THE ABSORPTION OF SUGAR ALCOHOLS FROM CLOSED INTESTINAL LOOPS IN DOGS WITH A METHOD FOR THE DETERMINATION OF THESE ALCOHOLS

by

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PART I

A METHOD OF DETERMINATION OF SUGAR ALCOHOLS

INTRODUCTION

A number of different methods have been used for the quantitative analysis of the various polyhydroxy sugar alcohols. Gravimetric, iodimetric, and polarimetric methods have all been used. The former has been employed chiefly to determine sorbitol in fruit wines by precipitating as hexa-acetyl sorbitol (1), dobenzalsorbitol (2), hexabenzoate (3), a ferric barium or ferric sodium complex (4), or as the chlorobenzaldehyde (5). The gravimetric method is not applicable to rapid routine analysis of the sugar alcohols or for use with minute quantities.

Polarimetric methods utilize the effect of different substances upon the optical properties of the alcohols. Methods have been described using sodium metaborate (6), alkaline arsenous oxide (7), and acidic ammonium molybdate (8). Such methods are not readily applied to physiological solutions.

Quantitative iodimetric determinations are based on the reducing

power of the sugar alcohols. The periodates (9), and periodic acid and alkaline periodates (10) have been used as oxidizing agents in this method. These procedures are not desirable for the present study due to the cost of periodic acid and the lengthy procedure involved.

Another iodimetric method employs an alkaline solution of potassium mercuric iodide for the determination of mannitol, inositol, and dulcitol (11). The excess iodine liberated in the reaction with the sugar alcohol is titrated with sodium thiosulphate. In previous work in this laboratory it was found that this method could not be used for accurate determination of small quantities of sorbitol such as were to be determined in the physiological work as presented later in this thesis.

As none of the above procedures could be readily used in the present study it was desirable to develop a rapid and accurate method which could be applied to minute quantities of the various sugar alcohols in physiological materials.

DEVELOPMENT OF A METHOD OF DETERMINING SUGAR ALCOHOLS

Basis of the Method

The method used for the determination of sugar alcohols as presented in this paper is based on the Hagedorn and Jensen glucose method in which an alkaline potassium ferricyanide solution is used as the oxidizing agent. During oxidation of the sugar the amount of potassium ferricyanide that is reduced to ferrocyanide is proportional to the amount of glucose present, and can be measured indirectly by determining the amount of unchanged potassium ferricyanide remaining after completion of the reaction. The unchanged potassium ferricyanide liberates iodine from an acid solution of potassium iodide. To prevent reversal of this reaction when the mixture is acidified zinc acetate is added and the ferrocyanide formed is precipitated as a zinc salt. After addition of potassium iodide and acid the liberated iodine is titrated with sodium thiosulphate, giving a value which is proportional to the amount of unchanged potassium ferricyanide, and this in turn measures the amount of glucose oxidized.

Oxidizing Agent

The first step in developing the present method was to find a satisfactory oxidizing agent. It was known that an alkaline solution

was necessary for oxidation of sorbitol. Sorbitol is not affected by the ordinary copper reagents used in the determination of sugars. The first reagent chosen in work previously carried out in this laboratory was a solution of mercuric acetate, potassium iodide, and potassium iodate in 5 N sodium hydroxide. After heating sorbitol with this reagent the solution was cooled and acidified with concentrated hydrochloric acid and the liberated iodine titrated against potassium thiosulphate. The titration difference given by this method was only 1-2 ml. per mg. of sorbitol. With such a small difference minute quantities of sorbitol could not be determined accurately. The method was, therefore, discontinued.

Alkaline potassium ferricyanide was then tried as the oxidising agent. The following equations indicate the reactions involved when sorbitol is oxidized by this method:-

(1) $K_3Fe(CN)_6$ + Sorbitol $\longrightarrow$ Glucose or other sugars $\longrightarrow$
$\longrightarrow$ Organic acids + $K_4Fe(CN)_6$

(2) $K_4Fe(CN)_6 + 2Zn(C_2H_3O_2)_2 \longrightarrow Zn_2Fe(CN)_6\downarrow + 4KC_2H_3O_2$

