ase (exo-PMG). Endo-PG attacks low-methoxy pectin by a random mechanism of hydrolysis which leads to intermediate oligogalacturonic acids and eventually to digalacturonic acid, whereas exo-PG removes terminal galacturonic units from the substrate and its intermediate polyuronides, and cleaves digalacturonic acid. Exo-PMG attacks pectin by a terminal mechanism of hydrolysis.

3. Viscometric measurement shows that myrobalans tannins exert a powerful inactivation on the early stages of the pectolysis of low-methoxy pectin, but only a slight retarding action on the pectolysis of pectin.

4. The fact that these enzymic hydrolyses go to completion in the presence of myrobalans tannins is consistent with the ripening of the fruit.

5. Gambir tannin has no effect on the pectolytic actions investigated.

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REFERENCES

Ashby, J., Brooks, J. & Reid, W. W. (1955). *Chem. & Ind.* p. 360.
Bate-Smith, E. C. & Westall, R. G. (1950). *Biochim. biophys. Acta*, **4**, 427.
Brooks, J. & Reid, W. W. (1955). *Chem. & Ind.* p. 325.
Demain, A. L. & Phaff, H. J. (1957). *Wallerstein Labs. Commun.* **20**, no. 69, pp. 119–140.
Freudenberg, K. (1921). *Z. angew. Chem.* **34**, 247.
Freudenberg, K. & Vollbrecht, E. (1921). *Hoppe-Seyl. Z.* **116**, 277.
Hathway, D. E. (1956). *Biochem. J.* **63**, 380.
Hathway, D. E. & Seakins, J. W. T. (1958). *Biochem. J.* **70**, 155.
Jermyn, M. A. & Tomkins, R. G. (1950). *Biochem. J.* **47**, 437.
Jones, J. K. N. & Reid, W. W. (1954). *J. chem. Soc.* p. 1361.
Jones, J. K. N. & Reid, W. W. (1955). *J. chem. Soc.* p. 1890.
Paniker, M. A. R. & Stiasny, E. (1911). *J. chem. Soc.* **99**, 1819.
Schubert, E. (1952*a*). *Nature, Lond.*, **169**, 931.
Schubert, E. (1952*b*). *Biochem. Z.* **323**, 78.
Trevelyan, W. E., Procter, D. P. & Harrison, J. S. (1950). *Nature, Lond.*, **166**, 444.

Distribution of Ascorbic Acid in Normal and Leukaemic Human Blood

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It has been known for some time that the buffy layer of normal human blood contains a high concentration of ascorbic acid (Butler & Cushman, 1940, 1941). The distribution between lymphocytes, granulocytes and platelets has, however, remained obscure and forms the subject of this present paper. Whereas previous workers have quoted their results on a wet-weight basis only, the data presented here are based on the analysis of individual cells determined by direct counting.

Leukaemic blood has been found to contain low concentrations of ascorbic acid, both in the serum (Hagtvet, 1945) and plasma and in the buffy layer (Waldo & Zipf, 1955). In contrast, Butler & Cushman (1940) found higher amounts than normal in the buffy layer. We have included analyses of chronic leukaemic human blood in this work, in order to investigate these claims and also to provide data on the relatively pure suspensions of lymphocytes and granulocytes obtainable in leukaemic diseases.

METHODS

Collection of blood. Blood (30–90 ml.) was aspirated from an antecubital vein with sterile paraffined all-glass syringes and no. 18 B.D. steel needles. It was immediately transferred to siliconized glass centrifuge tubes (10 ml.) containing as anticoagulant one part of disodium ethylenediaminetetra-acetate dihydrate (Sequestrene) soln. [1% (w/v) in 0·7% NaCl soln.] to nine parts of blood. The tubes were inverted several times to mix the contents.

Centrifuging. A horizontal refrigerated centrifuge, radius 14 cm., at a temperature of 4°, was used throughout.

