After all the salt had been washed through the column, the 60 % ethanol was added to elute the desired material, temporarily termed neuramin-X (N-X).

Upon analysis it was found that much hexose-containing material had been washed away with the salt (Fig. 3). The eluates containing the sialic acid material were combined, evaporated under reduced pressure to remove the alcohol, and lyophilized, yielding a white hygroscopic solid. Analysis of this material showed it to contain sialic acid, glucose and galactose, but no hexosamine. It contains components which give a reaction with ninhydrin after acid hydrolysis and chromatography, and it does not appear to be susceptible to neuraminidase action. It absorbs strongly in the u.v., showing a peak at 262 mμ; chromatography with the above solvent system reveals three spots which absorb strongly in the u.v. One of these spots reacts to stains for reducing sugar, sialic acid, phosphate and amino acids (ninhydrin). Further studies on this material are now in progress.

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[1] R. CAPUTTO AND R. E. TRUCCO, *Nature*, 169 (1952) 1061.
[2] R. E. TRUCCO AND R. CAPUTTO, *J. Biol. Chem.*, 206 (1954) 901.
[3] R. KUHN UND R. BROSSMER, *Angew. Chem.*, 68 (1956) 211.
[4] R. KUHN UND R. BROSSMER, *Ber.*, 89 (1956) 2013.
[5] A. GOTTSCHALK, *Biochim. Biophys. Acta*, 23 (1957) 645.
[6] R. KUHN UND R. BROSSMER, *Ber.*, 92 (1959) 1667.
[7] R. HEIMER AND K. MEYER, *Biochim. Biophys. Acta*, 27 (1958) 490.
[8] L. W. MAYRON AND M. E. RAFELSON, JR., *Federation Proc.*, 19 (1960) 408.
[9] G. HOGSTROM, *Acta Chem. Scand.*, 11 (1957) 743.
[10] I. WERNER AND L. ODIN, *Acta Soc. Med. Upsaliensis*, 57 (1952) 230.
[11] R. J. WINZLER, in D. GLICK, *Methods of Biochemical Analysis*, Vol. 2, Interscience Publishers, New York-London, 1955, p. 279.
[12] L. WARREN, *J. Biol. Chem.*, 234 (1959) 1971.
[13] E. SVENNERHOLM AND L. SVENNERHOLM, *Nature*, 181 (1958) 1154.

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Concentration of unbound amino acids in human platelets

The presence of unbound amino acids in platelets has been noted by several workers[1–3]. To our knowledge amino acid concentrations in the platelets have not been determined quantitatively. The results of five analyses are reported herein.

The platelets were supplied by the Protein Foundation, Jamaica Plain, Massachusetts. They were separated from blood collected in acid citrate-dextrose solution by the ADL-Cohn Fractionator (A. D. Little and Company, Cambridge, Massachusetts) by the method of TULLIS *et al.*[4]. The platelets were washed with two 25-ml portions of a 1 % mildly acetylated albumin in 0.15 *M* NaCl solution, then eluted from the fractionator bowl with 5 ml of the same solution. (The albumin was acetylated to prevent its binding of tryptophan, Expt. 135 DI, Table I, ref. 5.) Aliquots of

the platelet suspension were taken for platelet counts and for measurements of packed platelet volume. (The latter were conducted in microhematocrit tubes at a centrifugal force of 5,000 × *g* for 20 min.) Leukocyte contamination was apparently 1 per 10,000 platelets. The volume of the remaining solution was measured and then made up to a total of 10 ml with water. The suspended platelet solution was twice frozen to lyse the platelets and dialyzed by the thin-layer technique[6] (using Visking tubing size 23/32) against 40 ml water for 2 h. The dialysate was dried from the frozen state. The dried residue from the dialysate was dissolved in Tergitol-Safranin·dye solution[7] in the ratio of 0.43 ml/ml of the packed platelet volume dialyzed. Analyses were conducted by paper chromatography[7]. To express the concentrations of the amino acids on a kg of water basis the platelets were taken to be 81 % water[8].

