between the increased response and the time involved was observed.

The induction rate, i.e. the induction occurring per unit time, can either be logarithmic or linear, depending on whether the reaction product (R) is used as building material for the enzyme (E) or is taken out of the equilibrium, as shown in Figure 1. Since the end product, ammonia, is excreted in the urine and thereby taken out of the equilibrium, the observed linear response is consistent with MANDELSTAM's hypothesis. It may be concluded that the hypothesis of MANDELSTAM cannot only be applied to enzyme induction in microorganisms, but also to enzyme adaptation in mammals.

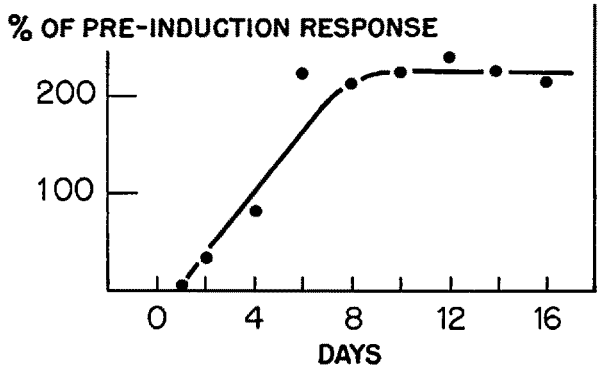

Fig. 3.—Increase in ammonia response during induction.

For the time being it is convenient to interpret adaptation from a teleological viewpoint, i.e. in the case of increased urinary ammonia excretion in chronic acidosis to consider this as a mechanism protecting the animal from an excessive loss of base. This phenomenon explains the loss of diuretic effect of ammonium chloride when this drug is administered for several days. Instead of sodium and potassium, ammonium is excreted, while the other cations are retained. This same phenomenon is probably important in renal diseases with acidosis. Further investigation of induction, experimental and spontaneously occurring in diseases, is warranted, since the prognosis of many metabolic diseases is possibly dependent on the degree of adaptation.

R. RICHTERICH-VAN BAERLE[14] and L. GOLDSTEIN

Department of Pharmacology, Boston University School of Medicine, Boston, Massachusetts, October 4, 1956.

Zusammenfassung

Chronische Verabreichung von Säure führt zu einer erhöhten Glutaminase-I-Konzentration in der Niere und zu einer gesteigerten Ammoniakausscheidung im Urin. Das ammoniakproduzierende biokatalytische System (Glutaminase I) ist besonders gut zum Studium der Enzymadaptation (Induktion) bei Säugetieren geeignet. Der Stand der Adaptation kann nicht nur durch die Bestimmung der Enzymveränderungen in der Niere, sondern auch durch den Nachweis des Reaktionsproduktes (Ammoniak) im Urin am lebenden Tier beurteilt werden.

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The Kinetics of Sulphatase A

The anomalous kinetics of the hydrolysis of dipotassium 2-hydroxy-5-nitrophenyl sulphate (nitrocatechol sulphate) by ox liver aryl sulphatase A (sulphatase A) were first noted in this laboratory[1] and similar anomalies have since been detected in the corresponding enzymes of rat[2] and human[3] liver. The reaction velocity is not directly proportional to the enzyme concentration but with incubation times of 1 h it is linearly related to the enzyme concentration raised to the power of 3/2[4]. It was suggested[4] that this effect might be due to a polymerisation of the enzyme. DODGSON and SPENCER[5] showed the fundamental anomaly to be that the reaction was not of zero order at low concentrations of enzyme and suggested an explanation for this effect in a series of competing reactions involving the enzyme, substrate and reaction products. Simultaneously, ROY[6] showed that nitrocatechol sulphate used in these studies was impure, containing up to 10% of nitropyrogallol disulphate[7] which could be removed by paper electrophoresis. It was also shown[6] that preparations of nitrocatechol sulphate obtained through its methylene blue salt did not exhibit these anomalous kinetics, the reaction being of zero order and the velocity directly proportional to the enzyme concentration. This seemed excellent evidence for the suggestion[6] that the anomalies might be due to the use of impure substrate preparations but more recent work has cast some doubt on this interpretation as specimens of nitrocatechol sulphate free from nitropyrogallol sulphate still give anomalous kinetics[8]. In view of the increasing use of nitrocatechol sulphate as a substrate in sulphatase assays it seems that a more detailed report of the observations might be of value.

In the present studies the nitrocatechol sulphate was free from nitropyrogallol disulphate and the experimental procedures were those already described in detail[9]. The enzyme was ox liver sulphatase A–3[4]. Assays were carried out at 37° in 0·15 M acetate buffer, pH 5·0, and at a substrate concentration of 0·003 M nitrocatechol sulphate.

