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DETERMINATION OF L-FUCOSE IN GLYCOPROTEINS

I. Effect of Nonfucose Moieties of Serum Glycoproteins

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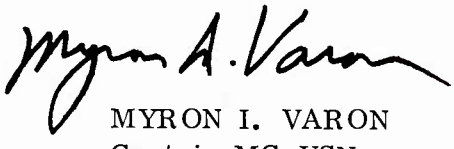

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ACKNOWLEDGMENT

The authors are grateful to A. S. Evans for his cooperation and for providing the human patient sera used in this study.

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FOREWORD
(Nontechnical summary)

The methylpentose L-fucose (6-deoxy-L-galactose) is a terminally bound carbohydrate on many glycoproteins. The determination of the fucose content of these glycoproteins is usually performed by one or more modifications to the cysteine-sulfuric acid reaction (CyR) in which sugars are converted to furfural derivatives and other degradation products with subsequent development of colored products which may be quantified by spectrophotometry. The shape and position of the absorption spectrum of the reaction products are the basis for calculating the concentration of fucose:

$$[\text{Fucose}] = \frac{(A_{396\ \text{nm}} - A_{430\ \text{nm}})_{SU} - (A_{396\ \text{nm}} - A_{430\ \text{nm}})_{B}}{(A_{396\ \text{nm}} - A_{430\ \text{nm}})_{ST}} \times [S]$$

where SU is the optical density increment (ODI) of the sample with cysteine added; B is the ODI of the sample without cysteine; and ST is the ODI of a known amount of fucose with cysteine [S]. Maximum absorption occurs at approximately 396 nm. The absorption at 430 nm is used as a correction for chromogens produced by nonfucose sugars.

The objective of this investigation was to determine the reliability of the fucose levels obtained by these methods since an elevated level of serum glycoprotein fucose has recently been suggested as a diagnostic aid for the detection of malignancy.

Our study reveals that the fucose values obtained by the method employing absorption at two wavelengths (396 nm and 430 nm) are a measure of both the methylpentose content and protein-bound hexose (e.g., galactose and mannose) content of

the glycoprotein material. This report presents information on (1) the nature of the protein-bound hexose interference, (2) the fucose levels obtained by various analytical methods, and (3) an analytical method which increases the specificity of the CyR.

ABSTRACT

The effect of galactose, mannose, and tryptophan on the determination of the methylpentose L-fucose in glycoproteins by the cysteine-sulfuric acid reaction is described. Under certain analytical conditions, protein-bound hexoses significantly contribute to the apparent fucose level. The true fucose level may be approximated under these same conditions by means of an empirically derived equation relating the concentration of serum protein-bound hexoses to the true fucose level. Fucose levels obtained with pathological and nonpathological sera by various methods are compared and specific conditions for the reliability of the statistics are defined and discussed.

I. INTRODUCTION

The methylpentose L-fucose (6-deoxy-L-galactose) is a terminally bound carbohydrate on many glycoproteins.[19] Recent studies have indicated that the level of serum glycoprotein fucose is elevated in malignancy[9,12] and after a glucose load in diabetes mellitus.[13] As a result it has been suggested that the fucose level may provide a useful diagnostic aid in these pathological conditions.[9,12] While considerable effort has been made with regard to the study of alterations of serum fucose levels in various pathological and nonpathological conditions,[18] very little attention has been directed toward defining the reliability of the analytical procedures involved in fucose determinations.

Rosato and Seltzer[12] have reported that the determination does not have the accuracy required of a screening procedure, e.g., they encountered 18 to 30 percent false-positives (elevated serum fucose levels associated with benign disease). Other evidence[10] suggests that the apparent fucose levels obtained after a glucose load may not be true fucose. The fucose levels in these studies[12,13] were determined by different modifications to the original cysteine-sulfuric acid reactions (CyR) described by Dische and Shettles.[3]

The modification proposed by Winzler[20] is the method usually employed for the determination of serum glycoprotein fucose in spite of the fact that nonfucose moieties contribute spurious chromogens to the CyR.[7,8,10] The assumption has been made that the absorption of chromogen produced in the CyR with glycoproteins at 430 nm is a measure of the nonfucose components and that the absorption increment between the methylpentose maximum of 396 nm and 430 nm is a reliable measure of the fucose

content of glycoprotein analogous to Dische and Shettles' findings with nonglycoprotein material.[3]

Recent evidence obtained in this laboratory from studies on fucose levels in patients with various pathological conditions suggested that the serum protein-bound neutral hexose level influences the apparent fucose level determined by Winzler's method[20] and that the specificity of the fucose method is a significant factor in interpreting the apparent fucose level. We have, therefore, investigated the CyR as applied to the analysis of glycoproteins to better define the sources of error. This report describes the effect of nonfucose glycoprotein moieties (galactose, mannose, and tryptophan) on the fucose analysis by CyR, introduces an empirical relationship between the apparent fucose level and the galactose-mannose concentration which may be used to give a more accurate estimate of "true" fucose levels, and finally compares the fucose levels obtained by various methods from pathological and nonpathological sera.

II. MATERIALS AND METHODS

Chemicals. Chromatographically pure L-fucose, D-galactose, D-mannose, and DL-cysteine HCl, L-tryptophan, β-nicotinamide adenine dinucleotide (β-NAD), Trizma base,* and reagent grade sulfuric acid† were used in this study. L-fucose dehydrogenase was a gift of Dr. H. Schachter.‡

Analytical methods. Fucose was determined either by: (1) Winzler's modification[20] of the CyR3 and CyR10 methods of Dische and Shettles using 50 μl of

* Sigma Chemical Company, St. Louis, Missouri
† Mallinckrodt Chemical Works, St. Louis, Missouri
‡ Department of Biochemistry, University of Toronto, Canada

serum,[3] (2) Gyorky and Houck's[7] acid hydrolysis method (modified by hydrolyzing glycoproteins obtained from 100 μl of serum for 75 minutes with 1.0 ml of 0.6 N sulfuric acid), (3) Sobocinski et al.'s automated method,[17] or (4) Finch et al.'s procedure employing L-fucose dehydrogenase.[5]

