Carbohydrates of Human and Bovine Platelets

By E. E. Woodside and W. Kocholaty

The lack of information on the polysaccharides and their corresponding monomers in human and bovine platelets has prompted this comparative study on the total bound monosaccharide units and their derivatives in these formed elements of blood. The determination of the monosaccharides in various chemically fractionated components (an acid mucopolysaccharide fraction, a glycoprotein fraction and the 5 per cent trichloroacetic acid-soluble and -insoluble fractions) has revealed the existence of numerous carbohydrate moieties.

Methods

Source and Preparation of Platelets

Human blood was collected by phlebotomy with the use of customary American National Red Cross equipment.* The standard evacuated glass bottles contained 110 to 120 ml. of an acid-citrate-dextrose anticoagulant solution, N.I.H. formula A or B. In a limited number of experiments, the freshly collected blood was immediately packed in ice so as to inhibit enzymatic breakdown of platelet constituents during the transportation of the blood to the laboratory. Fresh bovine blood was collected at the abattoir from the slashed neck veins of cattle.

The differential centrifugation procedure used was that outlined by Kocholaty and Horn.3 In a few experiments, human and bovine platelet-rich plasma was obtained from whole blood within 2 hours after collection. Platelets obtained by differential centrifugation were suspended in either saline, 2 per cent gelatin, or in the platelet preservation medium of Tullis.4† Thus, glycogen determinations could be carried out within 6 hours after the collection of blood. For prolonged incubation experiments, aliquots of the suspended platelets were distributed into appropriate tubes and incubated for the desired time at 37 or 4 C. The platelet suspensions, subsequently referred to as stock solutions, ranged from 1 to 2 × 10^10 platelets per milliliter for human cells and from 2 to 3 × 10^10 platelets per milliliter for bovine cells. One or 2.0 ml. of the stock platelet suspensions were dried to constant weight (±0.2 mg.) at 100 C. in tared aluminum dishes. The theoretical amount of NaCl (8.5 mg. per milliliter) was subtracted from the total dry weight, and these corrected values were correlated with the original cell counts.

Carbohydrate Standards

The following C. P. grade carbohydrates, obtained from Pfannstiehl Chemical Company, Waukegan, Ill., were used for both the analytical technic and paper chromatography: xylose, arabinose, rhamnose, mannose, galactose, glucose, levulose and glycogen. Fucose and galactosamine-HCl were obtained from Nutritional Biochemicals Corporation, Cleveland, Ohio; galacturonic acid and glucosamine-HCl, Eastman Organic Chemicals, Rochester, N. Y., and glucuronolactone, C. P., from Mann Research Laboratories, New York, N. Y.

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†Glucose 5 Gm. NaCl 0.85 Gm., sodium acetate 0.2 Gm. and gelatin 2.0 Gm. per 100 ml. distilled water.

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Chemical Determinations of Carbohydrates

Total hexoses, pentoses and methylpentoses.—The technic of Mokrasch² for the simultaneous determination of hexoses and pentoses was used. Aldohexoses and glycogen are reported as glucose equivalents and pentoses and methylpentoses as ribose equivalents.⁶ The individual sugars eluted from paper chromatograms are reported as equivalents of the corresponding sugars. For total hexose and total pentose (pentose and methylpentoses) determinations, 0.5 ml and 1.0 ml of 1:20 dilution of the stock platelet solutions were used.

Glucosamine and galactosamine determination.—The simultaneous determination of glucosamine and galactosamine of Tracey⁷ was employed. One ml. of the stock platelet solutions was used for the 3 N HCl hydrolysis. The hydrolyzed platelets or fractions were neutralized with 3 N NaOH and diluted with distilled water to a total volume of 6.0 ml. One and 2.0 ml aliquots were removed for the chemical determinations.

Sialic acid determination.—The diphenylamine reaction as modified by Winzler² was used. The alpha-1 glycoprotein* containing 10 per cent sialic acid was used as the standard. Only 0.2 ml of the stock platelet suspensions were needed for these determinations.

