rat No.	Wt. Gm.	Gm. CO_2 per 100 Gm. per hr.	Gm. O_2 per 100 Gm. per hr.	Polygalitol by Stomach Tube 40 per cent.	R.Q.	Run in hrs.
1	120	0.186	0.184	Fasting	0.735	2.5
		0.176	0.160	4 cc.	0.799	4.0
16	125	0.173	0.174	Fasting	0.726	2.5
		0.209	0.202	4 cc.	0.749	4.5
16	126	0.170	0.169	Fasting	0.728	2.8
		0.202	0.203	4 cc.	0.726	2.2
16	137	0.173	0.174	Fasting	0.725	2.9
		0.191	0.194	4 cc.	0.718	2.5
18	116	0.189	0.185	Fasting	0.739	2.5
		0.193	0.181	4 cc.	0.772	3.7
21	116	0.185	0.180	Fasting	0.748	2.8
		0.189	0.187	4 cc.	0.734	2.0
22	137	0.184	0.179	Fasting	0.740	2.1
		0.172	0.166	4 cc.	0.756	1.0
23	128	0.168	0.164	Fasting	0.738	3.6
		0.192	0.195	4 cc.	0.740	5.6
20	121	0.175	0.175	Fasting	0.728	2.7
		0.175	0.179	4 cc.	0.712	4.1
27	119	0.202	0.206	Fasting	0.713	2.5
		0.189	0.186	4 cc.	0.742	4.9
		Mean	0.179	Fasting	0.732	
		Mean	0.185	4 cc.	0.745	

TABLE XXXII

INFLUENCE OF POLYGALITOL ON BLOOD-SUGAR LEVEL OF RABBITS

Rabbit No.	Wt. Kg.	Polygalitol Gm.	Mgm. per 100 cc. Blood						
			Fasting	1/2 hr.	1 hr.	2 hr.	3 hr.	4 hr.	5 hr.
1	3.1	6.2	101		121	114	111	111	118
2	2.3	4.6	101	114	106	103	111	100	111
3	2.6	5.2	93	125	103	119	133		
4	2.3	9.2	118	125	112	125	123		
Mean			103	121	110	115	119	105	114

Influence of Polygalitol on Insulin Shock. In 19 animals fasted for 12 hours and injected with insulin, polygalitol, in amounts equal to that found effective with glucose was inacpable of relieving the convulsions. Even massive doses of this anhydride failed to relieve the hypoglycemia.

DISCUSSION OF THE METABOLISM OF THE ANHYDRIDES

The data assembled indicate that the anhydrides of sugar alcohols are in general not metabolized in the animal body. Erythritan, mannitan, dulcitan and isomannide to a limited extent deplete the glycogen stores of the liver. This action does not seem to be associated with acute toxicity. The compounds behave more as inert "foreign sugars", especially isomannide which is excreted almost quantitatively in the urine of animals. The non-toxic nature of these substances and their rapid excretion in the urine warrant further investigation as possible diuretic drugs. Mannitol, mannide and isomannide appear to exhibit no effect on the liver glycogen stores, when administered by stomach tube. Possibly this is because the liver does not synthesize glycogen from an unusual source until all normally available stores of carbohydrate are removed. In the feeding experiments with mannitol lasting over three to four days a constant supply of sugar alcohol was present and further acute diarrhoea was not produced as occurred in the stomach tube experiments. The anhydrides were not stored as glycogen under either condition and this is somewhat surprising when one considers that glycogen itself is made up of anhydride units. Throughout these investigations the occurrence of an occasional liver with a glycogen content of the order of magnitude of one per cent. while all other livers in the same group were uniformly low may be observed by

an inspection of the tables. The author is unable to explain this occurrence but suggests that perhaps in certain animals bacterial destruction of the sugar alcohol or anhydride occurred in the intestine with the formation of a metabolizable sugar. The occasional high R.Q. obtained after polygalitol, mannide and regularly after mannitan may be similarly explained. The capacity of members of the colon-aerogenes group to utilize the sugar alcohols has been studied by Dozois et al. (1935-1936).