The latter procedure was modified in the following manner: glycoproteins from a 100 μl serum sample were precipitated with 1.0 ml of 10 percent (w/v) trichloroacetic acid, centrifuged at 12,000 rpm (17,300 x g) for 10 minutes at 5°C and the supernatant discarded. The precipitate was washed twice with 5 ml portions of diethyl ether, centrifuged as above, air dried, and hydrolyzed with 1 ml of 0.6 N sulfuric acid for 75 minutes at 100°C, cooled and centrifuged as above. Fucose content of the acid hydrolysate (supernatant) was determined in a reaction mixture containing 0.5 ml of the acid hydrolysate, 1.0 ml of 0.67 M Trizma base (Tris [hydroxymethyl] aminomethane), 2 moles of β-NAD in 0.5 ml, and 0.05 ml L-fucose dehydrogenase.[5] The reaction mixture had a final pH of 8.0. Initial absorption at 340 nm was determined and the reaction mixture was allowed to stand at room temperature for 2 hours before the final absorption at 340 nm (production of NADH) was determined. Activity of our enzyme preparation was approximately one-tenth that used by Finch et al.[5] Serum protein-bound hexose content was determined by an automated modification of the manual method described by Evans et al.[4]

With the exception of the automated fucose method, all absorbance readings were determined with a Beckman Acta II spectrophotometer.* Absorption spectra

* Beckman Instruments, Inc., Fullerton, California

were obtained with the spectrophotometer connected to a Sargent MR recorder.* Unless stated otherwise in the figure legend, optical density is expressed in arbitrary units which represent 0 to 100 percent of the millivolt range of the recording instrument.

Statistical methods. Lines drawn through experimental points were obtained with a Wang Series 700 advanced programming calculator using the "nth order regression analysis" program No. 36.†

III. RESULTS

The absorption spectrum of the reaction products produced by an aqueous fucose solution (20 μg/ml) in the CyR3[3] is shown in Figure 1. A single symmetric peak occurs with an absorption maximum at 400 nm. The position of the maximum differs from the 396 nm reported by Dische and Shettles[3] but is the same as that reported by Aminoff and Morgan.[1] Very little absorption occurs at 430 nm.

Figure 2 shows the absorption spectrum of the reaction products produced by an equimolar aqueous solution of galactose and mannose containing 75 μg hexose/ml in the CyR3. Maximum absorption for this single peak occurs around 411 nm with a large amount of absorption at 430 nm. When the absorption spectra of various equimolar concentrations of galactose and mannose were examined, a small but measurable shift in wavelength of maximum absorption was detected. Results such as those shown in Figure 3A indicate that, with higher concentrations of these hexoses, the wavelength required to balance the optical density at 396 nm shifts toward

* E. H. Sargent and Company, Chicago, Illinois
† Wang Laboratories, Inc., Tewksbury, Massachusetts

higher wavelengths, i.e., toward 430 nm. Figure 3B shows that these hexoses do not react to form chromogens in the absence of cysteine. The correction for nonfucose sugars, absorption at 430 nm, employed by Dische and Shettles[3] depends on a symmetrical absorption peak where the absorption (OD) at 396 nm is the same as at 430 nm, i.e., increment of OD 396 nm - OD 430 nm equals zero. The wavelength actually required to balance the OD 396 nm for various concentrations of galactose and mannose is presented in Figure 4. The data indicate that the required wavelength varies with the concentration of nonfucose sugars present in the reaction mixture. For the concentrations used, the wavelength varies by 4 nm and was never observed to be 430 nm.

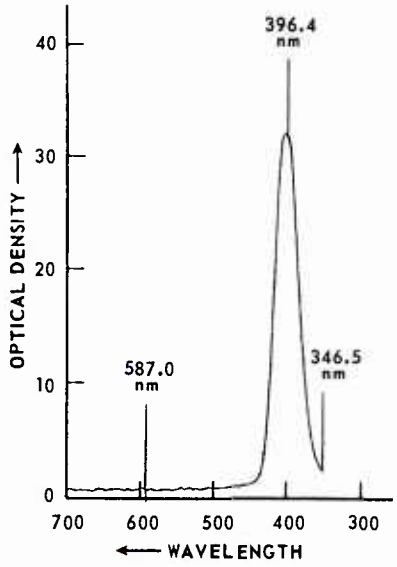


Figure 1. Absorption spectrum of products formed by 20 μg/ml of fucose in the CyR3

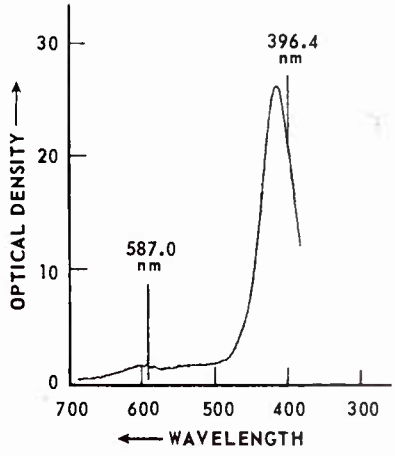


Figure 2. Absorption spectrum of products formed by an equimolar solution of galactose-mannose containing 75 μg/ml of hexose in the CyR3