Glucuronic acid determinations.—Only a qualitative chemical test was employed. The color reaction of glucuronic acid with thioglycolic and sulfuric acid in the presence of mannose, as described by Dische,⁵ was employed. Aliquots of 0.25 ml and 0.50 ml stock platelet suspensions were used.

Polysaccharide Fractionation Procedures

Fractionation with 5 per cent trichloroacetic acid (TCA).—To 1.0 ml of the sonorated¹ stock platelet suspension was added dropwise with agitation 1.0 ml of 10 per cent TCA. The mixture was allowed to stand at room temperature for 10 minutes and then centrifuged at 2000 rpm (510 x g) for 5 minutes at 4 °C. The supernatant solution was filtered through Whatman no. 42 filter paper and the precipitate resuspended in 2.0 ml of 5 per cent TCA and recentrifuged. This procedure was repeated two more times with the use of 3.0 ml of TCA, the final volume of the TCA soluble filtrate being 10 ml. Aliquots of 0.5 ml and 1.0 ml of the TCA soluble filtrate were analyzed for total hexose and pentose. The remainder of the filtrate was lyophilized and hydrolyzed according to the procedure of Glegg and Eidinger¹ for paper chromatography. The TCA insoluble residue was extracted twice with 10 ml portions of ethyl ether (to remove residual TCA) and hydrolyzed with 2 N H₂SO₄ at 100 °C. for 4 hours in sealed tubes prior to paper chromatography.

Glycogen determination.—Initially, glycogen was determined according to the TCA extraction technic described by Carroll, Longley and Roe.⁹ Glycogen was also determined by the 30 per cent KOH extraction technic of Fong, Schaeffer and Kirk.¹⁰ The latter procedure extracted approximately twice the amount of glycogen and required only half the initial volume of platelets. In the prolonged incubation experiments, aliquots were pipetted into centrifuge tubes at the 0 hours incubation in order to avoid clumping associated with prolonged incubation at either 4 or 37 °C.

Seromucoid determination.—The technic outlined by Winzler² was applied to 1.0 ml portions of sonorated stock platelet suspensions.

Mucopolysaccharide fraction.—The procedure outlined by Glegg and Eidinger¹ was applied to stock platelet suspensions (200 to 900 mg. dry weight of platelets). The acid mucopolysaccharide (F-I) fraction and the 84 per cent ethanol-insoluble fraction (F-II)

*A sample of alpha-1 glycoprotein was kindly supplied by Dr. K. Schmid, Department of Medicine, Harvard Medical School, Boston, Mass.

¹Platelet suspensions were treated with a Raytheon 10 KC oscillator cooled by running tap water. Treatment for 5 minutes at resonance resulted in a platelet disintegration of 95 per cent.
CARBOHYDRATES OF HUMAN AND BOVINE PLATELETS

were hydrolyzed by the cation exchange resin technic. The hydrolyzed monosaccharides and the eluted hexosamines were dried in vacuo at 40 C. for paper chromatographic analysis.

Paper Chromatographic Procedure

The ascending paper chromatographic technic of Hammond, Bartz and Reifte was employed. Due to the limited number of monosaccharides found to be present in platelets, it was necessary to run the paper chromatogram in the first dimension only. Two monosaccharides, fucose and ribose, could not be completely resolved by either one or two dimensional chromatography, however, the spray reagent, aniline hydrogen phthalate, readily differentiated between these two monomers. The spray reagent used for hexosamines was that described by Partridge. Whole platelets, TCA insoluble residues and some of the deproteinized fractions obtained in the Glegg and Eidingcr fractionation were found not to be completely hydrolyzed by the cation exchange resin. Consequently, these fractions were hydrolyzed with 2 N H2SO4 for 4 hours in sealed glass tubes at 100 C. The hydrolyzed material was then prepared for chromatography by the procedure of Partridge.