(3) $CH_3COOH + KI \longrightarrow HI + KC_2H_3O_2$

$K_3Fe(CN)_6 + 2HI \longrightarrow I_2 + K_4Fe(CN)_6 + 2H^+$

(4) $2H_2S_2O_3 + I_2 \longrightarrow H_2S_4O_6 + 2HI$

In reaction (1) the exact product of the oxidation of sorbitol is not known, but glucose or other sugar is probably formed as an intermediate product and may be used to illustrate oxidation of the sugar alcohol. The ferrocyanide formed during reactions (1) and (3) is precipitated as zinc salt by addition of zinc acetate as shown in reaction (2). This prevents reversal of reaction (3) when the mixture is acidified as zinc ferrocyanide is insoluble in acetic acid. The unchanged potassium ferricyanide reacts with the acidified iodide solution as shown in reaction (3). The reaction that occurs when the liberated iodine is titrated with sodium thiosulphate is shown in reaction (4).

Throughout the work on sugar alcohols aliquots of known solutions of the alcohols were measured into sugar tubes by means of a 5 ml. Ostwald-Folin blood pipette. All samples were run in triplicate. Three sugar tubes containing 5 ml. of distilled water were run as a blank with each set of determinations. To the sugar tubes containing the known amounts of sugar alcohol and the water blanks was added a definite amount of oxidising reagent by means of an Ostwald-Folin blood pipette. The tubes were closed by sealed glass bulbs and placed in a boiling water bath for the required period of heating.

The potassium ferricyanide reagent used was a 0.75 percent solution of potassium ferricyanide in 2.5 N sodium hydroxide. This oxidising reagent was added to aliquots of sorbitol solution in sugar tubes and heated for forty five minutes. It was later found that a thirty minute heating period was sufficient. After cooling, a solution of 30 per cent zinc sulphate and 10 per cent potassium iodide was added and the mixture acidified with glacial acetic acid. This

method was also discontinued as the potassium ferricyanide solution was not stable and the water blanks were found to use gradually less and less thiosulphate.

An attempt was made to work out a colorimetric method. Sorbitol was first oxidised with a solution containing 0.56 per cent potassium ferricyanide and 15 per cent sodium chloride in 2 N sodium hydroxide. The ferrocyanide produced in this reaction was converted to Prussian blue by the addition of an excess of ferric iron and the color measured against a standard solution in a colorimeter. The method was discontinued as it was not possible to develop a satisfactory color by this reaction.

Various ways were then tried to stabilise the potassium ferricyanide solution. The total alkalinity of the solution was reduced. 0.65 per cent potassium ferricyanide and 15 per cent sodium sulphate were made up in 2 N sodium hydroxide. The sodium sulphate was added to reduce the solubility of oxygen in the reagent and thus prevent its interference with oxidation during heating. This reagent did not give constant values for varying known amounts of sorbitol.

All reagents used were of reagent quality but it was possible that traces of impurities in the reagents or in the distilled water might cause some slight reduction of the potassium ferricyanide to ferrocyanide. To compensate for such a reduction 0.1 per cent potassium ferrocyanide was added to the solution of 0.65 per cent potassium ferricyanide and 15 per cent sodium sulphate in 2 N sodium hydroxide. By the mass action effect the small amount of potassium

ferrocyanide might prevent serious change in the total amount of ferricyanide present in the reagent. This ferricyanide-ferrocyanide reagent was found to be unstable after standing more than a week and did not give constant titration values for known amounts of sorbitol.

It was thought that decomposition of the potassium ferricyanide might be responsible for the variations in titration values. The ferricyanide radical slowly decomposes in the high alkalinity of the reagent with the production of ferric ions. After the reagent had stood for several days a fine precipitate was noticed on the bottom of the reagent bottle. On filtering off this precipitate it was found to consist of ferric hydroxide. To suppress these ferric ions sodium fluoride, potassium fluoride, potassium oxalate, and sodium pyrophosphate were each in turn added to the alkaline ferricyanide oxidizing reagent. The use of sodium fluoride to form unionized ferric iron complexes is not new. None of these salts added to the reagent increased its stability nor were the titration values for known amounts of sorbitol more constant when any of these salts was added.