Separation of the formed elements

Platelets. The whole blood was centrifuged at 800–1000 rev./min. for 10–15 min. The upper three-quarters of the supernatant platelet-rich plasma was carefully removed with siliconized Pasteur pipettes and transferred to polythene centrifuge tubes. (The blood remaining after removal of the platelet-rich plasma is called 'platelet-poor blood'.) After removal of a small measured quantity (about 0·01 ml.) for a direct platelet count (Barkhan, 1957), the measured volume of plasma was centrifuged at

3000 rev./min. for 15 min. to obtain a platelet 'button', which, after the removal of the supernatant plasma, was weighed. A platelet count on the platelet-poor plasma was performed as described above. The total number of platelets in the button was calculated from the difference between the platelet-rich and platelet-poor plasma counts and the total volume of the plasma. Microscopic examination of Leishman-stained films of the platelet-rich plasma showed a very occasional erythrocyte and the complete absence of leucocytes. The absolute number of erythrocytes was determined at the same time as the platelet count. The ratio erythrocytes:platelets was generally of the order of 1–3:1000; this small number would not contribute significantly to the weight of the platelet button.

Erythrocytes. About 10 ml. of platelet-poor blood was centrifuged in a Nylon centrifuge tube (1·5 cm. diam. × 10 cm.) for 30 min. at 3000 rev./min. The erythrocyte mass was obtained by piercing the base of the tube with a syringe needle and drawing off the erythrocyte layer, the top 1 cm. containing the leucocytes and remaining platelets being discarded. The number of erythrocytes was determined by direct counting. Examination of Leishman-stained films showed a concentration of less than 0·1% of leucocytes.

Leucocytes. The remaining platelet-poor blood was further centrifuged for 15 min. at 3000 rev./min. in several tubes (1·5 cm. × 10 cm.). After removal of three-quarters of the supernatant, the remaining plasma together with the top layers of cells containing the buffy layer was transferred with a Pasteur pipette to a narrow glass tube (15 cm. × 0·8 cm.) which was centrifuged at 3000 rev./min. for 1–2 hr. This produced an elongated buffy layer which could be separated from the underlying red-cell mass. The layer consisted of platelets and leucocytes but still contained a relatively large number of erythrocytes. This buffy-layer preparation was further suspended in a known volume of platelet-poor plasma (2–6 ml.) and the absolute number of each of these elements in the suspension was determined by direct counting, the platelets and erythrocytes as described above and the leucocytes by the standard method (Whitby & Britton, 1957). The measured volume of suspension was further centrifuged at 3000 rev./min. for 20 min. The packed cells were weighed after completely decanting the supernatant cell-free plasma. With normal blood the leucocytes consisted of both the poly- and mono-nuclear types.

Plasma. The platelet-poor plasma obtained after separation of the platelet button was used for the ascorbic acid estimation. This plasma contained some platelets (generally less than 10 000/mm.^{3}) but no leucocytes or erythrocytes.

Cell counts on whole blood

Leucocyte and platelet counts were made on each specimen of whole blood by the methods already described. An haematocrit value was also obtained (Wintrobe, 1956).

Ascorbic acid analyses

The platelet button and the buffy layer were extracted by stirring for a few minutes with metaphosphosphoric acid solution [3 ml. of 5% (w/v) solution/50 mg. of cells], and spinning till clear and removing the supernatant. The plasma and erythrocyte mass were mixed and allowed to stand with metaphosphoric acid solution [12 ml. of 5% (w/v) solution/4 ml. of cells or plasma] for a few minutes and then filtered. Portions (4 ml.) were used for ascorbic acid determinations by the 2:4-dinitrophenylhydrazine method of Roe & Kuether (1943) as modified by Bolin & Brook (1947). Most analyses were performed on the same day that the blood was taken. A few specimens were stored at $-20°$ for up to 3 days. Some additional estimations on the platelet extract were made by the 2:6-dichloroindophenol method of Mindlin & Butler (1937).

