THE NATURE OF THE REDUCING SUBSTANCE IN THE URINE OF INFANTS WITH NUTRITIONAL DISORDERS *

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The occurrence of a reducing substance in the urine of infants affected with gastro-enteric or nutritional disorders has long been recognized. Grosz¹ examined the urine of fifty breast fed infants and the urine of ten reduced Trommer's and Nylander's solutions. In no case did the urine ferment with yeast. Most of the positive results were from infants ill with gastro-enteric disorders. Grosz observed that melituria diminished when tea was substituted for breast milk, and again increased when the diet of breast milk was resumed. He thought that the reducing action of the urine was due to lactose or to a split product of lactose.

Orban² concluded that the reducing substance which commonly occurs in the urine of infants with gastro-enteric disturbances is lactose. Tests of the feces in such cases showed the absence of lactase, the ferment which splits lactose into glucose and galatose. It is well known that unaltered lactose cannot be utilized by the organism. If lactose enters the blood stream, it is eliminated in the urine. Due to the supposed absence of the specific lactose splitting enzyme (lactase), Orban considered that unchanged lactose was absorbed with resulting lactosuria.

Langstein and Steinitz³ examined the urine of thirty-five infants affected with gastro-enteric disorders. They concluded that the reducing action of the urine in such cases is always due, in part at least, to lactose. In a few cases they felt that they demonstrated the presence of galactose in addition to lactose. Contrary to Orban, they found that lactase was present in the intestinal contents of infants with

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1. Grosz, J.: *Jahrb. f. Kinderh.* **34**:83, 1892.

2. Orban: *Prag. med. Wchnschr.* **24**:427, 441, 454, 1899.

3. Langstein, L., and Steinitz, F.: *Beitr. z. Chem. Phys. u. Path.* **7**:575, 1906.

glycosuria. They, therefore, concluded that the presence of lactose in the urine was not due to absence of lactase but was dependent on some intestinal injury which permitted the absorption of unchanged lactose.

During the past seven years I have examined the urines of infants affected with severe gastro-enteric or nutritional disorders with the object of determining the nature of the reducing agent when such was present. In the beginning, the urines were examined by practically every method which might elucidate the problem. As the work progressed, it became evident that many of the procedures were useless. It was found that an accurate determination of the nature of the reducing substance offered great difficulties owing to the small amount present in the urine and also in many cases to the diminished secretion of urine which is common in the gastro-enteric disorders of infants. Many of the available tests for the identification of sugars in dilute solution are inexact and uncertain when applied to urine containing less than 0.2 per cent. of sugar. This is generally true, but applies particularly to the identification of osazones and to polariscopic examination when only a small percentage of sugar is present. The fermentation test is inexact when the sole criterion is the formation of visible gas. Carbon dioxide is soluble to a degree in urine. The test, however, is of distinct value when used to determine whether the reducing substance is partly or completely destroyed by yeast with the formation of alcohol. In this manner, the test is useful even though the amount of sugar is too small to give rise to visible gas.

Before considering the tests to determine the nature of the reducing substance in the urine of infants with gastro-enteric disorders, it is necessary to consider briefly the question of urine sugar in general. For some time it has been considered probable that all normal urines contain reducing sugar. Recently Benedict and his collaborators⁴ have shown that sugar is always present in the urine of normal adults and by means of fermentation with yeast can be divided into two fractions, one fermentable and one nonfermentable. They also found that the amount of urine sugar is dependent largely on the sugar intake. We have found that the same condition pertains to the urine of infants.⁵ Since sugar is always present in normal urine, an estimate of its significance must be made on a quantitative basis. The presence or absence of reduction of copper test solutions is not sufficient, as such tests are purely qualitative and may be misleading. If the secretion of urine is less than normal, it can contain sufficient sugar to reduce a copper

4. Benedict, S. R.; Osterberg, E., and Chenowith, I.: *J. Biol. Chem.* **34**: 217, 1918.

5. Unpublished results of Dr. E. Harrington and myself. Also Greenthal, R. M.: *Am. J. Dis. Child.* **20**:556 (Dec.) 1920.

test solution and yet the total sugar eliminated may be abnormally small. Reversely, if there is diuresis, the total sugar elimination may be increased in the presence of a negative qualitative test.

