Table 1. Color and spectral characteristics of amine-sugar reaction product. The absorption peak for maltose in the α region was taken as unity. (X), (S), and (I) refer to the maximum, shoulder, and minimum, respectively, in the indicated spectral region; (A) indicates absence of characteristic.

Saccharide	Color		Relative molar absorbency in spectral regions				Ratio α/δ
	Concd.	Dilute	α	β	γ	δ	
Maltose 1,4-	Blue	Purple	1.00(X)	0.75(S)	0.62(X)	0.51(I)	2.0
Maltotriose 1,4- + 1,4-	Purple	Purple	0.74(X)	0.63(S)	0.29(A)	0.27(I)	2.8
Panose 1,6- + 1,4-	Blue-black	Purple-brown	1.11(X)	0.90(X)	0.63(S)	0.52(I)	2.1
Isomaltose 1,6-	Slate	Green	0.48(X)	0.41(X)	0.37(I)	0.83(A)	1.2
Isomaltotriose 1,6- + 1,6-	Slate-brown	Green	0.46(X)	0.44(X)	0.37(I)	0.39(A)	1.3
Glucose	Slate	Green-brown	0.53(X)	0.50(X)	0.49(S)	0.49(S)	1.1

aniline sprays with a series of glucobioses was characteristic of the linkages between the bioses but independent of their anomeric configurations.

These qualitative observations also confirm and extend the findings of Giri *et al.* (*4*), who noted similar colors for glucose, maltose, and isomaltose with the Buchan-Savage mixture as a brush reagent.

In addition to the compounds listed in Table 1, confirmatory evidence for this color distinction was obtained from the reaction on paper chromatograms of a mixture of oligosaccharides of the maltose series and a corresponding mixture of the isomaltose series from bioses to octaoses.

Figure 1 illustrates some typical spectra obtained by placing a slip of reagent-treated paper containing the sugar spots in the path of light from a Beckman D.U. spectrophotometer. A summary of the spectral characteristics shown in Table 1 shows the presence of four spectral regions. All compounds tested showed an absorption peak in the α (610 to 640 mμ) and β (520 to 540 mμ) regions, except for the presence of a shoulder for maltose in the β region. The relative molar absorbencies of the maltose series and of panose were considerably higher in these regions than the corresponding absorbencies of glucose and the isomaltose series, whereas this difference was much less in the blue end of the spectrum.

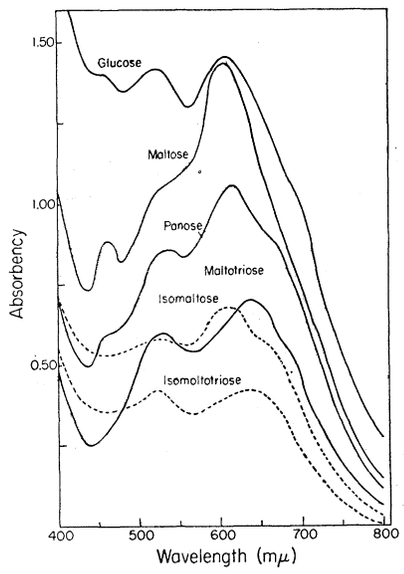

Fig. 1. Spectra (Beckmann D.U. spectrophotometer) of spots after reaction of saccharides on paper with diphenylamine-aniline-phosphoric acid reagent (*1*). Strips of paper 1 cm wide containing 150 μg of saccharide (spot 1 cm in diameter) placed in 1 cm optical cells with 0.9 cm cell spacer and held flat against optical wall of cell with proper insert. Blank cell contained paper strip cut from region adjacent to color spot. Broken lines represent isomaltose and isomaltotriose.

The presence of a minimum in the γ region (460 mμ) seemed to be characteristic of the isomaltose series, whereas only the substances containing an α, 1,4-linkage exhibited a minimum in the δ region (440 mμ). These characteristics are sharply different from those of the reaction product of diphenylamine and desoxyribose, which absorbs only at 380 mμ (*5*). Little or no differentiation in color could be obtained when the saccharides were allowed to react with the reagent in strong HCl solution. Two absorption maxima were observed, one at 510 mμ and the other at 620 mμ. Nor could large differences in color be found in the solutions obtained by extracting the spots from the paper with acetone. In the latter case, a yellow-orange residue remained on the papers from the spots of the isomaltose series, whereas no residue remained from the spots of the maltose series.

