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Determination of Serum Polysaccharides by the Tryptophane Reaction.*

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A number of investigators have described methods for the quantitative estimation of the polysaccharides associated with the serum proteins. Merten[1] estimated these bound sugars by determining the difference between the free sugar and the total reduction after acid hydrolysis of the serum. Lustig and Langer[2] applied the Tillman-Phillippi orcinol method to an alcohol precipitate of serum. Seibert and Atno[3] adopted the carbazole reagent of Dische[4] for the quantitative estimation of polysaccharide in serum, both directly on the proteins obtained by ethanolic precipitation and by difference between the reducing sugar and the total sugar determined with carbazole. Sheppard and Everett[5] described a reaction for the colorimetric determination of carbohydrates by means of tryptophane. The latter determination has the advantage that tryptophane arising from the hydrolysis of protein obviously does not interfere with it. It is the purpose of this paper to describe the application of this reaction to the rapid quantitative assay of non-glucosamine serum polysaccharides.

Experimental. Reagents: 1. Sulfuric acid solution, 77% by volume. 770 ml of C.P. concentrated sulfuric acid is added to 230 ml of distilled water.

2. Absolute ethanol.

3. Tryptophane solution, 1%. 1 g of *l*-tryptophane (Eastman Kodak) is dissolved in 99 ml of warm distilled water. The solution is filtered and stored in a glass stoppered bottle in the refrigerator until ready for use.

Method: The method for blood serum is as follows: The serum is diluted 1-2 with 0.9% sodium chloride. Two-tenths ml of the diluted serum is pipetted drop by drop into 10 ml of absolute ethanol in glass stoppered centrifuge tubes. The precipitate is centrifuged out and washed once with 10 ml of absolute ethanol. After centrifuging as before, the ethanol is decanted and the precipitate drained by inversion of the tube. One ml of distilled water and 7 ml of 77% sulfuric acid are then added, the tube is allowed to stand at room temperature for 10 minutes, to dissolve the precipitate, and it is then placed in an ice bath for 15 minutes. One ml of cold 1% aqueous tryptophane solution (filtered before using) is added to each tube without shaking. The contents of the tube are mixed and the tube placed immediately in a boiling water

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5 Sheppard, F., and Everett, M. R., *J. Biol. Chem.*, 1937, **119**, 1.

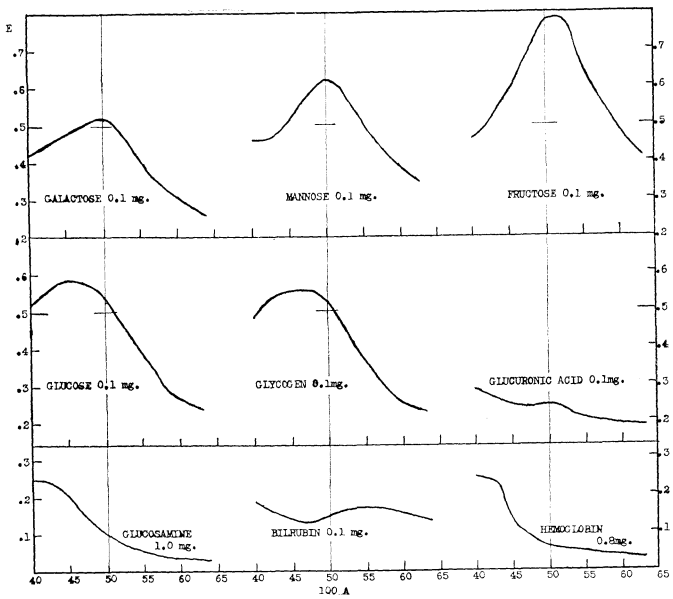


FIG. 1.

Absorption curves of single components after reaction with tryptophane.

bath for a period of 20 minutes. The tube is shaken once after it has been heated 10 minutes. After removing from the boiling water the tubes are cooled for 5 minutes in an ice bath and then allowed to stand 25 minutes at room temperature. The concentration is determined in a Coleman No. 11 spectrophotometer by reading against a blank (without the serum precipitate) which is run through the above procedure.

