was low. In one case (Table II, Case 1) with an ordinary balanced hospital diet there was a normal excretion in spite of administering large amounts of vitamin C. This may have been due to the restoration of vitamin C to the tissues depleted during illness. At no time did we note an 80% excretion of the vitamin C intake as described by Harris and Ray for normal individuals.

We have found that administration of crystalline cevitamic acid is preferable to the large amounts of orange juice required to furnish an equivalent quantity of vitamin C, as orange juice in very large quantities may cause diuresis and diarrhea.

Summary. Hypovitaminosis, as determined by delayed saturation, occurs while the temperature is elevated and especially during high fever in pneumonia. Urinary examination after oral administration of large quantities of cevitamic acid in divided doses permits rapid determination of the degree of saturation of the tissues.

8478 C

Color Reactions of Keturonic Acids and a Color Test Differentiating α- and β-Glucosides.*

FAY SHEPPARD AND MARK R. EVERETT.

From the Department of Biochemistry, University of Oklahoma Medical School, Oklahoma City.

Color reactions of the keturonic acids have recently become of importance to biology and medicine. In previous papers we have shown that a series of these acids can be prepared from carbohydrates by oxidation of their aqueous solutions with bromine.[1, 2] The solutions of the keturonic acids used in the experiments reported here were prepared as follows:

1% solutions of pure carbohydrates were placed in glass-stoppered flasks together with enough bromine to provide a small excess of liquid bromine throughout the experiments. These mixtures were kept in the dark for 42 days at 25°C. They were then aerated with washed air (and at times with carbon dioxide) to remove the excess bromine and then neutralized with potassium hydroxide to pH 7,

* Aided by a grant from the Research Appropriation of the University of Oklahoma Medical School.

1 Everett, M. R., Edwards, B. G., and Sheppard, F., *J. Biol. Chem.*, 1934, **104**, 11.

2 Sheppard, F., and Everett, M. R., *J. Biol. Chem.*, 1934, **105**, lxxx.

using a spot plate. The color tests were applied to aliquots, with the results given in Table I.

TABLE I.

Color Tests After Oxidation of 1% Solutions with Bromine for 6 Weeks at 25° C.

Carbohydrate	Molisch	Naphtho-resorcinol	Bial	Tashiro-Tietz	Selivan-off	Ferric Chloride
Glycerol	G	O[1]	LB	N	N	P
i-Erythritol	G	LP	GBr	O	N	LP
Adonitol	P	LR	G	P	LGBr	LBr
l-Arabitol	R	R	DG	OR	LGY	RBr
d-Sorbitol	P	Y[1]	O:Gp	P	R	Br
d-Mannitol	P	Y[1]	O:Gp	P	R	Br
Dulcitol	P	LY[1]	O:Gp	P	R	LBr
Perseitol	P	LBr	DG	P	R	LBr
Inositol	GBr	OR	N	N	N	DP:Br
d-Xylose	GBr	LP	N	N	N	B:P
d-Lyxose	G	LP	GBr	N	N	GaBr
l-Arabinose	GBr	LR	N	N	N	LG
l-Fucose	P	DB	G[4]	N	N	Br
l-Rhamnose	R:P	DB	G[4]	N	N	Br
d-Sorbose	P	G	O:Gp[4]	R	R	Br
d-Fructose	P	Y[1]	O:Gp[4]	P	R	Br
d-Glucose	R:P	DB	G	N	N	Br
d-Mannose	P	B	LG	N	N	LPBr
d-Galactose	R:P	B	LG	N	N	Y
d-Glucosamine	G:Ga[2]	LP[6]	N	N	N	LBr
d-Mannoketoheptose	P	Y	P:Gp[7]	P	LBr:R	Y
α-d-Glucoheptose	P	LP	N	R	LR	Y
α-d-Mannoheptose	P	G	LG	R	R	Y
β-Methylxyloside	GBr[3]	R	N	N[8]	N	B:P
α-Methylglucoside	BP[3]	LR	N:LG	N	N	Br
β-Methylglucoside	R:P[3]	DB	G	N	N	LBr
α-Methylmannoside	BP[3]	LR	O:G	N	N	LBr
Cellobiose	R:P	DB	G	N	N	Ga
Lactose	R	DB	G	N	N	Y
Maltose	P	Ga	N:LG	N	N	LBr
Sucrose	P	G	O:Gp[4]	P	R	LBr
Trehalose	P	LP	N:LG	N	N	Br
Melezitose	P	GaB	O:Gp[4]	P	R	Br
Raffinose	P	Br	O:Gp[4]	P	R	Y
Xylan	P	LP	G	N	N	Y
Inulin[5]	R:P	Y[1]	O:Gp[4]	P	R	Br
Dextrin[5]	P	LP	LG	N	N	Y
Soluble Starch[5]	P	LP	G	N	N	Br
Starch[5]	P	LR	G	N	N	Br
Glycogen[5]	P	LP	N	N	N	Br
α-Glucosan	P[7]	B[6]	G[6]	N[7]	N[8]	Br
Levoglucosan	P[7]	LR[6]	DG[6]	R[6]	N[7]	LBr
l-Ascorbic Acid	G:Br[3]	LR[7]	N:LG[7]	N[7]	N[7]	Y
Saccharic Acid	N	LP	N	N	N	Y
Mucic Acid	N	LR	N	N	N	Y