To eliminate the error that might arise as a result of the oxidation of sorbitol by the oxygen of air the reaction tubes were closed by rubber stoppers carrying about 12 mm. of capillary tubing instead of by the customary glass bulbs during the heating and cooling processes. The rubber stoppers were first boiled in potassium ferricyanide solution for twenty minutes. No appreciable advantage was noted when rubber stoppers replaced glass bulbs. Further attempts to reduce oxidation by the oxygen of air included covering the surface of the

solutions in the sugar tubes just before heating with a thin layer of toluene, of mineral oil, and of paraffin. The toluene was found to interfere with the end point during the titration with sodium thiosulphate. Neither the mineral oil nor the paraffin reduced the variations in titration values.

Citric acid and phosphoric acid were each used to acidify the oxidized sorbitol solution after cooling. The results obtained were unsatisfactory and similar to those obtained when acetic acid was used. It was not possible to use any of the mineral acids as they caused too much ionization of the zinc ferrocyanide compound formed on addition of the iodine-zinc reagent.

Iodine-Zinc Reagent

Varying amounts of zinc acetate and potassium iodide were used. It was found that 1 ml. of a 15 per cent zinc acetate solution was the minimum amount that could be used to remove the ferrocyanide from the reaction mixture. The proportion of zinc acetate to potassium iodide was also found to have a decided effect on the sharpness of the end point. At first these two solutions were added separately. The zinc acetate solution was added to the cooled, oxidized sorbitol mixture. Some ferricyanide was found to be carried down with the precipitate of zinc ferrocyanide, but the ferricyanide redissolved on addition of glacial acetic acid. The potassium iodide solution was then added, and the iodine liberated by the ferricyanide present was determined by

titration with sodium thiosulphate. It was found that glacial acetic acid could not be added before precipitating the ferrocyanide as a zinc salt. When glacial acetic acid was added directly to the cooled, oxidized sorbitol solution, a small amount of ferriferrocyanide, or Prussian blue, was formed which interfered with the determination.

To simplify the procedure a solution of 12 per cent potassium iodide and 15 per cent zinc acetate was made up together and added to the oxidized sorbitol solution before acidifying. This proved satisfactory. 10 per cent sodium sulphate was added to the potassium iodide-zinc acetate solution, but as this was of no value it was later omitted. To further simplify the procedure glacial acetic acid was added to the potassium iodide-zinc acetate reagent in the proportion of 1:1 and immediately added to the oxidized sorbitol solution. This eliminated the necessity of pipetting each solution separately and was found to be equally satisfactory. It was necessary to use this acidified reagent immediately as iodine was found to be liberated slowly if the solution was allowed to stand.

Reaction Time

The method used up to this time still gave considerable variation in the titration values for sorbitol from day to day, and the reagents used were not stable for more than a few days. It was then found that sorbitol was oxidized slowly by the alkaline ferricyanide reagent in the cold. An appreciable titration difference was found after allowing

sorbitol to stand 3 minutes with the reagent at room temperature. In running a series of 9 to 12 tubes or more, considerable time was consumed in pipetting the reagent into each tube. By the time the last tube was charged with the reagent and ready to place in the water bath the first tube containing sorbitol and oxidizing reagent had stood at room temperature for 10 to 15 minutes. During this time oxidation of the sorbitol had been slowly proceeding in each of the charged tubes.

To control the time of reaction of each tube, potassium ferricyanide in water solution, and sodium hydroxide were added separately. No reaction occurs between sorbitol and a water solution of potassium ferricyanide and it was, therefore, possible to charge each sugar tube containing sorbitol with this reagent and let all of the tubes stand while the next step was carried out. Immediately after addition of the alkali each tube was placed in the water bath and the time accurately noted, so that the time of reaction for each sugar tube was exactly the same. By thus controlling the reaction time satisfactory titration checks were obtained in dulpicate samples and there was less variation in the values obtained from day to day.

PROCEDURE

The following procedure was used for the determination of sorbitol and other sugar alcohols in the absorption studies reported later in this thesis.