Standard: Equal amounts of *d*-galactose and *d*-mannose were used as a standard since present evidence indicates that the polysaccharide of serum is composed of equimolecular amounts of glucosamine, mannose, and galactose.[6] As shown by absorption curves (Fig. 2) glucosamine has little influence on readings made at 500 mμ, increasing the absorption only 1%.

Results. Absorption Curves. Absorption curves for mannose, galactose, glucose, glycogen, fructose, glucosamine, glucuronic acid, bilirubin, and hemoglobin are shown in Fig. 1. The maximum absorption for galactose and for mannose is at 500 mμ, for fructose at 520 mμ, for glucose at 460 mμ. As expected, glucose and glycogen produce similar curves.

Absorption curves of mixed sugars are shown in Fig. 2. The optical densities of the individual constituents are additive within experimental error. A typical serum absorption curve, also absorption curves for ascitic fluid, and hydrocele fluid are included in Fig. 2. All curves have been calculated to the same height at 500 mμ. It is noteworthy that the serum, ascitic fluid, and hydrocele fluid curves differ from the galactose-mannose-glucosamine curve by their greater absorption in the 400-480 mμ range. However, absorption curves of partially purified serum polysaccharide (polysaccharide: total nitrogen ratio = 0.97 : 1) and a similar polysaccharide from hydrocele fluid (polysaccharide: total nitrogen ratio = 0.33 : 1) resemble closely the standard galactose-mannose-glucosamine curve. The enhanced absorption between 400 and 480 mμ is therefore due to the serum protein.

When the above described procedure is followed, except that 1 ml of distilled water is added to the serum precipitate in place of the tryptophane solution and the result is read against a blank containing 80 γ of tryptophane (the amount present in 0.0667 ml of

[6] Meyer, K., *Adv. in Protein Chemistry*, 1945, **11**, 249.

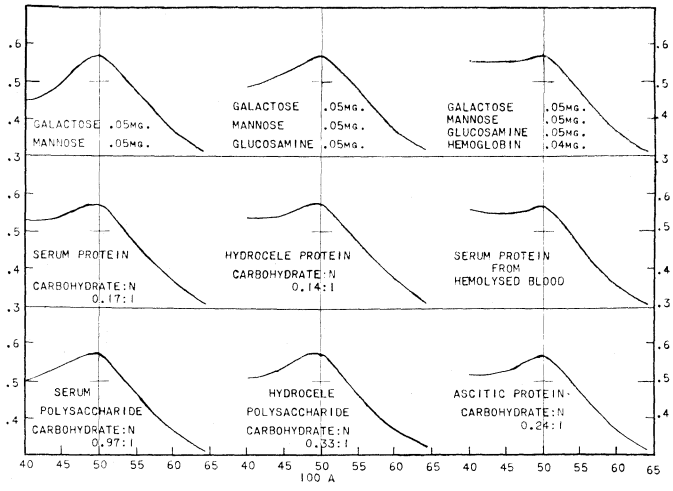


FIG. 2.

Absorption curves of sugar mixtures and of body fluids after reaction with tryptophane.

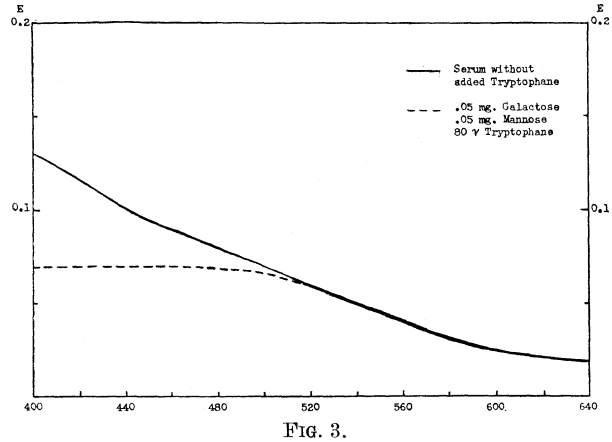


FIG. 3.