RESULTS AND DISCUSSION

By correlation of the dry weight with the platelet count, average dry weight values for both human and bovine platelets were obtained. The average of 21 separate dry weight determinations for 1 x 10^10 bovine platelets was 12.82 ± .58 mg. The average of 11 separate dry weight determinations for 1 x 10^10 human platelets was 20.64 ± .79 mg. The above values are given with their standard errors of the mean. Thus, bovine platelets would appear to be approximately one-third smaller than human platelets on a dry weight basis. The various carbohydrate values presented in the following experimental work have been calculated on a dry weight basis from the average values mentioned above.

Although Campbell, Small, Lopilato and Dameshek have demonstrated the presence of levulose in human platelets, this ketohexose could not be chromatographically identified in either human or bovine platelets subsequent to hydrolysis. It is possible that levulose does not exist in a polysaccharide-protein complex, but merely as the free phosphate ester which may be removed by diffusion during the repeated washings.

Contrary to the findings of Wagner, human platelets as well as bovine platelets were found to contain considerable amounts of glycogen. In fact, glycogen extracted by the KOH procedure accounted for nearly 50 per cent of the total hexose content in both human and bovine platelets. Upon hydrolysis and chromatography of the glycogen fractions (both TCA and KOH extractions), the only aldohexose identified was glucose. This finding would indicate that no co-precipitation of other polysaccharides which could contain such aldohexoses as mannose or galactose had occurred under the experimental conditions.

Upon hydrolysis and chromatography of both human and bovine platelets, the following monomers were identified: glucuronic acid, galactosamine, glucosamine, galactose, glucose, mannose, fucose and ribose. In addition to the above monomers, sialic acid was also found in both human and bovine platelets. This would further demonstrate the wide distribution of the sialic acids in mammalian tissues.
The glucuronic acid spot was invariably contaminated with another unidentified spot on the paper chromatograms of hydrolyzed human and bovine platelets. Elution of the overlapping spots with distilled water and chemical analysis, for glucuronic and galacturonic acids revealed the presence of only glucuronic acid. The unidentified spot would not exclude other uronic acids, such as iduronic, mannanuronic or guluronic acids. No attempts to identify these acids were undertaken in the above-mentioned solvent systems.

Excluding glucuronic acid, the total carbohydrate content of human or bovine platelets can be approximated by the addition of the individual carbohydrate components found to be present. The total bound carbohydrate content of human and bovine platelets, respectively, was calculated as 8.5 and 8.8 per cent of the dry weight (table 1).

Determination of the hexose and pentose distribution in platelets by various fractionations revealed significant differences between human and bovine platelets (table 2). The TCA-soluble fraction of human platelets accounted for 54 per cent of the total hexose, whereas it accounted for 68 per cent of the hexose of bovine platelets. Chromatography revealed the presence of ribose and glucose only.

Chromatography of the glycogen fraction revealed the presence of glucose with traces of ribose in some instances. By chromatographic identification, glucose was found to be the only aldohexose present in the TCA soluble fraction which could react with the anthracene reagent. Therefore, it seemed probable that not all of the glycogen was precipitated upon addition of alcohol (5:1 ratio). Upon increasing the alcohol concentration to 10:1, no additional glycogen could be precipitated. In view of the finding that KOH extraction of the glycogen was approximately twice that of the TCA method (30 per cent KOH would destroy free glucose and its oligosaccharides), it appeared that the TCA extraction procedure effected only partial glycogen extraction from platelet suspensions.

The seromucoid fraction, which could contain at least three carbohydrate components of different electrophoretic mobilities, accounted for 46 per cent of the total hexose of human platelets, but for only 23 per cent of the bovine total hexose content. With respect to human platelets, the 5 per cent TCA