Calculation of results

Plasma, erythrocyte and platelet values were calculated on the basis of their being pure preparations, since other components would not have contributed significantly to the amount of ascorbic acid estimated. For leucocytes, the value taken was that remaining after subtracting from the buffy-layer value the amount contributed by erythrocytes and platelets. Since the number of cells in each sample extracted had been determined it was possible to calculate the amount of ascorbic acid present in 10^{12} erythrocytes, leucocytes and platelets. Concentrations of ascorbic acid in units of mg./100 g. were calculated from the quantity of ascorbic acid in 10^{12} cells and then their weight. The weight of leucocytes was taken to be 894 g./10^{12} cells and was calculated from the known volume of leucocytes (1046μ^3 for granulocytes and 594μ^3 for lymphocytes; Blitzen & van den Berghe, 1945), assuming their specific gravity to be unity and the proportion of granulocytes to lymphocytes to be 2:1 (Wintrobe, 1956). For leukaemic leucocytes, the concentrations (mg./100 g.) were those obtained experimentally, since the buffy layers consisted almost entirely of leucocytes. The weight of platelets was taken to be 16 g./10^{12} platelets and was calculated from a mean platelet volume of 16 ml./10^{12} platelets determined experimentally and assuming a specific gravity of unity. The contribution of plasma and erythrocytes to the ascorbic acid concentration of whole blood was calculated from the haematocrit value and the ascorbic acid concentration in each constituent. The contributions of the leucocytes and platelets were calculated from the whole-blood cell count and the ascorbic acid in each cell.

Mean platelet volume

The mean platelet volume of six normal subjects was determined with a microhaematocrit adapted to hold a large volume of liquid. Platelet-rich plasma (2 ml.) containing a known number of platelets was spun at 3000 rev./min. until the volume of packed cells was constant (3–4 hr.). The length of the column of platelets was read off on the scale and the volume obtained by calibrating the microhaematocrit with water. The mean platelet volume was $16·0 \pm 0·7\mu^3$ and the range 13·3–18·1μ^3. The absolute value may have been lower than the results obtained owing to the difficulty of securing maximum platelet packing. Tocantins (1938) quotes 10–12μ^3; Franke & Horwitz (1938) found 12·5μ^3 for pregnant women. The value of 7·3μ^3 obtained by Olef (1937) is likely to be too low as the platelet counts were about twice as high as those considered to be normal.

Saturation of subjects with ascorbic acid

Normal subjects were dosed orally in the evening with 1 g. of ascorbic acid in water for 4 consecutive days and the blood was taken in the morning. Ascorbic acid analyses of urine by the method of Bessey & King (1933) indicated

that the subjects were saturated. Saturation tests on several leukaemic subjects were conducted by the method of Abassy, Harris, Ray & Marrack (1935).

RESULTS

Normal subjects: before and after saturation with ascorbic acid

Concentrations of ascorbic acid found in the constituents of human blood from subjects before and after saturation with ascorbic acid are shown in Table 1. The concentrations in the platelets and leucocytes were high and they contained approximately 20 times as much as the plasma or erythrocytes. In the platelets, ascorbic acid (in mg./100 g.) had almost the same concentration as in the leucocytes. For platelets, additional analyses were carried out by the 2:6-dichlorophenol method and the results agreed quite closely with those given by the 2:4-dinitrophenylhydrazine method.

On saturation of these subjects with ascorbic acid, the concentration was almost doubled in the plasma, erythrocytes and leucocytes and increased by 30% in the platelets.

Table 1 shows the ascorbic acid content of the cellular constituents expressed as mg./10^{12} cells. Leucocytes, because of their larger size, had by far the largest concentration per cell. Although the platelet is approximately one-tenth the volume of the erythrocyte there was about six times more ascorbic acid in each platelet.

The contribution of each constituent to the ascorbic acid content of whole blood is shown in Table 2. The results are expressed both as mg./100 ml. of blood and as percentages of the total amount. No determinations were made on whole blood, but the total ascorbic acid was calculated from the individual constituents.

The contribution of the plasma and erythrocytes accounted for about 80% of the total ascorbic acid in blood. This compared with 10% for the platelets and 10% for the leucocytes. On saturation with ascorbic acid, the increase was slightly more in the plasma than the other blood constituents.