Typical progress curves for the hydrolysis of nitrocatechol sulphate by sulphatase A are shown in Figure 1 and in Figure 2 is shown the relationship between enzyme concentration and reaction velocity. The progress curves consist typically of three parts: stage 1, in which the initial velocity decreases rapidly; stage 2, during which the velocity may fall to almost zero; and stage 3 when the velocity rises again to an almost constant value which is considerably less than the initial velocity. The relative proportions of these three stages vary considerably with changes in enzyme concentration and with those used in the original work[1] stage 2 disappears so that the reaction approximates to one of zero order. Preincubation of the enzyme in the absence of substrate did not significantly alter the shapes of these progress curves. Increasing the substrate concentration to 0·01 M caused an increased velocity during stage 1 and a pro-

[1] A. B. ROY, Biochem. J. *53*, 12 (1953).
[2] R. GIANETTO and R. VIALA, Science *121*, 801 (1955).
[3] K. S. DODGSON, B. SPENCER, and C. H. WYNN, Biochem. J. *62*, 500 (1956).
[4] A. B. ROY, Biochem. J. *55*, 653 (1953).
[5] K. S. DODGSON and B. SPENCER, Biochem. J. *62*, 30P (1956).
[6] A. B. ROY, Biochem. J. *62*, 35P (1956).
[7] A. B. ROY and L. M. H. KERR, Nature *178*, 376 (1956).
[8] A. B. ROY and L. M. H. KERR, Nature *178*, 376 (1956). – K. S. DODGSON and B. SPENCER, Biochim. biophys. Acta *21*, 175 (1956).
[9] A. B. ROY, Biochem. J. *53*, 12 (1953); *55*, 653 (1953).

longation of stage 2 but the velocity in stage 3 was apparently unaltered. Lowering the pH to 4·5 made no significant difference to the progress curves but at pH

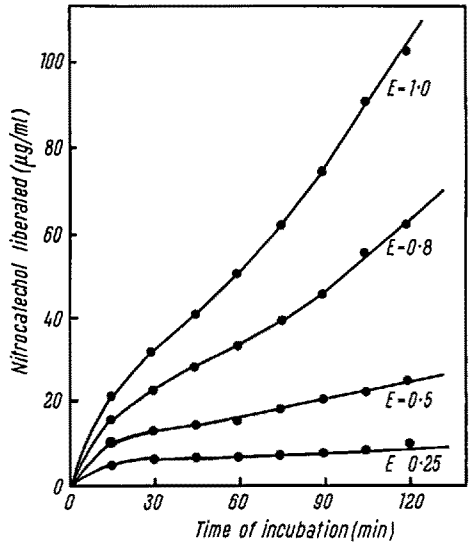

Fig. 1. — Progress curves for the hydrolysis of nitrocatechol sulphate (0·003 *M*) at pH 5·0 by sulphatase A. The relative enzyme concentrations are indicated on the appropriate curves.

6·0 the length of stage 2 was greatly prolonged, becoming important even at high concentrations of enzyme. The most interesting effect was that of SO_4^{2-}-ions: in the presence of 0·001 *M* SO_4^{2-}-ions stage 2 virtually disappeared, even at low enzyme concentrations, as shown in Figure 3. This effect was still obvious at pH 6·0 and is therefore presumably responsible for the pronounced activation of sulphatase A by SO_4^{2-}-ions at that pH[10].

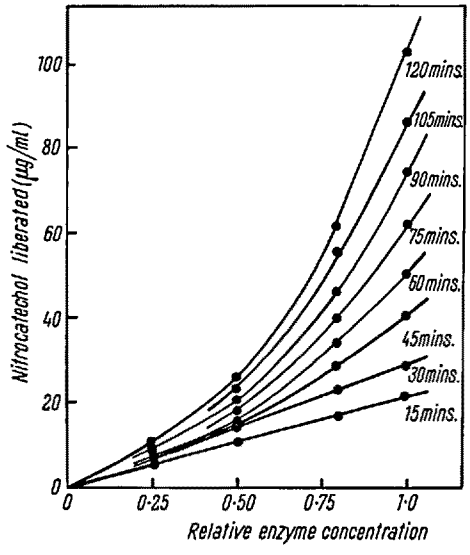

Fig. 2.—Effect of varying times of incubation on the relation between the concentration of sulphatase A and the velocity of the hydrolysis of nitrocatechol sulphate.

