THE UTILIZATION OF *d*-MANNOHEPTULOSE (*d*-MANNOKETOHEPTOSE) BY ADULT RABBITS

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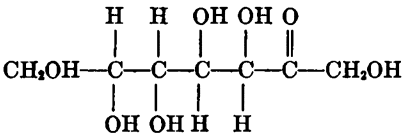
d-Mannoheptulose, a 7-carbon ketose, was isolated from the fruit of the avocado tree, *Persea gratissima*, by La Forge (1) in 1917. La Forge found the configuration of this sugar is as follows:



This ketoheptose occurs in the free state in the avocado fruit. The fact that this fruit has been regarded for centuries as an esteemed food made it seem probable that this sugar is assimilated by animals. Such a finding, if demonstrated, would be interesting because no 7-carbon monosaccharide has so far been observed to be utilized by the animal organism. A study of the physiological availability and metabolism of this sugar has therefore been undertaken.

d-Mannoheptulose Tolerance

Preparation of Mannoheptulose—The mannoheptulose used in the experiments reported in this paper was prepared from avocado pears by Miss Edna Montgomery at the National Institute of Health following the method of La Forge (1). The product was of the highest purity, being free from perseitol and *l*-arabinose, and having an $[\alpha]_D^{20}$ value of $+29.3°$ in water and a melting point of 152°.

Method for Determination of Mannoheptulose in Blood

Mannoheptulose reduces alkaline copper solutions and is not fermented by bakers' yeast. These properties were made the basis of a specific method for the determination of mannoheptulose in blood. In this procedure the blood is deproteinized by the $Zn(OH)_2$ method of Somogyi (2) and the filtrate is treated with thoroughly washed Fleischmann's yeast. The residual reducing substance after yeast fermentation is then determined by the copper reduction method of Benedict (3), a solution of mannoheptulose dissolved in saturated benzoic acid being used as a standard. Tests in which mannoheptulose was added to dog blood gave a 107 per cent recovery, which is very satisfactory in view of the fact that the Benedict procedure gives a slight blank with $Zn(OH)_2$ blood filtrates.

Procedure

Our first studies were of the nature of carbohydrate tolerance tests. Rabbits were fasted for 24 hours. After the urine was expressed from the animal's bladder and a control sample of blood was collected from the marginal ear vein, mannoheptulose was administered by mouth in some experiments and intraperitoneally in others. Samples of blood were then collected at hourly intervals for 4 hours, and the urine for 24 hours after administration was collected under toluene. The bloods were analyzed for total sugar by the Benedict method (3) and for mannoheptulose by the procedure described above. The total reducing substance in the 24 hour sample of urine was determined by the Shaffer-Somogyi method (4), with the latter authors' Reagent 50 with 5 gm. of KI.

Results of Tolerance Studies

The results of these experiments are shown in Table I. Following the administration of mannoheptulose, some mannoheptulose appeared in the blood as such and there was a marked increase in the total blood sugar. The concentrations of mannoheptulose in the blood were comparatively small, except in the experiment with Rabbit 5, in which a very large dose of the sugar was administered intraperitoneally. The mannoheptulose also stayed at a fairly constant level, while the total blood sugar showed a gradual increase, the last sample of blood collected having the highest value.

The mannoheptulose appearing in the blood will not account for the increments in total blood sugar; hence, the increases in total blood sugar are increases in reducing substance which is fermentable with yeast. These data therefore show that mannoheptulose undergoes a metabolic transformation in the rabbit in which fermentable reducing substance is formed.