Reagents

I. 1.06% potassium ferricyanide

II. 5% sodium sulphate in 3.33 N sodium hydroxide

III. 15% zinc acetate and 12% potassium iodide in combined solution

IV. glacial acetic acid

Pyrex sugar tubes were charged with 5 ml. of sorbitol solution containing 0.1 to 0.7 mg. sorbitol per 5 ml. solution, by means of a 5 ml. Ostwald-Folin blood pipette. Three water blanks were run simultaneously. To each sugar tube was added 3 ml. of Reagent I using a 3 ml. Ostwald-Folin blood pipette. 3 ml. of Reagent II were quickly blown in from a 3 ml. Ostwald-Folin blood pipette, charging 3 tubes at a time. Each of the three tubes was covered with a sealed glass bulb, mixed by lateral shaking and all three tubes immediately placed in a boiling water bath. The time required to fill three tubes with Reagent II and place them in the water bath did not exceed one minute. After heating the required time, each set of three tubes was plunged

immediately into a bath of cold water and cooled for five minutes. Reagents III and IV were mixed in the proportion of 1:1. 5 ml. of this solution were added to each tube, blown in rapidly by means of a fast flowing 5 ml. pipette. Each tube was mixed by lateral shaking. After five minutes the excess iodine was titrated with 0.005 N sodium thiosulphate, with a few drops of starch indicator added near the end point.

With the reagent and conditions above, 0.3 mg. of sorbitol produced a titration difference of 2.59 ml. of 0.005 N thiosulphate. Each ml. of titration difference would then represent 0.3/2.59 or 0.116 mg. of sorbitol. The factor 0.116 was used to calculate the amount of sorbitol present in solutions that gave a titration difference of 2 to 3 ml. 0.5 mg. of sorbitol gave a titration difference of 4.17 ml. of 0.005 N thiosulphate, or a factor of 0.5/4.17, or 0.120. The factor 0.120 was used to calculate the amount of sorbitol present in solutions that gave a titration difference of 4 to 5 ml.

When mannitol was determined by the above procedure 0.3 mg. of mannitol gave a factor of 0.112, and 0.5 mg. gave a factor of 0.115.

DETERMINATION OF SUGAR ALCOHOLS IN PHYSIOLOGICAL SOLUTIONS

Precipitating Agent

To apply the sorbitol method to physiological work it was necessary to remove interfering reducing substances as much as possible from the physiological solutions by some suitable precipitating agent. A 20 per cent solution of mercuric sulphate in 2 N sulphuric acid was chosen and its effect on sugar alcohols determined by adding it to known amounts of sorbitol and mannitol in water solution and neutralizing with solid barium carbonate.

Procedure

5 ml. of mercury sulphate reagent were added to the solutions of sorbitol or mannitol of known concentration and mixed by shaking. The solution was neutralized by adding about 3 gm. of barium carbonate. The flask was thoroughly shaken to blow off carbon dioxide, then stoppered and shaken cautiously, releasing the stopper often, until no further pressure developed, and the mixture was no longer acid to blue litmus. If necessary additional barium carbonate was added. The mixture was filtered through coarse paper. 1 gm. of zinc dust was added for each 10 ml. of filtrate for the removal of all traces of mercury, and the solution carefully filtered through No. 2 Whatman paper.

Determinations of the sugar alcohol were run on aliquots of this filtrate. Table I shows the recoveries obtained after treatment by this procedure.

TABLE I
Recovery of Sorbitol and Mannitol after Treatment with Mercury Sulphate Reagent

Mg.	Sugar Alcohol	Total ml. solution	Per cent Recovery
3.0	Sorbitol	35	106
5.5	Sorbitol	60	96
5.5	Sorbitol	60	98
			100% Average
3.0	Mannitol	35	110
5.0	Mannitol	50	99.6
5.0	Mannitol	100	108
			100.6% Average

Since the recoveries of sorbitol and mannitol were sufficiently accurate for our purpose the mercury sulphate precipitation was used on all physiological solutions before analysis.

Determination of Sorbitol and Mannitol in Urine

Known amounts of sorbitol and mannitol were added to urine and determinations made on the solutions after treating with mercury sulphate reagent. The following procedure was used:

Procedure for Urine

Solution I

10 ml. of urine were mixed with the appropriate volume of sugar alcohol solution of known concentration and this treated with 15 ml. of mercury sulphate. The volume was made to 100 ml. and after shaking thoroughly the mixture was neutralised with 24 gm. of barium carbonate and filtered. Zinc dust was added and the solution filtered as in the previous procedure. 10 ml. of this filtrate were diluted to 100 ml. in a volumetric flask.

Solution II

As not all of the reducing substances in urine are removed by this method it was necessary to determine the reduction value of urine without added alcohol to be used as a correction. The urine solution was prepared as follows:- To 10 ml. of urine was added 75 ml. of distilled water and 15 ml. of mercury sulphate reagent. The mixture was neutralised with barium carbonate and treated with zinc dust as above. 10 ml. of the final filtrate were diluted to 100 ml. in a volumetric flask.