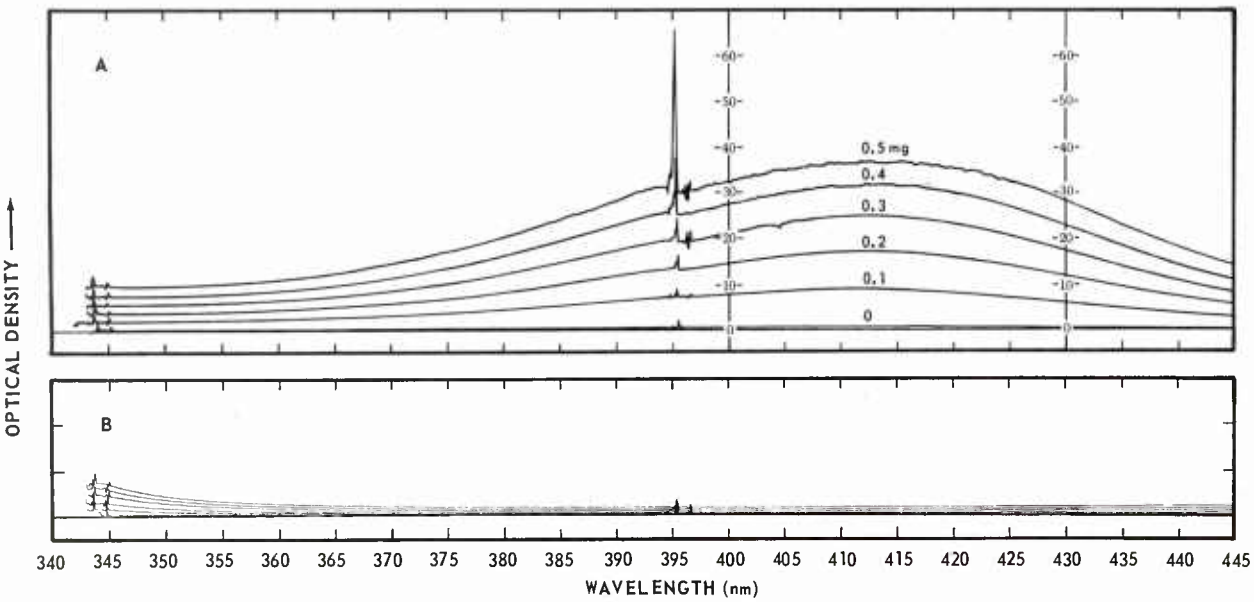


Figure 3. Absorption spectra of products formed by various concentrations (0 - 0.5 mg/ml) of equimolar solutions of galactose-mannose in the CyR3. A, with cysteine; and B, without cysteine. Scaled vertical lines were drawn at 400 nm and 430 nm to facilitate comparison of the amount of absorption at these two wavelengths. The erratic spikes occurring at approximately 395 nm are due to a filter change in the spectrophotometer.

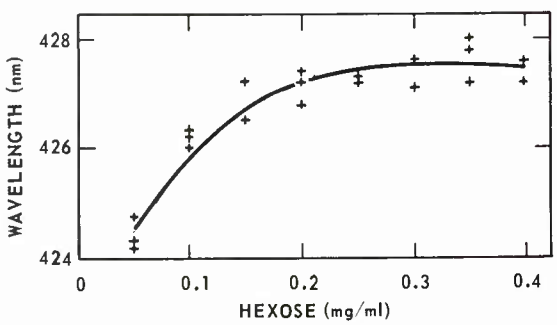


Figure 4.
Effect of various concentrations of hexoses on the wavelength required to produce an absorption equal to that observed at 396 nm in the CyR3. Experimental values (+) were obtained with either galactose or mannose. Line drawn through experimental points represents best fit (see text).

The effect of various concentrations of equimolar mixtures of galactose and mannose on the OD of 5 and 10 μg/ml fucose standards when the CyR3 procedure is used and measurements are made at 430 nm and 396 nm is shown in Figure 5. These results indicate that there is a linear relationship between OD increment and the amount of galactose and mannose in the reaction mixture and that the chromogens

produced by these hexoses add to the fucose chromogens. An empirical relationship was derived from the data presented in Figure 5 to correct the observed increment in OD in the presence of nonfucose sugars to an increment in OD due to fucose alone. The relationship may be expressed as:

$$A_F = Y - 0.452\ (X) \qquad (1)$$

where A_F is the corrected increment (396 nm - 430 nm) in OD due to fucose, Y is the observed increment, and X is the amount (mg) of hexose present as galactose-mannose in the reaction mixture.

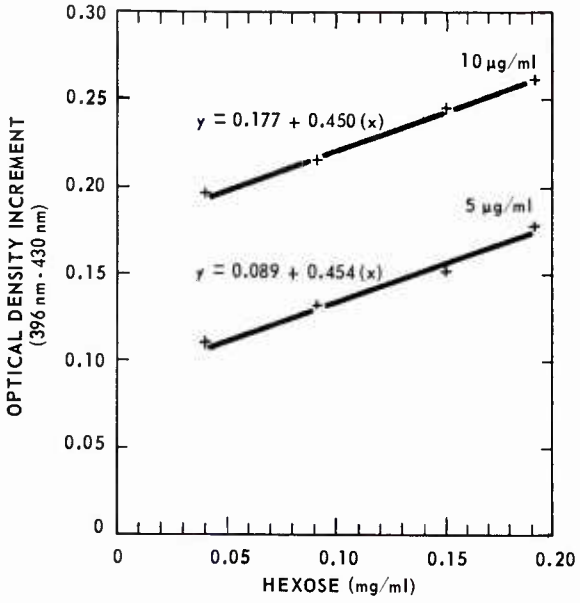


Figure 5.
Effect of various concentrations of hexoses on the absorption increment obtained for fucose standards in the CyR3. Equimolar solutions of galactose-mannose were used. Lines drawn through experimental points (+) are best fit (see text). Equations for each line are shown.

Figure 6 shows the change in absorption increment, OD 396 nm - OD 430 nm, with time for a 20 µg/ml fucose solution. Maximum absorption occurs 1-1/2 to 2 hours after the addition of cysteine to the reaction mixture. The absorption remains stable for at least 5 hours, contrary to a report made by Gibbons[6] but in agreement with that of Dische and Shettles.[3] However, in a reaction mixture containing fucose

and galactose-mannose the absorption increment increases with time. These results are shown in Figure 7A. The increase in absorption increment is due to a decrease in absorption of chromogens at 430 nm and a slight increase in chromogens measured at 396 nm shown in Figure 7B.

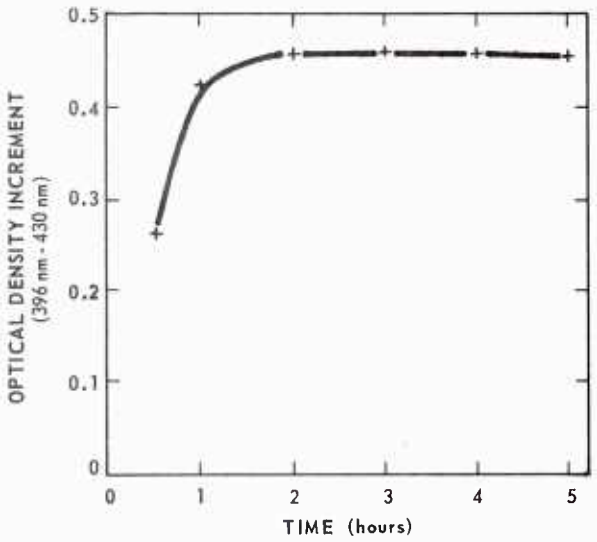


Figure 6.
Absorption increment for fucose at various times after CyR3. The reaction mixture contained 20 μg/ml fucose.