For this reason, as pointed out by Benedict,⁴ the use of the term glycosuria in its generally accepted meaning is no longer accurate. It has been used to designate the reduction of a copper test solution with the idea that sugar was an abnormal constituent of urine and was detected in this manner. Owing to the inaccuracy of the term glycosuria, Benedict has proposed the term glycuressis to designate an increased sugar elimination.

TABLE 1.—DETERMINATION OF THE REDUCING SUBSTANCE BY THE QUANTITATIVE METHOD OF BENEDICT

Name	Age, Mos.	Diet*	Reducing Substance in Urine, Calculated As Glucose, per Cent.	Fermentation With Yeast for 24 Hours		
				Gas Production	Reduction of Benedict's Qualitative Reagent after Fermentation	Nonfermentable Reducing Substance, Calculated as Glucose, per Cent.
A. T.	3½	P. M.	1.1	++	+	Too small to determine
J. A.	4	B. W.	0.95	0	—	Too small to determine
J. Q.	5	P. M.	1.8	++	+	Too small to determine
W. Z.	5	W.	0.6	0	—	Too small to determine
A. B.	6	P. M.	1.63	++	+	Too small to determine
B. Y.	6½	P. M.	2.1	+++	+	0.68
T. J.	7	P. M.	0.63	0	—	0.72
C. M.	8	P. M.	0.22	0	—	Too small to determine
N. C.	8	B. W.	1.16	++	+	Too small to determine
W. T.	8½	P. M.	0.19	0	—	Too small to determine

* The diets given were the ones given during the period of urine collection. P. M. = protein milk (Finkelstein's formula); B. W. = 2 per cent. barley water; W = water.

Eighty-two patients were observed, from whom 196 specimens of urine were examined. All of the patients were affected with severe nutritional disturbances characterized by diarrhea, refusal of food, vomiting or loss of weight. The greater number of patients showed the presence of toxic symptoms. The urine of sixty-three infants reduced Benedict's qualitative reagent. The amount of the reducing substance was determined in sixty-five specimens of urine from thirty-eight infants by Benedict's quantitative reagent, Bertrand's method or the micro-method of Benedict and Osterberg.⁶ The amount of reduc-

6. Benedict, S. R., and Osterberg, E.: J. Biol. Chem. **34**:195, 1918.

ing substance calculated as glucose varied from 0.12 to 2.1 per cent. Some of the results are shown in Tables 1 and 2.⁷

The glycosuria in these cases is of relatively short duration, so that repeated examinations of the reducing substances in the urine of the same patient is often impossible. As a rule, glycosuria occurs only during the period of acute illness, and the patient either succumbs or improves within a few days. With improvement, the glycosuria diminishes markedly and subsides. The duration of glycosuria varies greatly, but in most cases covers a period of from one to four days.

TABLE 2.—DETERMINATION OF THE REDUCING SUBSTANCE BY THE MICRO-METHOD OF BENEDICT AND OSTERBERG

Name	Age, Mos.	Diet*	Reducing Substance in Urine, Calculated As Glucose, per Cent.	Fermentation With Yeast for 24 Hours		
				Gas Production	Reduction of Benedict's Qualitative Reagent after Fermentation	Nonfermentable Reducing Substance, Calculated as Glucose, per Cent.
S. C.	3	P. M.	0.582	0	—	0.184
M. D.	3	P. M.	0.163	0	—	0.063
D. W.	4	P. M.	0.52	0	+	0.192
N. T.	4½	B. W.	0.338	0	—	0.097
A. T.	4½	P. M.	0.479	0	—	0.106
T. J.	5	W.	1.267	++	+	0.410
B. W.	5	P. M.	0.534	0	+	0.146
A. Z.	5	P. M.	0.293	0	—	0.072
D. W.	6½	P. M.	0.736	+	+	0.214
P. J.	7	P. M.	0.496	0	—	0.076
B. D.	7	P. M.	0.638	0	+	0.180
W. M.	7	B. W.	1.347	++	+	0.430
A. E.	8	B. W.	0.920	+	+	0.215
F. J.	8½	W.	0.464	0	—	0.093

* The diets given were the ones given during the period of urine collection. P. M. = protein milk (Finkelstein's formula); B. W. = 2 per cent. barley water; W = water.

