SUMMARY

1. The substrate specificities of the esterases in rat pancreas and brain have been studied with organophosphorus compounds as selective esterase inhibitors.

2. The pancreas contains at least two enzymes with esterase activity which are relatively resistant to inhibition by diethyl *p*-nitrophenyl phosphate (E 600). The data obtained suggest that the E 600-resistant activity towards salicyl butyrate is due to carboxypeptidase or an enzyme with similar properties, whereas the E 600-resistant activity towards ethyltyrosine is probably due to chymotrypsin.

3. The enzyme in pancreas identified previously as cholesterolesterase (Fodor, 1950; Myers *et al.* 1955*b*) appears to be capable of hydrolysing a variety of esters including the ethyl esters of tyrosine, phenylalanine and leucine. The low activity towards these amino acid esters probably reflects the broad substrate specificity of this enzyme.

4. Three ali-esterases with distinct substrate-specificity patterns could be distinguished in brain homogenates. These enzymes are distinct from the cholinesterases and from the cathepsin in brain, described by Krimsky & Racker (1949).

5. The possible physiological functions of these enzymes and the differentiation of esterases by means of selective inhibitors are discussed.

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REFERENCES

Aldridge, W. N. (1953). *Biochem. J.* **53**, 110.
Aldridge, W. N. (1954). *Biochem. J.* **57**, 692.
Balls, A. K. & Jansen, E. F. (1952). *Advanc. Enzymol.* **13**, 321.
Curtius, T. & Muller, E. (1904). *Ber. dtsch. chem. Ges.* **37**, 1261.
Easson, E. H. & Stedman, E. (1937). *Biochem. J.* **31**, 1723.
Erlenmeyer, E. (1866). *Liebigs Ann.* **47**, 250.
Fodor, P. J. (1950). *Arch. Biochem.* **26**, 331.
Hartley, B. S. & Kilby, B. A. (1952). *Biochem. J.* **50**, 672.
Hofstee, B. H. J. (1952). *J. biol. Chem.* **199**, 365.
Johnston, R. B. (1955). *Fed. Proc.* **14**, 232.
Kilby, B. A. & Youatt, G. (1954). *Biochem. J.* **57**, 303.
Krimsky, I. & Racker, E. (1949). *J. biol. Chem.* **179**, 903.
Krimsky, I. & Racker, E. (1952). *J. biol. Chem.* **198**, 721.
Kunitz, M., Northrop, J. H. & Herriot, R. M. (1948). *Crystalline Enzymes.* New York: Columbia University Press.
Mendel, B., Myers, D. K., Uyldert, I. E., Ruys, A. C. & Bruyn, W. M. de (1953). *Brit. J. Pharmacol.* **8**, 217.
Mendel, B. & Rudney, H. (1943). *Biochem. J.* **37**, 59.
Mounter, L. A. & Whittaker, V. P. (1953). *Biochem. J.* **54**, 551.
Myers, D. K. (1949). M.A. Thesis: University of Toronto.
Myers, D. K. (1954). Ph.D. Thesis: University of Amsterdam.
Myers, D. K. (1956). *Biochem. J.* **62**, 556.
Myers, D. K. & Mendel, B. (1949). *Proc. Soc. exp. Biol., N.Y.*, **71**, 357.
Myers, D. K., Schotte, A. & Mendel, B. (1955*a*). *Biochem. J.* **60**, 481.
Myers, D. K., Schotte, A., Boer, H. & Borsje-Bakker, H. (1955*b*). *Biochem. J.* **61**, 521.
Neurath, H. (1952). In *Modern Trends in Physiology and Biochemistry*, p. 453. New York: Academic Press.
Neurath, H. & Schwert, G. W. (1950). *Chem. Rev.* **46**, 69.
Ord, M. G. & Thompson, R. H. S. (1951). *Biochem. J.* **49**, 191.
Racker, E. & Krimsky, I. (1948). *J. biol. Chem.* **173**, 519.
Richter, D. & Croft, P. G. (1942). *Biochem. J.* **36**, 746.
Smith, E. L. (1951*a*). In *Enzymes and Enzyme Systems*, p. 47. Cambridge, Mass., U.S.A.: Harvard University Press.
Smith, E. L. (1951*b*). *Advance. Enzymol.* **12**, 191.
Suzuoki-Ziro & Suzuoki-Tuneko (1954). *Nature, Lond.*, **173**, 83.
Whittaker, V. P. (1951). *Physiol. Rev.* **31**, 312.
Wilson, I. B. (1952). *J. biol. Chem.* **197**, 215.
Zeller, E. A. (1945). *Helv. physiol. acta*, **3**, C47.

