790. *The Hydrolysis of Aspirin in Guinea-pig Plasma.*

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The hydrolysis of aspirin has been measured in guinea-pig plasma at substrate concentrations of 10^{-2} to $2{\cdot}5 \times 10^{-4}$M and plasma concentrations of 1% to 50%, a spectrophotometric method being used. The esterase-catalysed reaction is very much faster than the normal hydrolysis rate in a buffer at 37°, pH 7·4. Reaction velocity varies linearly with enzyme concentration at the higher concentrations; at lower concentrations there is a marked reduction in the relative activity. Activity is unchanged by storage for a week at 4° but is destroyed by 1 hour's heating at 57°. Calcium ions do not affect the reaction rate appreciably. Physostigmine retards the reaction only when present in relatively large doses at low plasma concentrations. It is estimated that the life of aspirin in the blood-stream would be between 5 and 10 minutes.

In earlier papers (Edwards, *Trans. Faraday Soc.*, 1950, **46**, 723; 1951, **47**, 1191; 1952, **48**, 696) the kinetics of hydrolysis and dissolution of aspirin in aqueous solution were studied at temperatures from 10° to 50° over the whole pH range, a spectrophotometric method being used for the simultaneous estimation of acetylsalicylic and salicylic acid.

The present study deals with measurements *in vitro* of the esterase-catalysed hydrolysis of aspirin in guinea-pig plasma at 37°, pH 7·4. Substrate concentrations from 10^{-2} to $2{\cdot}5 \times 10^{-4}$M and plasma concentrations from 1% to 50% were investigated. These limits represent the extremes within which the reaction could be followed by the spectrophotometric technique with reasonable accuracy.

Experimental

The blood from each guinea-pig was collected, citrated, and centrifuged for 15 minutes to yield the plasma which, except where otherwise indicated, was used in the kinetic experiments within 30 minutes of the killing of the animal.

The aspirin, calcium aspirin, and physostigmine sulphate solutions were always freshly prepared immediately before a run, M/10-sodium citrate solution being used as solvent and adjusted to pH 7·4 whenever necessary before being mixed with the plasma. If further dilution of the reaction mixture was desired, the M/10-sodium citrate buffer was used. When physostigmine was used it was added to the plasma 30 minutes before addition of the substrate solution.

Reaction mixtures were placed in a thermostat at 37° and samples were withdrawn at intervals for spectrophotometric estimation by aid of the Hilger Medium Quartz instrument. Preliminary experiments, not reported here, established the approximate hydrolysis rates, and the sampling times were chosen accordingly to cover as much of the course of reaction as was practicable. In general, it was found that the hydrolysis of mixtures containing up to 20% of plasma could be followed by drawing samples from the thermostat bath as required, but for high plasma concentrations the reactions were carried out in quartz cells contained in a jacketed cell-holder described elsewhere (Edwards, *Chem. and Ind.*, 1952, 779). These fast reactions were followed by the fixed-density technique (*idem*, *Trans. Faraday Soc.*, 1950, **46**, 723; 1952, **48**, 696) in which the spectrophotometer is used as a transit instrument, a time base replacing the usual extinction base.

In estimations of the degree of hydrolysis the following extinction values at 296·5 mμ were used.

	ϵ		ϵ
Aspirin at pH 7·4 (fully ionized)	30	Physostigmine sulphate at pH 7·4	3000
Salicylic acid at pH 7·4 (fully ionized)	3520		

Plasma from :	$E^{10\%}_{1\text{ cm.}}$	Plasma from :	$E^{10\%}_{1\text{ cm.}}$
Guinea-pig no. 1	1·94	Guinea-pig no. 5 after 1 week in refrigerator	1·28
,, ,, 2	1·60	Guinea-pig no. 5 after 5 hours at 37°	1·29
,, ,, 3	2·00		
,, ,, 4	1·48		
,, ,, 5	1·28		

The density drum was calibrated against potassium chromate, $\varepsilon_{max.}$ 4816 at 371 mμ being taken as standard.

Results.—The relevant experimental data and the observed velocity constants for each hydrolysis are set out in Tables 1 and 2. The constant $k_{obs.}$ is expressed in day^{-1} as in earlier papers.

TABLE 1. *Hydrolysis rates for aspirin in plasma at* 37°, pH 7·4.

Guinea-pig no.	Aspirin molarity	Plasma, vol. %	$k_{obs.}$	Guinea-pig no.	Aspirin molarity	Plasma, vol. %	$k_{obs.}$
—	10^{-2}	0	0·92	1	5×10^{-4}	2·5	13·6
—	5×10^{-3}	0	0·915	,,	$2·5 \times 10^{-4}$	2·5	12·4
—	5×10^{-4}	0	0·92	,,	10^{-2}	20	205
—	$2·5 \times 10^{-4}$	0	0·925	,,	5×10^{-3}	20	212
1	10^{-2}	2·5	14·8	,,	5×10^{-4}	20	185
,,	5×10^{-3}	2·5	14·8	,,	$2·5 \times 10^{-4}$	20	170

In the following expts. the aspirin molarity was 5×10^{-3}.