Absorption curve of serum protein without added tryptophane read against 60% sulfuric acid.

serum containing 7% protein) the upper absorption curve in Fig. 3 is obtained. The lower curve was obtained by allowing a standard solution of galactose, mannose, and glucosamine to react with 80 γ tryptophane under the above conditions. The difference between the two curves should then represent the result of chromophoric reactions of the system other than the tryptophane-sugar reaction. When this derived curve is used to correct the serum absorption curve, they approach the absorption curves of the galactose-mannose-glucosamine mixtures within experimental error. It is noteworthy that no appreciable correction is necessary for the absorption at 500 mμ. The nature of the substance or substances in serum protein which increase absorption in the region between 400 and 480 mμ was not discovered. Addition to standard sugar solutions of tyrosine, cystine, and histidine at levels found in serum protein failed to influence the shape of the galactose-mannose-glucosamine curve.

Hemolysis of the blood sample, if extensive, results in a positive error; the effect of hemoglobin on the absorption curve (Fig. 2) is most striking in the 400-480 mμ range and small at 500 mμ. Therefore, the polysac-

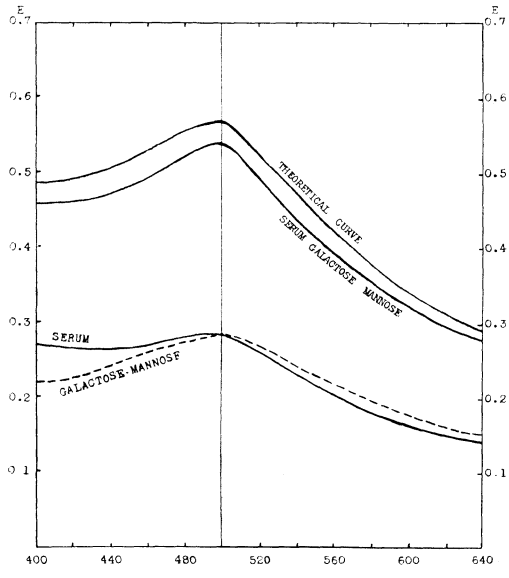
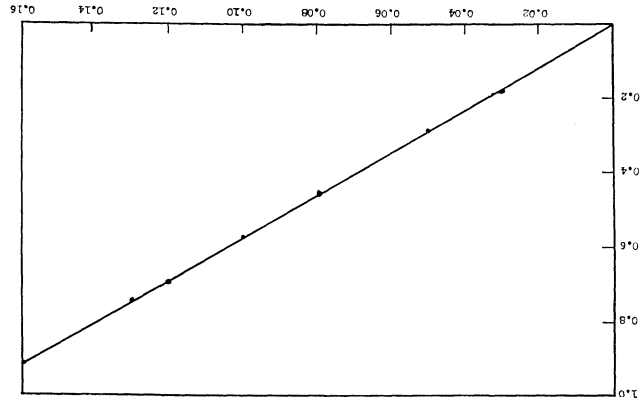


FIG. 4.
Effect of added sugar upon serum-tryptophane absorption curve.

solutions of the precipitates of several serums using the Folin-Wu procedure with a Somogyi (zinc hydroxide) precipitation. No glucose was found in any of these precipitates.

To determine the completeness of the polysaccharide precipitation the total carbohydrate and the polysaccharide were determined by the tryptophane method on the same samples of serum. Free sugar was also determined on these serums by the Folin-Wu procedure using the Somogyi precipitation. When the difference in absorption between the total carbohydrate and the polysaccharides was calculated as glucose, the results are as follows:

	Serum 1	Serum 2
Polysaccharide by tryptophane method	132	118
Glucose by Folin-Wu method	97	102
Total carbohydrate (calculated)	229	220
Total carbohydrate by tryptophane method	228	224



Milligrams of Galactose and Mannose.
FIG. 5.
Concentration curve of the sugar-tryptophane system at 500 mμ.

charide of slightly hemolysed samples may be determined with reasonable accuracy.