Nucleotides of Human Blood Platelets

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Blood platelets take part in several reactions connected with haemostasis, and haemorrhagic conditions have been recognized in which platelet abnormalities appear to be the cause of the disturbance. The diagnosis of the defect is supported by microscopic examination of platelets and by laboratory tests designed to determine platelet activity in haemostatic reactions. During a study of the latter, it was found necessary to obtain information about purine and pyrimidine derivatives in platelets. There is considerable uncertainty in the literature concerning the presence of nucleic acids and their breakdown products in blood platelets. The earlier literature has been reviewed by Maupin (1954). Platelets are believed to contain ribonucleic acid (RNA) by Tinacci & Cessi (1952) on the basis of

microscopic staining reactions, whereas Maupin (1953) finds evidence to the contrary. Judging from chemical analysis, Wagner (1946) found that human platelets contain RNA, and Greene (1954) reported the isolation of ribonucleoprotein from platelets and stated it to be a major nitrogenous constituent. On the other hand, Maupin, Schneebeli & Ardry (1954) detected only traces of RNA in platelets. Results of an investigation concerning the purine and pyrimidine content of human-blood platelets carried out in this laboratory are presented below.*

EXPERIMENTAL

Isolation of platelets. Platelets were separated from the blood of persons who, on clinical evidence, were thought not to be suffering from any platelet abnormality. Blood was taken from an arm vein through a 1·4 mm. bore needle. The first 10 ml. was discarded. The collecting vessels contained either 0·13 M trisodium citrate solution or a solution of 0·027 M disodium ethylenediaminetetraacetate (EDTA) and 0·12 M-NaCl according to Dillard, Brecher & Cronkite (1951). One volume of anticoagulant solution was mixed with 9 vol. of blood. Approximately 400 ml. blood was taken from each person. Platelet-rich plasma was obtained by centrifuging the blood for 15 min. at 200 *g* (at the bottom of the tube), followed by 2 min. at 900 *g*. To remove erythrocytes and leucocytes as completely as possible the plasma was pipetted off and centrifuged again in the above manner. The platelet-containing plasma was then centrifuged for 20 min. at 2000 *g*. The supernatant plasma was discarded. The sediment was resuspended in a solution of 0·03 M trisodium citrate in 0·15 M-NaCl, or in 0·0027 M EDTA in 0·15 M-NaCl. Dispersion of the platelets was usually far easier in the latter solution. The platelet suspension was centrifuged for 10 min. at 2000 *g* and this process was repeated. Two more washings followed, with 0·15 M-NaCl. Plasma proteins and anticoagulants were thus removed. Finally the platelets were suspended in 10 ml. of 0·15 M-NaCl. The above operations were carried out at 5°. The concentration of the suspension was estimated after appropriate dilution with 0·15 M-NaCl by counting the platelets in a haemocytometer. Erythrocytes and leucocytes in platelet preparations were estimated by techniques given by Whitby & Britton (1953). The yield of platelets was 25–40 %, based on counts.

Analytical procedures

Total nitrogen was determined by a micro-Kjeldahl technique (Miller & Houghton, 1945). Protein was determined by a biuret technique (Robinson & Hogden, 1940). However, platelets did not give a clear solution in NaOH and the turbidity could not be removed by the usual procedures without interference with the biuret colour. Therefore it was necessary to deduct the light-absorption of a platelet solution from that after development of the biuret colour. Tyrosine and tryptophan were determined by the ultraviolet-absorption technique of Goodwin & Morton (1946).

The separation of protein from water-soluble material was carried out by the procedure of Sevag, Lackman & Smolens (1938). A mixture of 4 vol. of chloroform and 1 vol. of redistilled commercial *sec.*-octanol was used. The removal of protein was complete after six extractions.

The extraction of nucleic acids was carried out by the procedure of Schneider (1945). After removal of trichloroacetic acid by exhaustive extraction with peroxide-free diethyl ether, ultraviolet absorption was measured at 260 mμ., and the concentration of nucleic acid was calculated on the assumption of an extinction coefficient of 7000 for one nucleotide unit of 340 g./l.

Phosphorus was determined by the method of Berenblum & Chain (1938). Ribose was determined by the method of Militzer (1946). Deoxyribose was determined by the method of Stumpf (1947).

Paper chromatography of nucleotides was performed with 5 % (w/v) aqueous KH_2PO_4 and *iso*pentanol according to Carter (1950). The technique of Eggleston & Hems (1952) was also used.

Ionophoresis was carried out by a technique similar to that of Davidson & Smellie (1952). Strips of Whatman no. 2 paper, 57 cm. × 8 cm., were used and a potential gradient of 9·5 v/cm. was applied for 8 hr. at a temperature of 20–22°.

The nucleotides were hydrolysed with N-HCl according to Smith & Markham (1950) and chromatographed with 65 % (v/v) propan-2-ol–water, 2 N with respect to HCl (Wyatt, 1951). Whatman no. 1 paper was washed with the solvent to be used for chromatography, then with water, and dried at room temperature. Developed chromatograms were dried in a draught of air at room temperature, and were photographed on reflex document paper by exposure to a Chromatolite ultraviolet lamp (Hanovia, Slough, England) by a technique similar to that of Markham & Smith (1949).

Countercurrent distribution of adenine and guanine was carried out according to the method of Tinker & Brown (1948), except that the lower phase was transferred instead of the upper phase.

Adenosine triphosphate (ATP) was determined by the technique of Kornberg (1950) and adenosine diphosphate (ADP) by the technique of Slater (1953).

Leucocytes were isolated from blood according to the procedure of Dausset, Nenna & Brecy (1954).