<table>
<thead>
<tr>
<th>Carbohydrate fraction</th>
<th>Human platelets</th>
<th>Bovine platelets</th>
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<tbody>
<tr>
<td></td>
<td>No. of exper.</td>
<td>% of dry weight*</td>
</tr>
<tr>
<td>Pentose and methylpentose</td>
<td>16</td>
<td>0.67 ± .11</td>
</tr>
<tr>
<td>Hexose—total</td>
<td>16</td>
<td>4.41 ± .28</td>
</tr>
<tr>
<td>Glycogen-TCA† extraction</td>
<td>6</td>
<td>1.16 ± .17</td>
</tr>
<tr>
<td>Glycogen-KOH† extraction</td>
<td>6</td>
<td>2.34 ± .17</td>
</tr>
<tr>
<td>Glucosamine</td>
<td>10</td>
<td>0.10 ± .04</td>
</tr>
<tr>
<td>Galactosamine</td>
<td>10</td>
<td>2.57 ± .16</td>
</tr>
<tr>
<td>Sialic acid</td>
<td>14</td>
<td>0.72 ± .05</td>
</tr>
</tbody>
</table>

*Including the standard error of the mean.
†Trichloroacetic acid.
‡Potassium hydroxide.
Table 2.— **Distribution of the Total Carbohydrate in Chemically Fractionated Platelets***

<table>
<thead>
<tr>
<th>Carbohydrate fraction</th>
<th>Human platelets</th>
<th>Bovine platelets</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of exper.</td>
<td>Pentose and methylpentose</td>
</tr>
<tr>
<td>Total carbohydrate</td>
<td>17</td>
<td>0.60 ± .11</td>
</tr>
<tr>
<td>5% TCA soluble fraction</td>
<td>15</td>
<td>0.34 ± .08</td>
</tr>
<tr>
<td>Glycogen-TCA extraction</td>
<td>16</td>
<td>0.08 ± .02</td>
</tr>
<tr>
<td>Seromucoid fraction</td>
<td>6</td>
<td>0.39 ± .11</td>
</tr>
</tbody>
</table>

*All values are expressed as % of dry weight including the standard error of the mean.

†Trichloroacetic acid.

Insoluble residue was, in essence, the seromucoid fraction since chemical analysis and chromatography of the sugars revealed similar monosaccharides in both fractions. With respect to bovine platelets, approximately 9 per cent of the TCA insoluble residue could not be attributed to the precipitated seromucoid fraction; however, this difference could be due to co-precipitation of the seromucoid fraction with the platelet proteins. ²

Hydrolysis and chromatography of the TCA-insoluble residues yielded mannose, glucose, galactose, and fucose in both human and bovine platelets (hexosamine determinations were not included in this series of experiments). In addition to containing twice the amount of hexose, the human platelet seromucoid fraction also contained twice as much fucose (methylpentose) as the bovine platelet seromucoid fraction on a dry weight basis. Fractionation of the TCA-insoluble residue by the Glegg and Eidinger¹ procedure revealed the corresponding polysaccharide fractions, namely F-I and F-II, in this residue also.

In the above mentioned glycogen determinations, considerable discrepancies between values obtained either by the KOH or by the TCA extraction procedures (table 1) were observed in sonorated human and bovine platelets. Some investigators¹⁰,²⁴,²⁵ have shown 5 or 10 per cent TCA to extract considerably less glycogen from liver and skeletal muscle than the KOH procedure. On the other hand, Carroll, Longley and Roe⁰ maintained that all of the glycogen could be extracted from liver cells and muscle by employing proper homogenization and re-extraction with TCA. Following their procedures, it was found that 45 to 55 per cent more glycogen was extracted from sonorated platelets than from whole platelets. However, glycogen values for whole or sonorated platelets remained essentially the same when extracted with KOH.

Table 3.— **Variations in Extractable Glycogen, as Influenced by the Suspending Medium**

<table>
<thead>
<tr>
<th>Suspending medium</th>
<th>Glycogen standard (100 µg.)</th>
<th>Human platelets ex. glycogen per ml.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TCA* µg. extracted</td>
<td>KOH µg. extracted</td>
</tr>
<tr>
<td>Saline</td>
<td>98.9</td>
<td>100</td>
</tr>
<tr>
<td>2% gelatin</td>
<td>7.05</td>
<td>98.9</td>
</tr>
<tr>
<td>Tullis medium</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

*Trichloroacetic acid extraction technic of Carroll et al.²⁵
¹Potassium hydroxide extraction technic of Fong et al.²⁶
In addition, the KOH values were approximately 50 per cent higher than those obtained for sonorated platelets extracted by the TCA procedure.