Reduction values on aliquots of Solutions I and II were determined. The amount of reduction given by Solution I was due to sorbitol plus the reducing substances of urine. The reduction by Solution II was due to substances of urine alone, and gave a titration difference of 1 to 2 ml. of 0.005 N thiosulphate. To determine the sorbitol present in the urine solution the titration value given by Solution II was subtracted from the value for Solution I. This figure represented

the reduction due to the added sugar alcohol.

Table II gives the percentage recoveries of added sorbitol and mannitol from urine solutions treated by the above procedure.

TABLE II

Recovery of Sorbitol and Mannitol from Urine Solutions

	SORBITOL in 100 ml.			
	50 mg.		40 mg.	30 mg.
Per cent Recovery	94	78	94	93
	93	81	85	
	85	81	86	
	97	90	92	
	97	93		
	97	90		
	87	95		
	96	93		
	96			
Average	90.7%		89%	

	MANNITOL in 100 ml.		
	50 mg.		40 mg.
Per cent Recovery	79		94
	75		104
	95		92
	91		
	90		
	92		
Average	87%		96.7%

The results of the series of experiments shown in Table II indicate that sorbitol and mannitol in urine can be determined by the method with an accuracy of about ten per cent.

Determination of Sorbitol and Mannitol in Blood Solutions

Known amounts of sorbitol and mannitol were added to blood and determinations made on the mercury sulphate filtrates. As blood filtrates give a titration difference of 2 to 3 ml. by the method it was necessary to run determinations on blood and blood plus sugar alcohol. The following procedure was used:-

Solution I

3 ml. of blood and 84 ml. of distilled water were treated with 3 ml. of mercury sulphate. The mixture was shaken thoroughly, neutralized with about 7 gm. of barium carbonate, and filtered. Zinc dust was added and the solution filtered.

Solution II

Known amounts of sorbitol and mannitol containing 1 mg. of sugar alcohol per ml. of solution were added to blood and appropriately diluted with water. Mercury sulphate reagent was added in volume equal to that of the blood used. The solutions were neutralized with barium carbonate and treated with zinc dust as above.

Reducing values of 5 ml. aliquots of Solutions I and II were determined. The difference in reduction between Solutions I and II was calculated as sugar alcohol.

Table XII gives the percentage recoveries of sorbitol and mannitol from blood after treatment by the above procedure.

TABLE XII
Recovery of Sorbitol and Mannitol From Blood

ml. Blood	ml. Total Sol.	Added Sorbitol				
		2.0 mg.	3.0 mg.	4.0 mg.	5.0 mg.	7.0 mg.
5	60					
	Per cent Recovery	96	85	76	98	87
		59	69	79	87	
		130	100			
			75			

ml. Blood	ml. Total Sol.	Added Mannitol		
		2.0 mg.	3.0 mg.	4.0 mg.
4	60			
	Per cent Recovery	75	73	57
		72		51

ml. Blood	ml. Total Sol.	Added Sorbitol	Added Mannitol
		10 mg.	10 mg.
3	90		
	Per cent Recovery	62	48
		60	60
		60	54
		55	68
		76	72

Considerable variation was shown in determinations of sorbitol and mannitol. The mercury sulphate reagent was apparently unsatisfactory for use in blood solutions. The total dilution of blood and the amount of sugar alcohol added are both important factors in the determination. More work is needed before the method can be

successfully applied to the determination of sorbitol or the other sugar alcohols in blood.

PART II

THE ABSORPTION OF SUGAR ALCOHOLS FROM CLOSED LOOPS IN DOGS

INTRODUCTION

Recently some of the sugar alcohols, sorbitol and mannitol notably, have become available in quantities at a low price and sorbitol has been found to be an efficient physical diuretic (12). Other work in this laboratory has shown that sorbitol administered orally causes an increase in the liver glycogen of fasted rats. In view of the increasing interest in the physiology of the sugar alcohols a study of the rates of intestinal absorption of these compounds in comparison with glucose was undertaken.