Optical-density measurements were made with a Hilger Uvispek spectrophotometer with 0·5 cm. cells. Extinction coefficients of purine and pyrimidine derivatives were taken from Beaven, Holiday & Johnson (1955). Most of the analytical reagents were British Drug Houses Ltd. preparations.

RESULTS

The total nitrogen content of human-platelet preparations is given in Table 1.

It appears from the figures given in Table 1 that platelets separated from plasma have practically the same nitrogen content whether citrate or EDTA has

* After the completion of this paper the following additional information became available. According to Bestetti & Crosti (1955), large amounts of adenosine triphosphate (ATP) are present in platelets. Small, Campbell & Dameshek (1956) separated ATP and adenosine diphosphate (ADP) from human platelets. Born (1956) reports that human platelets contain 1·2–1·9 μmole of ATP/g. moist wt. On the assumption of 5×10^{10} platelets/g. moist wt. our results correspond to 1 μmole of ATP/g. moist wt.

been used as anticoagulant. Erickson, Williams, Avrin & Lee (1939) found that platelets prepared with sodium metaphosphate have a higher nitrogen content than those prepared with sodium citrate.

Maupin (1953) found 0·31 mg. of nitrogen in 10^9 human platelets separated from citrated blood.

The ultraviolet-absorption spectrum of human platelets dissolved in 0·01 N-NaOH gave no indication of the presence of any known tissue component. On the other hand, a quantitative biuret reaction carried out on alkaline platelet extracts gives the results shown in Table 2. From the results given in Table 2 it is apparent that 83–92 % of the total nitrogen of platelets is present in protein. Erickson *et al.* (1939) and Maupin (1953) obtained similar results by other techniques.

The quantitative protein determinations of the various workers are in agreement. It is therefore remarkable that the ultraviolet-absorption curve of platelet extracts did not indicate the presence of protein. Obviously the characteristic absorption curve of protein was masked by other ultraviolet-absorbing components. To obtain information about these components platelet protein was isolated from 7·6 ml. of a platelet preparation containing 2×10^6 platelets, 1×10^2 erythrocytes and 4×10^2 leucocytes/mm.^{3}, by the process of Sevag *et al.* (1938). The separating gels from the extractions were combined, treated with absolute ethanol and dried over P_2O_5 *in vacuo* at 100°. This material contained 15·7 % of nitrogen and was recovered in an amount corresponding to approximately 75 % of the total platelet nitrogen. The biuret and ultraviolet-absorption curves indicated that the material was a protein containing 4·4 % of tyrosine and 1·7 % of tryptophan.

The aqueous solution remaining from the exhaustive treatment of platelets with chloroform–octanol had the ultraviolet-absorption spectrum shown in Fig. 1.

The curves shown in Fig. 1 are characteristic of purine and pyrimidine derivatives. After dialysis of the aqueous layer against 0·15 M-NaCl at 6° the ultraviolet-absorbing material disappeared. From the results so far obtained it would seem that platelets contain protein and purine and pyrimidine derivatives.

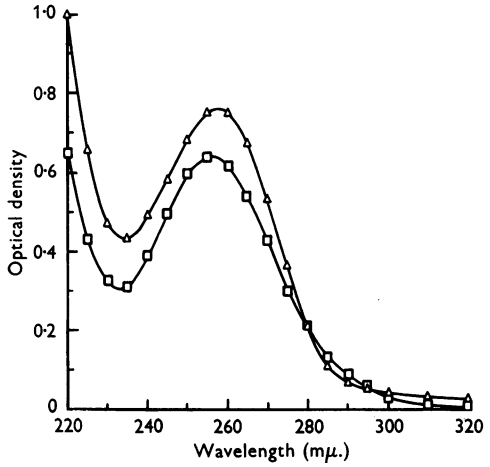

Fig. 1. Ultraviolet-absorption curves of aqueous layer from extraction of platelet suspension with $CHCl_3$–octanol. Suspension (11 ml.) contained, per mm.^{3}: $4·8 \times 10^6$ platelets, 3×10^2 erythrocytes and $2·5 \times 10^2$ leucocytes. The final aqueous layer was diluted 13·3 times: □, in 0·1 N-HCl; △, in 0·1 N-NaOH.

Table 1. *Total nitrogen content of isolated human blood platelets*

Anticoagulant	Composition of suspension/mm.^{3}: Platelets (millions)	Erythrocytes	Leucocytes	N in suspension containing 10^9 platelets (mg.)
Citrate	2	400	700	0·39
	2·3	100	900	0·37
EDTA	3	200	500	0·40
	2	700	550	0·45
	3·1	300	600	0·37
	4·7	550	500	0·37

Table 2. *Quantitative biuret reaction of human platelets (EDTA as anticoagulant)*

	Composition of suspension/mm.^{3}: Platelets (millions)	Erythrocytes	Leucocytes	Suspension containing 10^9 platelets: Protein N (mg.)	Total N (mg.)
Suspension 1	1·8	300	550	0·34	0·41
Suspension 2	3·1	300	600	0·34	0·37

Nucleic acids and nucleotides of platelets

Nucleic acids were determined by ultraviolet absorption of an extract of 18 ml. of a preparation containing (per mm.^{3}): $2{\cdot}5 \times 10^6$ platelets, $2{\cdot}5 \times 10^2$ erythrocytes and $1{\cdot}5 \times 10^2$ leucocytes; 125 μg. of nucleic acids was calculated to be present.