The short duration of the sugar elimination in this variety of case is in marked contrast to the continuous glycosuria associated with the regular ingestion of large amounts of sugar. A consideration of the latter type will be the subject of a separate communication.

As shown in the tables, fermentation by yeast always caused a marked diminution or entire disappearance of the reducing substance. In only nine specimens, however, was there visible gas production. These were the ones which contained the largest amount of reducing substance. In order to estimate correctly the amount of the reducing substance destroyed by yeast, it is necessary to consider the delicacy of the tests used. The method of Benedict and Osterberg is sufficiently delicate to show that the reducing power is practically never completely destroyed by yeast. Copper reduction tests are much less delicate and will be negative in the presence of amounts of sugar easily demonstrable

7. These results are from infants showing the largest amounts of urine sugar.

by the method of Benedict and Osterberg. The relation is largely quantitative. As a rule, tests with Benedict's qualitative copper solution are positive when the amount of sugar (estimated as glucose) is approximately 0.1 per cent. This variation in the results obtained with the two methods is apparent by comparing Tables 1 and 2.

These results show definitely that the greater amount of the reducing substance is fermentable and, therefore, they do not agree with the observations of Grosz, Langstein and Steinitz and others who found that the reducing substance was not fermented by yeast. The fact that in my cases the reducing substance was largely destroyed by yeast, indicates definitely that it was not lactose. Whether the fraction of the reducing substance not fermented by yeast was lactose is impossible to say. I am aware of no facts which bear directly on this question. This substance is usually present in amounts too small to determine its nature by the ordinary tests. Moreover, it always occurs in normal urine. It is of interest that in a number of cases the urine after fermentation still reduced Benedict's qualitative reagent (Tables 1 and 2). It is possible that part of this reducing action may have been due to the presence of a small amount of lactose in addition to the usual nonfermentable sugar of urine. Definite tests bearing on this question were not made.

Additional evidence that the reducing substance is not lactose was obtained by preparing osazones from ten specimens of urine. These specimens all contained 1 per cent. or more of reducing substance calculated as glucose so that sufficient osazones were obtained for purification. Their solubility, appearance and melting point indicated that the sugar was not lactose but was probably glucose (Table 3).

TABLE 3.—APPEARANCE, SOLUBILITY AND MELTING POINT OF OSAZONES

Name	Percentage of Reducing Substance Calculated as Glucose	Appearance of Osazone	Melting Point of Osazone, Degrees C.	Solubility of Osazone in Hot Water
A. J.	0.962	Glucosazone	205.6	Not perceptably soluble
T. P.	1.1	Glucosazone	203.8	Not perceptably soluble
W. A.	1.3	Glucosazone	204.0	Not perceptably soluble
T. J.	0.873	Glucosazone	204.6	Not perceptably soluble
O. M.	1.6	Glucosazone	205.0	Not perceptably soluble
J. W.	1.25	Glucosazone	206.2	Not perceptably soluble
W. S.	0.92	Glucosazone	206.0	Not perceptably soluble
O. W.	1.4	Glucosazone	205.2	Not perceptably soluble
F. A.	0.775	Glucosazone	205.8	Not perceptably soluble
A. L.	0.18	Glucosazone	204.4	Not perceptably soluble