A great deal of work has been done on the rates of absorption of various sugars from the intestinal tract. Cori in 1925 (13) made a study of the rates of absorption of hexoses and pentoses using the whole intestinal tract of rats. He found that 50 per cent solutions of the hexoses :- glucose, galactose, fructose and mannose - are absorbed from the intestinal tract of rats at a rate which is constant for each sugar. The following order in the rate of absorption was found:- galactose > glucose > fructose > mannose > xylose > arabinose. Cori also found the rate of absorption to be independent of the

absolute amount and also of the concentration of the sugar present in the intestine (14).

Magee and Reid (15) studied the absorption of glucose from the alimentary canal in the rat, cat, and rabbit and found a 0.75 M solution to be the optimal concentration for absorption of glucose by the living animal. They found this to be the optimal concentration for excised intestine as well. They advanced the theory that solutions stronger than 0.75 M were diluted to approximately this concentration before the bulk of the sugar was propelled into the duodenum and absorbed.

Mavdin, Johnston, and Morrison (16) used the modified Thiry loop in the unanesthetized animal for study of the absorption of glucose from the intestine. They found the rate of absorption of glucose from these loops was dependent in large measure on the concentration and volume of the solution used; increasing amounts were absorbed with increasing concentrations of glucose. Increasing the volumes of the same concentration increased the total amount absorbed. When the amount of sugar absorbed was plotted against time no linear relationship could be demonstrated. They found the amount of glucose absorbed in increasing time periods was dependent on the concentration and volume of the solution present during any given period. When the volume and concentration of the solutions introduced into the loop were kept constant and the time interval increased, no linear relationship between time and the amount absorbed could be demonstrated. From these studies they concluded that absorption from jejunal loops is affected by both the concentration and the volume of the solution in the

segment at any time period. When solutions of glucose varying in concentration from 3.5 to 50 per cent were placed in the dog's stomach they found the concentrations of the solutions recovered from the jejunum and ileum at the end of one hour to be remarkably constant. From these experiments they felt that it was impossible to compare data obtained where the gastro intestinal tract is used as a whole with that obtained from a single segment of this unit.

By introducing glucose directly into the intestine of the dog and continuously maintaining an excess of sugar at that location, Trimble and Maddock (17) found that the rate of absorption is not increased beyond the maximum obtained when the sugar was given by mouth. They did not find a 0.75 M (13.5%) solution to be the optimal one for absorption of glucose by the living animal, contrary to the reports of Magee and Reid (15).

The relative absorption rates of glucose and fructose were determined by Burget, Moore, and Lloyd (18), using three different methods: the closed intestinal loop in dogs (19), (20); loops of small intestine of anesthetized rabbits; and the gastro-intestinal tract of normal rats. The results of all three methods were in close agreement; glucose was absorbed slightly more rapidly than fructose. The closed intestinal loop as devised by Burget, Wartzloff, Suckow, and Thornton (20), (21) provides a method of studying absorption using loops that are viable, in a normal environment, and that can be used repeatedly. The unabsorbed residue of solution injected into such loops was found to be recoverable to within approximately

1 ml. of its total volume (19). In a later study Burget (22) found that repeated experiments on the closed loop of the same animal, using constant concentrations of sugar, showed quite constant results.

In the present study it was desired to make a comparison of the rates of intestinal absorption of sugar alcohols and glucose. Inasmuch as the various investigators are not fully in agreement as to the possibility of comparing data obtained by different methods it was necessary to choose one method for use throughout the study. The closed intestinal loop in dogs was the method chosen as these loops, barring complications, have been found to remain normal histologically and physiologically, and they digest and absorb food substances at normal rates (21). The dogs can be kept alive for periods of from several months to more than a year by proper care of the loop and the same animal can be used for repeated experimentation.

PROCEDURE

Absorption by Dog Loops

Injection of the sugar alcohol solutions into dog loops and removal of the solutions after the period of absorption were done by members of the Physiology Department. Equimolecular solutions of the sugar alcohols and glucose were used for comparison. The dog loops were tested for distention before carrying out each experiment and used for absorption only when they were found to be free of fluid. Before injecting the solution to be tested the loop was washed with warm, isotonic saline. 5 or 10 ml. of the sugar alcohol solution, depending on the size of the loop, were injected by hypodermic into the loop. After one hour the loop was aspirated and washed with warm, isotonic saline three times. The aspirated material and saline washings were carefully collected and determination of unabsorbed sugar alcohol made on this material.