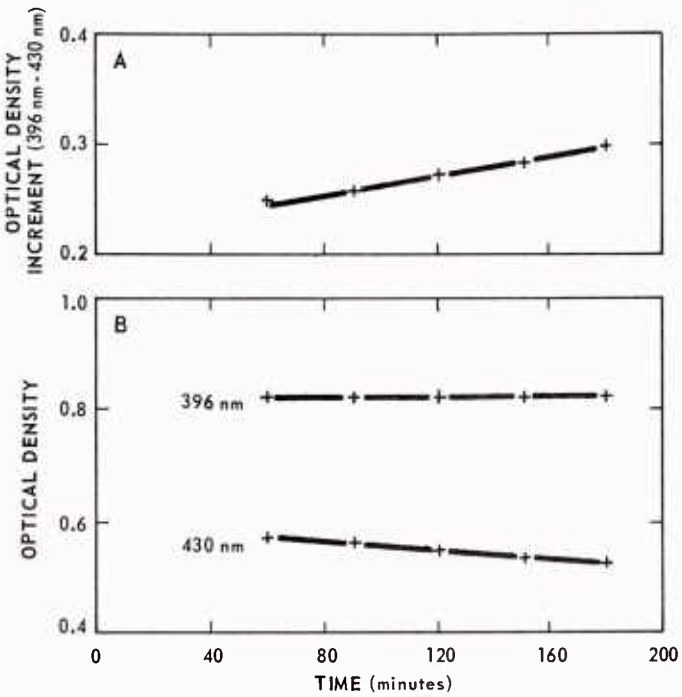


Figure 7.
A, Absorption increment, and B, absorption at 396 and 430 nm for fucose in the presence of galactose and mannose at various times after CyR3. The reaction mixture contained 10 μg of fucose and 150 μg of an equimolar mixture of galactose and mannose. Lines drawn through experimental points (+) are best fit (see text).

Figure 8 shows the increase in absorption of the reaction products liberated from a serum glycoprotein precipitate (human serum pool) after hydrolysis in 0.6 N sulfuric acid for varying time intervals. Hydrolysis of reactive moieties is complete after 75 minutes. According to the data presented in Figure 8, the 60-minute time interval employed by Gyorky and Houck[7] results in only 75 percent hydrolysis. Heating beyond 105 minutes resulted in a rapid breakdown of reactive moieties when absorption was measured at 396 nm and 430 nm. Figure 9 shows the absorption spectrum of the reaction products when an aliquot of an acid hydrolysate of a human serum pool was assayed for fucose by the CyR3 method. Figure 10 is the absorption spectrum obtained for the reaction products when the same human serum pool was assayed for fucose using Winzler's[20] modification of the CyR3. The use of acid hydrolysis lowers the absorption at 396 nm and greatly reduces the absorption measured above 400 nm especially

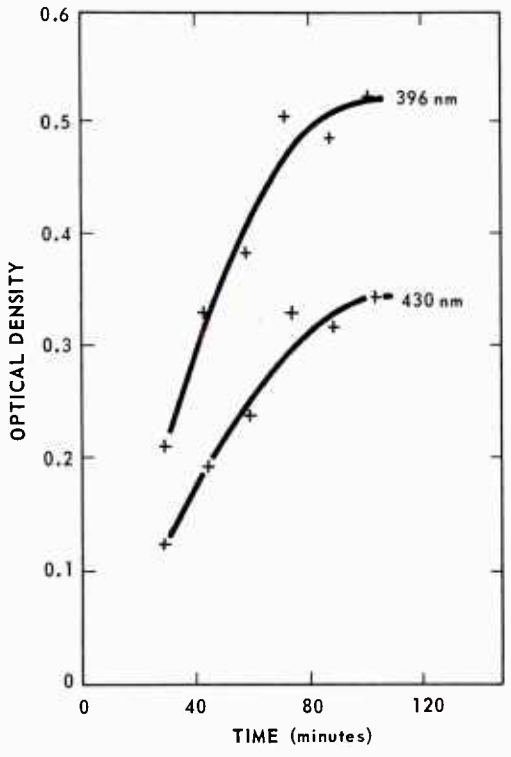


Figure 8.
Absorption of an acid hydrolysate of pooled human serum measured at 396 and 430 nm at various times after CyR3. Conditions for hydrolysis are described in the text. Lines drawn through experimental points are best fit (see text).

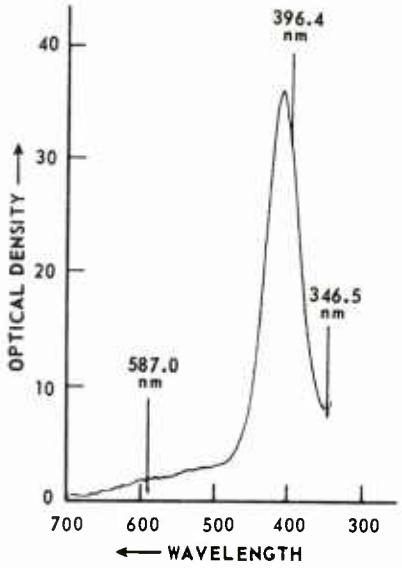


Figure 9. Absorption spectrum of products obtained with an acid hydrolysate of pooled human serum after CyR3.

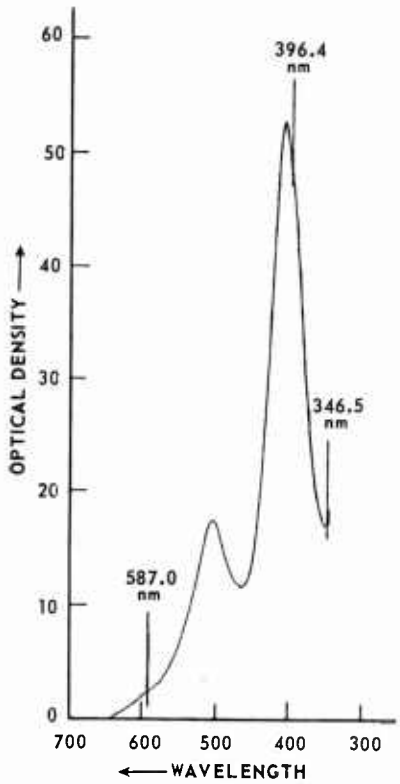


Figure 10. Absorption spectrum of products obtained with an unhydrolyzed sample of pooled human serum after CyR3.

the peak observed at 510 nm with the Winzler procedure. This latter peak is due primarily to chromogens produced by tryptophan in the presence of galactose and mannose.