These observations suggest a competition between two reaction series, one leading to the formation of a blue product, the appearance of which does not depend on the presence of a free hydroxyl at the C-4 position of the reducing glucose, the other involving a less specific "browning" reaction that requires the presence of free C-4 hydroxyl. In this connection, Hodge has proposed that the nonenzymatic browning reaction of amines with reducing sugar goes through a dehydration reaction involving the C-4 hydroxyl (*6*).

This reagent has proved useful for distinguishing panose from isomaltose on chromatograms irrigated with the solvent mixture of Gjeisten (*7*). We have also found that the oligosaccharide spots obtained with this reagent are readily amenable to direct reflectance measurements on the developed chromatogram so that a fairly precise quantitative estimation of the oligosaccharide can be obtained. Finally, it is suggested that the spectrophotometric results and interpretations presented herein may be of value in interpreting color differences obtained with other carbohydrate series and other developing agents.

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2. We wish to thank the following individuals for generous gifts of the chromatographic standards: J. H. Pazur, maltotriose; H. M. Tsuchiya, isomaltose, isomaltotriose, and dextranase hydrolyzate of dextran [A. R. Jeanes *et al., J. Am. Chem. Soc.* 75, 5911 (1953)]; R. J. Dimler, panose [M. L. Killey *et al., ibid.* 77, 3315 (1955)]; and amylase hydrolyzate of amylose [R. J. Dimler *et al., Cereal Chem.* 27, 488 (1950)].
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20 September 1955

On the Rule for Leap Year

George W. Walker has suggested two changes [*Science* **123**, 25 (6 Jan. 1956)] in our present rule for leap year: first, that leap years be skipped from now until the year 2000 to bring the solstice to approximately 1 Jan.; and second, that our rule for insertion of leap year be changed. The first proposal is of academic interest only, since there is no chance of calendar reform by 1960, the first year in which he proposes that the leap year be dropped; but it is ingenious.

Walker's proposed revision of the rule for insertion of leap years is based on the assumption that the present rate of decrease in the length of the tropical year, or year of seasons, about half a second per century, will continue indefinitely. This, however, is not correct. In the course of time, the length of the year will be increasing. Astronomers working on the length of the year remind us that reasonably accurate observations are available for only about 150 years; that their work is complicated by irregularities in the length of day, some of which

are unexplained as yet; and that the error in the present Gregorian rule should amount to a day only after some 3000 years. They suggest that by that time the length of the year, and changes in it, should be known much more accurately than at present. Hence, they have recommended leaving revision of the Gregorian rule to future generations.

The Gregorian rule for leap year is "Years divisible by 4 are to be leap years unless they are also divisible by 100, in which case they are to be leap years only if divisible by 400." This rule obviously expresses the length of the year in days as

$$365 + 1/4 - 1/100 + 1/400$$

or in decimals as

$$365 + 0.25 - 0.01 + 0.0025$$

Combining, we obtain 365.2425 as the length of the Gregorian year.

The true length of the tropical year is 365.24220. Comparing with the Gregorian year, we see that the Gregorian rule is in error by 0.0003 day per year, or 3 days in 10,000 years.

Walker proposes the rule "Years divisible by 4 are to be leap years unless they are also divisible by 120, in which case they are to be common years." This expresses the length of the year in days as

$$365 + 1/4 - 1/120$$

or in decimals

$$365 + 0.25 - 0.00833 \ldots$$

Combining, we obtain 365.24166 . . . as the length by the proposed rule. Comparing this length, 365.24166 . . . , with the adopted length 365.24220, we see that it is too short by more than 5 days in 10,000 years.

Since both the Gregorian rule and the proposed rule start with "Years divisible by 4 are to be leap years . . ." one applying either rule starts with a mental division of the last two figures of the year number, to see whether it is divisible by 4. For the next step, almost any high-school student who has read the Gregorian rule recently should be able to figure mentally that the century years 1800, 1900, and 2100 are not divisible by 400 and, hence, are leap years; but 2000 is divisible by 400 and, hence, is not a leap year. The Gregorian rule can be applied mentally, but how many high-school students would be able to figure without pencil and paper which years between 1900 and 2100 are divisible by 120 and, hence, would not be leap years by the proposed rule?

There have been many proposals for correcting the Gregorian rule, but the only one usually mentioned in textbooks on astronomy was proposed several generations ago. It is that "but years divisible by 4000 shall not be leap years" be added to the Gregorian rule. This would make the length of the calendar year 365.24225 days and, on the assumption that the present figure for mean length of the year of seasons is correct, reduce the error to half a day in 10,000 years. This rule also continues the use of the division by 4, and has no effect on the present rule before the year 4000.