Probably the most important contributing factor preventing total precipitation of glycogen from TCA solutions was the nature and amount of proteins present in the TCA-soluble and insoluble fractions. From table 3 it can be seen that glycogen in 0.85 per cent saline solutions could be completely recovered by the TCA as well as the KOH procedure. However, when glycogen was suspended in 2 per cent gelatin only 7 per cent of the glycogen was recovered by the TCA procedure. In contrast, the KOH procedure resulted in complete recovery of the glycogen from the glycogen-2 per cent gelatin suspension (the 30 per cent KOH most likely degrades the gelatin to the point where polysaccharide-protein bonding is negligible).

At the beginning of the investigations, large variations in the glycogen and hexose values of human platelets were encountered (table 4). These variations were found to be related to the varying time intervals between the collection and isolation of platelets. The influence of time of collection and of treatment prior to carbohydrate determination revealed some of the major sources of variation (table 4). Sonoration of platelets presumably allowed for greater endogenous metabolism of the carbohydrates of platelets since whole platelet suspensions maintained higher total hexose and glycogen values. Even more striking were the higher values obtained when the platelets were removed from the blood within 2 hours after collection. Furthermore, it was found that prolonged incubation of isolated whole platelets at 4 C. in saline did not result in as low values as obtained when the platelets were left suspended in whole blood for extended periods of time. It would appear that the carbohydrate metabolism of platelets was more accelerated in whole blood than in saline, implying that either plasma or the other formed elements of blood, or both, could exert biochemical influences resulting in an increased catabolism of the platelet carbohydrates.

The total hexose values for both the human and bovine platelets listed in table 5 varied in a manner similar to those previously described in table 4. Thus, it is evident that one of the important factors governing the loss of total

| Preliminary treatment | Hours incubation 4 C. | Total hexose | Glycogen† | Ratio: prolonged incubation; 0 hr. control | Hexose | Glycogen
|-----------------------|-----------------------|--------------|----------|-----------------------------|--------|--------|
| Sound-treated platelets | 1 day in whole blood plus 1 day in saline at 4 C. | 0 | 2.32 | 0.98 | 1.02 | 0.91
| Whole platelets | 1 day in whole blood plus 1 day in saline at 4 C. | 24 | 2.36 | 0.89 | 1.01 | 0.88
| Whole platelets | 3 hours in whole blood plus 6 hours in saline | 0 | 5.88 | 2.44 | 0.87 | 0.63

*Values expressed as per cent of dry weight.
†Glycogen extracted by the procedure of Fong et al.26
Table 5.—Effect of Storage at 4 C. on the Carbohydrate Content of Platelets

<table>
<thead>
<tr>
<th>Human platelets</th>
<th>Bovine platelets</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hours exposure</strong></td>
<td><strong>Total hexose</strong></td>
</tr>
<tr>
<td><strong>whole blood</strong></td>
<td><strong>Time in saline</strong></td>
</tr>
<tr>
<td>24 days</td>
<td>1-2</td>
</tr>
<tr>
<td>24 hours</td>
<td>6</td>
</tr>
<tr>
<td>3 hours</td>
<td>6</td>
</tr>
</tbody>
</table>

*All values reported as per cent of dry weight with their corresponding standard error of the mean.

hexose as well as glycogen, is the exposure of platelets to whole blood. These observations may be compared with the studies on leukocyte glycogen by Wagner, who reported a rapid loss of the glycogen from leukocytes isolated from blood and stored at 4 C. Since initial glycogen determinations on human and bovine platelets were done 9 hours after collection (3 hours in whole blood), the total glycogen of whole blood, as reported by Wagner, still not accounted for (32 per cent) may possibly be losses attributable to platelets (table 5). If this were the case, an upper limit for the glycogen content would be 3.66 mg./100 ml. of whole blood or double the highest glycogen value obtained (66 per cent of the total glycogen in whole blood).