Figures 11 and 12 show the absorption spectra obtained in the CyR3 with tryptophan (50 μg/ml) and an equimolar concentration of galactose and mannose (75 μg/ml) in the presence of tryptophan (50 μg/ml), respectively, in the CyR3.

Figure 13A and B shows the absorption spectra obtained with a solution containing 20 μg/ml of fucose and 75 μg/ml of equimolar galactose-mannose in the CyR3 with 30 μg/ml and 50 μg/ml of tryptophan added, respectively. There is no apparent

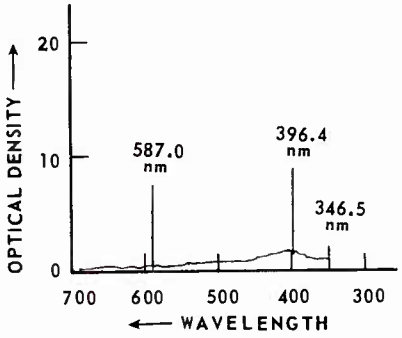


Figure 11. Absorption spectrum of products obtained from tryptophan after CyR3. The reaction mixture contained 50 μg of tryptophan.

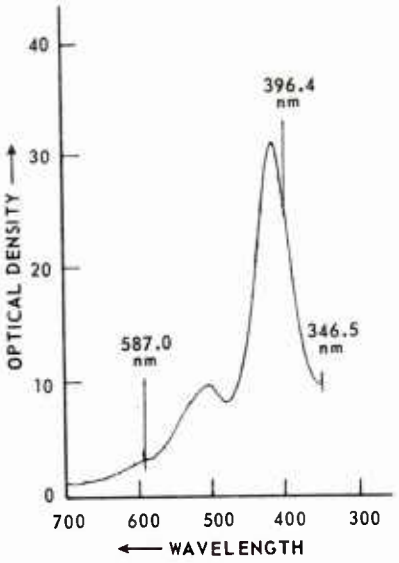


Figure 12. Absorption spectrum of products obtained from tryptophan in the presence of galactose and mannose after CyR3. The reaction mixture contained 50 μg of tryptophan and 75 μg of an equimolar solution of galactose-mannose.

effect on the absorption of the major peak but there is an increase in absorption at 510 nm due to the presence of an increased amount of tryptophan. Figure 14 shows that fucose (20 μg/ml) and tryptophan (50 μg/ml) do not interact in the CyR3 to produce an absorption peak at 510 nm as previously shown for hexoses and tryptophan.

Figure 15 shows the results obtained when a sample from a human serum pool (50 μl) was assayed by CyR3 in the absence of added tryptophan (A-1), and when 50 μg (A-2) and 100 μg (A-3) of tryptophan were added to the reaction mixture. The B series represented in Figure 15 was obtained without cysteine but with the corresponding amounts of tryptophan. Tryptophan added in the reaction mixture with

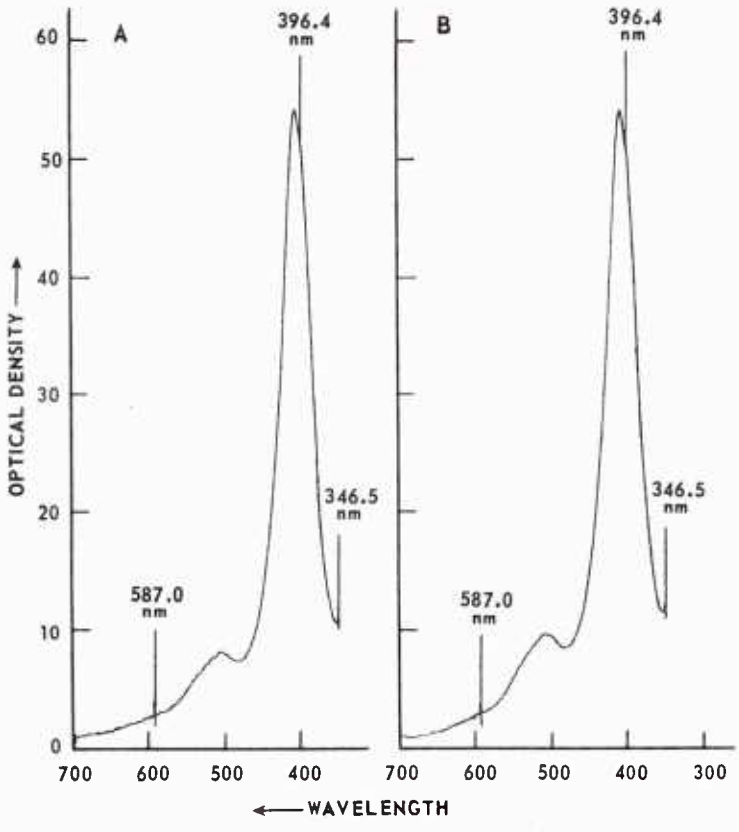


Figure 13. Absorption spectra of products obtained from fucose, galactose, mannose and tryptophan after CyR3. The reaction mixture contained 20 μg fucose, 75 μg hexose (equimolar solution of galactose-mannose), and A, 30 μg tryptophan, or B, 50 μg tryptophan.