Recovery Curves. Mixtures of standard galactose and mannose with serum gave the absorption curve shown in Fig. 4. The height of this curve varies between 94% and 97% of the calculated value obtained by adding the absorption curve for the sugars to that of the serum.

Efficiency of the Precipitation Process. Since it was necessary to learn if any free sugar is carried down in the ethanolic precipitation, glucose was determined on aqueous

Since the total carbohydrate figures determined directly or by calculation agree quite closely and since the precipitate contains no glucose, the serum polysaccharides are apparently completely precipitated by the procedure described.

Calculations. In the concentration range, 0.02 mg to 0.16 mg of an equimolecular mixture of galactose and mannose, the Lambert-Beers law applies,as indicated in Fig. 5. Calculation of unknown concentrations may be made by either reading from the concentration curve or by the following formula:

$$\frac{Ex}{Es} \times .1 \times \frac{100}{.0667} = \text{mg\% polysaccharide as galactose and mannose}$$

where Ex is the extinction (log 1/transmission) of the unknown, and Es is the extinction of 0.1 mg of an equimolecular mixture of galactose and mannose.

Effect of Aging on Absorption Curves. When the solutions were read at various times after development of color, absorption curves similar to those shown in Fig. 6 were obtained. During the first 30 minutes, absorption increased in the 400-480 mμ range. No significant change in the curve occurred between 30 and 60 minutes. Consequently, for quantitative results the samples were read between 30 and 60 minutes after removal from the hot water bath.

Effect of Heating Time on Color Development. When the heating time in the boiling water bath was varied between 10 and 30 minutes, the absorption curves shown in Fig. 7 resulted. There is a progressive increase in the absorption at both ends of the curve as the heating time is increased. Little variation occurred at 500 mμ except in the 25-minute curve for the galactose-mannose mixture.

Quantitative Results on Human Sera. The concentrations of polysaccharide, exclusive of glucosamine, in the sera of 59 normal persons representing both sexes and all ages varied between 80 mg % (fetal blood) and 139 mg % (blood from a senile man). These values are in general agreement with those of Siebert.[3] Detailed results from studies of normal and pathological subjects will be given in another paper. The method described has considerable accuracy, the average difference between duplicates being 2.4% in a large number of blood serum samples.

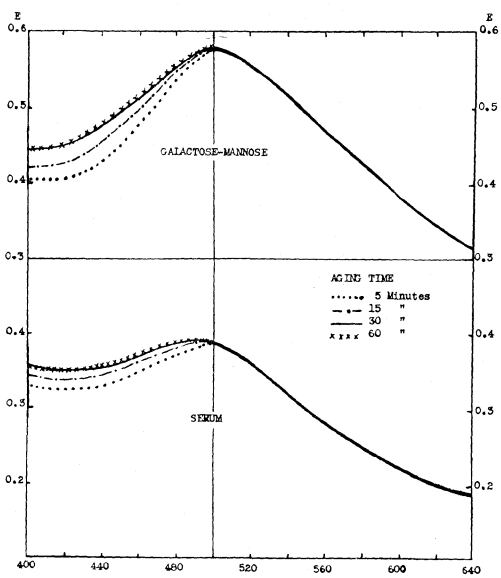


FIG. 6.
The effect of aging after removal from the boiling water bath upon the absorption of the sugar-tryptophane system.