The variations in pentose and methylpentose illustrated in table 5 can be attributed to normal variation in ribose content and/or leakage of ribose derivatives from the platelets upon prolonged storage. These variations ranged from 0.38 to 0.68 per cent for human platelets and from 0.64 to 0.73 per cent for bovine platelets.

In the fractionation procedure of Glegg and Eidinger, two distinct fractions were obtained by varying the alcohol concentration. Fraction I (F-I), which was precipitated by 63 per cent ethanol, contained the acid mucopolysaccharides and glycogen. Fraction II (F-II), which contained one or more polysaccharides composed of aldohexoses, methylpentoses, hexosamine and a considerable amount of protein, was precipitated by 84 per cent ethanol. Application of this fractionation procedure to human and bovine platelets revealed the presence of glucuronic acid, galactosamine, galactose, glucose and ribose in F-I fractions of both human and bovine platelets. Glucosamine, galactose, mannose, fucose, ribose and sialic acid were present in F-II fractions of both human and bovine platelets. In addition, F-II fractions of human platelets also contained galactosamine (table 6).

Cytochemical evidence has been presented for the absence of acid mucopolysaccharides and for the presence of these constituents in platelets. Identification of galactosamine and glucuronic acid with the concurrent absence of glucosamine in F-I fractions of both human and bovine platelets would tend to eliminate the possible presence of either hyaluronic acid or heparin (table 6). These results would confirm the preliminary findings of Kerby and
Table 6.—Monosaccharides Present in Isolated Fractions I and II After Hydrolysis and Paper Chromatography

<table>
<thead>
<tr>
<th>Source of Fractions</th>
<th>Fraction I</th>
<th>Fraction II</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GA</td>
<td>Ga-N</td>
</tr>
<tr>
<td>Human platelets</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Bovine platelets</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Human RBC</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Prothrombin</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

+ , monomer always present; −, monomer always absent; ±, monomer absent but traces occasionally present; 0, monomers not determined.

GA: glucuronic acid; Ga-N: galactosamine; Gl-N: glucosamine; Ga: galactose; Gl: glucose; M: mannose; F: fucose; R: ribose; SA: sialic acid (determined chemically on aliquots of unhydrolyzed fractions).

Langley\textsuperscript{34} with respect to the existence of chondroitin sulfate B in human platelets. In addition, the tentative identification of chondroitin sulfate in rat platelets by Odell, Jr., and Anderson\textsuperscript{31,32} is in agreement with the findings of the monomers of chondroitin sulfate (table 6) in F-I fractions of both human and bovine platelets.

The ribose derivatives in the F-I fractions of both human and bovine platelets have been identified as adenosine-phosphate and its derivatives.\textsuperscript{14,28} Most of the glucose present in F-I is probably derived from glycogen. In addition, part of the glucose present could exist as a polysaccharide in combination with the galactose and/or galactosamine which were also present in this fraction. The biologic significance of the polysaccharide(s) present in F-II has not been elucidated. Recently Anderson and Odell, Jr.,\textsuperscript{32} have obtained from rat platelets a polysaccharide which was free of uronic acids and which could be similar to the F-II fractions listed in table 6. From the similarity of the monomers isolated from F-II fractions of human platelets and human red blood cells (table 6) one may speculate on the presence of immunologically specific carbohydrate-protein complexes responsible for the platelet antigenicity.\textsuperscript{35,36}

Bestetti and Crosti\textsuperscript{37} have identified at least two aldohexoses and glucosamine on paper chromatograms of hydrolyzed human platelets and have suggested the possible existence of physiologically active glycoproteins which may influence the blood clotting mechanism. Subsequent studies of washed human\textsuperscript{38} and bovine\textsuperscript{39} platelets revealed the presence of fibrinogen. Human and bovine platelets and prothrombin contain similar monomers in F-II fractions (table 6).

