METABOLISM OF *d*-MANNOHEPTULOSE. EXCRETION OF THE SUGAR AFTER EATING AVOCADO

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Roe and Hudson (1) administered *d*-mannoheptulose[1] to rabbits and concluded from their experiments that this sugar is available to this animal. The same authors (2) later concluded that the rat and the dog were unable to utilize the sugar. Unpublished experiments performed in this laboratory gave no evidence of glycogen formation when mannoheptulose was given by stomach tube to rats. In view of the fact that this sugar occurs free in the avocado, and as this fruit is becoming more popular, it seemed important to study the metabolism of mannoheptulose in man. Consequently, we have observed the excretion of fermentable and non-fermentable sugars after the ingestion of avocado or solutions of mannoheptulose.[2]

Methods

The subjects for the experiments were normal men and women employed in the laboratory. The experiments began at 9.30 a.m., when the fruit or sugar was ingested. Following this, 2 hour specimens of urine were collected up to the end of the 6th hour. An untimed preliminary specimen was included to show that abnormal quantities of sugar were not present. In the first two series of experiments breakfast was allowed and the usual midday meal came within the 2 to 4 hour period. In the last series of

[1] This sugar will be referred to simply as mannoheptulose throughout the paper.

[2] We are indebted to Dr. Carl L. Alsberg for calling our attention to the possibility that sugar might appear in the urine after avocado was eaten.

experiments the subjects had breakfast but did not have the midday meal, and the preliminary urine specimen was timed.

Approximate determination of the urine sugar concentration was made by Benedict and Osterberg's picrate method (3) and then appropriate dilutions were made for the determination by the method of Shaffer and Somogyi (4). Interfering substances were removed before this determination by shaking the diluted urine with Lloyd's reagent and permutit. The non-fermentable sugar values were obtained by fermentation with washed yeast (5). In several cases, mannoheptulose was determined by a modification of Roe's fructose method (6). This method[3] was carried out as follows: 2 cc. of urine were diluted 1:10 with 1 per cent acetic acid, treated with 0.2 gm. of acid-washed norit for 5 minutes, and filtered. To 2 cc. of this filtrate were added 4 cc. of a 0.1 per cent alcoholic resorcinol solution and 5 cc. of concentrated HCl solution containing about 1 per cent $SnCl_2$. The mixture was heated in a water bath at a temperature of 80° and then read in a colorimeter against a 0.02 or 0.03 per cent mannoheptulose standard treated in the same way. The color produced in the Seliwanoff reaction by mannoheptulose is much less than that given by fructose.

Mannoheptulose was isolated from the Trapp variety of avocado by the methods of La Forge (7) and Montgomery and Hudson (8).

EXPERIMENTAL

The Trapp variety of avocado was eaten by ten subjects in quantities ranging from 137 to 214 gm. Avocados are reported to contain from 3 to 7 per cent carbohydrate, but we do not have any values for their mannoheptulose content. The results of the experiments are given in Table I. It is seen that in every case there was a marked increase in the excretion of non-fermentable sugar which generally reached the maximum value in the 2 to 4 hour period. There were at least seven instances in which the concentration of non-fermentable sugar was enough to give a marked reduction with Benedict's qualitative copper reagent. The highest concentration obtained was 0.32 per cent

[3] We are greatly indebted to Dr. Joseph H. Roe for allowing us to use his unpublished method for mannoheptulose determination.

TABLE I

Excretion of Urine Sugar after Ingestion of Avocado

Subject	Avocado eaten	Time	Urine volume	Total fermentable sugar	Non-fermentable sugar		Total mannoheptulose as glucose
					Per 100 cc.	Total	
	gm.	*hrs.*	*cc.*	*mg.*	*mg.*	*mg.*	*mg.*
M. E.	138	Before	166	13	20	33	
		0–2 after	136	16	53	72	
		2–4 "	138	80	93	129	
		4–6 "	288	16	44	127	
L. McC.	156	Before	32	1	28	9	
		0–2 after	155	14	64	99	
		2–4 "	67	10	278	186	146
		4–6 "	103	8	121	125	
S. S.	214	Before	460	8	6	28	
		0–2 after	270	21	35	95	
		2–4 "	240	116	76	182	
		4–6 "	326	36	51	166	
M. C. E.	152	Before	117	43	52	61	
		0–2 after	208	26	61	127	
		2–4 "	80	23	211	169	157
		4–6 "	66	8	165	109	85
H. C.	137	Before	48	7	105	50	
		0–2 after	90	9	132	119	77
		2–4 "	82	21	306	251	228
		4–6 "	72	20	238	171	162
L. S.	190	Before	87	11	49	43	
		0–2 after	91	7	141	128	
		2–4 "	76	16	297	226	
		4–6 "	113	14	143	162	
A. L.	136	Before	125	10	38	48	
		0–2 after	112	9	139	156	
		2–4 "	124	15	205	254	225
		4–6 "	131	3	132	173	
J. Q.	167	Before	88	7	45	40	
		0–2 after	44	3	110	48	
		2–4 "	104	23	211	219	196
		4–6 "	142	2	113	160	
J. T.	197	Before	69	12	64	44	
		0–2 after	102	8	148	151	
		2–4 "	148	11	109	161	
		4–6 "	145	10	120	174	
B. C.	153	Before	6	1	49	3	
		0–2 after	77	11	154	119	93
		2–4 "	79	11	318	251	214
		4–6 "	93	8	162	151	115