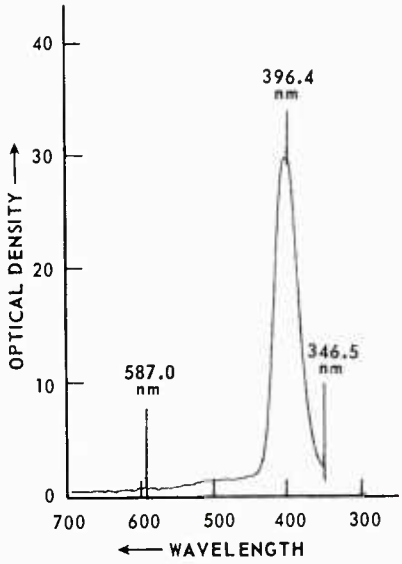
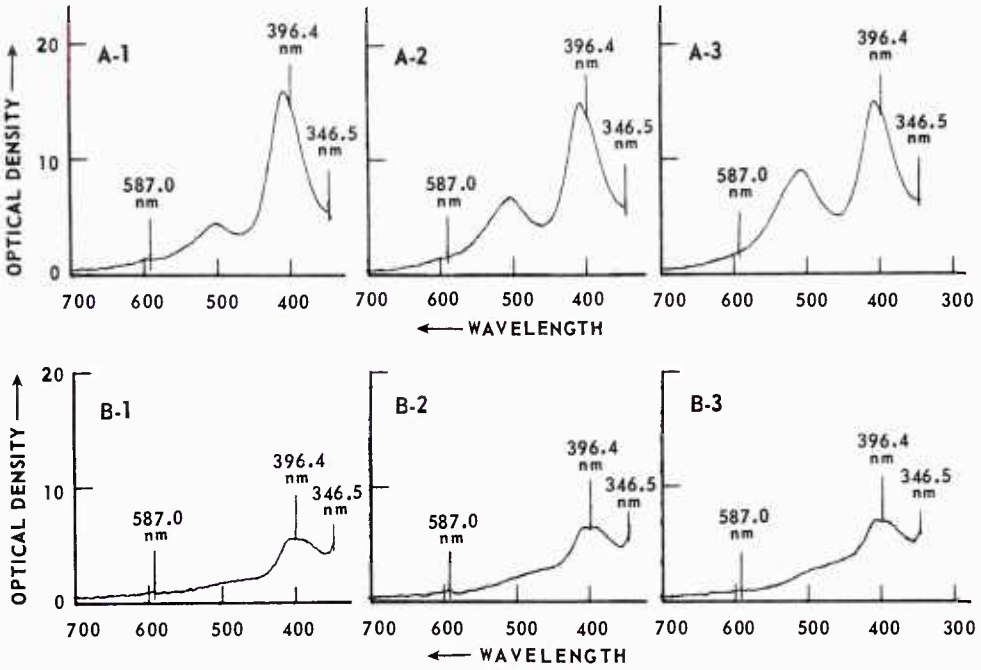


Figure 14. Absorption spectrum of products obtained from fucose in the presence of tryptophan after CyR3. The reaction mixture contained 20 μg fucose and 50 μg of tryptophan.

cysteine slightly lowers the absorption spectrum of the major peak and increases the absorption at 510 nm. Under these experimental conditions the absorption at 510 nm is linearly related to the tryptophan concentration. The decrease in absorption increment (396 nm – 430 nm) in the presence of either 50 μg or 100 μg of added tryptophan is approximately 5 percent.



SAMPLE IDENTIFICATION	OPTICAL DENSITY			
	396 nm	430 nm	396 nm - 430 nm	510 nm
A-1 B-1	0.428 0.159	0.248 0.074	0.095	0.086
A-2 B-2	0.403 0.179	0.236 0.102	0.090	0.152
A-3 B-3	0.410 0.194	0.243 0.117	0.090	0.210

Figure 15. Absorption spectra of products obtained after CyR3 from a pooled human serum (PHS) with and without the addition of tryptophan and cysteine. A represents PHS with cysteine; A-1, no added tryptophan; A-2, 50 μg added tryptophan; A-3, 100 μg added tryptophan. B represents PHS without cysteine but with tryptophan as described for A. Optical density measurements at various wavelengths are shown in the table. Fifty microliters of PHS were used for each experiment.

Since reference to the fucose procedure of Dische and Shettles[3] may cite either CyR3 or CyR10 (the number indicates the time of incubation at 100°C), it was pertinent to compare the results obtained on a single serum sample by these two methods. Figure 16 shows the absorption spectra obtained when a patient serum sample (N-25) and a 20 μg/ml fucose standard were assayed by CyR3 and CyR10. Use of CyR3 with glycoprotein samples results in an absorption spectrum showing increased absorption of the major peak when compared to CyR10 (Figure 16A and C). The decrease in absorption at 396 nm and 430 nm for N-25 in the CyR10 was 37 and 52 percent, respectively, when compared to CyR3. The decrease in absorption at 396 nm for a fucose standard (20 μg) is only 14 percent and at 430 nm it is negligible (Figure 16E and F). The differential in destruction of chromogens in CyR10 at 430 nm and 396 nm results in a higher optical density increment for N-25 with CyR10 than with CyR3, i.e., higher apparent fucose levels are obtained with CyR10. Figure 16B and D represents absorption spectra obtained for N-25 by CyR3 and CyR10 without added cysteine, respectively.

The results obtained when a human serum pool was assayed by five methods are shown in Table I. If the value obtained for fucose by the L-fucose dehydrogenase procedure represents the true fucose level, no significant difference between mean fucose values exists when values obtained by automated[17] or CyR3[20] corrected by equation (1) are compared. The highest values were obtained with Winzler's modification of the Dische and Shettles CyR3.[20] The use of the internal standard method suggested by Dische and Shettles[3] yields results comparable to those obtained by Winzler's modifications.[20]

