bited this inactivation. The concentrations of pantooyltaurine required for inhibition of the pantothenate metabolism by different organisms varied over a 300-fold range but were correlated with the concentrations required for inhibition of growth of the respective organisms.

2. Of 14 compounds structurally related to pantothenate, seven, which inhibited growth of haemolytic streptococci, inhibited their inactivation of pantothenate. The activities of the compounds in inhibiting the inactivation of pantothenate were correlated with their activities in inhibiting growth. Five compounds affected neither process; two com-

pounds which inhibited growth and which were not antagonized by pantothenate did not affect pantothenate inactivation.

3. Of the compounds examined, those which inhibited pantothenate inactivation were all amides of pantoic acid; those not containing an intact and unsubstituted pantoic acid portion did not affect pantothenate inactivation.

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The Activation of Jack-bean Arginase by Cobalt, Manganese, and Iron

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The hydrolysis of arginine by preparations of jack-bean 'urease' in the presence of cobaltous ions was reported by Hellerman & Perkins (1935). Stock, Perkins & Hellerman (1938) showed that an arginase present in jack-bean extracts was strongly activated by cobalt, by manganese, and to a less extent by nickel. Cobalt produced a shift in the optimal pH from 9.0 to 7.7, and at the latter reaction the metal was necessary for activity. The present paper deals with the activation of this enzyme by cobalt, manganese, and iron, and with a preliminary investigation into the nature of the substance activated.

METHODS

Enzyme preparation. Jack-bean urease powder (Peters & Van Slyke, 1932) was used unless otherwise stated. The powder formed a stable, reproducible source of arginase and urease combined. A weighed amount of the powder was dissolved in distilled water afresh for each experiment.

Buffers. Clark & Lub's phosphate buffers and Sorensen's glycine buffers were used, allowances being made for the temperature coefficient of the latter (Clark, 1928). Clark & Lub's borate buffer could not be used, as borate inactivates urease. In a few experiments the methylglyoxaline buffers

of Kirby & Neuberger (1938) were used, but their high temperature coefficient made them less suitable.

Hydrolysis. The reaction was carried out in the apparatus for the determination of urea described by Kay & Sheehan (1934). In the reaction tube were placed: 1 ml. urease solution, 5 ml. buffer, 1 ml. activating metal solution; the racks of tubes were placed in a bath at 40°; after 10 min. 1 ml. of a solution of *D*-arginine hydrochloride (2.4 g./100 ml.) was added, and the tubes containing 8 ml. total volume were stoppered and connected to the receiving tubes. The reaction mixture was stirred at intervals by drawing a gentle current of air through the apparatus. At the end of 1 or 2 hr. the tubes were cooled rapidly, and the reaction was stopped by adding 20 ml. of saturated potassium carbonate solution to each tube. The ammonia formed was then aerated and determined by titration in the usual manner.

RESULTS

The activation by cobaltous ions. Preliminary experiments confirmed the finding of Stock *et al.* (1938) that the hydrolysis of arginine by jack-bean urease in the presence of cobaltous ions is a pseudomonomolecular reaction. Two factors in the reaction were determined: first, the relationship between the concentration of cobalt ion and the concentration of

enzyme preparation, and secondly, the optimum hydrogen-ion concentration for the hydrolysis with varying concentrations of cobalt.

Fig. 1 shows that at pH 7.5 hydrolysis increased rapidly to a maximum, then fell off practically to zero as the concentration of cobalt was increased; also that there was a direct proportionality between the amount of enzyme preparation used and the amount of cobalt required to produce full activation, the total volume remaining 8 ml. These curves were

lysis at pH 7.5 was obtained with 0.16mm-cobalt chloride, at pH 8.0 with 0.42mm, and at pH 9.0 with 3mm.

Activation by manganese. The optimum pH with varying metal concentrations, and the effect of incubating the enzyme and manganese for varying times before addition of the substrate were investigated. The curves obtained with increasing concentrations of manganese were of a more usual form, rising to a maximum and falling only slowly with

Fig. 1. Activation by cobalt at pH 7.5. Time 60 min.

Fig. 2. Effect of hydrogen-ion concentration on cobalt activation, 10 mg. urease. Time 60 min.

Fig. 3. Larger amounts of cobalt at pH 9.0. Time 60 min.

obtained with phosphate buffers, but a curve similar in shape was obtained with 25 mg. urease in 4 (or 5)-methylglyoxaline buffer giving a pH of 7.6 at 40°. In control experiments with urea in place of arginine, amounts of cobalt salt up to 1mm had no demonstrable effect at pH 7.5 on the activity of the urease in 10 mg. of the powder. Schmidt (1928) found that larger amounts of cobalt inactivated urease. When the hydrolysis was carried out at other hydrogen-ion concentrations, it was found that the maxima were obtained with varying amounts of cobalt, as will be seen from Figs. 2 and 3, where the maximum hydro-

lysis at pH 7.5 was obtained with 0.16mm-cobalt chloride, at pH 8.0 with 0.42mm, and at pH 9.0 with 3mm.

Further increase in concentration of manganese. The optimum pH was in the neighbourhood of 8.8, and the maximum hydrolysis was obtained with the same concentration of manganese at all reactions from pH 8.2 to 8.8 (Fig. 4). If the hydrolysis was continued for 2 hr. larger quantities of manganese produced some inhibition in a manner similar to that found with cobalt.