Guinea-pig no.	Plasma, vol. %	$k_{obs.}$	Guinea-pig no.	Plasma, vol. %	$k_{obs.}$	Guinea-pig no.	Plasma, vol. %	$k_{obs.}$
2	1	3·73	4 *	1	4·71	5 †	2·5	10·5
,,	2·5	9·74	,,	2·5	12·3	,,	5	25·1
,,	5	21·9	,,	5	27·2	,,	10	59·9
,,	10	49·1	,,	10	68·0			
,,	20	124	,,	20	162	5 ‡	2·5	0·93
,,	35	269	,,	35	321	,,	5	0·91
,,	50	400	,,	50	500	,,	10	0·93
						,,	20	0·985
3	1	6·22	5	2·5	10·0			
,,	2·5	16·0	,,	5	25·9			
,,	5	38·5	,,	10	61·4			
,,	10	91	,,	20	159			
,,	20	249	,,	35	328			
,,	35	450						

* Ca salt.
† Plasma kept for 1 week at 4° before use.
‡ Plasma heated at 57° for 1 hour before use.

The value of $k_{obs.}$ for the hydrolysis catalysed by the water and hydroxyl ion present was about 0·92 at all concentrations of substrate. This is in agreement with values already reported

TABLE 2. *Effect of physostigmine sulphate on esterase-catalysed hydrolysis of* 5×10^{-3}M-*aspirin at* 37°, pH 7·4.

Guinea-pig no.	Plasma, vol. %	Physostigmine molarity	$k_{obs.}$	Guinea-pig no.	Plasma, vol. %	Physostigmine molarity	$k_{obs.}$
2	1	0	3·73	2	5	0	21·9
,,	,,	10^{-7}	3·70	,,	,,	10^{-4}	22·1
,,	,,	10^{-6}	3·81	,,	,,	10^{-3}	20·8
,,	,,	10^{-5}	3·65				
,,	,,	10^{-4}	3·07	3	10	0	91
,,	,,	10^{-3}	2·03	,,	,,	10^{-4}	91·5
,,	2·5	0	9·74	,,	,,	10^{-3}	90
,,	,,	10^{-5}	9·70				
,,	,,	10^{-4}	8·49				
,,	,,	10^{-3}	7·05				

(Edwards, *Trans. Faraday Soc.*, 1952, **48**, 696). At pH 7·4, $k_{obs.} = 55{\cdot}5k_5 + k_6c_{OH}$ where, at 37°, $k_5 = 1{\cdot}64 \times 10^{-2}$ and $k_6 = 1{\cdot}18 \times 10^5$, giving $k_{obs.} = 0{\cdot}92$.

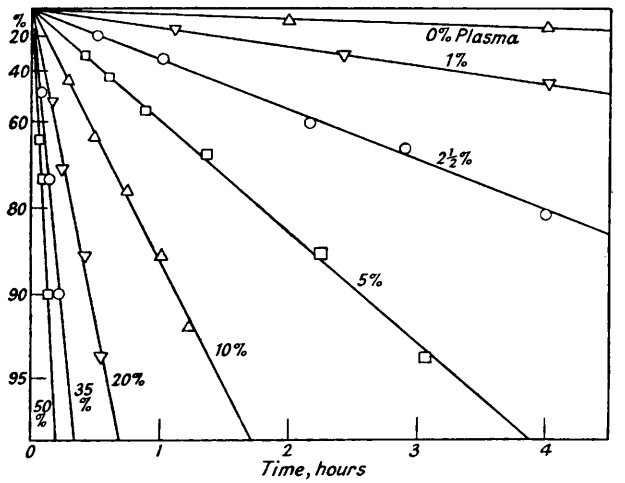

FIG. 1. *First-order rates of hydrolysis of aspirin at various plasma concentrations; plasma from guinea-pig no.* 2, 37°, *sodium citrate buffer* pH 7·4.

At substrate concentrations of 5×10^{-3} and above, the enzyme activity appeared to be at a maximum for plasma from guinea-pig no. 1. In subsequent experiments the substrate concentration was kept at this level. Esterase activity varied for different animals, as may be seen by comparing the $k_{obs.}$ values at the same plasma level.

In all cases the hydrolysis followed a first-order course and the experimental results for no. 2 plasma, illustrated in Fig. 1, are typical for the series.

When calcium aspirin was used in place of aspirin as substrate, the rate of the hydrolysis was much the same as before, although Ca^{++} is often a positive catalyst for esterase-catalysed reactions.

The enzyme activity was unaltered if the plasma was stored in the refrigerator for a week, but an hour's heating at 57° substantially destroyed the esterases and reduced the hydrolysis rate to the normal water-catalysed level (plasma no. 5).