The water-soluble non-protein fraction of the platelets was obtained in most experiments by the following procedure. A platelet suspension, soon after preparation, was centrifuged, the supernatant discarded and the sediment frozen and thawed with cold water. An equal volume of ice-cold 10 % (w/v) trichloroacetic acid was added, the cold mixture stirred, then centrifuged at 2°. In the clear supernatant solution determinations of total P, inorganic P, P liberated by hydrolysis with N acid at 100° for 7 min., ribose and deoxyribose were made. After exhaustive extraction of trichloroacetic acid from the solution with peroxide-free diethyl ether the ultraviolet absorption was measured. In Table 3 are presented the results of the above determinations.

The results in Table 3 indicate that extracts of platelets in trichloroacetic acid contain on the average (per 10^9 platelets): 0·066 μmole of inorganic P, 0·19 μmole of easily hydrolysable P (7 min. P), and 0·235 μmole of ribose. From the ultraviolet absorption it is calculated that 10^9 platelets contain on the average 0·16 μmole of purine and pyrimidine derivatives, taking the molecular extinction coefficient of platelet substance soluble in cold trichloroacetic acid as 14 000 at 260 mμ. in 0·1 N-HCl. The average of total P was 0·57 μmole/10^9 platelets. From the results given later (Table 4) it is calculated that the nucleotides contain only 0·29 μmole of P/10^9 platelets.

For comparative purposes erythrocytes as well as leucocytes were isolated from human blood and extracted with cold trichloroacetic acid in a similar manner to the extraction of platelets. From the ultraviolet absorption of the extracts the material with an absorption peak in the region of 260 mμ. was calculated at ATP. Thus one erythrocyte contained $1{\cdot}82 \times 10^{-10}$ μmole and one leucocyte 6×10^{-9} μmole.

The extracts after removal of trichloroacetic acid were reduced to a small volume by evaporation at room temperature over solid KOH under reduced pressure, and hydrolysed with N-HCl at 100° for 1 hr. Chromatography was carried out according to Wyatt (1951). Photographs of the chromatograms are represented schematically in Fig. 2, in which the circles indicate the relative positions of the compounds on the chromatogram.

The circles have been drawn so that the areas are proportional to the number of moles of the corresponding compound found by elution and ultraviolet absorption of each spot. For the characterization of each compound twenty or more strips were chromatographed and the spots corresponding to each compound combined and eluted with 0·1 N-HCl. From the ultraviolet spectrum of the eluates

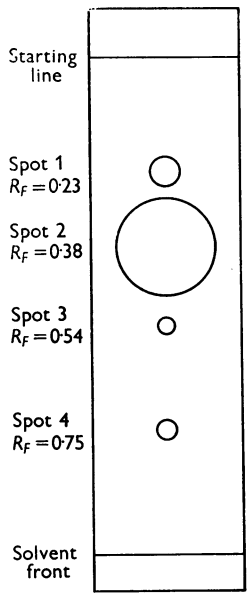

Fig. 2. Diagram of a paper chromatogram of a trichloroacetic acid extract of platelets hydrolysed with N-HCl and run in propan-2-ol–aq. 2 N-HCl. Platelet suspension used contained, per mm.^{3}: $2{\cdot}5 \times 10^6$ platelets, 5×10^2 erythrocytes and $3{\cdot}5 \times 10^2$ leucocytes.

Table 3. P, *ribose, deoxyribose and ultraviolet-absorbing material in cold trichloroacetic acid (TCA) extracts of platelets*

Composition of suspension/mm.^{3}			μmole in TCA extract from 10^9 platelets		Ribose in TCA extract from 10^9 platelets (μmole)	Deoxyribose in TCA extract from 10^9 platelets (μmole)	$E_{1\,cm.}$ at 260 mμ. (0·1 N-HCl) of TCA extract from 10^9 platelets/ml.
Platelets (millions)	Erythrocytes	Leucocytes	Inorg. P	7 min. P			
2·8	250	150	0·052	0·152	0·235	0·0054	2·15
2·2	400	450	0·066	0·229	0·249	—	2·44
1·8	500	450	0·055	0·203	0·217	—	2·15
1·8	400	250	0·091	0·174	0·249	—	2·21

of each of the four spots and from the R_F values it appears that the compound in spot 1 is guanine and the compound present in spot 2 is adenine. Spots 1 and 2 were further characterized by countercurrent distribution according to Tinker & Brown (1948). Results for spot 1 are given in Fig. 3.

As can be seen from Fig. 3 the maximum concentration of the main component in spot 1 (Fig. 2) occurs at tube 13, which is the same as that calculated for guanine. However, there are impurities present. The one causing a shoulder in the curve at tube 9 was present in too low a concentration for further characterization. The other impurity was probably extracted from the filter paper.