CONCLUSIONS REGARDING THE METABOLISM OF THE ANHYDRIDES

1. The removal of one or two molecules of water from one molecule of the sugar alcohols destroys the capacity of these substances to be stored as glycogen in the liver by the white rat.
2. Dulcitan and isomannide do not increase the respiratory quotient of the fasting white rat. Mannitan, mannide and polygalitol produce a slight increase in R.Q.
3. The anhydrides of the sugar alcohols are ineffective in relieving insulin shock in mice.
4. The anhydrides of the sugar alcohols are incapable of raising the fasting blood-sugar level in rabbits.
5. The anhydrides of the sugar alcohols are absorbed from the intestines of rats and rabbits and are partially excreted unchanged in the urine.

SUMMARY AND DISCUSSION OF EXPERIMENTAL PROBLEM

Carbohydrates have three primary functions in nutrition, i.e., to furnish energy and heat, to synthesize glycogen as a reserve food and to form fat. The ability of a class of chemical compounds related to simple carbohydrates to furnish energy and to synthesize glycogen in animals has been studied in this investigation. Related physiological phenomena have also been explored. The experience gained by these studies permit a few generalizations to be made regarding the metabolism of the sugar alcohols and their anhydrides.

There appears to be no relation between the number of carbon atoms in the molecule in this series and the degree of utilization by the body. The three carbon atom compound, glycerol, and the six carbon atom alcohols, sorbitol, mannitol and dulcitol are utilized most readily. This result might have been anticipated if one considers the apparent preference of the body cells for 3 and 6 carbon atom compounds among the carbohydrates. No 7 carbon atom sugar alcohol was available at the time these studies were made. Recently primulitol has been extracted in a large quantity and experiments will be undertaken shortly to determine its fate in the animal body although experience offers little that would point to a possible utilization of this compound. A ketone or aldehyde grouping seems to be essential for metabolism. The direct utilization of ethyl alcohol seems to be an exception. In evidence of this hypothesis the utilization of the 7 carbon monosaccharide, d-mannoketophetose by rabbits, as shown by Roe and Hudson (1936) may be pointed out. The fact that this compound is the only 7 carbon sugar so far observed to be utilized by the animal organism is of interest, not because other similar

sugars have been studied and found inert but because it is the first sugar with more than 6 carbon atoms that has been made in sufficient quantity for study. One is tempted to speculate as to the possible information that may be made available when all the isomeric hexoses have been so studied.

The removal of one or two molecules of water from the sugar alcohols with the formation of their first and second anhydrides respectively converts the sugar alcohol into a substance which is chemically and physiologically stable and which behaves as a foreign substance when administered to animals.

The ability of mannitan and dulcitan to completely strip the liver of glycogen is interesting. Other experimental measures which can produce this effect are adrenalectomy or freezing of the animal after strychnine convulsions. This result was unexpected and somewhat difficult to reconcile with the anhydride linkage in starch, sucrose and glycogen itself. Physical properties and chemical reactivity seem to be very important factors in determining the degree and speed of metabolism of sugars. There was no evidence in this investigation of a compound with more than 2 carbon atoms in the molecule that was metabolized in a manner different from that of glucose. Of extreme interest in this connection are the studies that have been carried out on glucosan, the anhydride of glucose. This compound likewise has been found to be inert physiologically.

Styracitol, the 1-5 anhydride of sorbitol, has likewise been found to be physiologically non-metabolizable in a very cursory experiment carried out by Freudenberg (1933). One hundred grams of styracitol has been synthesized in this laboratory and a complete study of this anhydride is contemplated.

CONCLUSIONS

1. The sugar alcohols are absorbed from the intestinal tract of animals and many of them serve to a limited degree as available carbohydrate.
2. These compounds apparently follow the intermediary metabolism of glucose, i.e., glycogen formation - blood glucose - insulin and tissue catabolism.
3. The members of this series may be classified according to their availability as carbohydrate food, glycerol > mannitol, > sorbitol > dulcitol > erythritol > ethylene glycol > methyl alcohol.
4. The anhydrides of the sugar alcohols are absorbed from the intestinal tract of animals and excreted unchanged in the urine.
5. Mannitan and dulcitan possess the unusual capacity of depleting the liver of residual glycogen stores.
6. The anhydrides are no more poisonous than the sugar alcohols when administered orally. The anhydrides and sugar alcohols produce diarrhoea in high concentration by stomach tube.
7. Anhydride formation, in general, destroys the capacity of a substance to be metabolized in the animal body. There is no evidence that these anhydride compounds might be metabolized in a manner different from that of glucose.
8. Aldehyde or ketone groupings seem to be essential for prompt and efficient utilization of a carbohydrate-like substance. Physical properties and chemical reactivity are important factors in this scheme.

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