Reports

Specificity of a Glucose Oxidase Test for Urine Glucose

Most tests for urine glucose depend on the reduction of cupric or bismuth compounds. Such tests are not specific, for other reducing sugars as well as nonsugar reducing substances will give positive reactions when they are present in the urine in sufficient concentration. Recently, a test for urine glucose has been devised which employs the enzyme glucose oxidase.

In this test, glucose is oxidized to gluconic acid and hydrogen peroxide, the reaction being catalyzed by the enzyme. The hydrogen peroxide oxidizes orthotolidine in a reaction catalyzed by peroxidase. The enzymes and indicator are impregnated on a stick of stiff filter paper (*1*). The detection of glucose in urine is readily accomplished by dipping the test stick in the urine sample and observing at the end of 1 minute. Development of a blue color denotes the presence of glucose; the test is negative if no blue is seen at 1 minute. One of the outstanding features of the test composition is its specificity in detecting glucose as differentiated from other reducing sugars or nonglucose reducing substances. This report describes studies that establish the high specificity of this test.

The minimum concentration of sugar which gave a positive reaction with the enzyme test was established by testing various dilutions of the sugars in urine and water. Since most sugars prepared from natural sources may contain variable small amounts of glucose, it was essential also to test the sugar after removal of the glucose. Glucose can readily be removed from either urine or an aqueous solution by adding powdered glucose oxidase and aerating the mixture.

Twenty-percent solutions of the various sugars were prepared, using both distilled water and normal urine as the solvent. Serial dilutions of the sugar were then made at twofold intervals, using water as the diluent with the aqueous solutions and urine as the diluent with urine solutions. For removal of glucose, a 20-ml aliquot of sugar solution was mixed with 500 mg of glucose oxidase (Takamine-crude powder), and the mixture was aerated by passing approximately 400 ml of air per minute for periods ranging from ½ to 4 hours, depending on the amount of glucose to be removed. New serial dilutions were then prepared from the aerated solutions. The fact that the glucose oxidase had not significantly changed the amount of nonglucose-reducing substance was confirmed with a quantitative copper-reduction method.

Table 1 shows the results obtained with solutions of 11 different sugars, when mixed with urine. Quite comparable results were obtained when the same sugars were studied in aqueous solution, but in water the enzyme test is at least five times more sensitive. Two samples of galactose were included in the study, one of which was labeled practical and the other C.P. A useful control which was also included was a mixture of 95 parts of galactose (C.P.) and 5 parts of glucose (C.P.). Commercial galactose, fructose, mannose, maltose, and xylose reacted at fairly low concentrations with the enzyme test (0.05 to 1.0 percent in water, 0.2 to 5 percent in urine).

It is evident that the reactivity of the glucose oxidase test sticks with the sugar solutions was due to contaminating glucose, since treatment with glucose oxidase abolished the ability of all of the solutions except galactose to react at even a 20 percent concentration of sugar. With the chemically pure galactose, a reaction with the glucose oxidase test stick did occur at 20-percent concentration, but since the ratio of activity of this material before and after treatment is comparable to that of the more impure galactose, it may well be that this is caused by residual glucose impurities. That no significant amount of nonglucose-reducing sugar disappeared is shown by the values obtained with the copper-reduction method after treatment with glucose oxidase.

When an attempt was made to confirm further the presence of glucose as a contaminant by applying the classical method of yeast fermentation, an unexpected limitation was found. Galactose solutions aerated along with glucose oxidase gave negative enzyme stick tests, but after they had been mixed with washed yeast for a few minutes, they gave trace reactions with the stick test. The same result to an even greater extent was obtained with fructose solution. Since washed yeast gave negative tests with the enzyme composition, it appears that minute amounts of these sugars are converted to glucose by the yeast. While the amounts of glucose formed are quite small, this suggests possible limitations of the classical yeast method.

Data in this report differ somewhat from those reported by Keilin and Hartree (*2*). Using a manometric method, these workers found that a glucose oxidase from *Penicillium notatum* catalyzed the oxidation of xylose and galactose at a rate about 1 percent of that of glucose. Eleven other sugars were oxidized to a minute degree. It is evident that their methodology was different, and accordingly the difference in the results obtained in this study as compared with the observations of Keilin and Hartree may be due to a difference in the enzyme, to a difference in the conditions used for

Table 1. Reactivity of various sugars in urine with glucose oxidase test sticks.

Sugar	Minimum concn. giving positive test (%)	
	Untreated	Aerated with glucose oxidase
Galactose (practical)	0.2	10
Galactose (C.P.)	0.5	20
Fructose	2	Neg. at 20
Lactose	Neg. at 20	
Mannose	2	Neg. at 20
Maltose	1	Neg. at 20
Sucrose	Neg. at 20	
Xylose	5	Neg. at 20
D-Ribose	Neg. at 20	
D-Arabinose	Neg. at 20	
L-Arabinose	Neg. at 20	
L-Xylulose	Neg. at 20	
95 parts galactose (C.P.) and 5 parts glucose (C.P.)	0.1	5

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the reaction, or possibly to the presence of minute amounts of contaminating glucose in their samples.

The minimum concentration of glucose which reacts with the enzyme stick is about 0.002 percent in water and 0.01 to 0.05 percent in urine. This indicates the small amounts of glucose that would have to be present in the commercial sugar samples to give the results obtained. It is impressive that, after glucose removal, none of the sugars in urine gave a positive reaction at a concentration less than 20 percent. This concentration is much more than is ever encountered in any of the benign meliturias (*3*). Accordingly, the specificity of the test is demonstrated.