Leukaemic blood

Determinations were made on the blood from three patients with chronic myeloid leukaemia and two with chronic lymphatic leukaemia (Table 3). The leucocyte counts of these patients were high (50 000–500 000/mm.^{3}), and the cells consisted of at least 95% of the granulocytic or lymphatic types respectively. One of the chronic myeloid leukaemia patients and the two chronic lymphatic leukaemia patients had no measurable ascorbic acid in the plasma ($<0{\cdot}02$ mg./100 ml.), and only very small amounts or none in the erythrocytes; the other two cases of myeloid leukaemia had fairly low values in the plasma and erythrocytes. The platelets and leucocytes of all patients, however, contained an appreciable amount of ascorbic acid. Because of these findings, one of the myeloid and the two lymphatic leukaemic patients were given an ascorbic acid-loading test. It was found that a longer time (6–14 days) was needed to attain saturation than in normal subjects, who usually reach saturation in 2 days (Abassy *et al.* 1935). After saturation, the ascorbic acid contents of the constituents of the blood were within the normal range for saturated subjects (Table 3). This applied particularly to the leucocytes when the concentration was expressed in mg./10^{12} cells. Chronic myeloid and lymphatic leukaemic leucocytes contained about the same amount of ascorbic acid per cell. Since the volume of the granulocytes is nearly twice that of the lymphocytes (Tivey, Li & Osgood, 1951), the concentration expressed as mg./100 g. is larger in the lymphocytes than in the granulocytes.

DISCUSSION

From the above results it is clear that platelets and leucocytes contribute almost equally to the high concentration of ascorbic acid in the buffy layer of human blood. Whilst it is generally believed that leucocytes have a high content, the possibility that platelets are also rich in ascorbic acid has received little attention. We have found that the concentration in the platelets is about the same as that in the leucocytes.

From a functional aspect, ascorbic acid may play an important role in the activity of leucocytes. Thus in scorbutic guinea pigs the phagocytic activity of leucocytes is said to be lowered (Lawrynowicz, 1931; Cottingham & Mills, 1943). Nungester & Ames (1948) have also claimed that the phagocytic activity of leucocytes of peritoneal exudates bears a direct relationship to their ascorbic acid content. For platelets, no evidence has so far been obtained that ascorbic acid influences their activity. Thus in scorbutic guinea pigs the activity of platelets in thromboplastin formation and in syneresis is normal (P. Barkhan & A. N. Howard, to be published). One of the functions of platelets may be as a carrier of pharmacologically active materials (Humphrey & Jaques, 1954). This concept has been used to explain the large quantities of adrenaline (Weil-Malherbe & Bone, 1954), serotonin (Bigelow, 1954) and histamine (Humphrey & Jaques, 1954) which they contain. Platelets could also be considered as a means of vitamin C transport to sites of injury.

There is increasing support for the concept that intravascular blood coagulation is a continuous

Table 1. *Ascorbic acid concentration in the constituents of human blood of normal and saturated subjects*

Mean values with the standard errors of the means and the ranges are given. Ascorbic acid results were obtained by the 2:4-dinitrophenylhydrazine method, with the exception of results in column *B*, where the 2:6-dichloroindophenol method was used.

		Ascorbic acid							
		(mg./100 ml.)		(mg./100 g.)			(mg./10^{12} cells)		
				Platelets					
Subjects	No.	Plasma	Erythrocytes	*A*	*B*	Leucocytes	Erythrocytes	Platelets	Leucocytes
Normal	6	0·69±0·06 (0·50–0·89)	0·69±0·05 (0·53–0·83)	18·3±1·4 (13·2–22·8)	18·6±1·2 (14·8–22·8)	16·5±2·14 (12·1–25·1)	0·55±0·04 (0·42–0·66)	2·91±0·22 (2·11–3·65)	148±19 (108–224)
Saturated with ascorbic acid	6	1·24±0·11 (0·85–1·62)	1·15±0·07 (0·92–1·41)	24·3±1·8 (20·6–32·8)	—	27·7±1·9 (20·3–32·5)	0·92±0·06 (0·73–32·5)	3·90±0·29 (3·32–5·24)	248±17 (182–290)

Table 2. *Contribution of each constituent to the ascorbic acid concentration of whole blood*

Mean values per 100 ml. of blood and per cent. of total ascorbic acid, with the standard error of the mean, are given. Ascorbic acid was estimated by the 2:4-dinitrophenylhydrazine method.

