REFERENCES

1 A. B. Roy, *Biochem. J.*, 77 (1960) 380.
2 E. Mehl and H. Jatzkewitz, *Biochim. Biophys. Acta*, 151 (1968) 619.
3 A. B. Roy, *Biochem. J.*, 55 (1953) 653.
4 H. Baum, K. S. Dodgson and B. Spencer, *Biochem. J.*, 69 (1958) 567.
5 K. S. Dodgson, B. Spencer and C. H. Wynn, *Biochem. J.*, 62 (1956) 500.
6 A. A. Farooqui and B. K. Bachhawat, *J. Neurochem.*, 18 (1971) 635.
7 A. B. Roy, *Biochim. Biophys. Acta*, 227 (1971) 129.
8 H. Baum and K. S. Dodgson, *Biochem. J.*, 69 (1958) 573.
9 R. G. Nicholls and A. B. Roy, *Biochim. Biophys. Acta*, 242 (1971) 141.
10 W. Bleszynski, *Enzymology*, 32 (1967) 169.
11 H. Baum, K. S. Dodgson and B. Spencer, *Clin. Chem. Acta*, 4 (1959) 453.
12 O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. Biol. Chem.*, 193 (1951) 265.
13 A. B. Roy, *Experientia*, 13 (1957) 32.
14 M. Dixon and E. C. Webb, *Enzymes*, Longmans, Green and Co., London, 1959, p. 179.
15 A. B. Roy, *Biochem. J.*, 59 (1955) 8.

APPENDIX (Received March 20th, 1972)

KINETIC PROPERTIES OF ARYLSULPHATASE A—THEORETICAL TREATMENT

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In the foregoing paper it was shown empirically that the relationship between the amount of nitrocatechol produced *(u)* by the action of sulphatase A on nitrocatechol sulphate in time *(t)* had the following form:

$$\frac{1}{u} = A + B\frac{1}{t} \tag{1}$$

Eqn 1 accounts for the inactivation of sulphatase A which occurs during its reaction with its substrate. A relationship of this form can be rather simply derived if the enzymic reaction is assumed to be

$$E + S \rightleftharpoons ES \xrightarrow{k_3} E + \text{products} \tag{2}$$

$$ES \xrightarrow{\alpha k_3} \text{inactive enzyme} \tag{3}$$

In this scheme α is small: k_3 is approximately 30 s^{-1} (Roy, unpublished observations) whereas the half-time for the inactivation of sulphatase A is about 5 min, corresponding to a velocity constant of about 0.003 s^{-1} so that α must be of the order of 10^{-4}. When the substrate concentration (*s*) is much greater than K_m, then

$$v_0 = k_3 e' = k_3 e_0 \tag{3a}$$

where v_0 is the initial velocity of the enzymic reaction and e_0 and e' the initial total enzyme concentration and the concentration of the enzyme–substrate complex *(ES)*, respectively.

From Eqn 3a it follows that

$$\frac{de'}{dt} = -ak_3e'$$

$$\therefore e'_t = e'_0e^{-akt}$$

$$= e_0e^{-ak_3t} \text{ (when } s \gg K_m)$$

From Eqn 1

$$\frac{du}{dt} = k_3e_t'$$

$$= k_3e_0e^{-ak_3t}$$

$$\therefore u = \frac{e_0}{a} - \frac{e_0}{a} \cdot e^{-ak_3t} \tag{4}$$

$$\frac{1}{u} = \frac{a}{e_0 - e_0e^{-ak_3t}}$$

$$= \frac{a}{e_0} + \frac{ae^{-ak_3t}}{e_0(1 - e^{-ak_3t})}$$

$$= \frac{a}{e_0} + \frac{a}{e_0}\left\{\frac{e^{-ak_3t}}{1 - e^{-ak_3t}}\right\} \tag{5}$$

Consider now the term in brackets in Eqn 5. This may be simplified by expanding the exponentials as a power series in the usual way: by neglecting terms of power 3 and greater it may be written

$$\frac{1 - ak_3t + \frac{a^2k_3^2t^2}{2}}{ak_3t - \frac{a^2k_3^2t^2}{2}}$$

which may be further simplified by the method partial fractions and by rearranging to give

$$\frac{1}{ak_3t}\left\{\frac{2}{2 - ak_3t} - ak_3t\right\}$$

but when a is very small the term in brackets will tend to unity so that under these conditions the above expression simplifies to $1/ak_3t$. Substituting this in Eqn. 5 gives

$$\frac{1}{u} = \frac{a}{e_0} + \frac{a}{e_0} \cdot \frac{1}{ak_3t}$$

but from Eqn 3a, $k_3e_0 = v_0$ and from Eqn 4, when $t \to \infty$, $u \to u_{max} = e_0/a$ so that

$$\frac{1}{u} = \frac{1}{u_{max}} + \frac{1}{v_0} \cdot \frac{1}{t}$$

This is of the form of Eqn 1 where $A = 1/u_{max}$ and $B = 1/v_0$, as was deduced in the accompanying paper.

A theoretical explanation of Eqn 1 is therefore available: the relationship is

only approximate but will be valid when $s \gg K_m$ and when α is small. The first condition is easily satisfied experimentally and the second seems to be true under the conditions so far examined (see the accompanying paper and ref. 8 therein). It must be stressed that this derivation does not allow any conclusions to be drawn as to the precise nature of Reaction 3, the inactivation of sulphatase A in the presence of its substrate. The only assumption is that it is the enzyme–substrate complex, not the free enzyme, which undergoes inactivation, an assumption justified by the experimental findings. Further, Eqn 1 will be valid if Reaction 3 is reversible when αk_3 will be an apparent velocity constant for the attainment of the equilibrium between ES and the inactive species.

I am deeply indebted to Dr Stinshoff for allowing me to see his paper prior to its publication and to publish this possible interpretation of his findings.