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References and Notes

1. These sticks for detection of urinary glucose are supplied by the Ames Company of Elkhart, Ind., under the registered trademark of Clinistix.
2. D. Keilin and E. F. Hartree, *Biochem. J.* (*London*) 42, 221 (1948).
3. J. C. Bock, *Physiol. Revs.* 24, 169 (1944).

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Effect of Bone Marrow Treatment on Mortality of Mice Irradiated with Fast Neutrons

Lethality following acute whole-body x-ray irradiation of laboratory animals can be greatly reduced by injecting a suspension of normal bone marrow cells into the animals after irradiation (*1*). Increases in the probability of recovery of guinea pigs following chronic gamma-ray irradiation and following the mixed radiation from an intravenous injection in mice of a sodium citrate solution containing radon in equilibrium with its decay products have also been reported for the same bone marrow treatment (*2*). A further test of this treatment for mice following irradiation by fast neutrons is described in this report (*3*).

The mice, usually 12 weeks old, were placed in individual perforated plastic tubes at a distance of 15 cm from the target of a Cockcroft-Walton accelerator. They were then irradiated with 14.1-Mev neutrons produced by the $H^3(d,n)He^4$ nuclear reaction. The dose rate in wet tissue was 100 to 200 rad/hr. There was little gamma contamination (*4*). In each experiment, the dose was determined by the use of two simultaneous monitors. Isologous bone marrow was taken from the femurs of normal mature animals, suspended in Tyrode's solution, and injected intravenously into half of the irradiated animals within a few hours after the completion of irradiation. In all but one experiment reported here, each treated mouse received the bone marrow taken from the shafts of eight femurs. Control and test animals were identically caged, 3 to 6 to a cage. Purina laboratory chow and water were supplied *ad libitum*. In all experiments, survival was checked daily up to 30 days, and in some experiments average body weights of treated and control groups were followed.

Table 1 gives the mortality results for seven experiments and the totals for all experiments. In every experiment, the 30-day mortality was greater among the control animals than among the animals treated with bone marrow. The most pronounced effect of the treatment was the 5/1 reduction in the totals in the probability of mortality during the second week of animals surviving the first week. Ninety-seven percent of all deaths occurred within 2 weeks after irradiation.

Applications of the chi-square statistic to the totals indicated that the reduction in mortality following bone marrow treatment was significant at the 1-percent level for 30-day mortality, 14-day mortality, and mortality during the second week, and at the 4-percent level for 7-day mortality, but was insignificant for 5-day mortality. Applications of the three-dimensional chi-square test (*5*) to the totals of LAF_1 and $101 \times C_3HF_1$ mice indicated that: (i) at the 10-percent level, bone marrow treatment of LAF_1 mice was more effective than treatment of $101 \times C_3HF_1$ mice, but this may be because of differences in the mortality levels in controls for the two strains; and (ii) that there was no significant difference in the effectiveness of treatment of males versus females.

Curves of the average weights of the survivors at each day for the first three experiments showed that the control animals reached a minimum of about 73 percent of their initial weight at 8 days postirradiation but asymptotically approached the initial weight at 30 days. The bone marrow treated animals reached a minimum of about 76 percent of their initial weight at 6 days and asymptotically approached their initial weight at 30 days. The effects of smaller doses of bone marrow are being investigated. The mortality among control animals exposed at these dose rates to 14-Mev neutron irradiation was somewhat lower and later than the reported values for equal irradiation by lower energy neutrons at higher dose rates (*6*).

The fast neutron doses administered were chosen to produce more than 50 percent mortality at 30 days in control animals. The dose required to meet this condition is a function of the dose rate, average linear energy transfer and probably the ratio of dose to bone marrow to that to the gastrointestinal tract. An investigation of the relative biological effectiveness for acute lethality in the RF strain of mice as a function of these parameters is in preparation (*7*). The higher concentration of elements with atomic weights greater than 20 (for example, calcium and potassium) and lower concentration of hydrogen in bone than in wet tissue tend to make the ratio of bone marrow dose to tissue dose other than unity. The ratio of the first collision dose rate in a large homogeneous bone to the dose rate in wet tissue exposed to the same 14-Mev neutron flux is estimated as about 0.75. This value is only an approximation to the value ob-

Table 1. Effect of bone marrow treatment on mortality from fast neutrons.

Strain	Sex	Dose (rad)	Treatment	Mortality* during days		
				0–7	8–14	0–30
RF	M	715	Bone marrow†	3/10	1/ 7	6/10
RF	M	715	None	1/10	9/ 9	10/10
$101 \times C_3HF_1$	M	837	Bone marrow	2/12	1/10	3/12
$101 \times C_3HF_1$	M	837	None	3/11	2/ 8	5/11
$101 \times C_3HF_1$	M	906	Bone marrow	7/12	1/ 5	8/12
$101 \times C_3HF_1$	M	906	None	7/12	4/ 5	11/12
$101 \times C_3HF_1$	F	877	Bone marrow	3/11	0/ 8	3/11
$101 \times C_3HF_1$	F	877	None	9/12	2/ 3	11/12
$101 \times C_3HF_1$	F	902	Bone marrow	0/12	1/12	1/12
$101 \times C_3HF_1$	F	902	None	3/12	1/ 9	5/12
LAF_1	M	900	Bone marrow	1/12	3/11	4/12
LAF_1	M	900	None	6/12	6/ 6	12/12
LAF_1	F	800	Bone marrow	1/10	0/ 9	1/10
LAF_1	F	800	None	2/12	6/10	8/12
Total			Bone marrow	17/79	7/62	26/79
Percentage			Bone marrow	21.5	11.2	32.9
Total			None	31/81	30/50	62/81
Percentage			None	38.3	60.0	**76.5**

* The numerator gives the number of mice that died during the interval and the denominator gives the number living at the start of the interval.
† Each of these mice received bone marrow from only 3 donor femurs.