		Ascorbic acid								
		Plasma		Erythrocytes		Platelets		Leucocytes		Total
Subjects	No.	(mg./100 ml.)	(%)	(mg./100 ml.)	(%)	(mg./100 ml.)	(%)	(mg./100 ml.)	(%)	(mg./100 ml.)
Normal	6	0·34±0·04	40·2±3·2	0·35±0·04	40·7±3·0	0·08±0·003	9·5±0·8	0·08±0·009	9·7±1·1	0·84±0·05
Saturated with ascorbic acid	6	0·68±0·06	45·9±2·1	0·52±0·03	36·4±3·7	0·11±0·02	7·7±1·0	0·15±0·01	10·1±1·3	1·46±0·08

Table 3. *Ascorbic acid in the blood of leukaemic subjects*

				Ascorbic acid						
				(mg./100 ml.)		(mg./100 g.)		(mg./10^{12} cells)		
Subject	Type of chronic leukaemia	Ascorbic acid status	Time to saturate (days)	Plasma	Erythrocytes	Platelets	Leucocytes	Erythrocytes	Platelets	Leucocytes
Ba	Myeloid	Unsaturated	—	0·00	0·00	13·8	11·5*	0·00	2·2	101
Te	Myeloid	Unsaturated	—	0·56	0·20	15·6	23·9	0·16	2·5	162
Wa	Myeloid	Unsaturated	—	0·37	0·50	20·6	8·5	0·39	3·3	87
Wa	—	Saturated	8	1·04	1·36	24·4	22·1	0·83	3·9	298
Gi	Lymphatic	Unsaturated	—	0·10	0·36	6·9	21·7	0·29	1·1	62
Gi	—	After 10 days on saturation dose	—	0·00	—	16·9	38·3	—	2·7	190
Gi	—	Saturated	15	0·90	1·02	26·2	67·6†	0·82	4·2	274
Wd	Lymphatic	Unsaturated	—	0·00	0·16	19·2	61·6	0·13	3·1	265
Wd	—	Saturated	6	1·26	1·11	24·4	67·1	0·88	3·9	289

* 11·4 mg./100 g. by method of Mindlin & Butler (1937).

† 68·8 mg./100 g. by method of Mindlin & Butler (1937).

process under physiological conditions (Roos, 1957). This would explain the normal maintenance of haemostasis, the continuous utilization of the various clotting factors and the tendency to haemorrhage when the coagulation mechanism becomes defective either through deficiency of one or more clotting components or the presence of inhibitory substances. The platelet is an important component of the haemostatic mechanism. How it reacts with the vascular wall to prevent haemorrhage is not known; possibly it helps to form the fibrin film on the endothelium. If this involves deposition of platelets on the vascular wall, then this would constitute a means of carrying ascorbic acid to the site where it is believed to play an important part in the formation of the ground substance of the vascular membranes (Wolbach & Howe, 1926) or in the tissue sheath (Penney & Balfour, 1949), which are also important in maintaining vascular integrity.

Another pointer to the function of vitamin C in platelets may be seen in the similarity between the petechial haemorrhages observed in scurvy and those seen in thrombocytopenic purpura. The abnormality in scurvy, however, is not due to a quantitative deficiency of platelets since the platelet count is actually increased (Hess, 1920; P. Barkhan & A. N. Howard, to be published). These vascular defects may have a common origin in the failure of sufficient vitamin C to reach the vascular and capillary walls.

The ascorbic acid content of erythrocytes and plasma is low and the results are in agreement with those of other authors (Butler & Cushman, 1940). Owing to the much larger mass of erythrocytes and plasma compared with the buffy layer, they contribute up to 80% of the total in the blood.