Preparation of Loop Contents

The loop contents were diluted to 100 ml. in a graduate. 80 ml. of distilled water and 10 ml. of mercury sulphate reagent were added to 10 ml. of the diluted loop contents. After mixing well the solution was neutralised with about 15 gm. of barium carbonate and filtered

through coarse paper. One gm. of zinc dust was added to the filtrate for each 10 ml. of solution and the mixture carefully filtered through No. 2 Whatman paper. 10 ml. of this filtrate were diluted to 100 ml. in a volumetric flask and determination of sugar alcohol made on 5 ml. aliquots of this dilution.

When it was necessary to preserve the loop contents for a few hours or a day a few drops of toluene were added and the solution kept in the ice box. To remove the toluene before making the determination the loop contents were diluted to 100 ml. and 10 ml. of this dilution carefully pipetted, passing the pipette through the surface layer of toluene. 60 ml. of distilled water were added to the 10 ml. of loop contents and moist air was blown through the solution until all odor of toluene was removed. The solution was then treated as in the procedure above.

Upon several occasions saline loop washings were treated by the above procedure and gave no reduction. Added sorbitol was recovered quantitatively from them.

RESULTS

Table IV gives the absorption of sorbitol, glucose and erythritol by the loop of Dog #1 in a series of experiments carried out in previous work in this laboratory.

TABLE IV
Absorption of Sorbitol, Glucose, and Erythritol by the loop of Dog #1

	ml. given	gm. given	gm. Absorbed	% Absorption
Sorbitol				
10.46%	10	1.05	0.33	33
	10	1.05	1.32	31
19.24%	10	1.92	0.62	31
14.01%	10	1.40	0.48	34
13.44%	10	1.34	0.42	31.5
		Average	0.44 gm.	32%
Glucose				
10.74%	10	1.07	0.35	32.6
9.94%	10	0.99	0.32	31.9
		Average	0.33 gm.	33%
Erythritol				
10%	10	1.00	0.48	52
	10	1.00	0.46	54
	10	1.00	0.47	53
		Average	0.47 gm.	53%

Table V gives the absorption of mannitol, glucose, dulcitol, and erythritol by the loop of Dog #5. Determinations of glucose were made by the Shaffer-Hartmann method.

TABLE V
Absorption of Mannitol, Glucose, Dulcitol, Erythritol by the Loop of Dog #5

	ml. given	gm. given	gm. Absorbed	% Absorption
Mannitol 10%	5	0.47	0.16	36
	5	0.50	0.16	31
	5	0.50	0.25	50
	5	0.50	0.22	44
	5	0.50	0.24	48
	5	0.50	0.24	48
		Average	0.22 gm.	44%
Glucose 10%	5	0.46	0.35	75
	5	0.46	0.36	78
	5	0.50	0.35	70
		Average	0.35 gm.	74%
Dulcitol 6.2%	5	0.31	0.16	52
	5	0.31	0.20	61
	5	0.31	0.12	39
	5	0.31	0.14	45
	5	0.31	0.16	52
		Average	0.16 gm.	51%
Erythritol 6.8%	5	0.34	0.28	82
	5	0.34	0.27	79
	5	0.34	0.30	88
	5	0.34	0.30	88
		Average	0.29 gm.	84%

Table VI shows the absorption of mannitol, glucose, and duleitol by the loop of Dog #6.

TABLE VI
Absorption of Mannitol, Glucose, Duleitol
by the loop of Dog #6

	ml. given	gm. given	gm. Absorbed	% Absorption
Mannitol 10%	10	1.00	0.12 *	12 *
	10	1.00	0.19 *	19 *
	5	0.50	0.12	24
	5	0.50	0.10	20
	5	0.50	0.14	28
	5	0.50	0.11	22
	5	0.50	0.14	28
		Average	0.12 gm.	25%
Glucose 10%	5	0.50	0.27	54
	5	0.46	0.32	69
		Average	0.30 gm.	61%
Duleitol 6.2%	5	0.31	0.14	45
	5	0.31	0.13	42
	5	0.31	0.13	42
		Average	0.13 gm.	43%

* Not included in average.

Table VII shows the absorption of glucose and dulcitol by the loop of Dog #9.