It has been suggested also that, if it develops that our present figure for the mean length of the year is appreciably too large, "but years divisible by 2000 shall not be leap years" be added to the Gregorian rule. This should be applied first in the year 2000; with our present marvelously accurate crystal clocks, astronomers may well know before the year 2000 whether this addition would improve the rule.

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Determination of Phosphocreatine and Other Phosphorylated Intermediates with Dowex-1 Resin

The analysis of phosphocreatine (PCr) in animal tissues has long been beset with certain difficulties. Interfering labile substances such as phosphopyruvate and triose phosphates are usually present in various amounts. In addition, the problem of isolating phosphocreatine chemically for radioactive studies is particularly acute since the laborious technique of removing orthophosphate (P) by magnesium precipitation (*1*) results in a loss of PCr and other compounds. Paper chromatography (*2*) has been used, but resolution is poor and spontaneous hydrolysis is a problem. The present report describes the use of the anion exchange resin Dowex-1 in the quantitative separation of PCr. Dowex-1 resins have been extensively applied to the analysis of nucleotides (*3, 4*), but little attention has been given PCr and other phosphorylated intermediates.

After the discovery that hydrolysis of labile phosphates occurred more readily with finer mesh resins (200 to 400 mesh), a porosity of 100 to 200 mesh Dowex-1 X8 resin was employed and the elution was performed at 0°C. The resin, which was stored in concentrated formic acid, was washed in 6 by 0.7-cm columns with 10 volumes of concentrated formic acid (plus 1*M* ammonium formate if other intermediates were to be separated) and then with distilled water until the effluent was neutral. After the neutralized extract was run through, the column was washed with 10 ml of water. Elution of the phosphorylated intermediates was begun with 0.05*N* formic acid, and the collection rate was 5 ml/10 minutes per tube, using the automatic fraction collector.

A typical separation of some intermediates is presented in Fig. 1. With the exception of hexose phosphates, most of the other substances are clearly separable. There is a tendency for most hexose phosphates as well as phosphoglyceric acid to be eluted simultaneously. At present, some success in separation has been achieved by combining silicic acid with Dowex-1. When only PCr was to be

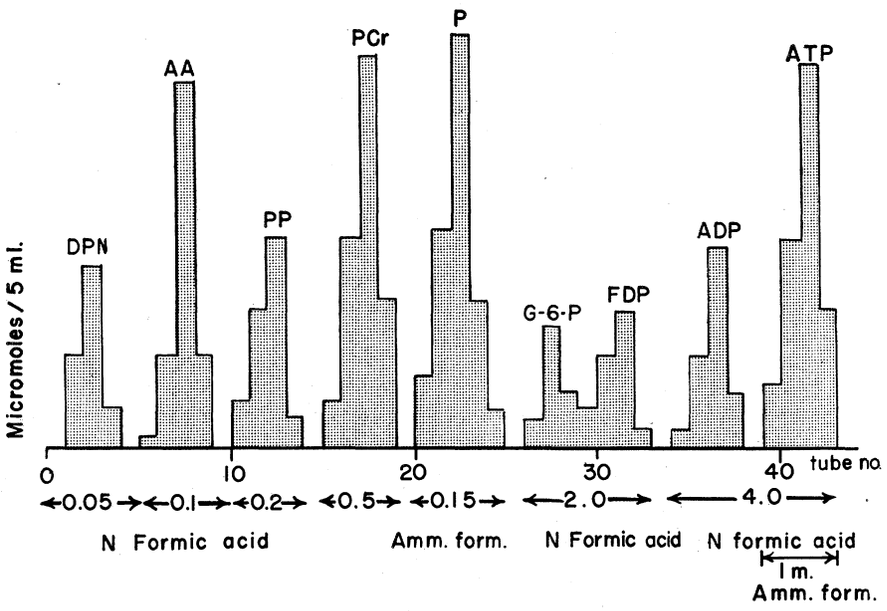

Fig. 1. Separation of some phosphorylated intermediates by ion-exchange chromatography. DPN, diphosphopyridine nucleotide; AA, adenylic acid; PP, inorganic pyrophosphate; PCr, phosphocreatine; P, orthophosphate; G-6-P, glucose-6-phosphate; FDP, fructose-1,6-phosphate; ADP, adenosinediphosphate; ATP, adenosinetriphosphate. Further details of the method are described elsewhere (*5*).