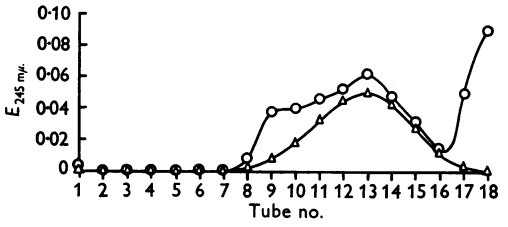

Fig. 3. Countercurrent distribution of material in spots 1 (Fig. 2) pooled from twenty strips, dissolved in 0·6 ml. of 0·1 N-HCl, to which was added 5·4 ml. of M phosphate buffer, pH 6·5, saturated with *n*-butanol, temp. 18–19°. (*a*) Curve from spots 1 (○); (*b*) calculated curve for 0·565 μmole of guanine, partition coefficient = 0·45 (△).

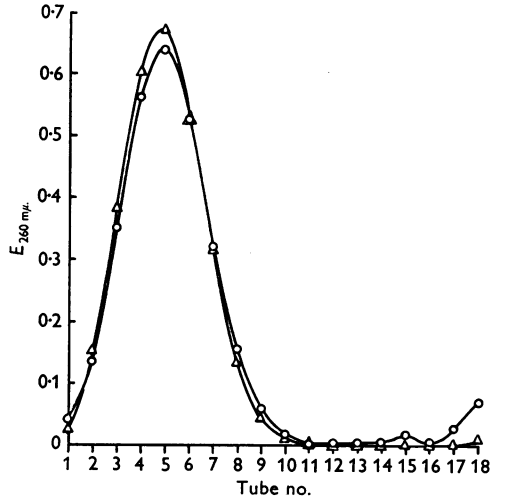

Fig. 4. Countercurrent distribution of material in spots 2 (Fig. 2) pooled from twenty strips, dissolved in 0·6 ml. of 0·1 N-HCl, to which was added 5·4 ml. of phosphate buffer, pH 6·5, saturated with *n*-butanol, temp. 18–19°. (*a*) Curve from spots 2 (○); (*b*) curve for 5·52 μmoles of adenine, B.D.H. Ltd. (△).

The result of the countercurrent distribution of the material in spot 2 is shown in Fig. 4.

From Fig. 4 it is apparent that the maximum concentration of the substance in spot 2 (Fig. 2) occurred in tube 5, as did authentic adenine.

The substance in spot 3 (Fig. 2) is cytidylic acid (R_F 0·54, maximum ultraviolet absorption at 280 mμ. in 0·1 N-HCl; 271 mμ. in 0·1 N-NaOH. Found: 2·8 μg. of P; calc. 4·1 μg. of P). Substance in spot 4 (Fig. 2) is uridylic acid (R_F 0·75, maximum ultraviolet absorption at 262 mμ. in 0·1 N-HCl; 263 mμ. in 0·1 N-NaOH. Found: 4·2 μg. of P; calc. 4·4 μg. of P).

Resolution of the platelet extract into the individual components was attempted by chromatography according to Carter (1950) and Eggleston & Hems (1952), and by ionophoresis according to Davidson & Smellie (1952).

The result of chromatography in 5% (w/v) aqueous KH_2PO_4 saturated with *iso*pentanol is shown in Fig. 5.

Comparison of the position of the platelet extract on the chromatogram with the positions of the known compounds used and with the R_F values given by Carter (1950) indicates the absence of free purines, pyrimidines and adenosine, and the possible presence of adenosine polyphosphates. Results obtained by ionophoresis are given in Fig. 6.

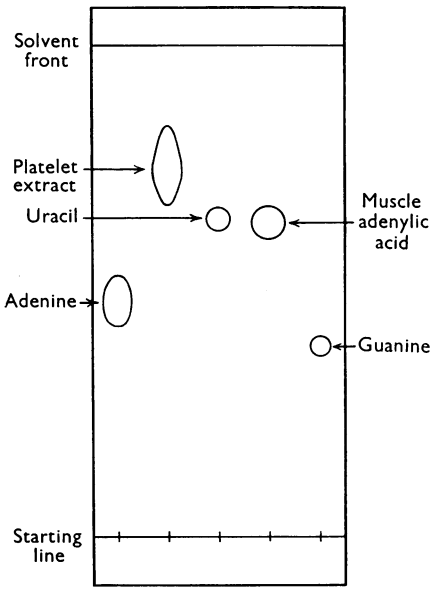

Fig. 5. Diagram of paper chromatogram of a cold trichloroacetic acid extract of platelets run in 5% aq. KH_2PO_4–*iso*pentanol. Platelet suspension used contained, per mm.^{3}: $1{\cdot}4 \times 10^6$ platelets, 10^2 erythrocytes and 50 leucocytes.