The fact that the main portion of the reducing substance is fermentable indicates that it is glucose or galactose. The speed with which fermentation occurs points to the former. Galactose is fermented by yeast but more slowly than glucose. Definite data bearing on this

question were obtained in six cases. In these the urine contained more than 1 per cent. of reducing substance calculated as glucose. Quantitative determinations by means of Bertrand's method were compared with the rotation of polarized light. The values always corresponded much more closely with the specific rotation of glucose (52.5) than with galactose (81). For example: a specimen of urine gave a copper precipitate which was equivalent to 2.12 per cent. glucose. Calculated as galactose the percentage was 2.14 per cent. In a 2 decimeter polarimeter tube the rotation was 2.02 degrees, which calculated as glucose was equivalent to 1.92 per cent. sugar. Calculated as galactose the percentage was 1.24.

TABLE 4.—INFLUENCE ON URINE SUGAR OF CHANGE OF DIET FROM PROTEIN MILK TO WATER. URINE COLLECTED FOR PERIOD OF 8 HOURS AND SUGAR OUTPUT FOR 24 HOURS CALCULATED FROM THIS

Name	Protein Milk	Water
	Sugar Output, Mg. per 24 Hours Calculated as Glucose	Sugar Output, Mg. per 24 Hours Calculated as Glucose
Gerald.....	640*	128*
Ellwood.....	506*	420*
Miller.....	610†	615†
Geyer.....	395*	340*
Cereale.....	820*	195*
Kroph.....	560†	470†
Batchford.....	718†	230†
McLean.....	483†	406†
O'Neill.....	540*	204*
Lunetti.....	762†	607†
Smith.....	491†	602†
Peny.....	480*	163*
Barrows.....	623*	201*
Kintas.....	350*	350*
Miller.....	963*	770*
Sweeney.....	820	695*

* Determined by micro-method of Benedict and Osterberg.

† Determined by the quantitative copper solution of Benedict.

The results of these determinations also offer additional evidence that the reducing agent was not lactose. Using the example cited, if the reducing action of the urine was due to lactose, the percentage would have been about 2.9. Calculated by the rotation, the percentage of lactose would have been 1.29. The mucic acid test was applied to these urines with uniformly negative results.

In addition to the chemical evidence there are a number of clinical observations which have a direct bearing on the nature of the reducing substance. If this substance is lactose or galactose it should be present in the urine only when lactose is ingested. This is not the case. Six infants with severe nutritional derangement (intestinal intoxication), whose urine showed marked reducing action, had received water or barley water alone for from sixteen to twenty-four hours.

Sixteen infants whose urine exerted a marked reducing action on copper solutions while receiving protein milk (Finkelstein's formula) as food were given water alone for from ten to eighteen hours. In six cases the reducing power of the urine was markedly decreased. In ten cases a quantitative determination showed only slight diminution of the reducing action of the urine (Table 4).

In consideration of these clinical tests alone, it seems inconceivable that the reducing action of the urine was due to lactose. No lactose was ingested and so far as is known the infant organism is incapable of storing or synthesizing lactose. On the basis of such evidence it seems that the reducing agent could only be glucose, the sugar of the body fluids.

While the results cited seem to warrant the conclusion that the reducing action of the urine of infants with nutritional disorders of the type observed is due to glucose and not lactose, it is not my intention to imply that alimentary lactosuria does not occur. Most of the patients on whom my investigations were conducted received food containing little lactose. This may perhaps explain to some degree the difference in my results and those of Grosz, Orban, Langstein and Steinitz and others.

In my experience, lactosuria has occurred only in infants who were not thriving and who were ingesting considerable lactose. The following case will serve as an example.

REPORT OF CASE

Baby F., aged 3 months, received in twenty-four hours 28 ounces of a milk and whey formula containing about 12.5 per cent. lactose. Just preceding a nutritional disturbance, the urine reduced Benedict's qualitative reagent, and a quantitative determination by Bertrand's method showed a reducing power equivalent to 1.95 per cent. glucose. Incubation with yeast for twenty-four hours showed no gas formation, and a second quantitative determination showed only a slight diminution of reducing power, a glucose equivalent of 1.58 per cent. After hydrolysis with 1 per cent. hydrochloric acid incubation with yeast for twenty-four hours caused distinct gas formation and a marked impairment of reducing action. Quantitative determination after fermentation for thirty hours showed an equivalent of about 0.3 per cent. glucose. The osazone had the appearance of lactosazone and dissolved almost completely in hot water.