Preincubation of the enzyme with SO_4^{2-}-ions in the absence of substrate did not alter the progress curves from those in Figure 3. The addition of nitrocatechol (0·1 *mM*) to the reaction mixture caused an overall increase in the reaction velocity. When both nitrocatechol and SO_4^{2-}-ions were added in equivalent amounts their effects were additive. For comparison with these results Figure 4 shows curves obtained using substrate preparations purified via the chloroform-soluble methylene blue salt: the completely normal kinetics of this hydrolysis are obvious.

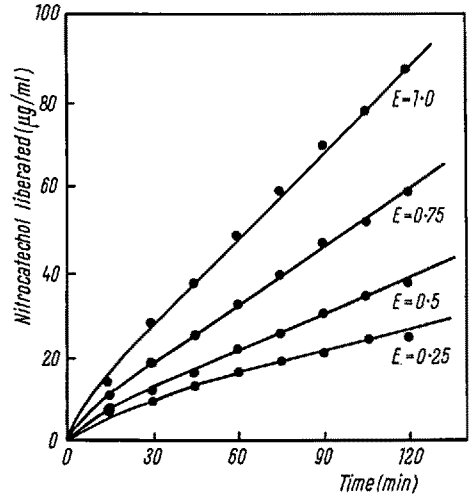

Fig. 3. — Effect of 0·001 *M* K_2SO_2 on the progress curves of the hydrolysis of nitrocatechol sulphate by sulphatase A.

One point of considerable importance was whether these anomalous kinetics were shown for the hydrolysis of substrates other than nitrocatechol sulphate. The high specificity[4] of sulphatase A made detailed study of this point difficult as to obtain results of immediate significance it was essential to use the enzyme under the same conditions for the hydrolysis of all the substrates. Figure 5 illustrates the hydrolysis of 0·05 *M* *p*-nitrophenyl sul-

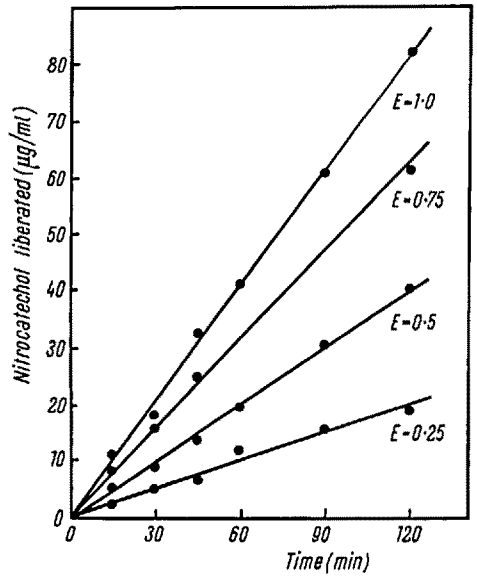

Fig. 4. — Progress curves for the hydrolysis by sulphatase A of nitrocatechol sulphate obtained via the methylene blue salt.

phate by sulphatase A. It is obvious that the reaction does not show the anomalies characteristic of the hydrolysis of nitrocatechol sulphate, the progress curves being simply those to be expected if the enzyme were unstable under the experimental conditions. It can also be seen that the reaction velocity is not directly related to the enzyme concentration. The addition of 0·001 *M* SO_4^{2-}-ions

[10] A. B. Roy, Biochem. J. *59*, 8 (1955).

did not alter the progress curves although it caused considerable inhibition. These results are not in agreement with those obtained for human sulphatase A by DODGSON and SPENCER[11] who state that the hydrolysis of *p*-nitrophenyl sulphate exhibits the same anomalies as that of nitrocatechol sulphate.

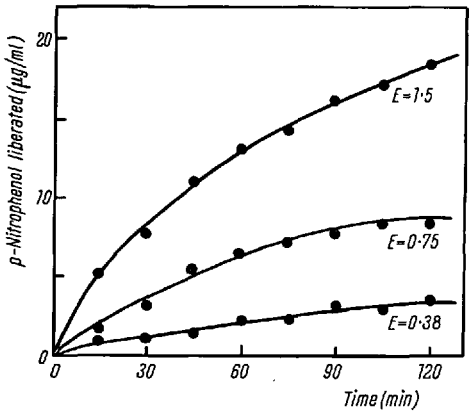

Fig. 5. – Progress curves for the hydrolysis of *p*-nitrophenyl sulphate (0·05 *M*) at pH 5·0 by sulphatase A.

These results are extremely difficult to interpret and any explanation must account for the following facts:

(1) During the hydrolysis of nitrocatechol sulphate the reaction velocity rapidly falls and then rises again. Under identical conditions with *p*-nitrophenyl sulphate the velocity falls but does not subsequently rise.