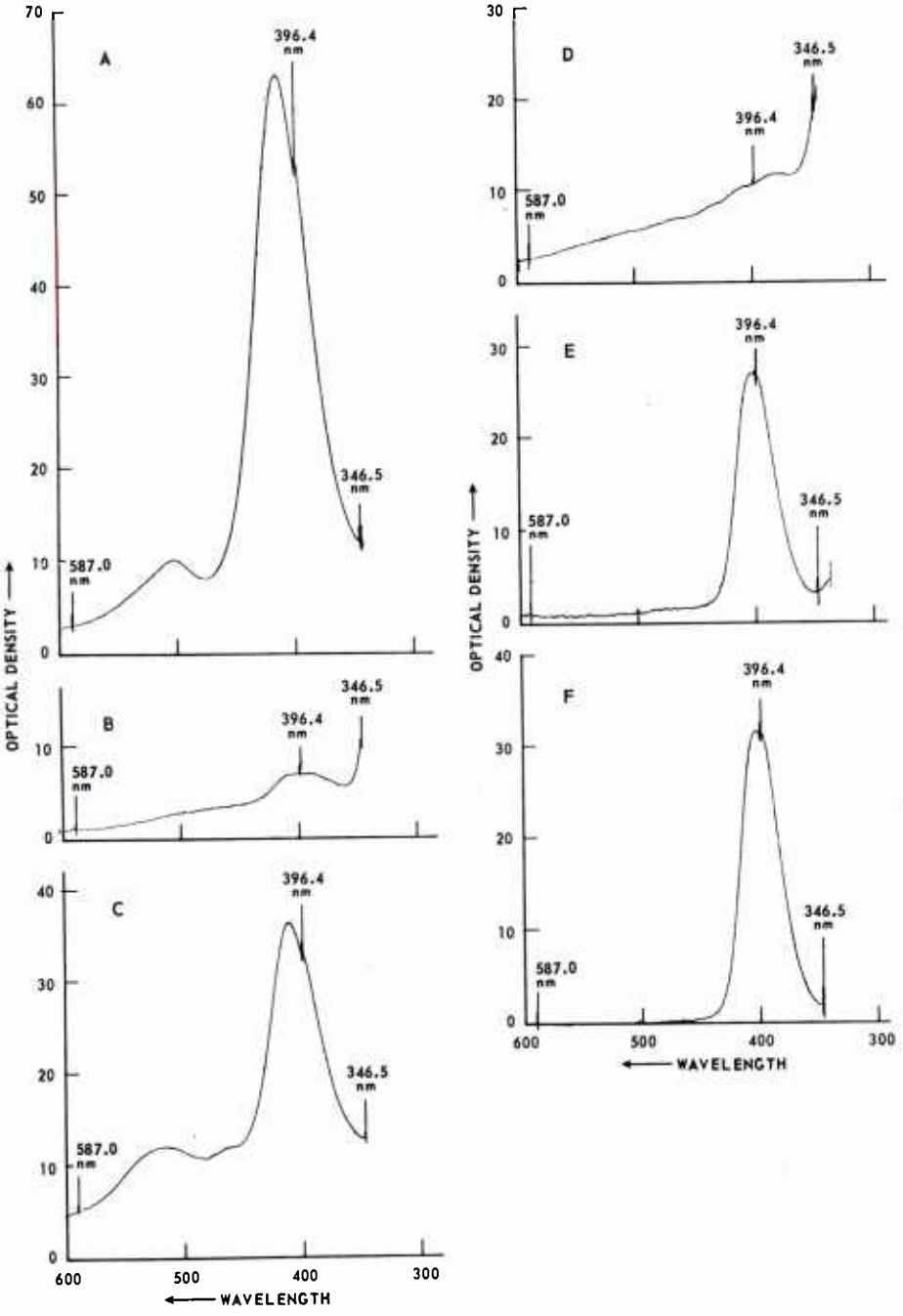


Figure 16. Absorption spectra of products obtained from a human serum sample and a fucose standard after CyR10 and CyR3. Identification of spectra: A, patient N-25, CyR3 with cysteine; B, patient N-25, CyR3 without cysteine; C, patient N-25, CyR10 with cysteine; D, patient N-25, CyR10 without cysteine; E, 20 μg fucose, CyR10; F, 20 μg fucose, CyR3.

Table I. Serum Fucose Levels Obtained by Various Methods

Specimen	Analytical method*				
	Automated[17]	Manual (Winzler[20])	Enzymic	Manual (corrected by equation (1))	Internal standard[3]
NHP†	7.2 ± 0.7 (10)	9.5 ± 1.1 (9)	6.6 ± 1.4 (10)	5.9 ± 1.1 (9)	9.5 ± 1.6 (6)

* Methods are described in the text; fucose levels are shown as the mean mg/100 ml ± S.D.

† NHP = normal human serum pool; number of determinations are shown in parentheses

Figures 17 and 18 show the results obtained when serum fucose levels from 20 patients were determined by the automated and manual Winzler techniques and compared, respectively, before and after the manual results were corrected by equation (1). A linear correlation exists between the two methods whether the manual results are corrected or uncorrected; however, as shown in Figure 17, the manual results are, with the exception of one determination, higher than the automated results. Adjusting the manual results with respect to the protein-bound hexose content of the serum lowers the fucose value and a correlation coefficient close to unity is obtained (Figure 18). One of the serum samples, patient N-25, was icteric and contained a highly elevated level of protein-bound hexose (450.9 mg/100 ml). The protein-bound hexose content of the 20 serum samples ranged from 99.6 to 450.9 mg/100 ml with a mean of 176.4 ± 73.2 mg/100 ml (S.D.).

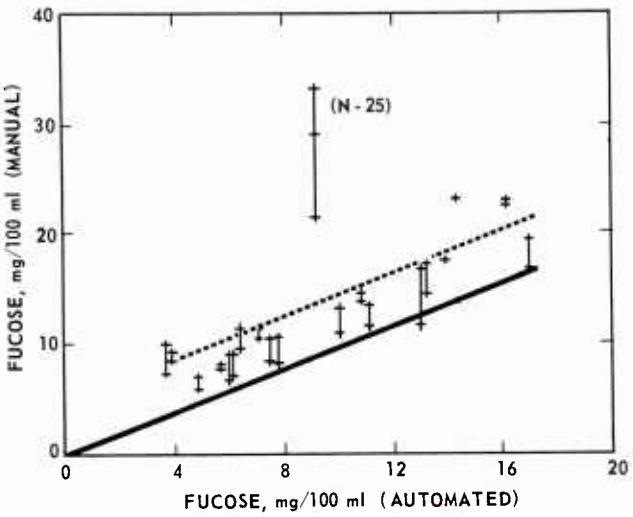


Figure 17. Serum fucose levels of human sera obtained by two different methods. Vertical lines represent the range of values obtained by the manual CyR3 method described in the text. The solid line represents a correlation coefficient of unity. The dashed line represents best fit (see text). Experimental points (+) labeled N-25 represent values obtained for a human serum containing an extremely elevated protein-bound carbohydrate level (see text).