That the activation is not instantaneous and increases with time was shown by incubating the enzyme with manganese for varying periods up to 2 hr. before adding the substrate. A steady increase

in activation with increase in time was found under these conditions (Fig. 5).

Activation by ferrous ions. In order to obtain any activation by ferrous sulphate, relatively large quantities (3mm) had to be employed at pH 8.8 (Fig. 6), and it will also be seen that ferrous ions were inhibitory at pH 8.2. These observations, and also the reported activation of liver arginase by cysteine and ferrous salt (Purr & Weil, 1934), suggested the

The nature of the activated substance

Hellerman & Perkins (1935) found that a crude preparation of crystalline jack-bean urease could be activated to attack arginine, but later Stock *et al.* (1938) reported that the recrystallized urease is inactive. During the present investigation it was noticed that preparations of soya-bean urease were inactive toward arginine.

Fig. 4. Effect of hydrogen-ion concentration on activation by manganese. Time 60 min.

Fig. 5. Incubation of manganese and enzyme for varying times before the addition of substrate. Hydrolysis for 60 min.

Fig. 6. Activation by ferrous iron. Time 120 min.
○—○ pH 8.8; ●—● pH 8.2.

Fig. 7. Activation by ferrous iron + cysteine. Time 120 min.

addition of cysteine to the reaction mixture. The addition of freshly neutralized solutions of cysteine hydrochloride to the buffered enzymes before the addition of ferrous salt activated strongly, and curves resembling in shape those seen with cobalt were obtained (Fig. 7). Control experiments without the addition of iron showed that cysteine alone in amounts greater than 2 mg. reduced the small activity of the preparation itself at pH 8.8, and that this inactivation was practically complete with 20 mg. cysteine.

An attempt was made to fractionate the crude jack-bean urease. The active substance was found to be associated with the globulin fraction and was not removed by dialysis, although a very slow irreversible inactivation took place in solution even at 0°. The jack-bean globulins were therefore investigated separately. With the methods given by Sumner (1919) and Sumner & Graham (1925), crystalline preparations of 'concanavalin A' and 'concanavalin B' were prepared, which were found to be quite inactive in the presence of manganese.

The activity was found in the residual amorphous 'canavalin'. Sumner & Howell (1936) described the preparation of a fourth crystalline globulin from the amorphous 'canavalin' by the action of trypsin. A preparation of this globulin gave crystals identical in shape with those described by Sumner, but this also was found to be inactive and was not activated by manganese. In these experiments a preparation of soya-bean urease was added to those protein solutions which were devoid of urease action.

Further attempts to isolate the substance responsible have so far been unsuccessful.

DISCUSSION

The variation in the optimum pH with changes in the concentration of cobaltous ions explains the finding of Stock *et al.* (1938) of a shift in the optimal pH from 9.0 for the enzyme alone to pH 7.7 in the presence of cobalt. In their experiments the same concentration of cobalt was used throughout. The explanation of the inhibition by larger concentrations of cobalt and manganese, or by ferrous iron at pH 8.2, is not obvious. Such an inhibition by ferrous iron has already been reported for rat-liver arginase by Weil (1935). The concept of arginase as a complex of a protein carrier and a metal ion has been suggested by Edlbacher & Zeller (1936) and Edlbacher & Baur (1938). This hypothesis is supported by the work of Hellerman and his collaborators (Hellerman & Perkins, 1935; Hellerman & Stock, 1938). The finding reported here, that the activation by manganese can be increased by a preliminary incubation of the preparation with manganese, would appear to support this hypothesis. The same effect has been reported recently by Hunter & Downs (1944) for the activation of liver arginase by cobalt.

The arginase of jack-bean is very feebly active and may be compared to liver arginase after subjection to prolonged dialysis until only slightly active; such a preparation can be activated by the addition of manganese, etc. One may postulate that in the jack-bean the protein carrier is present in large excess with very little of the active metal complex.

A determination of the reduction potentials of these systems might throw light on the mechanism involved, and it is hoped to undertake work on these lines in the future. The actual component of the jack-bean extracts which is activated is still undetermined and all that can be deduced from the present experiments is that it is associated with the globulin fraction, but that it does not appear to be any of the globulins so far isolated.

SUMMARY

1. In the activation of jack-bean arginase by cobaltous ions there was a direct proportion between the amount of enzyme and the concentration of cobalt required to produce full activation. As the cobalt concentration was increased the extent of hydrolysis increased to a maximum and then fell off. The optimum pH was found to vary with the concentration of cobalt used, increasing from 7.5 to 9.0 with rising cobalt concentration.

2. With manganous ions the optimum pH was in the neighbourhood of 8.8, and was independent of the concentration of manganese. Large quantities of manganese produced some inhibition. Incubation of the enzyme with manganese before the addition of substrate caused the activity to increase with the time of incubation.

3. Ferrous salt activated only in comparatively high concentration at pH 8.8, and at pH 8.2 was inhibitory. Cysteine markedly increased the activation by ferrous ions at pH 8.8, and produced an activation at pH 8.2. The activity-concentration curves obtained with cysteine and ferrous ions were similar in shape to those obtained with cobalt.

4. The substance activated is associated with the globulin protein of the jack-bean extract, but is not concanavalin A, concanavalin B, or the fourth crystalline globulin described by Sumner & Howell (1936).

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