with any confidence. Terminal (β phase) half-lives were determined by least squares linear regression fitting to the semi-logarithmically transformed data. Area under the plasma level curve was determined by trapezoidal rule integration, with area under the curve from the last measured data point to infinity being calculated by dividing the last measured plasma concentration (C_t) by β (Gibaldi & Perrier, 1975). Where indicated, the immediate pre-dose plasma concentration (resulting from previous dexamethasone administration) was also divided by β and subtracted from the area under the curve, to compensate for the effect of the previous drug dose. Dose divided by area under the curve yielded the whole plasma clearance of the drug, and clearance values divided by β allowed estimation of the apparent volume of distribution ($V_d\,\beta$).

The values of the pharmacokinetic parameters in individual patients are shown in Table 1. Although there was substantial variation in the individual values, and the terminal half-life value for Case 10-b was so widely deviant that it must be suspect, the mean half-life of 2.95 h (2.29 h if the deviant value is excluded) agreed reasonably well with the mean value of the dexamethasone half-life obtained with a specific HPLC assay by Tsuei *et al.* (1979) *viz.* 2.86 h (2.37 h in females and 3.35 h in males). In the present study the difference in half-life between the sexes either including (F = 2.15 h, M = 3.53 h) or excluding the deviant half-life value (F = 2.15, M = 2.39 h) were not statistically significant ($t = 0.905$; $P > 0.30$ and $t = 0.324$, $P > 0.70$, respectively). Regression analysis showed no statistically significant effect of age on dexamethasone half-life. Clearance and volume of distribution values obtained in the present study were both higher than those obtained by Tsuei *et al.* (1979) (Table 1). These differences may reflect consequences of prior exposure to drugs which may induce the hepatic mixed oxidase system, as well as effects of intraoperative administration of drugs and fluids.

It would then appear that in the pharmacokinetically complex situation in which dexamethasone is used during neurosurgery, the disposition parameters of the drug do not differ to any marked degree from those which apply when the drug is given for the first time to healthy volunteers. The significance of the higher dexamethasone clearance values is uncertain.

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J. McCAFFERTY, T.R.O'R. BROPHY, J.D. YELLAND, B.E. CHAM, F. BOCHNER* & M.J. EADIE

Departments of Medicine and Surgery, University of Queensland, Royal Brisbane Hospital, Brisbane 4029, Australia

*Present address: Department of Clinical and Experimental Pharmacology, University of Adelaide, Adelaide, Australia

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ERYTHROCYTE AND PLASMA ASPIRIN ESTERASE

Enzymes able to hydrolyse aspirin are present in blood and have been termed 'aspirin esterases'. Rainsford *et al.* (1980) have shown that the main plasma aspirin esterase activity is associated with the plasma cholinesterases.

Most publications in this field seem to ignore the contribution of the red cell to aspirin hydrolysis. Admittedly several previous reports (Morgan & Truitt, 1965; Mulinos & Ardam, 1950; Harthon & Hedstrom, 1971) have mentioned in passing that whole blood has a higher enzymatic activity than serum or plasma but the contribution from the cellular fraction has not been extensively studied.

In the present study a u.v. method was used to measure aspirin esterase activity (Rylance & Wallace, 1980). In this, phosphate buffer (0.139 M; pH 7.0; 9.5 ml), aspirin solution (7.2 mM in isotonic saline; 5.0 ml) and the sample to be tested (0.5 ml) were mixed, incubated at 37°C and samples removed at 5 min and suitable intervals thereafter. The samples were centrifuged for 10 min and the absorbance of the clear solutions read at 300 nm. The

reading at 300 nm gave the amount of salicylic acid formed by hydrolysis. Appropriate blanks (a) without test sample, and (b) without aspirin were included in the assay system. The rate of spontaneous hydrolysis was deducted from the total rate to give the enzymic hydrolysis rate. The method is simple to carry out and, provided the blood has been carefully taken, there is no haemolysis which would interfere with the estimation at 300 nm. Plasma and serum can be estimated without centrifuging.

The phosphate buffer used in the incubation mixture is iso-osmotic with plasma (osmolality = 297 mosmol/kg; plasma range 280–295 mosmol/kg on the same instrument — an Osmette) and *no* haemolysis takes place during the reaction. When testing whole blood, cells or plasma no sample showing haemolysis was used. Any haemolysis would of course give a high initial absorbance reading at 300 nm.

In experiments to determine whether the esterase activity was mainly in the red cells, the plasma or the cell membranes, the following procedure was followed. Whole blood was taken, its activity determined and the plasma separated by centrifugation. The activity of the plasma was estimated after dilution to the original total blood volume with phosphate buffer (0.139 M; pH 7.0). The red cells were washed three times with the above buffer and the enzyme activity determined after dilution to the original volume. The buffy coat was discarded.

To prepare red cell stroma, the cells from 5 ml of blood were lysed by adding a very dilute acetic acid-acetate solution (4 mM acetic acid/0.8 mM sodium acetate) to give a total volume of 10 ml. After mixing, the suspension was centrifuged at 2,000 rev/min for 30 min and this procedure repeated until the supernatant was completely colourless. This procedure is essentially that of Watt (1961) but without the inclusion of Hibitane (chlorhexidine diacetate) in the lysing solution. The stroma was suspended in 0.139 M phosphate buffer to the original blood volume before testing. Unfortunately with the method used the estimation of the enzyme activity in the cell contents was not possible; the high absorbance at 300 nm due to haemoglobin making the assay impracticable.