The mean concentrations of 22 substances in platelets and the ratio which these concentrations bear to the normal plasma concentrations are listed in Table I. For comparison the ratio of the leukocyte-plasma concentrations of the unbound amino acids in normal human blood[7] are also given. The concentrations of glutamic acid, taurine, O-phosphoethanolamine and proline were approximately the same in the platelets (3,600, 23,800, 2,300 and 600 μmoles/kg water respectively) as in normal leukocytes (2,745, 28,683, 2,651 and 862 μmoles/kg water respectively)[7]. With

TABLE I

UNBOUND AMINO ACID CONCENTRATIONS IN PLATELETS

	Number of subjects	*μmoles/kg water*			*Platelet-plasma ratio**	*Leukocyte plasma ratio***
Alanine	5	1,160	±	69***	2.9	7.5
α-Amino-n-butyric acid	4	120	±	24	4.2	—
Arginine	4	120	±	14	1.7	4.2
Glutamic acid	5	3,600	±	895	high	high
Glutamine	5	850	±	196	1.7	5.2
Histidine	5	300	±	155	3.3	9.8
Lysine	5	410	±	127	2.0	10.2
Methionine	4	160	±	25	7.4	14.5
Ornithine	5	870	±	311	10.2	20.6
Phenylalanine	4	270	±	30	5.0	13.6
Proline	5	600	±	54	3.2	4.8
Threonine	5	930	±	50	5.6	14.4
Tryptophan	4	100	±	18	5.5	11.4
Tyrosine	5	280	±	23	4.0	12.0
Valine	5	550	±	16	2.7	6.6
Ergothioneine	5	50				
Ethanolamine	5	350				
Leucine plus isoleucine	5	920	±	81	4.5	10.4
Serine plus glycine	5	5,000	±	1,460	8.2	22.1
Taurine	5	22,800	±	940	high	high
Urea	4	4,900	±	780	0.9	—
O-phosphoethanolamine	5	2,300	±	460	high	high
Serotonin	3	140	±	80§	high	—
Platelet volume	5	0.014	±	0.0007	—	—

* The platelet–plasma ratio was computed using the average plasma concentrations found for 15 normal fasting subjects[7].

** Taken from ref. 7.

*** Standard deviation of the mean.

§ Serotonin creatinine sulfate was added to the standard solution for this estimation.

the exception of these four substances and lysine, whose concentration was one-fifth the concentration found in the leukocytes, the concentrations of the remaining compounds were one-third to one-half the leukocyte values. The concentrations of the basic amino acids, histidine, lysine and ornithine varied more in the analyses than the other amino acids.

The mean serotonin concentration found in the platelets was 140 μmoles/kg water. (The R_F value of serotonin identified with EHRLICH's reagent was approximately that of leucine.) On the basis of a platelet count of 300,000/mm^3 whole blood this value, *i.e.*, 140 μmoles/kg platelet water, was slightly less than the value 220 $\pm$ 80 (0.16 $\pm$ 0.06 μg/ml blood) reported by HARDISTY AND STACEY[9] or the range of values 140–400 (0.1–0.3 μg/ml blood) reported by UDENFRIEND, WEISBACK AND CLARK[10] for normal human subjects.

A prominent unknown zone, positive to the PtI_4 reagent but negative to ninhydrin, with an R_F value of approximately that of alanine in Solvent A was found in all platelet analyses. Treatment of the zone with H_2O_2 destroyed its response to the PtI_4 test. The substance was not otherwise identified. Traces of this zone have occasionally been observed in leukocyte analysis, but not in plasma or red-cell analyses[7].

Amino acid values obtained from platelets which had been packed by centrifugation, but not washed, were similar to those reported in Table I when compared on a platelet basis.

The average packed platelet volume 0.014 ml/10^9 platelets, which was obtained by using a centrifugal force of 5,000 $\times$ *g*, compares favorably with the value of 0.017 ml/10^9 platelets obtained by HARDISTY AND STACEY[9] in which a centrifugal force of 2,000 $\times$ *g* was used.

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1 C. MAURI, U. TORELLI AND G. GROSSI, *Boll. soc. ital. biol. sper.*, 34 (1958) 152.
2 W. GEROK AND R. GROSS, *Thromb. Diath. Haem.*, 3 (1959) 654.
3 J. FRENDO, A. KOJ AND J. M. ZGLICZYNSKI, *Nature*, 183 (1959) 685.
4 J. L. TULLIS, D. M. SURGENOR, R. J. TINCH, M. D'HONT, F. L. GILCHRIST, S. DRISCOLL AND W. H. BATCHELOR, *Science*, 124 (1956) 792.
5 R. H. MCMENAMY AND J. L. ONCLEY, *J. Biol. Chem.*, 233 (1958) 1436.
6 R. H. MCMENAMY, G. C. LUND AND J. L. ONCLEY, *J. Clin. Invest.*, 36 (1957) 1672.
7 R. H. MCMENAMY, C. C. LUND, G. J. NEVILLE AND D. F. H. WALLACH, *J. Clin. Invest.*, 39 (1960) 1675.
8 B. MAUPIN, *Rev. hématol.*, 8 (1953) 302.
9 R. M. HARDISTY AND R. S. STACEY, *J. Physiol.*, 130 (1955) 711.
10 S. UDENFRIEND, H. WEISSBACK AND C. T. CLARK, *J. Biol. Chem.*, 215 (1955) 337.

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