tion, which has been observed in many tissues as well(8), has any significance, especially with reference to the various physiologic functions of platelets, remains to be further investigated.

Summary. (1) Platelets are a source of glutamic oxalacetic transaminase in human blood. (2) Platelets, however, contain only traces of glutamic pyruvic transaminase in normal and pathologic conditions. This finding is not explained by presence of enzyme inhibitors or by lack of a co-enzyme. The significance of the dissociation between GO-T and GP-T activity in platelets, as for other tissues, remains obscure.

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A Method for Assaying for Glycoprotein in Column Fractionation. (24240)

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In the course of experiments involving chromatographic and electrophoretic separation of proteins on a column with subsequent collection of as many as 400 tubes containing eluate from the column, we have been faced with the task of assaying each of these tubes for glycoprotein. Several methods have been described(1-4) for determining qualitatively, semi-quantitatively, and quantitatively carbohydrate; but none can be used conveniently for repeated routine assay purposes. We adapted a staining technic for carbohydrate, which can be managed with a minimum of practice and a maximum of convenience and which will give quantitative determinations with an estimated accuracy of 5%.

Method. The method involves the use of Köiw's stain devised for use with paper electrophoretic or chromatographic strips. The technic for its use is described elsewhere(5). Aliquot samples from each tube are withdrawn and applied to strip of paper by means of a striper supplied with the Spinco hanging-strip paper electrophoresis apparatus. Samples of 10 λ may be applied in a single application, but larger samples are best applied in multiple applications. The paper strip should be that supplied in the Spinco paper electrophoresis apparatus. The samples should be applied about ¼ inch apart at prelocated points on the strip marked by pencilled dots, starting about 3½ inches from one end. About 25-30 samples may be applied to one strip.

Application of sample to the paper is critical inasmuch as there will be an accumulation of carbohydrate at edges of the band formed by the sample, if it is left to dry in air at room temperature after application. Should this happen, quantitative determination is impossible. We placed the paper strip on a slide warmer at temperature about 80°C, applying the samples to the strips, and immediately thereafter passing a flow of warm air over them from a hair dryer. In this way, the sample is dry within 10 seconds. As many as 8 such strips may be stained simultaneously with Köiw's stain, and the resultant strips, after drying, should be passed immediately through the Spinco Analytrol for a permanent record and quantitative determination. If

necessary, a known amount of glycoprotein or other standard may be applied to each strip by way of calibration reference. Integration under the curves obtained on the Analytrol is readily performed, but the height of peaks may be used instead for quantitative determination. The Analytrol should be made to scan a sufficient distance along the strip where no samples have been applied, to obtain adequate determination of the background. It may be possible to obtain improved results by use of green light filters (*e.g.*, Klett 52) instead of standard blue filters found in the Analytrol.

Results. Fig. 1 shows the kind of results obtained by assaying for carbohydrate in the region of a peak eluted from a calcium phosphate column and detected by absorption at 280 mμ. The material placed on the column was a bovine fraction VI and the eluent was a 0.06 M phosphate buffer at pH 6.8.

Fig. 2 shows results of applying this method to the location of a glycoprotein fraction in the column electrophoresis of bovine fraction VI to which had been added I^{131} labeled thyroxine. The LKB column electrophoresis apparatus packed with Solka Floc (cellulose), 200 mesh, was used. Two hundred mg of protein was placed on the column and electrophoresed at 5 ma and 770 volts for 29 hours in a barbital buffer medium at pH 8.6, 0.0125 M. The column was eluted at 60 seconds/drop with barbital buffer, and 1 ml fractions collected. It is worth noting that the glycoprotein peak coincides with the radioactivity peak but not exactly with the OD 280 mμ peak, and this result has been confirmed.

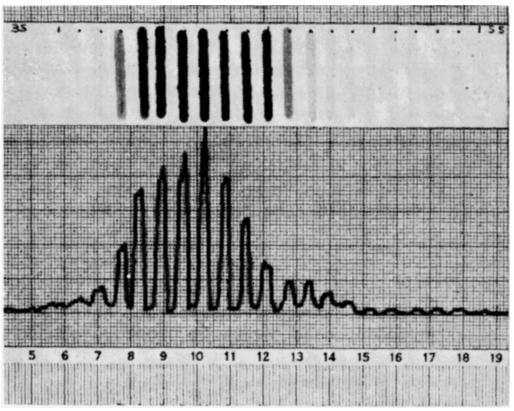

FIG. 1. Developed paper strip and Analytrol scan. Assay for glycoprotein in a chromatographic separation of fraction VI.

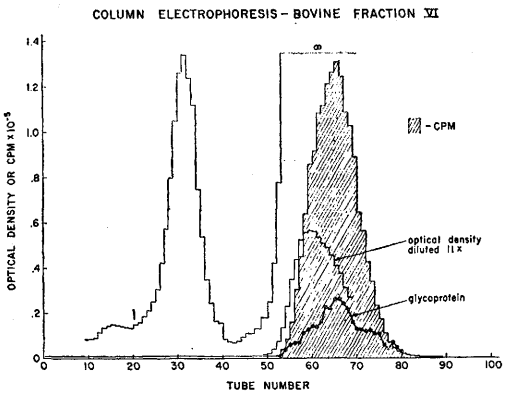

FIG. 2. Column electrophoresis of bovine fraction VI. Shaded area represents location of thyroxine.

Summary. A convenient method for qualitative or quantitative determination of carbohydrate in many samples has been worked out. The method involved the deposition of micro quantities of the samples on paper strips, staining with Köiw's stain and analysis of the results with the Spinco Analytrol apparatus. The method, applied to the fractions obtained from the column electrophoresis of bovine fraction VI on a cellulose column, demonstrated the presence of a glycoprotein component which binds I^{131} labeled thyroxine.

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