The inhibitory action of physostigmine was examined at various concentrations. Although there was a certain amount of retardation of the hydrolysis at low plasma concentrations, yet comparatively massive doses of the inhibitor were required; at higher plasma concentrations amounts of physostigmine of up to 10^{-3}M had little effect on the reaction velocity.

DISCUSSION

By choosing the appropriate scale along the plasma-concentration axis the values for the esterase-catalysed reaction ($k_{obs.} - 0{\cdot}92$) could all be made to lie on a smooth curve (Fig. 2). In a system where

$$\text{enzyme} + \text{substrate} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \text{complex} \xrightarrow{k_3} \text{products}$$

the reaction rate is theoretically

$$-dc_s/dt = k_3c_ec_s/(K + c_s)$$

K being the Michaelis constant $(k_2 + k_3)/k_1$. When the substrate concentration, c_s, is fixed, the rate should be proportional to the enzyme concentration, c_e. Fig. 2 shows that this relation is obeyed at plasma concentrations above *ca.* 10—15%. At the lower concentrations there is considerable departure from linearity. This is not unusual; Kraupp (*Z. Vitamin-, Hormon- u. Fermentforsch.*, 1949, **2**, 179) reported that large dilutions lower the activity of choline esterase, for example.

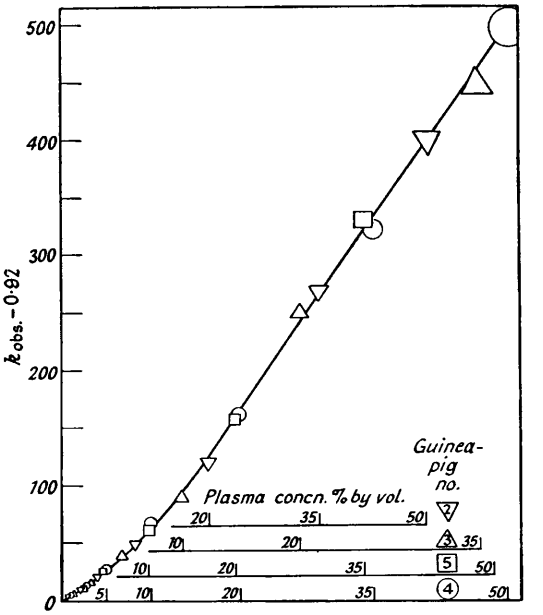

FIG. 2. *First-order velocity constants for the esterase-catalysed hydrolysis of aspirin as a function of plasma concentration : animals 2, 3, 5—substrate aspirin ; animal 4—substrate calcium aspirin.*

Some idea of the relative esterase concentrations in different individuals may be obtained by comparing the scale factors in Fig. 2. The mean value being taken as unity, the relative activities of the four plasma samples are :

Guinea-pig no.	2	3	4	5
Relative esterase activity	0·79	1·30	0·97	0·94

indicating that a deviation of at least 30% from the mean can be expected in different individuals.

If the linear portion of Fig. 2 is extrapolated to 100% plasma concentration, the corresponding reaction rates are :

Guinea-pig no.	2	3	4	5
$k_{obs.}$	860	1420	1060	1020
$t_{90\%}$ (min.)	3·8	2·3	3·1	3·2

It is noteworthy that to obtain a reaction velocity of this magnitude at 37° in the absence of catalysts other than those molecular and ionic species normally present in water it would be necessary to use a buffer solution of pH 12.

In applying these results to the hydrolysis *in vivo* of aspirin in the blood-stream some caution is needed. It must be borne in mind that (i) the whole blood of a living animal is being compared with the plasma from a dead animal; (ii) the plasma is citrated; (iii) the extrapolation from 50% to 100% plasma concentration assumes a linear relation which, although theoretically probable, has not been experimentally demonstrated. It seems likely, however, that the hydrolysis rate *in vivo* would not be less than that determined *in vitro*. If this argument is accepted it would follow that the life of aspirin in the blood-stream is quite short, 99% of the initial concentration being hydrolysed in about 5—10 minutes.

Lester, Lolli, and Greenberg (*J. Pharmacol.*, 1946, **87**, 329) recorded that aspirin is

rapidly hydrolysed on incubation at 37° with plasma. They gave no values for the reaction rate under these conditions but attempted to determine the aspirin concentrations in the plasma after oral administration by subtracting the free salicylate from the total salicylate (by hydrolysis of plasma). They found that 30 minutes after ingestion of 0·65 g. of aspirin 27% of the total blood salicylate was acetylsalicylate; at 120 minutes none could be detected. 70 Minutes after the ingestion of 2·60 g., 13% of the total was present as acetylsalicylate; at 160 minutes none could be detected. When allowance is made for the time taken for the aspirin to pass into the blood-stream and the possibility that their differential method of analysis might have overestimated the aspirin content of the blood, their findings are not inconsistent with the conclusions made from the present study.

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