That mannoheptulose was excreted in the urine in the experiments of Table I was demonstrated by boiling the urine with HCl

TABLE I

Data Showing Mannoheptulose Tolerance of Normal Rabbits

Rabbit No.	Weight	Method of administration	Dose	Blood sugar, mg. per 100 cc. as glucose						Urine sugar Reducing substance in urine collected for 24 hrs. after administration, as mannoheptulose	
				Determination	Hrs. after administration						
					0	1	2	3	4		Excretion
	kg.		*gm. per kg.*							*gm.*	*per cent*
1	2.37	By mouth	2	Total sugar	91	116	126	132			
				Mannoheptulose		19	21	20		0.312	6.5
2	2.50	" "	5	Total sugar	92	115	125	131	135		
				Mannoheptulose		22	21	21	24	0.750	6.0
3	2.39	" "	5	Total sugar	60	100	115	121	126		
				Mannoheptulose		17	16	17	17	0.267	2.2
4	2.52	Intraperitoneally	2	Total sugar	68	80	89	92	91		
				Mannoheptulose		15	14	14	15	0.315	6.2
5	2.32	"	5	Total sugar	101	428	485	502	509		
				Mannoheptulose		212	212	207	204	4.588	39.5

and testing for furfural, a reaction for mannoheptulose to which La Forge (1) called attention. The true mannoheptulose excretion was actually less than that indicated by the values shown in Table I, however, since there is some reducing carbohydrate in normal rabbit urine and the Shaffer-Hartmann reagent is sensitive to non-sugar reducing moieties of urine.

The urinary excretion of mannoheptulose in these experiments was small, considering the dosages used. In four of the experiments the total reducing substance in the urine ranged from 2.2 to

6.5 per cent of the mannoheptulose administered. In one experiment the urine for a second 24 hours after mannoheptulose administration was examined and did not show any more reducing substance than normal rabbit urine, thus eliminating the possibility of a delayed excretion of sugar. In the experiment with Rabbit 5 there was considerable excretion of mannoheptulose (39.5 per cent), but this result was obtained by intraperitoneal injection of a large dose of the sugar, an exaggerated experimental procedure in which the animal's body was rapidly flooded with mannoheptulose. The two experiments in which doses of 5 gm. per kilo of body weight were administered by mouth and not over 6 per cent of the sugar was excreted in the urine are especially significant and show that the rabbit has a high tolerance for mannoheptulose.

The above data obtained from an examination of the blood and urine following mannoheptulose administration thus seem to indicate that mannoheptulose is well utilized by the rabbit.

Rôle of Liver

Procedure

An experimental procedure was developed to determine the concentration of sugar of the afferent and efferent blood to the liver after mannoheptulose administration. In the experiments of Table II, Rabbits 1 and 2 were given mannoheptulose in 10 per cent solution by mouth and approximately 1 hour after administration the animals were anesthetized with nembutal and small amounts of ether. A longitudinal slit was made in the rabbit's abdomen and samples of blood were collected simultaneously from the portal vein and from one of the hepatic veins. In the experiments with Rabbits 3 and 4, the animals were anesthetized similarly and, after the abdomen was opened, mannoheptulose solution was injected into the duodenum. The animal's abdomen was then closed by means of clamps and, 30 minutes after injection of the sugar, samples of blood were collected simultaneously from the portal vein and a hepatic vein. The samples of blood were analyzed for total sugar and for mannoheptulose.

Results

The data of Table II show a low concentration of mannoheptulose in the portal blood. This was due either to slow absorption

of this sugar from the intestinal tract, or to a metabolic conversion of mannoheptulose into fermentable reducing substance in passing through the walls of the intestine. As the total sugar determinations reveal that there was a considerable increase in the total sugar of the portal blood, which was fermentable, the latter postulation is strongly suggested. These results also are of interest in that they show an apparently negative part played by the liver. The differences in the concentrations of mannoheptulose of the simultaneously collected samples of portal and hepatic blood are within the limits of experimental error and therefore seem to indicate that the liver does not participate in the metabolic transformation of mannoheptulose in the rabbit.

TABLE II

Rôle of Liver in Mannoheptulose Metabolism

The total sugar and mannoheptulose of afferent and efferent blood to the liver after administration into the alimentary tract of mannoheptulose are measured in mg. per 100 cc. as glucose.