Results in Fig. 6 show that at least four bands of ultraviolet-absorbing material could be separated by ionophoresis at pH 3·5. The bands were cut out, eluted with water, the solutions evaporated and the residue was hydrolysed with N-HCl at 100° for 1 hr. and chromatographed according to Wyatt (1951). Band *B* in Fig. 6 had a mobility varying from 0·31 to 0·35 cm.^{2} hr.$^{-1}$v^{-1} with an average of 0·33 cm^2. hr.$^{-1}$v^{-1}. Hydrolysates contained adenine and in some cases uridylic acid in a molar ratio of 14 of adenine to 1 of uridylic acid. ADP used as a marker had a mobility of 0·34 cm.^{2} hr.$^{-1}$v^{-1}. The mobility of uridylic acid was 0·30 cm.^{2} hr.$^{-1}$v^{-1}. Smith (1955) observed that uridylic acid had the same mobility as adenine uracil dinucleotide at pH 3·5. The mobility indicates that band *B* contained mainly ADP, but it is undecided in which form uridylic acid was present. Band *C* had a mobility varying from 0·40 to 0·44 cm.^{2} hr.$^{-1}$v^{-1}, with an average of 0·41 cm.^{2} hr.$^{-1}$v^{-1}. Hydrolysates contained adenine, guanine and uridylic acid in a molar ratio of 11:7:4. Judging from the mobility, band *C* may contain several di- or tri-nucleotides. Band *D* had a mobility varying from 0·43 to 0·49 cm.^{2} hr.$^{-1}$v^{-1}, with an average of 0·45 cm.^{2} hr.$^{-1}$v^{-1}. Hydrolysates contained adenine, and in some cytidylic acid in a molar ratio of 50 of adenine to 1 of cytidylic acid. The mobility of ATP as a marker was 0·48 cm.^{2} hr.$^{-1}$v^{-1}. The concentration of adenine in this band is much higher than that in band *B*. On the other hand, specific tests for ATP and ADP in platelet extracts (Table 4) indicated a higher concentration of the latter than the former. The material may therefore contain some ATP but the constitution of the bulk of adenine derivatives in this band remains unknown. Band *E* had a mobility varying from 0·46 to 0·52 cm.^{2} hr.$^{-1}$v^{-1} with an average of 0·47 cm.^{2} hr.$^{-1}$v^{-1}. Hydrolysates contained adenine, guanine and uridylic acid, in the approximate molar proportion of 13:14:3. The mobility suggests that band *E* may contain ATP and a number of di- and tri-nucleotides and guaninosine diphosphate. In addition, in some platelet extracts a faint band (*A*, Fig. 6) with a mobility of 0·14 cm.^{2} hr.$^{-1}$v^{-1} was detectable. The hydrolysate contained adenine and cytidylic acid in a molar ratio of 3:2. Adenosine 5′-phosphate (AMP) as a marker had a mobility of 0·16 cm.^{2} hr.$^{-1}$v^{-1}. Judged from the mobility, band *A* could therefore contain AMP and cytidylic acid or dinucleotides containing these components.

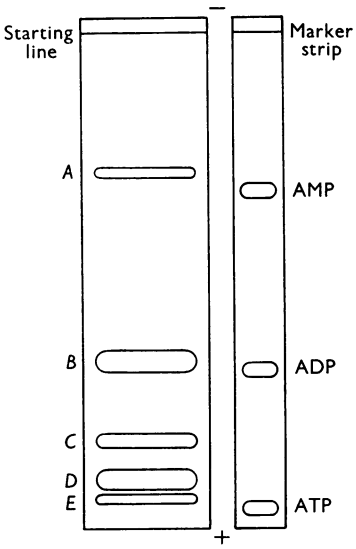

Fig. 6. Diagram of paper ionophoresis of trichloroacetic acid extract of platelets. 0·02M citrate buffer, pH 3·5, 9·5 v/cm., 8 hr. 20–22°. Suspension used contained, per mm.^{3}: $4{\cdot}3 \times 10^6$ platelets, 3×10^2 erythrocytes and 5×10^2 leucocytes.

The concentration of ATP and ADP as determined by the techniques of Kornberg (1950) and Slater (1953) respectively, together with the total purine and pyrimidine content of platelets, are given in Table 4.

DISCUSSION

Concerning the composition of platelets, the purity of the preparation has to be considered. Even with sacrifice of yield it is difficult to obtain a platelet preparation free of blood cells. Therefore a comparison between the dimensions of platelets and blood cells is essential. The average volume of one erythrocyte is 90 μ^3, that of one leucocyte approximately 1000 μ^3, calculated from the diameters given by Whitby & Britton (1953), and that of one platelet 11 μ^3, according to Tocantins (1938). Expressed as volumes, one erythrocyte corresponds to eight platelets and one leucocyte to approximately ninety platelets. One of our most contaminated preparations consisted of $2{\cdot}5 \times 10^6$ platelets, 7×10^2 erythrocytes and 10×10^2 leucocytes per

Table 4. *ATP, ADP, total purine and pyrimidine content of human blood platelets*

Composition of suspension/mm.^{3}			μmole/10^9 platelets				
Platelets (millions)	Erythrocytes	Leucocytes	Total guanine	Total adenine	Total pyrimidine	ATP	ADP
2·5	500	350	0·016	0·16	0·013	—	—
4·2	500	300	—	—	—	0·02	0·051

mm.^{3}: the blood cells in 1 mm.^{3} of that preparation have therefore a volume corresponding to 95×10^3 platelets. On the assumption of equal distribution of the compounds present in blood cells and platelets the error introduced by the contaminating blood cells is approximately 4 %.