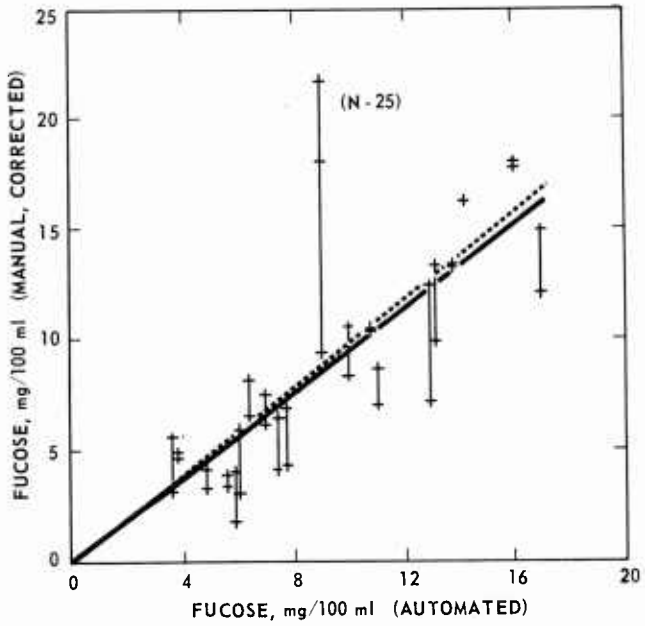


Figure 18. Serum fucose levels of human sera obtained by two different methods after correction for hexose interference. Manual values shown in Figure 17 were corrected by use of equation (1) (see text).

IV. DISCUSSION

Evidence presented in this study clearly demonstrates that the fucose levels obtained for glycoproteins by Winzler's modification[20] of the Dische and Shettles CyR3[3] are not true fucose levels but rather a measure of the protein-bound methylpentose and hexose content. The nonspecificity of the method is attributable to the erroneous use of CyR3 absorption increment (AI) between the two wavelengths 396 nm and 430 nm as a measure of the absorption due specifically to fucose. This absorption increment is linearly related to both fucose and hexose concentrations, i.e., AI is positive for hexoses in the absence of fucose and not zero as generally assumed from the work of Dische and Shettles.[3] In fact, it was pointed out by Dische and Shettles[3] that the wavelength (W) at which absorption of CyR3 due to hexoses equals absorption at 396 nm must be experimentally determined when fucose analyses are performed in the presence of hexose because of small variations in time necessary to bring reaction mixtures to 100°C. Our results indicate that the value for W depends on the concentration of hexoses present in the CyR3 which in turn is dependent on the protein-bound hexose content of the serum glycoprotein when the analytical method proposed by Winzler[20] is used. It is easy to see how false-positive results, e.g., elevated fucose levels in benign disease, could be found since elevated protein-bound hexose levels have been reported in various nonmalignant diseases.[14] We suggest that when fucose levels are determined by Winzler's technique false-positives may be detected by use of equation (1); however, values used in this equation may vary with different experimental conditions and should be determined by each laboratory. The use of an internal standard as suggested by Dische and Shettles[3] would not correct falsely

elevated fucose levels for the following reason: when a known amount of fucose is added to an unknown solution it displaces the AI by an increment equal to that observed by adding the same amount of fucose to an aqueous standard fucose solution. What is important to determine is which standard fucose curve pertains to the specimen. This determination is made according to the hexose content of the specimen.

The role of tryptophan in the CyR3 appears to be the production of chromogens in the presence of hexoses with maximum absorption at 510 nm. A similar interaction of tryptophan and sugars under the catalytic influence of acid and its possible interference with absorption measurements made at 505 nm has been well documented.[11] The finding that 50 μg or 100 μg of tryptophan decreases the absorption increment to the same extent (5 percent) suggests that the absorption at 510 nm is not the result of the conversion of much of the hexose-cysteine chromogen at 400 nm to a new chromogen with an absorption maximum at 510 nm as suggested by McMillan et al.[10] but rather that degradation products of hexoses and tryptophan interact to form new chromogens whose absorption at 510 nm is independent of the absorption of chromogens between 396 nm and 430 nm. In our studies, 50 μg of tryptophan in the CyR3 is within the range that would be expected to be present in 50 μl of normal serum,[2] the volume used in this study for the Winzler[20] procedure. In view of this, higher levels of protein-tryptophan encountered in serum samples from patients with neoplasms[16] would be expected to produce increased absorption at 510 nm with the CyR3. Shetlar et al.[15] proposed the tryptophan-sugar reaction as a method for the determination of serum polysaccharide and showed that bilirubin in the presence of sulfuric acid and tryptophan contributes chromogens with absorption at 400 nm.

This latter finding may explain why we were unable to correct the fucose levels obtained for patient N-25 by adjusting for hexose (equation (1)).

Another area of concern in interpreting fucose analyses performed by investigators using CyR is that the citation of Dische and Shettles[3] as a reference could mean either the CyR3 or CyR10 procedure, two methods which do not produce identical results with glycoprotein material, i.e., the fucose value obtained by CyR10 > CyR3. This observation may be pertinent to the fucose levels reported by Shaw et al.[13] in that they used a modification of CyR10. Their observation of elevated fucose levels in early diabetes after a glucose load has been criticized by McMillan et al.[10] as being due to glucose interference in the methodology. Although this may explain the elevated fucose levels in early diabetes when glucose levels differ from normals (after a glucose load) it fails to explain the elevated fucose levels where glucose levels of early diabetics are the same as normals. We suggest that a possible explanation for the latter finding resides in either (1) the protein-bound carbohydrate level of the early diabetic is elevated at this time and because of the peculiarities of CyR10 the apparent fucose levels are elevated, or (2) the apparent rise in fucose level is not artifactual in these patients; however, both require further investigation.

We believe that the use of L-fucose dehydrogenase (FDH) as suggested by Finch et al.[5] provides the most specificity and is the method of choice; however, the enzyme is not commercially available and its preparation is beyond the capability of most clinical laboratories. Prior acid hydrolysis of terminal fucose residues of glycoproteins followed by application of CyR3 as suggested by Gyorky and Houck[7] appears to be the best practical method available for fucose determinations since it

eliminates a large amount of interfering hexoses. We have automated this procedure[17] to provide (1) a better approximation of "true" fucose levels, and (2) a rapid method for screening large numbers of samples. The FDH procedure is easily adaptable to an automated procedure and should be the method of choice if the enzyme becomes commercially available in large quantities.

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13. ABSTRACT

The effect of galactose, mannose, and tryptophan on the determination of the methylpentose L-fucose in glycoproteins by the cysteine-sulfuric acid reaction is described. Under certain analytical conditions, protein-bound hexoses significantly contribute to the apparent fucose level. The true fucose level may be approximated under these same conditions by means of an empirically derived equation relating the concentration of serum protein-bound hexoses to the true fucose level. Fucose levels obtained with pathological and nonpathological sera by various methods are compared and specific conditions for the reliability of the statistics are defined and discussed.

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