High values for an indophenol-reducing substance have been reported in leukaemic blood (Stephen & Hawley, 1936; Cuttle, 1938), and the ascorbic acid concentration of leukaemic leucocytes is apparently higher than normal (Butler & Cushman, 1940). Our observations show that the ascorbic acid concentration of leucocytes is higher than normal in chronic lymphatic leukaemia but not in chronic myeloid leukaemia. Owing to the smaller volume of lymphocytes compared with granulocytes, the ascorbic acid content per cell is the same in both types of leukaemia. Data presented by Butler & Cushman (1940) are in accord with our results; they also found very high ascorbic acid concentrations in chronic lymphatic leukaemia. Whether or not this difference in ascorbic acid concentration exists between normal lymphocytes and granulocytes is as yet unknown. Although Waldo & Zipf (1955) found that the buffy layer in both acute and chronic leukaemia is low in ascorbic acid, their apparently conflicting results could be explained by the differences between the ascorbic acid nutrition of their subjects and ours.

We have confirmed the report of Waldo & Zipf (1955) that the ascorbic acid content of leukaemic plasma is low. In three of our cases no measurable ascorbic acid was found in the plasma, although the leucocytes and platelets did contain considerable amounts. Plasma ascorbic acid is considered a poor index of ascorbic acid nutrition (Sebrell & Harris, 1954) and for this reason three patients were submitted to the saturation test of Abassy *et al.* (1935). These subjects took a much longer period to saturate than is normal. Cuttle (1938) obtained similar results with two cases of acute and two of chronic myeloid leukaemia.

SUMMARY

1. The concentration of ascorbic acid in the plasma, erythrocytes, platelets and leucocytes of human blood has been determined in normal subjects, before and after saturation with ascorbic acid.

2. Both the platelets and leucocytes were found to have high concentrations (before saturation 15 mg./100 g.; after saturation 25 mg./100 g.) but the plasma and erythrocytes were comparatively low (before saturation 0·7 mg./100 ml.; after saturation 1·2 mg./100 ml.).

3. Because of the large mass of erythrocytes and plasma, they contributed about 80% of the total ascorbic acid in the blood. About 10% was contributed by the platelets and 10% by the leucocytes.

4. The concentration of ascorbic acid was higher in the chronic leukaemic lymphocyte (60 mg./100 g.) than in either the normal leucocyte (20 mg./100 g.) or leukaemic granulocyte (20 mg./100 g.); the total quantity in each cell was, however, the same in all these cell types.

5. It was confirmed that in chronic lymphatic (two cases) and myeloid (three cases) leukaemia the ascorbic acid concentration in the plasma is lower than normal.

REFERENCES

Abassy, M. A., Harris, L. J., Ray, S. N. & Marrack, J. R. (1935). *Lancet*, ii, 1399.

Barkhan, P. (1957). *J. clin. Path.* **10**, 26.

Bessey, O. A. & King, C. G. (1933). *J. biol. Chem.* **103**, 687.

Bigelow, F. S. (1954). *J. Lab. clin. Med.* **43**, 759.

Blitzen, I. & van den Berghe, L. (1945). *Rev. belge Sci. méd.* **16**, 322.

Bolin, D. W. & Brook, L. (1947). *Science*, **106**, 451.

Butler, A. M. & Cushman, M. (1940). *J. clin. Invest.* **19**, 459.

Butler, A. M. & Cushman, M. (1941). *J. biol. Chem.* **139**, 219.

Cottingham, E. & Mills, C. A. (1943). *J. Immunol.* **47**, 493.