Normal human blood contains 1·5 μmole of ATP/ml. according to Steyn-Parvé (1953). Assuming 5×10^9 erythrocytes in 1 ml. of blood, one erythrocyte contains 3×10^{-10} μmole of ATP. From ultraviolet absorption of trichloroacetic acid extracts of normal human erythrocytes we find $1{\cdot}82 \times 10^{-10}$ μmole of ATP/erythrocyte. Accepting the higher figure, 1 ml. of the above platelet preparation contains $2{\cdot}1 \times 10^{-4}$ μmole of ATP as impurity present in the erythrocytes and 60×10^{-4} μmole of ATP present in the leucocytes. The optical density at 260 mμ. of the purine and pyrimidine bases of the erythrocytes and leucocytes soluble in cold trichloroacetic acid present in the above platelet suspension is approximately 0·087. However, the platelet suspension gave an optical density of 5·5. From these calculations it is apparent that the error introduced by the contaminating blood cells cannot be greater than 1·6 %.

One other point must be considered in this connexion and that is the fact that platelets have the ability to adsorb substances from the surrounding fluid. For instance, adrenaline and noradrenaline (Weil-Malherbe & Bone, 1954), and 5-hydroxytryptamine (Hardisty & Stacey, 1955), can be adsorbed on human platelets. Since cell-free plasma is free of nucleotides the nucleotides found can be considered as components of platelets.

The nucleic acid content of isolated human platelets as determined in this paper was approximately $2{\cdot}8 \times 10^{-9}$ μg. of nucleic acid/platelet. This is on a volume basis approximately forty times less than that found per leucocyte by Davidson, Leslie & White (1951). The nucleic acid nitrogen in platelets is approximately 0·11 % of the total platelet nitrogen. It is therefore obvious that nucleic acids are only present as a minor constituent in human-blood platelets. On the other hand, platelets contain purine and pyrimidine derivatives. Their nitrogen content is 2·5–3·5 % of the total platelet nitrogen. The evidence that these compounds are not nucleic acids but rather nucleotides is given by the fact that the bulk of material showing the characteristic ultraviolet-absorption spectrum of purine and pyrimidine derivatives is extractable from freshly prepared platelets by cold trichloroacetic acid and is dialysable. According to ionophoretic behaviour and specific reactions the nucleotides consist of 12 % of ATP, 30 % of ADP and a complex mixture of polyribonucleotides.

Sufficient evidence is available to indicate that Wright's view (1910) that blood platelets are derived from megakaryocytes in the bone marrow is correct (Humphrey, 1955). The deoxyribose concentration found in platelets is for practical purposes negligible. This excludes their nuclear derivation. They therefore originate from the cytoplasm of megakaryocytes and in the light of this it is remarkable that there is a preponderance of adenine over the other bases. The significance of the nucleotides in the platelets is not apparent. The presence of ATP and ADP suggests a connexion with energy metabolism. However, Tullis (1953) found that isolated platelets have a very low oxygen consumption, approximately one-hundredth of that of leucocytes. It has been found by Libber (1955) that 0·0025 M ATP inhibits blood clotting. Further it has been suggested, in analogy to muscle contraction, that ATP is essential for the contraction of fibrin gel (Budtz-Olsen, 1951). However, platelets accelerate blood clotting and although they are essential for the contraction of fibrin gel their ATP content is far too low to be of significance in these processes. Perhaps the release of platelets from the megakaryocytes is a process which requires the energy liberated from ATP.

SUMMARY

1. Platelets were isolated from normal human blood and the following constituents were found in the approximate concentrations given as percentage of total nitrogen: (*a*) 80–90 % in protein; (*b*) 0·1 % in the nucleic acid fraction; (*c*) 1·2 % in adenosine diphosphate plus adenosine triphosphate; (*d*) 1·8 % in adenine, guanine, cytosine and uracil, as ribose phosphates in undetermined combination.

2. Traces of deoxyribose were detected in human blood platelets.

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REFERENCES

Beaven, G. H., Holiday, E. R. & Johnson, E. A. (1955). In *The Nucleic Acids*, vol. 1, chap. 14, p. 493. Ed. by Chargaff, E. & Davidson, J. N. New York: Academic Press.

Berenblum, I. & Chain, E. (1938). *Biochem. J.* **32**, 295.

Bestetti, A. & Crosti, P. F. (1955). *Atti Soc. lombarda Sci. med. biol.* **10**, 284. [Cited in *Chem. Abstr.* (1956). **50**, 2728*a*.]

Born, G. V. R. (1956). *Biochem. J.* **62**, 33 P.

Budtz-Olsen, O. E. (1951). *Clot Retraction.* Oxford: Blackwell.

Carter, C. E. (1950). *J. Amer. chem. Soc.* **72**, 1466.

Dausset, J., Nenna, A. & Brecy, H. (1954). *Blood*, **9**, 696.