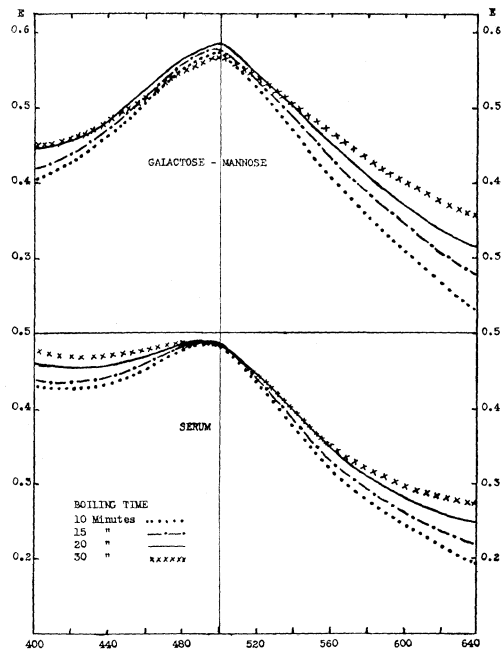


FIG. 7.
The effect of heating time on the absorption of the sugar-tryptophane system.

Summary. A colorimetric method based on the reaction of tryptophane with carbohydrates is described for the determination of serum non-glucosamine polysaccharide. The absorption maximum for the carbohydrate-tryptophane compound is at 500 mμ for galactose and for mannose, 460 mμ for glucose, and 520 mμ for fructose. Glucuronic acid, glucosamine, and hemoglobin have maxima at wave lengths below 400 mμ. Glucuronic acid also exhibits a weak band at 480-500 mμ.

The absorption curves for alcohol precipitates from serum, ascitic fluid, and hydrocele fluid differ from postulated galactose-mannose-

glucosamine curves by showing greater absorption in the 400-480 mμ range. This difference diminishes when the polysaccharide is partly freed from protein.

Hemolysis, if marked, introduces a positive error in the method. The color produced by the carbohydrate tryptophane reaction obeys the Lambert-Beers law within the limits investigated.

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Oxygen Uptake of Human Placental Tissue as Affected by Selected Substrates and Drugs.*

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Very few human tissues other than the placenta are readily available for *in vitro* studies. Despite this fact, little is known about the influence of metabolites or of pharmacologic agents upon this tissue. Any knowledge that may be gained about the behavior of this complex organ, furthermore, may assist in understanding some unsolved problems of human reproduction. We have selected for study the substrates commonly used for the *in vitro* study of tissue metabolism, and those drugs most frequently employed in obstetrics.

Early reports upon the oxygen uptake of human placental tissue[1-4] gave very low Q_{O_2} values, perhaps attributable to their methods. Loesser[5] studied the oxygen consumption, anaerobic and aerobic glycolysis and in placentas two months of age found values for Q_{O_2} of 5.0, $Q_L^{N_2}$ of 7.0 and $Q_L^{O_2}$ of 5.1. At term, these values were 1.4, 3.5, and 0.57, respectively. Wang and Hellman[6] demonstrated changes in placental Q_{O_2} with relation to age in a manner corresponding well with the histologic changes in the villi. The Q_{O_2} was found to decrease from 5.3 at the second month to 1.7 at term, and the addition of glucose to the medium did not alter this value. No reports have been found regarding the influence of any other agents upon this tissue.

Methods. Placentas were placed in an ice-packed container immediately following delivery. The tissue was prepared for respiration studies in a cold box at 5°C.[7] In choosing the experimental material necrotic, highly calcified, or traumatized areas were avoided. Thick sagittal slices were immersed in Ringer's solution and the villi teased free with dissecting needles. Samples of villi weighing from 200 to 250 mg were blotted on filter paper, weighed, and transferred to Warburg flasks. Conventional manometric methods were used throughout these studies. The medium consisted of 2.0 ml of Krebs-Ringer-phosphate solution (pH 7.3) modified to conform more closely to the composition of interstitial fluid.[8] The substrate was 0.2% glucose unless otherwise specified, and the gas phase was oxygen. The oxygen consumption is expressed as microliters of oxygen per milligram dry weight tissue per hour (Q_{O_2}). In testing the effect of drugs upon oxygen uptake, a control period of 80 minutes preceded the experimental

* Aided by grants from the John and Mary Markle Foundation, New York City, and the National Institute of Health, Bethesda, Md.

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