Cuttle, T. D. (1938). *Quart. J. Med.* **31**, 575.
Franke, M. & Horwitz, S. (1938). *Z. Geburtsh. Gynäk.* **103**, 318.
Hagtvet, J. (1945). *Skr. norske VidenskAkad.* **2**, 1. Quoted in *Chem. Abstr.* (1947). **41**, 796c.
Hess, A. F. (1920). *Scurvy—Past and Present.* Philadelphia: Lippincott and Co.
Humphrey, J. H. & Jaques, R. (1954). *J. Physiol.* **124**, 305.
Lawrynowicz, A. (1931). *J. Physiol. Path. gén.* **29**, 270.
Mindlin, R. C. & Butler, A. M. (1937). *J. biol. Chem.* **122**, 673.
Nungester, W. J. & Ames, A. M. (1948). *J. infect. Dis.* **83**, 50.
Olef, I. (1937). *J. Lab. clin. med.* **23**, 166.
Penney, J. R. & Balfour, B. M. (1949). *J. Path. Bact.* **61**, 171.
Roe, J. H. & Kuether, C. A. (1943). *J. biol. Chem.* **147**, 399.
Roos, J. (1957). *Thromb. Diath. Haemorr.* **1**, 471.
Sebrell, W. H. & Harris, R. S. (1954). *The Vitamins*, vol. 1, p. 362. New York: Academic Press Inc.
Stephen, P. J. & Hawley, E. F. (1936). *J. biol. Chem.* **115**, 653.
Tivey, H., Li, J. G. & Osgood, E. E. (1951). *Blood*, **6**, 1013.
Tocantins, L. M. (1938). *Medicine, Baltimore*, **17**, 155.
Waldo, A. L. & Zipf, R. E. (1955). *Cancer Res.* **8**, 187.
Weil-Malherbe, H. & Bone, H. D. (1954). *Nature, Lond.*, **174**, 557.
Whitby, L. E. H. & Britton, C. J. C. (1957). *Disorders of the Blood*, p. 713. London: Churchill.
Wintrobe, M. M. (1956). *Clinical Haematology*, 3rd ed. London: Henry Kimpton.
Wolbach, S. B. & Howe, P. R. (1926). *Arch. Path.* (*Lab. Med.*) **1**, 1.

Thetin–Homocysteine Transmethylase

SOME FURTHER CHARACTERISTICS OF THE ENZYME FROM RAT LIVER

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In previous communications (Maw, 1955, 1956) it was shown that as the enzymic methylation of homocysteine by dimethylthetin, $(CH_3)_2\overset{+}{S}\cdot CH_2\cdot CO_2^-$, at pH 7·4 involves the appearance of one proton for each molecule of methionine formed, the reaction can therefore be followed manometrically in a bicarbonate medium by measuring the carbon dioxide evolved. The enzyme responsible has been named thetin–homocysteine transmethylase, and has been partially purified from rat liver. The effect on the activity of the rat-liver transmethylase of varying the concentrations of the two substrates at pH 7·4 has been studied.

The present paper deals with some further characteristics of the enzyme. A brief survey of its substrate specificity has been made, and the effects on its activity of metal ions and a number of the more common enzyme inhibitors have been examined. Homocysteine is specifically required as a methyl acceptor. This is in contrast with the less specific requirement for a methyl donor, for in addition to dimethylthetin chloride nine other sulphonium salts have so far been shown to have some activity as methyl donors.

Some properties of the thetin–homocysteine transmethylase from pigeon liver, including its inhibition by mercaptoacetate (thioglycollate), have been briefly reported by Sloane, Boggiano, Coulomb & Hutchings (1956), and Durell & Anderson (1956) have described the characteristics of the enzyme from horse liver. In these studies enzymic activity was followed by colorimetric determination of the methionine formed. During the completion of the experiments reported here, Durell, Anderson & Cantoni (1957) published details of the purification of the horse-liver transmethylase, in which methionine determinations were used as a measure of enzymic activity. Many of the characteristics of the enzyme described by these workers are in substantial agreement with those given below for the rat-liver enzyme.

MATERIALS AND METHODS

Substrates. The various thetins under study were prepared in the form of their chlorides or bromides by interaction of the corresponding chloro- or bromo-fatty acids with the appropriate thio ether (see Maw, 1953). Sulphocholine iodide (2-hydroxyethyldimethylsulphonium iodide) was prepared according to the method of Maw & du Vigneaud (1948*c*). The methyl ester of dimethylthetin iodide was obtained by the interaction of methyl methylthioacetate with methyl iodide. The methionine methylsulphonium chloride used was a gift from Professor F. Challenger, to whom the author wishes to express his thanks.

DL-Homocysteine was prepared by the reduction of DL-homocystine with sodium in liquid ammonia (Riegel & du Vigneaud, 1935–36).

Manometric procedure. The preparation of active fractions of thetin–homocysteine transmethylase from rat liver has been described in a previous communication (Maw, 1956). An account of the manometric method used to study the transmethylase has also been given in this earlier paper.