(2) The rise in velocity of the hydrolysis of nitrocatechol sulphate is greatly enhanced by 0·001 *M* SO_4^{2-} ions although higher concentrations are inhibitory. The hydrolysis of *p*-nitrophenyl sulphate is not activated.

(3) Nitrocatechol increases the rate of hydrolysis of its sulphate.

(4) Preincubation of the enzyme in the absence of substrate, with or without nitrocatechol or SO_4^{2-}ions, makes no difference to the progress curves.

(5) Purification of the nitrocatecholsulphate through its methylene blue salt gives preparations which do not exhibit anomalous kinetics.

From these data it is impossible to decide whether or not the anomalies are due to some peculiarity of the nitrocatechol sulphate or to some inherent property of the enzyme. The differences in behaviours between nitrocatechol sulphate and nitrophenyl sulphate suggests the former, but on the other hand this difference might simply be due to the much lower affinity and rate of hydrolysis of nitrophenyl sulphate[4]. As preincubation of the enzyme in the absence of substrate does not affect the progress curves it seems that the fall in velocity in stage 1 of the hydrolysis is due not to a simple inactivation of the enzyme, but to some complex reaction involving the substrate. Further, as the reaction velocity increases again in stage 3 it must be presumed that the inactivation in stage 1 is reversible. This increase in velocity in stage 3 cannot be directly due to the action of nitrocatechol and SO_4^{2-}ions liberated by the hydrolysis as if this were so then a similar effect would be expected on adding SO_4^{2-}ions to the system hydrolysing nitrophenyl sulphate.

DODGSON and SPENCER[5] proposed an explanation based on the substrate inhibition occurring with nitrocatechol sulphate. They suggested that this substrate inhibition differed from the normal type in that it was a slow reaction, so accounting for the drop in velocity during stage 1. They then suggested that the later rise in velocity was due to a reversal of the substrate inhibition by the reaction products. Such an explanation seems improbable as it is difficult to see how the reaction products could reverse the substrate inhibition.

The following explanation seems a possible alternative. If it is supposed that the enzyme-substrate complex (ES) can react in two ways, one reforming the active enzyme (E) and the other giving an inactive enzyme (E′), then the following equilibria must be considered during the reaction:

$$E + S \rightleftharpoons ES \begin{cases} \rightarrow E + \text{Products} \\ \rightarrow E' + \text{Products.} \end{cases}$$

The rate at which the inactivation of the enzyme will occur during the hydrolysis of nitrocatechol sulphate will therefore be governed by the kinetics of the various reactions. Further, as the reaction products accumulate, or are added, it is possible that the active form of the enzyme, E, could be reformed from the inactive one, E′, if the appropriate equilibrium constants were of the correct magnitude. In the case of the hydrolysis of nitrophenyl sulphate, on the other hand, the equilibrium

$$ES \rightleftharpoons E' + \text{Products}$$

may lie so far to the right that the addition of the reaction products might make relatively little difference to the rate of formation of E′. Not all the experimental findings can be explained on this basis. It might be expected from the above that the addition of SO_4^{2-}ions at the beginning of the reaction would prevent the initial rapid formation of E′ but as seen from Figure 3 this does not occur. Again the results using the substrate obtained via the methylene blue salt cannot be explained on the above basis.

These points suffice to show the extremely complex nature of the kinetics of sulphatase A and although the hypothesis proposed may be incorrect it provides a basis for further investigation. They also show the dangers inherent in the use of nitrocatechol sulphate in the assay of sulphatases of the type of sulphatase A: other sulphatases apparently do not show these anomalies[12] so that the use of this substrate in their assay is straighforward.

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Résumé

Les cinétiques de l'hydrolyse du sulfate de nitrocatéchol par la sulfatase A sont très anormaux parce qu'il n'y a aucune relation linéaire entre la vitesse de la réaction et la concentration de l'enzyme. La réaction tient trois phases: dans la première, la vitesse décroît rapidement, dans la deuxième, elle diminue de plus en plus tandis qu'elle s'accroît dans la troisième. La réaction est très sensible à la composition du mélange réactionel, et l'addition des produits de la réaction, particulièrement le sulfate inorganique, augmente la vitesse de l'hydrolyse. En utilisant comme substrat le *p*-nitrophénylsulfate, ces irrégularités ne sont pas constatées.

Une interprétation de ces résultats est présentée!

[11] K. S. DODGSON and B. SPENCER, Biochim. biophys. Acta *21*, 175 (1956).

[12] K. S. DODGSON, B. SPENCER, and K. WILLIAMS, Biochem. J. *61*, 374 (1955). – D. ROBINSON, J. N. SMITH, B. SPENCER, and R. T. WILLIAMS, Biochem. J. *51*, 202 (1952).