and distilled water for 5 minutes each. They are then stained for 8 minutes in 1-percent cresyl echt violet (*3*) (British Drug House) and are differentiated in 95-percent ethyl alcohol for 3 to 5 seconds. The stained smears are rinsed in absolute ethyl alcohol until nuclear detail is clear on examination under the high dry objective of the microscope. They are then mounted in Permount.

The mounted smears are studied under the oil-immersion objective of the microscope. The sex of the newborn infant has been established on the basis of external physical examination.

Many of the nuclei in amniotic fluid debris are not suitable for identification of the sex chromatin body. Some are markedly pyknotic, while other take the stain poorly or are badly fragmented. White blood cells may also appear in the fluid obtained from patients in labor.

The incidences stated in Table 1 are based only on examination of well-stained healthy nuclei that are assumed to be desquamated from the amnion, skin, or mucosae of the fetus. Such a nucleus with a sex chromatin body is shown in Fig. 1.

Observer 1 counted 100 nuclei in each instance. Observer 2, who studied some of the smears counted by observer 1, counted 50 nuclei. Neither observer knew the sex of the infant or the other observer's results at the time the counting was done.

It will be noted that there is no overlap between the maximum counts on male fetuses and the minimum on females. The means are separated by a gap 4 to 5 times the mean for male fetuses; and the extremes, by a gap 2 to 3 times the male fetus mean. Since there are no erroneous identifications of anatomic sex in the group, these observations are overwhelmingly significant.

The amniotic fluid was obtained at or near full term, with the exception of one instance of fetal death *in utero* of unknown etiology at 32 weeks. The incidence of nuclear pyknosis was increased in this case, but a sufficient number of satisfactorily stained nuclei were found nevertheless.

One smear not included in Table 1 requires comment. It was prepared from amniotic fluid obtained at cesarean section in a patient who was fully dilated with ruptured membranes and a fecal fistula in the vaginal vault. The fluid was grossly contaminated with maternal feces. The smear includes many polymorphonuclear leukocytes and unstained digested vegetable matter. It was originally counted by observer 1 as having 54 percent of nuclei with the sex chromatin body and later by observer 2 as 22 percent. A third observer found a count of 20 percent. The fetus was a male. The count by observer 1 was made at 2 A.M.

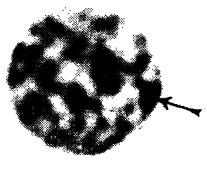

Fig. 1. Nucleus of a cell in a stained smear of amniotic fluid debris (× 3300). The arrow points to the sex chromatin body along the nuclear membrane.

Table 1. Incidence of sex chromatin body in amniotic fluid cell nuclei.

	Observer 1			*Observer 2*	
	No.	Range (%)	Mean (%)	No.	Range (%)	Mean (%)
Males	14	6–17	10	6	6–22	13
Females	16	49–71	59	6	42–70	54

Decisions on which nucleus to count and which to ignore in these amniotic fluid smears will vary from time to time, from observer to observer, and will depend in part on the quality of the stained smear and the density of the cell populations. In the present study, frequent consultation between observers on the appearance of individual nuclei served to limit the range of these variables, but the operation of chance produced some variation between two counts on the same smear (*4*). It is, therefore, not to be expected that repetition of these observations by others will produce results of an identical order of magnitude. The counts obtained on oral mucosa by Marberger *et al.* have lower means but the same wide separation between the means without overlap (*1*). The likelihood of finding a count on a genetic male that even approaches the lowest count on a genetic female is virtually nil by either method, provided that the absolute size of the counts is determined by conditions held constant for any group of counts made.

That the smears must be obtained and read for this purpose under ideal conditions is emphasized by the exceptional case cited in a foregoing paragraph. The unusual amount of debris of maternal origin in this amniotic fluid with a fecal fistula and late labor with ruptured membranes resulted in a smear that was misread by a fatigued observer.

It has been assumed that the infants identified as female after birth do not include any instances of gonadal agenesis who might be genetic males.

The techniques of exfoliative cytology have been applied to the determination of genetic sex of the fetus *in utero* by the incidence of the sex chromatin body in the cellular debris of amniotic fluid.

The mean incidence of this body in the nuclei found in the fluid of female fetuses is several times that found in male fetuses, and the minimum incidence in females in this series is approximately twice the maximum incidence among male fetuses. There are no erroneous determinations. This result is highly significant.

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4. E. G. Bertram, instructor in anatomy at Marquette University School of Medicine, provided valuable advice in the technique of staining these smears.

28 December 1955

Reagent for Differentiation of 1,4- and 1,6-Linked Glucosaccharides

Buchan and Savage have employed a mixture of aniline, diphenylamine, and phosphoric acid as a spray reagent for the detection and estimation of sugars on paper chromatograms (*1*). In applying this reagent to characterize the end products of enzymic degradation of starchlike substrates, we have observed variations in the color of oligosaccharide spots on paper chromatograms. Further investigation with known 1,4- and 1,6-linked glucosaccharides (*2*) revealed that these differences correlated to a certain extent with variations in the structure of these compounds.

Those saccharides in which the carbon-4 hydroxyl proximal to the reducing end of the molecule was combined in glucosyl linkage yielded blue to purple colors, whereas those saccharides in which this hydroxyl was uncombined yielded slate, green, or yellow spots, depending on the concentration of the substance per unit area of the paper chromatogram (Table 1). Aso and Yamauchi (*3*) have recently observed independently that the color of the reaction products of other

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.

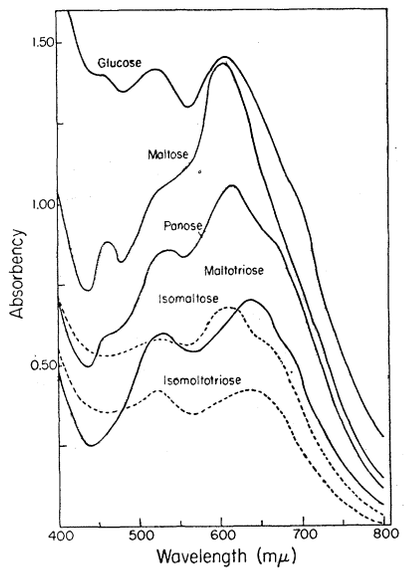

Fig. 1. Spectra (Beckmann D.U. spectrophotometer) of spots after reaction of saccharides on paper with diphenylamine-aniline-phosphoric acid reagent (*1*). Strips of paper 1 cm wide containing 150 μg of saccharide (spot 1 cm in diameter) placed in 1 cm optical cells with 0.9 cm cell spacer and held flat 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