non-fermentable sugar in the 2 to 4 hour period of subject B. C. Increased excretion of fermentable sugar was observed seven times in the 2 to 4 hour period. The values for mannoheptulose by Roe's method are seen to agree well with the non-fermentable

TABLE II

Excretion of Urine Sugar after Ingestion of d-Mannoheptulose

Subject	Mannoheptulose ingested	Time	Urine volume	Total fermentable sugar	Non-fermentable sugar		Total mannoheptulose as glucose
					Per 100 cc.	Total	
	gm.	*hrs.*	*cc.*	*mg.*	*mg.*	*mg.*	*mg.*
L. S.	10	Before	61	7	37	23	
		0– 2 after	95	25	203	193	175
		2– 4 "	61	22	272	166	145
		4– 6 "	82	21	221	181	151
		6– 7 "	36	3	125	45	
H. C.	5	Before	54	4	69	37	
		0– 2 after	85	14	173	147	140
		2– 4 "	96	24	195	187	167
		4– 6 "	74	20	160	118	99
		6– 7 "	36	5	103	37	
J. T.	5	Before	63	10	66	42	
		0– 2 after	91	16	202	184	169
		2– 4 "	145	22	133	193	
		4– 6 "	134	8	88	118	
		6– 7 "	60	5	66	40	
J. Q.	10	Before	48	8	42	20	
		0– 2 after	64	26	280	179	176
		2– 4 "	148	40	152	225	217
		4– 6 "	134	20	92	123	
		6– 7 "	62	6	61	38	
B. C.	5	0– 2 "	73	51	185	135	127
		2– 4 "	58	28	330	191	175
		4– 6 "	63	23	233	147	131
		6– 8 "	102	18	104	107	
		8–10 "	60	7	111	67	
		10–12 "	83	13	80	66	

values when account is taken of the normal content of these substances. 100 mg. of glucose are equivalent to 151 mg. of mannoheptulose when determined by the Shaffer-Somogyi method.

Mannoheptulose in quantities of 5 and 10 gm. was ingested by five of the same subjects who ate avocado. The results are

found in Table II. Again the peak of the excretion of mannoheptulose occurred late, either in the 2 to 4 or the 4 to 6 hour period. In four cases, most of the sugar was excreted by the end of the 6th hour, as indicated by the drop in non-fermentable sugar values to the preliminary levels. In the other case, the fall in non-fermentable sugar content took place in the 8 to 10 hour period. It is interesting that the two subjects who ingested 10 gm. of mannoheptulose excreted no more sugar than did those who took 5 gm. Furthermore, the mannoheptulose excreted after the sugar was taken was comparable in amount to that put out after the avocado was eaten. In this series of experiments, there were at least ten specimens containing enough non-fermentable sugar to give unmistakable reduction of Benedict's sugar reagent. The highest concentration was 0.33 per cent in the 2 to 4 hour specimen of subject B. C. Calculation of the amount of mannoheptulose recovered in the urine shows that about 5 per cent was thus accounted for when 5 gm. were ingested. Small increases in the excretion of fermentable sugar were again obtained.

Another series of experiments on the same subjects as in the second series was undertaken to learn more about the excretion of fermentable sugar after the ingestion of mannoheptulose. In these experiments the subjects went without the midday meal. The results are recorded in Table III. Again it is seen that several of the specimens contained enough non-fermentable sugar to reduce Benedict's qualitative sugar reagent strongly. The highest concentration of non-fermentable sugar occurred in the 6 to 7 hour period of subject B. C. Four of the subjects had the greatest rate of non-fermentable sugar excretion in the 2 to 4 hour period, while the other had practically identical rates in the 2 to 4 and 4 to 6 hour periods. In no case did the rate of non-fermentable sugar excretion of the 6 to 7 hour period approach the preliminary value, showing that the excretion of mannoheptulose was not yet completed. All of the subjects apparently excreted more fermentable sugar after the ingestion of mannoheptulose. However, we believe that these increases are fictitious and are dependent upon the method used in determining fermentable and non-fermentable sugar by yeast. For instance, when approximately 0.2 per cent mannoheptulose was added to a normal urine con-

taining 5 mg. of fermentable sugar per 100 cc., there was an apparent increase of 24 mg. per 100 cc. in this fraction. Similar results were found after the addition of 0.4 per cent mannoheptulose, and 0.2 and 0.4 per cent *l*-xylose to normal urine. It seems probable that the apparent increases in fermentable sugar