Rabbit No.	Dose	Vein	Total sugar	Mannoheptulose	Change in mannoheptulose
	gm.				
1	10	Portal	94	20	
		Hepatic	99	25	+5
2	12	Portal	130	16	
		Hepatic	190	16	0
3	5	Portal	178	26	
		Hepatic	180	26	0
4	12	Portal	178	27	
		Hepatic	199	24	−3

Effect of Insulin

Crystalline insulin, of a potency of 18 units per mg., dissolved in 0.01 N HCl was used in these experiments. 3 units of the solution used produced convulsions in fasted 2 to 2.5 kilo rabbits within $1\frac{1}{2}$ to $2\frac{1}{2}$ hours after subcutaneous injection.

Two rabbits, fasted for 24 hours, were given 3 units of insulin subcutaneously. When the animals had convulsions, a solution containing 10 gm. of mannoheptulose was injected intraperitoneally. One animal survived 2 hours and the other one $3\frac{1}{2}$ hours after the onset of convulsions. Samples of blood were collected from the heart immediately after the death of the animals and were

analyzed for total sugar and for mannoheptulose. In the blood of one rabbit the total sugar and mannoheptulose were 296 and 302 mg. per 100 cc., respectively; for the other rabbit the total sugar and mannoheptulose were 325 mg. per 100 cc. of blood. Thus the analyses of postmortem blood from these two animals showed mannoheptulose present in large amounts, but *complete absence of fermentable sugar.* In the experiment with Rabbit 5 of Table I, an exactly parallel procedure except that insulin was not administered, there were present in the blood around 200 mg. of fermentable sugar per 100 cc. at approximately the same time after intraperitoneal injection of mannoheptulose. *Thus, when insulin was not injected, mannoheptulose administration brought about a marked increase in the fermentable sugar of the blood; and, when insulin was injected, fermentable sugar completely disappeared from the blood after mannoheptulose administration.* These data reveal that mannoheptulose is not a direct physiological antagonist to insulin, as there was a high concentration of this sugar in the blood of the two rabbits at the time of death; and they appear to indicate that insulin accelerates the oxidation of the fermentable substance which results from the metabolic transformation of mannoheptulose.

Further experiments were performed which corroborate the above findings. Two rabbits of approximately the same size as the two in the above experiment were fasted for 24 hours. 3 units of insulin were injected into each rabbit and at the same time 5 gm. of mannoheptulose in 10 per cent solution were given by mouth and 5 gm. of the sugar in a solution of the same concentration were injected intraperitoneally. One rabbit had convulsions twice, but recovered completely and survived; the other rabbit survived without any convulsions. It thus appeared from these experiments that mannoheptulose gives some protection against toxic doses of insulin, and if given at the same time that the insulin is administered, it may completely protect the animal against a minimum lethal dose of insulin.

In another experiment a rabbit was given 6 units of insulin and 10 gm. of mannoheptulose by mouth at the same time. This rabbit did not survive. In this experiment the rate of mannoheptulose conversion into the protective metabolite was not rapid enough to protect against a large dose of insulin.

DISCUSSION

The data of this report show that mannoheptulose, when administered to rabbits, gives rise to the formation of a yeast-fermentable, copper-reducing substance in the blood of the animals. The removal of this substance from the blood is stimulated by insulin. The identity of this metabolite is obviously of considerable interest. In view of its behavior with yeast, with alkaline copper solution, and with insulin, properties corresponding to those exhibited by glucose and fructose, an interesting physiological finding, the metabolic transformation of a 7-carbon sugar into a sugar of lower carbon content, seems a possibility. Further work is planned to determine the identity of this substance.

SUMMARY

1. Data have been obtained which show that mannoheptulose is physiologically available to the rabbit and that rabbits have a high tolerance for this sugar.

2. In the rabbit mannoheptulose is converted into a yeast-fermentable, copper-reducing metabolite. The removal of this substance from the blood is stimulated by insulin.

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