While this work was in progress, 2 papers appeared whose authors proved that intraperitoneal insemination is feasible in the bovine(7), although the results were not encouraging, and in the guinea pig(8), where only epididymal semen was used. Further work on this problem is still in progress.

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## Platelets XXI. Glutamic Pyruvic Transaminase Activity of Human Platelets.* (24239)

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Previous work has shown that human blood platelets represent a source of glutamic oxalacetic transaminase (GO-T)(1). Observations have now been extended to the related enzyme, glutamic pyruvic transaminase (GP-T). The study appears justified since both enzymes have been found in serum and in homogenates of many tissues with wide differences of activity from tissue to tissue and from health to disease.

*Materials and methods.* The activity of GO-T and GP-T was investigated in serum, platelet-rich and platelet-poor plasma, washed platelets, washings of isolated platelets and water soluble platelet extracts. All materials were of human source. Serum was obtained by centrifugation from clotted blood, collected in sterile syringes and incubated at 37° for one hour. To prepare platelet-rich and platelet-poor plasma, blood was collected in Silicone-coated test tubes, using Sequestrene as anticoagulant(2). To obtain platelet-rich plasma, the blood was centrifuged at 1,000 rpm/10′/4°C; and at 3,000 rpm/30′/4°C for platelet-poor plasma. The upper two-thirds of supernatant plasma were transferred to fresh Silicone-coated test tubes. Platelets were prepared by the technic previously described(2). They were washed 3 times with sterile isotonic saline solution using, each time, a volume identical to that of the platelet-rich plasma originally used. A discrete suspension was obtained, the platelets counted by the method previously described(3) using phase contrast microscope and their number adjusted to various levels by adding sufficient isotonic saline to the original platelet suspension. Water soluble platelet extracts were prepared exactly as described(1). Both GO-T and GP-T activity were determined within 2 hours of preparation of the reagents. The spectrophotometric technic of Karmen *et al.* (4) was used for determination of GO-T and that of Wróblewski and LaDue(5) for determination of GP-T. A minor modification was introduced since 0.03 ml of malic acid reagent was used rather than 0.1 ml as suggested in the original technics. All experiments were done in triplicate. The reacting mixture was incubated for 10 minutes and readings taken at 5, 10 and 15 minutes after adding alpha-ketoglutaric acid reagent. Therefore, the final figures reported represent the average of 9 values obtained for each sample. Duplicate determinations were also done using the colorimetric method of Reitman and Frankel(6).

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TABLE I. Glutamic Oxalacetic and Glutamic Pyruvic Transaminase Activity in Serum, Plasma and Various Platelet Preparations.*

| Preparation | No. of platelets per mm³ ($10^3$) | GO-T activity, units/ml | GP-T activity, units/ml |
|---|---|---|---|
| Serum | — | 47.6 ± 4.9 | 40.6 ± 5.8 |
| Platelet-rich plasma | 356 ± 12.3 | 46.3 ± 6.1 | 37.3 ± 6.3 |
| " -poor " | 13 ± 1.4 | 25.3 ± 3.2 | 36.2 ± 4.7 |
| " suspensions | 2,000 ±240 | 265 ±15.3 | <1 |
| | 480 ± 27 | 59.3 ± 4.0 | |
| | 190 ± 19 | 47.2 ± 2.7 | |
| | 50 ± 3 | 29.4 ± 1.3 | |
| Washings of sedimented platelets | | 12.5 ± .9 | 8.3 ± .9 |
| Water soluble platelet fraction | | 109.7 ± 9.9 | 3.2 ± .3 |

* Avg of 5 experiments and stand. dev.

This technic appeared adequate, although less sensitive than the spectrophotometric procedure.

*Results.* (Table I). The figures obtained for GO-T activity in serum and platelets agreed closely with those previously reported (1). The serum GP-T activity was within the ranges reported by previous investigators. There was however an insignificant difference in GP-T activity between platelet-poor and platelet-rich plasma. Also platelet suspensions and water soluble platelet extracts contained negligible GP-T activity. These results suggested that human platelets contain no GP-T activity. Since, however, slight GP-T activity was detected in washings of sedimented platelets, it is possible that minute amounts of enzymatic activity are absorbed from serum on platelets. Two patients with acute infectious hepatitis and one with myocardial infarction were studied since serum GP-T activity is higher in these diseases. GO-T activity was greatly increased in serum, plasma and various platelet preparations. As expected, GP-T activity was very high in serum, platelet-poor and platelet-rich plasma, but only traces were present in platelet preparations and in water soluble platelet extracts (Table II).

To eliminate the presence of an inhibitor of GP-T activity in human platelets, equal volumes (0.1 ml) of untreated human serum and of a suspension of homologous platelets in isotonic saline (containing 190,000 platelets/cu mm) were incubated at 37°C for 3 hours. Also, in other experiments 100 mg of pyridoxal phosphate were added to the reacting mixture, since it has been shown that this reagent is a coenzyme of transaminases(7). Because of the possibility that platelets may not be permeable to the added coenzyme or that an inhibitor may not diffuse out of intact platelets these experiments were repeated using water soluble platelet extracts. There was no change in GP-T activity of serum beyond that accounted for by dilution.

*Discussion.* It appears that human platelets contain considerable GO-T but very little or no GP-T activity. The very low values of GP-T detected may even indicate that washing of platelets was insufficient to remove contaminating plasma. Whether this dissocia-

TABLE II. Comparative Glutamic Oxalacetic and Glutamic Pyruvic Transaminase Activity in Serum, Plasma and Platelet Suspensions in 3 Patients.

| | Infectious hepatitis | | Infectious hepatitis | | Acute myocardial infarction | |
|---|---|---|---|---|---|---|
| | GO-T | GP-T | GO-T | GP-T | GO-T | GP-T |
| | Units/ml | | | | | |
| Serum | 245 | 500 | 220 | 390 | 184 | 142 |
| Platelet-rich plasma | 240 | 475 | 210 | 382 | 178 | 136 |
| " -poor " | 182 | 475 | 137 | 378 | 132 | 135 |
| " suspensions (200,000 platelets/mm³) | 72 | 10 | 45 | 11 | 62 | 2 |

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