TABLE III

Excretion of Urine Sugar after Ingestion of 5 Gm. of d-Mannoheptulose

Subject	Time	Urine volume	Fermentable sugar	Non-fermentable sugar	
	hrs.	*cc.*	*mg. per hr.*	*mg. per 100 cc.*	*mg. per hr.*
L. S.	1.5 before	96	4	38	24
	0–2 after	117	7	130	76
	2–4 "	100	16	200	100
	4–6 "	82	11	183	75
	6–7 "	35	6	165	58
H. C.	1.66 before	37	6	76	17
	0–2 after	72	17	202	73
	2–4 "	79	14	233	92
	4–6 "	60	10	215	65
	6–7 "	24	10	190	46
J. T.	1.66 before	82	6	28	14
	0–2 after	111	22	112	62
	2–4 "	110	30	206	113
	4–6 "	86	13	214	92
	6–7 "	33	12	200	66
J. Q.	2.5 before	66	2	43	11
	0–2 after	72	5	135	49
	2–4 "	76	4	220	84
	4–6 "	94	5	124	58
	6–7 "	30	1	120	36
B. C.	2.0 before	43	5	66	14
	0–2 after	100	23	180	90
	2–4 "	87	20	205	89
	4–6 "	62	13	231	72
	6–7 "	26	9	269	70

under such conditions may be due to adsorption on the yeast which is present in great excess.

The fluid intake in the above experiments was purposely restricted in the hope that highly concentrated specimens of urine would be obtained, so that the osazone of mannoheptulose could be isolated and recrystallized. We were successful in obtaining

the characteristic spherulites described by Wright (9) but failed to recrystallize them.

Mannoheptulose has the property of reducing alkaline copper sugar reagents at temperatures under the boiling point. This property serves the useful purpose of helping to identify the sugar. The test of Lasker and Enklewitz (10) for xylulose is thus positive for mannoheptulose, as well as for xylulose and fructose. Mannoheptulose and fructose are positive in the Seliwanoff test but the first is not fermented by yeast while the latter does ferment. Neither mannoheptulose nor xylulose is fermented by yeast and both are positive in Bial's test, while fructose is negative. Mannoheptulose in Bial's test gives a reaction which may be easily mistaken for xylulose, differing in that a fleeting red color is produced before the blue-green color appears. The final reaction product for the two sugars is practically indistinguishable. Application of Tauber's (11) color test for pentoses enables one to decide which sugar is present. Mannoheptulose reduces the common sugar reagents much less strongly than does glucose. We found that 100 mg. of glucose are equivalent to 151 mg. of mannoheptulose when determined by the Shaffer-Somogyi method.

DISCUSSION

Our results show that roughly 5 per cent of the mannoheptulose ingested appeared in the urine within 6 hours after 5 gm. had been taken. No greater amount appeared when 10 gm. were ingested. This would indicate that 5 gm. were enough to furnish the quota required by the low coefficient of absorption of the sugar. Excretion of the sugar began to decline after 6 hours. The bulk of the sugar seems to have been disposed of otherwise than through absorption and excretion. It may have been converted to a utilizable carbohydrate, as suggested by the experiments of Roe and Hudson on rabbits, or it may have suffered change in the gastrointestinal tract. Unpublished experiments on rats performed in this laboratory did not show that the sugar was stored as such in the tissues, so we do not consider it likely that such storage occurred in the experiments on man. The results fail to show whether or not mannoheptulose is available to man.

The experimental findings reveal that mannoheptulose may appear in the urine after the avocado is eaten. Furthermore, the concentration of the sugar in the urine may be sufficiently high to

cause reduction of the alkaline copper sugar reagents and lead to a false diagnosis of diabetes mellitus. The simple test of adding 1 cc. of urine to 5 cc. of Benedict's qualitative sugar reagent and allowing the mixture to stand overnight at room temperature will detect xylulose, fructose, and mannoheptulose. Glucose will reduce under these conditions only when its concentration is 2 per cent or higher. This test should be made whenever sugar is found in the urine of a patient seen for the first time. It should perhaps be recalled that xylulose will always be found in the urine of persons having essential pentosuria, but that fructose, in cases of essential fructosuria, and mannoheptulose will occur only when the appropriate foods have been eaten.

SUMMARY

It was shown that mannoheptulose appears in the urine of normal persons after the avocado is eaten. The concentration of sugar was high enough to cause reduction of the ordinary sugar reagents, thus becoming another source of confusion in the diagnosis of diabetes mellitus. The peak of excretion of mannoheptulose usually occurred in the 2 to 4 hour period after the avocado was eaten or after the ingestion of the sugar. The rate of sugar excretion began to decrease 6 hours after consumption of the sugar. Approximately 5 per cent of the sugar appeared in the urine during a period of 6 hours after 5 gm. of the sugar were ingested. The fate of the remainder of the sugar was not determined.

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