Symbols: B, blue; Br, brown; D, deep; G, green; Ga, gray; L, light; N, negative; O, orange; P, purple; p, precipitate; R, red; Y, yellow; :, upon standing changes to; 1, green in aqueous layer; 2, at 4 days P, original sugar N; 3, original sugar RP; 4, original sugar O:Gp; 5, oxidized sugar gives no color with iodine; 6, original sugar N; 7, original sugar same; 8, original sugar R.

Results obtained too late for tabulation: Oxidized d-ribose and d-arabinose give the same colors as l-arabinose; d-gulose as d-mannose; gentiobiose as lactose; melibiose as trehalose; and tartaric acid as saccharic acid, except a GBr Molisch and Br naphthoresorcinol test with tartaric acid.

In the ferric chloride test of Fenton and Jones[3] we added one drop of 10% ferric chloride solution and 3 drops of 10% potassium hydroxide solution to 5 cc. of neutral sugar solution. The purple color obtained from oxidized glycerol solution indicates that hydroxypyruvic acid is formed[3] and the similar colors from oxidized erythritol, inositol and the pentoses suggest similar products in these cases. These keturonic acids, and also those from glucosamine and ascorbic acid, give green Molisch[4] tests. The Molisch colors from oxidized α- and β-methylhexosides are distinctly different.

Oxidation products from triite and tetrite alcohols give no ketose reactions; those from pentites, the Tashiro-Tietz reaction[4]; those from hexites and heptites give both the Selivanoff[4] and Tashiro-Tietz reactions. Only keturonic acids from aldoheptoses give positive ketose reactions. Oxidized ketoses, together with their oligo- and polysaccharides, still give positive ketose reactions. The behavior of glucosans and ascorbic acid with these reagents is noteworthy.

Many keturonic acids give Bial's test,[4] but not those from pentoses, glucosamine, glucoheptose or α-glucosides. Oxidized ketoses behave like original ones, giving late green precipitates, and oxidized hexite alcohols act similarly. Intense colors are given by oxidized levoglucosan, perseitol and mannoketoheptose.

Classical red or purple colors are given by most keturonic acids in the naphthoresorcinol test of Neuberg and Kobel,[5] while oxidized ketoses and hexites give yellow to green colors. Especially interesting are the deep blue colors given by oxidized methylpentoses, aldohexoses and β-hexosides. Since α-hexosides do not give a similar blue color, we have devised the following rapid differentiating test: Heat 1% methylhexoside solution with excess bromine 4 hours at 65°C., using a flask with ground-in 6 ft. reflux condenser surrounded by an ice-salt cooling mixture. Remove the excess bromine by aeration and neutralize the solution to pH 7 with potassium hydroxide. Only β-hexosides so treated give a deep blue naphthoresorcinol test. α- and β-disaccharide hexosides show much better differentiation by oxidizing them with bromine at 25°C. for 6 weeks as described in this paper. This reaction is limited to hexosides.

---

[3] Fenton, H. J. H., and Jones, H., *J. Chem. Soc.*, 1900, **77**, 72.

[4] Hawk, P. B., and Bergeim, O., Practical Physiological Chemistry, P. Blakiston's Son and Co., Philadelphia, 10th Edition, 1931.

[5] Neuberg, C., and Kobel, M., *Biochem. Z.*, 1931, **243**, 435. (We used 2 N hydrochloric acid and ether.)