Davidson, J. N., Leslie, I. & White, J. C. (1951). *J. Path. Bact.* **63**, 471.
Davidson, J. N. & Smellie, R. M. S. (1952). *Biochem. J.* **52**, 594.
Dillard, G. H. L., Brecher, G. & Cronkite, E. P. (1951). *Proc. Soc. exp. Biol., N.Y.*, **78**, 796.
Eggleston, L. V. & Hems, R. (1952). *Biochem. J.* **52**, 156.
Erickson, B. N., Williams, H. H., Avrin, I. & Lee, P. (1939). *J. clin. Invest.* **18**, 81.
Goodwin, T. W. & Morton, R. A. (1946). *Biochem. J.* **40**, 628.
Greene, R. W. (1954). *Proc. Soc. exp. Biol., N.Y.*, **87**, 412.
Hardisty, R. M. & Stacey, R. S. (1955). *J. Physiol.* **129**, 24P.
Humphrey, J. H. (1955). *Nature, Lond.*, **176**, 38.
Kornberg, A. (1950). *J. biol. Chem.* **182**, 779.
Libber, L. M. (1955). *J. cell. comp. Physiol.* **45**, 103.
Markham, R. & Smith, J. D. (1949). *Biochem. J.* **45**, 294.
Maupin, B. (1953). *Rev. Hémat.* **8**, 302.
Maupin, B. (1954). *Les Plaquettes Sanguines de l'Homme.* Paris: Masson and Co.
Maupin, B., Schneebeli, J. & Ardry, R. (1954). *C.R. Soc. Biol., Paris*, **148**, 441.
Militzer, W. E. (1946). *Arch. Biochem.* **9**, 85.
Miller, L. & Houghton, J. A. (1945). *J. biol. Chem.* **159**, 373.
Robinson, H. W. & Hogden, C. G. (1940). *J. biol. Chem.* **135**, 727.
Schneider, W. C. (1945). *J. biol. Chem.* **161**, 293.
Sevag, M. G., Lackman, D. B. & Smolens, J. (1938). *J. biol. Chem.* **124**, 425.
Slater, E. C. (1953). *Biochem. J.* **53**, 157.
Small, W. J., Campbell, E. W. & Dameshek, W. (1956). *Clinical Research Proceedings IV*, p. 5.
Smith, J. D. (1955). In *The Nucleic Acids*, vol. 1, p. 273. Ed. by Chargaff, E. & Davidson, J. N. New York: Academic Press.
Smith, J. D. & Markham, R. (1950). *Biochem. J.* **46**, 509.
Steyn-Parvé, E. P. (1953). *Biochim. biophys. Acta*, **10**, 121.
Stumpf, P. K. (1947). *J. biol. Chem.* **169**, 367.
Tinacci, F. & Cessi, C. (1952). *Boll. Soc. ital. Biol. sper.* **28**, 283.
Tinker, J. F. & Brown, G. B. (1948). *J. biol. Chem.* **173**, 585
Tocantins, L. M. (1938). *Medicine, Baltimore*, **17**, 155.
Tullis, J. L. (1953). *Amer. J. med. Sci.* **226**, 191.
Wagner, R. (1946). *Arch. Biochem.* **11**, 249.
Weil-Malherbe, H. & Bone, A. D. (1954). *Nature, Lond.*, **174**, 557.
Whitby, L. E. H. & Britton, C. J. C. (1953). *Disorders of the Blood*, 7th ed. London: Churchill.
Wright, J. H. (1910). *J. Morph.* **21**, 263.
Wyatt, G. R. (1951). *Biochem. J.* **48**, 584.

Movements of Water and Ions in Mitochondria

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The osmotic environment can affect the metabolic activity of mitochondria (Kielley & Kielley, 1951; Potter & Recknagel, 1951; Raaflaub, 1953*b*; Lehninger & Kennedy, 1948). Harman & Feigelson (1952*a*, *b*) concluded from visual comparisons that the morphological state of swelling not only influenced, but was in turn influenced by, the rates of oxidation and oxidative phosphorylation. Another relation, that oxidative phosphorylation in isolated mitochondria can be coupled to active transport, was shown by Bartley & Davies (1952, 1954) and Macfarlane & Spencer (1953), who also found that these particles maintained a low water content in the presence but not the absence of an adenosine phosphate.

This paper describes experiments on the nature of the water and ion movements in respiring rat-liver mitochondria (Macfarlane & Spencer, 1953). One finding of the present study, the extrusion of water by mitochondria as distinct from the maintenance of the initial water content, has been obtained by Chappell & Perry (1954) with non-respiring suspensions of sarcosomes under conditions widely different from ours. Part of this work has been communicated to the Biochemical Society (Price & Davies, 1954).

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EXPERIMENTAL

Abbreviations. These are as follows: adenosine monophosphate, AMP; adenosine diphosphate, ADP; adenosine triphosphate, ATP; aminotrishydroxymethylmethane, tris; 2:4-dinitrophenol, DNP; diphosphopyridine nucleotide, DPN; ethylenediaminetetraacetic acid, EDTA.

Preparation of mitochondria and sarcosomes. Albino rats were made unconscious by stunning and then their throats were cut. The livers were removed, cooled for several minutes in partially frozen 0·25 M sucrose or partially frozen 0·9% KCl, weighed and passed through a Fischer mincer (Broyeur de Fischer à Main; Jouan, Paris) and then a Craigie pressure mincer with a finely corrugated plug (Craigie, 1949). The homogenate formed was suspended in