Table 7 reveals that only very small amounts of the total hexoses are accounted for in both F-I and F-II fractions of human and bovine platelets (7.3 per cent and 8.1 per cent of the total hexose, respectively). In other experiments, yields up to two and one-half times greater than those listed in table 7 were obtained. A similar variation in yield has been reported by Glegg and Eidinger.\textsuperscript{1} The above data show that from 75 to 92 per cent of the total hexoses are not present in either F-I or F-II preparations. In addition, a highly soluble carbohydrate moiety was found in the supernatant solution after 84 per cent ethanol precipitation of F-II. After drying this supernatant solution in vacuo at 40 C. and extraction of the residue 3 times with 12 to 15 ml. of 95 per cent ethanol, the remaining residue was found to contain additional hexoses. The
large variety of hexose-containing protein fractions further exemplified the probable complexity of the carbohydrate protein moieties existing in both human and bovine platelets.

**Summary**

1. Average values for the total carbohydrate content of human and bovine platelets were 8.47 and 8.77 per cent of the dry weight, respectively. On a dry weight basis, human platelets were calculated to be approximately one-third larger than bovine platelets. By chemical and chromatographic analysis, glucose, galactose, mannose, fucose, ribose, glucosamine, galactosamine, glucuronic acid and sialic acid were detected in both human and bovine platelets.

2. Glucose and ribose were found to be the only monomers present in the 5 per cent TCA-soluble fractions of both human and bovine platelets. The TCA-insoluble residue contained glucose, galactose, mannose, fucose, ribose, glucuronic acid, glucosamine, galactosamine and sialic acid. The acid mucopolysaccharide fraction of both human and bovine platelets contained galactosamine and glucuronic acid monomers as well as galactose, glucose, and ribose.

3. Glycogen was found in both human and bovine platelets. Total glycogen was extracted by 5 per cent TCA from sonorated human and bovine platelets; however, subsequent ethanol precipitation of the glycogen was incomplete. In contrast, 30 per cent KOH extraction followed by ethanol precipitation resulted in complete recoveries of the glycogen.

4. Variations in both total hexose and glycogen contents were found in both human and bovine platelets. These variations were partially dependent on the length of storage of the platelets in whole blood or in 0.85 per cent NaCl after separation from whole blood.

**Summario in Interlingua**

1. Valores medie pro le contento total de hydrato de carbon in plachettas human e bovin esseva 8,47 e 8,77 pro cento del peso sic, respectivamente. Caleulate super le base de pesos sic, plachettas human se revelava como approximativamente un tertio plus grande que le plachettas bovin. Le analyse chimic e chromatographic demonstrava le presentia in plachettas human e bovin de glucosa, galactosa, mannosa, fucosa, ribosa, glucosamina, galactosamina, acido glucuronic, e acido sialic.
2. Glucosa e ribosa esseva le sol monomeros trovate, tanto in le caso del plachettas human como etiam bovin, in le fractiones solubile in 5 pro cento de acido trichloroacetic. Le non assi solubile residuo contineva glucosa, galactose, mannosa, fucosa, ribosa, acido glucuronic, glucosamina, galactosamina, e acido sialic. Le fraction de mucopolysaccharido acide del plachettas human e bovin contineva monomeros de galactosamina e acido glucuronic e etiam galactosa, glucosa, e ribosa.

3. Glycogeno esseva trovate in ambe typos de plachetta. Glycogeno esseva extrahite per 5 pro cento de acido trichloroacetic ab sonorate plachettas human e bovin. Tamen, le subsequente precipitation ethanolic de glycogeno esseva incomplete. Per contrasto con isto, extraction con 30 pro cento de hydroxydo de kalium sequite per precipitation ethanolic resultava in le complete recuperation del glycogeno.

4. Variationes in le contentos total de hexosa e de glycogeno esseva trovate in le caso del plachettas human e etiam bovin. Iste variationes dependeva in parte del intervallo de immagasinage del plachettas in sanguine total o in 0,85 pro cento de NaCl post lor separation ab le sanguine total.

REFERENCES


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