In a small initial study involving 9 males (age range 24–65 years, each decade being represented) and 12 females (age range 19–58 years, each decade being represented) no correlation between age and enzyme level was observed. Thereafter no special effort was made to match for age the groups examined. Where comparisons were made between different groups, the sexes were compared separately and the numbers are stated in the appropriate table.

Whole blood and plasma enzyme levels were determined on samples obtained from healthy individuals attending the Blood Transfusion Centre, the blood being collected in heparinised tubes. From Table 1 it can be seen that about 80% of the aspirin esterase activity of whole blood was located in the cellular fraction. There was no significant difference between male and female plasma levels from these healthy controls. However, the mean male whole blood esterase activity was significantly higher than the mean female value ($P < 0.05$).

There was no significant correlation between the enzyme levels in erythrocytes and plasma suggesting that the enzyme inside the red cell and plasma was different.

This was illustrated again when the results from patients with rheumatoid arthritis were examined (Table 1). These patients were taking aspirin only at the time of this study and no other drugs. They had all been taking aspirin for many years; from a minimum of 2 years to a maximum of 20 years. The ages ranged from 34 to 76 years for males and 26 to 76 years for females.

The plasma enzyme activity was lowered in these patients compared to controls but not significantly so, whereas the total whole blood values were elevated, significantly so for the females ($P < 0.05$). Thus the red cell enzyme activity has risen compared to controls, whereas the plasma enzyme activity is slightly lowered, suggesting again two different enzymes. This also draws attention to the question whether the

Table 1 Aspirin esterase levels in blood and plasma.
Comparison of blood and plasma enzyme levels in normal individuals and a further comparison with patients with rheumatoid arthritis on long-term aspirin therapy

		*Blood**	*Plasma**
Male	Controls	24.46 ± 3.55 (37)	5.48 ± 3.22 (25)
	Patients	25.11 ± 4.66 (22)	3.17 ± 2.34 (10)
Female	Controls	22.61 ± 4.12 (37)	5.82 ± 3.09 (23)
	Patients	25.80 ± 4.52 (21)	5.41 ± 2.29 (10)

* Aspirin esterase values in i.u./l of sample (37°C); mean ± s.d. (number of individuals).

Table 2 Aspirin esterase activity in whole blood and its components

	Exp. 1	*Exp. 2*	*Exp. 3*	*Exp. 4*	*Exp. 5*
Whole blood	34.00	25.05	26.86	20.57	23.41
Plasma	7.14	2.42	2.18	2.66	2.70
Cells (before lysis)	25.05	16.94	ND	ND	19.81
Red cell stroma	ND	ND	0	0	0

Aspirin esterase values in i.u./l of sample (37°C)
ND = not determined

plasma or red cell enzyme type is of major importance in the metabolism of aspirin, assuming that both types are represented in the liver — the main site of aspirin hydrolysis (Rowland & Riegelman, 1968). It must be added that the above results with patients with rheumatoid arthritis do not allow any conclusions to be drawn as to whether the prolonged treatment with aspirin or the disease has caused this change in enzyme level.

In a few experiments the contribution from the intact cells and cell stroma was determined separately (Table 2), the red cell enzyme activity being located inside the erythrocyte with none in the stroma.

The effect of a cholinesterase inhibitor on the cellular and plasma enzymes was examined by the method used in an earlier study (Rylance & Wallace, 1980). Physostigmine was added to the 0.139 M (pH 7.0) phosphate buffer in which plasma or erythrocytes were diluted and incubated at 37°C for 5 min to allow any drug binding to take place, prior to addition of the aspirin solution. The enzyme activities for plasma and erythrocytes with and without added physostigmine (1.66 μmol/l in final test system) were compared and tested for significance, using the modified Student's *t*-test for unpaired samples and small numbers (Hill, 1967). With plasma, physostigmine gave complete inhibition in all of six determinations; with cells (diluted back to the original blood volume) there was no significant difference ($P = 0.05$) in hydrolysis rates with and without added physostigmine (six determinations). Obviously the esterase activity in the cellular fraction was much higher than in plasma and therefore additional experiments were carried out in which the cells were diluted with saline to give the same level of enzyme activity as plasma. Again no inhibition was found with physostigmine (1.66 μmol/l); in fact, a slight non-significant stimulation was observed. % inhibition = -10.4 ± 9.8 (four determinations). Purified human cholinesterase (Boehringer Corporation) exhibited high aspirin esterase activity.

This study demonstrated that an enzyme inside the red cell will hydrolyse aspirin and that the cellular enzyme contribution is several fold greater than that of plasma. The cellular enzyme is *probably* a 'non specific' esterase whereas the plasma enzyme activity is due to cholinesterase as previously demonstrated by Rainsford *et al.* (1980).

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H.J. RYLANCE & RUTH C. WALLACE

Rheumatic Diseases Unit, Northern General Hospital, Edinburgh EH5 2DQ

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