These results would seem to indicate that the greater portion of the reducing substance was lactose although there was also in all probability a small amount of glucose. Similar results were obtained in the case of six other infants who were receiving moderate or large amounts of lactose in their food.

During investigations of lactose tolerance, lactosuria occurred in three cases. These cases, however, were exceptional. As a rule, when the urine reduced Benedict's qualitative reagent following the ingestion

of large amounts of lactose it was due to a readily fermentable sugar which was apparently glucose.

Infants with severe nutritional disorders usually secrete much less urine than normal which in consequence is concentrated. The question naturally arises whether the reduction of copper solutions by the urines of these patients actually represents an increased sugar output. Since normal urine always contains reducing sugar, it seems possible that with diminished urine secretion the concentration of sugar may be sufficient to reduce copper solutions without there being an actual increase in the amount of sugar eliminated. There seems little question that the increased concentration of the urine may be a factor in the frequency with which such urines reduce copper test solutions. If this were the only factor concerned, there might be only an increase in the percentage of urine sugar but no true glycosuria.

TABLE 5.—COMPARISON OF SUGAR OUTPUT IN URINE DURING PERIOD OF ACUTE SYMPTOMS AND AFTER RECOVERY. URINE COLLECTED FOR A PERIOD OF 8 HOURS AND THE 24-HOUR OUTPUT CALCULATED FROM THIS

Name	Period of Acute Symptoms		After Recovery	
	Protein Milk, Amount Ingested in 24 Hours, Ounces	Sugar per 24 Hours as Glucose, Mg.	Food, Ounces	Sugar per 24 Hours as Glucose, Mg.
Ellwood.....	9	506	Milk..... 11 Lactose..... ½ Breast milk. 14	206 180
Cerecle.....	7	820	Protein milk 23 Maltose..... 1 Milk..... 14	207 240
Lunetti.....	16	762	Lactose..... ½ Protein milk 13 Cane sugar.. ½	193
Kinetas.....	2	380		
Miller.....	8	963		

Evidence on this question is given by quantitative determination of the total sugar output. Such determinations were made on the urine voided during eight hours, and the twenty-four hour elimination was calculated from this period. The total sugar per twenty-four hours calculated in this manner ranged from 280 to 963 mg. Some of the results are shown in Table 4. These figures indicate a greatly increased sugar elimination as normal infants on such a restricted sugar intake rarely put out more than 150 mg. Sugar eliminations comparable to these are found in normal infants only when very large amounts of sugar are ingested. Additional evidence was obtained in five cases in which the sugar output during the period of acute symptoms was compared with that after recovery. Despite the much greater sugar ingestion after recovery the sugar output was much less (Table 5). It,

therefore, seems evident that infants with nutritional disorders may show a true glycuresis.

Finally, the origin and significance of the increased sugar output must be considered. Is it due to a disturbance of sugar metabolism or is it merely of renal origin? Blood sugar determinations have been made in sixteen cases, and in all but two there was distinct hyperglycemia. The blood sugar after four hours' fasting ranged from 140 to 210 mg. per 100 c.c. This would seem to indicate a disturbance in the metabolism of sugar rather than the presence of a purely renal glycuresis. Increased blood sugar in the presence of increased urine sugar is generally assumed to indicate a disturbance in sugar metabolism.

As to the ultimate cause of the glycuresis, there is no direct evidence on which to base a definite opinion. There are a number of general observations on sugar metabolism which might have a bearing on this particular subject and which open a tempting field for discussion. It is my belief, however, that such discussion would go but a little way toward elucidating the problem and should be deferred until evidence bearing directly on the subject is available.