TABLE VII
Absorption of Glucose and Dulcitol
by the Loop of Dog #9

	ml. given	gm. given	gm. Absorbed	% Absorption
Glucose 10%	5	0.50	0.46	96
	5	0.50	0.46	92
		Average	0.47 gm.	94%
Dulcitol 6.2%	5	0.31	0.16	52
	5	0.31	0.20	65
	5	0.31	0.13	42
	5	0.31	0.08	26
	5	0.31	0.12	39
		Average	0.14 gm.	45%

Table VIII shows the absorption of Mannitol by the loop in Dog #7.

TABLE VIII
Absorption of Mannitol by Loop of Dog #7

	ml. given	gm. given	gm. Absorbed	%Absorption
Mannitol 10%	5	0.50	0.14	28
	5	0.50	0.14	28
	5	0.50	0.12	24
	5	0.50	0.18	36
	5	0.50	0.16	32
	5	0.50	0.13	25
		Average	0.15 gm.	29%

Table IX shows the absorption of mannitol by the loop of Dog # 2.

TABLE IX

Absorption of Mannitol by the Loop of Dog #2

	ml. given	gm. given	gm. Absorbed	% Absorption
Mannitol 10%	10	0.98	0.18	19
	10	1.00	0.12	12
	10 *	1.00	0.16	16
	10 *	1.00	0.28	28
		Average	0.19	19

* Determinations made in previous work.

DISCUSSION

Sorbitol, mannitol, and dulcitol are isomeric hexahydric alcohols. Erythritol is a tetrahydric alcohol related to the sugar erythrose. It was possible to prepare equimolecular solutions of sorbitol, mannitol, and erythritol, but dulcitol is not sufficiently soluble to prepare a 10 per cent solution. At 40 degrees Centigrade a 6.2% solution of dulcitol can be prepared. This is the temperature at which the solutions were injected into the dog loops.

Comparison of the average rates of absorption of the sugar alcohols studied is shown in Table X.

TABLE X
Average Percentage Absorption of Sorbitol, Mannitol, Dulcitol, Erythritol, and Glucose

	Dog #1	Dog #5	Dog #6	Dog #7	Dog #7	Dog #8
10% Sorbitol	32%	---	---	---	---	---
10% Mannitol	---	44%	25%	---	29%	19%
6.2% Dulcitol	---	51%	43%	45%	---	---
10% Glucose	33%	74%	61%	94%	---	---
6.8% Erythritol	53%	84%	---	---	---	---

From Table X it is evident that individual animals differ considerably in their ability to absorb the various sugar alcohols. A comparison of relative rates of absorption must, then, be made on the same animal. Burget, Moore, and Lloyd (18) report a similar variation in the ability of individual animals to absorb levulose in a series of experiments on the relative absorption rates of glucose and levulose by closed loops in dogs.

Comparing the relative rates of absorption in each individual dog it is seen that in Dog #1 erythritol is absorbed more rapidly than glucose, and glucose more rapidly than sorbitol. In Dog #5 the order of absorption is as follows:- erythritol > glucose > dulcitol > mannitol > sorbitol. Dog #9 absorbs glucose more rapidly than dulcitol. No comparison was made in Dog #7 nor Dog #2. These series of experiments indicate a possible order of rate of absorption which can be shown as follows:- erythritol > glucose > dulcitol > mannitol > sorbitol.

As comparisons of rates of absorption were made in only four animals no definite conclusions can be drawn. The experiments indicate that there probably is a more or less definite order of rates of absorption for the various sugar alcohols such as has been found to hold true for the hexose and pentose sugars. The rate of absorption for any given sugar alcohol varies considerably with different animals. This is due partly to the variations in the size of the loops in the different animals.

Further work on the sugar alcohols is necessary to definitely establish their rates of absorption by the intestinal tract. It would be necessary in such a study to compare the rates of absorption of the various sugar alcohols in the same dog loop. A series of such studies made on a number of dogs would determine whether or not the relative rates of absorption of equimolecular solutions of the sugar alcohols are constant for the dog.

SUMMARY

1. A method of determining sugar alcohols, based on the Hagedorn Jensen glucose method, is given.

2. A mercury sulphate precipitation method for the preparation of physiological solutions for analysis is given.

3. Recoveries of sorbitol and mannitol from urine are shown.

4. The results of a study of the rates of absorption of the sugar alcohols from closed loops in dogs are shown.

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