LVI. THE RELATION BETWEEN THE ACTIVITY OF UREASE AND THE OXIDATION-REDUCTION POTENTIAL.

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AMONGST the substances which exercise accelerating or inhibitory actions on the activity of enzymes, particular interest has recently been attached to compounds possessing reducing or oxidising properties such as hydrogen sulphide, cysteine, glutathione, iron and other metallic complexes, and more recently also ascorbic acid [Karrer and Zehender, 1933; Edlbacher *et al.*, 1933, 1, 2]. These substances may react chemically with the enzyme-substrate system or they may influence the enzyme reaction by stabilising the oxidation-reduction potential of the medium. In the first case the activity will be related to the particular properties of the system added, whilst in the latter case every oxidation-reduction system will maintain the same potential and have the same influence. Mainly Waldschmidt-Leitz *et al.* [1929; 1930; 1933] and Grassmann *et al.* [1930; 1930-31] have indicated a relationship between the oxidation-reduction potential and the activity of the enzyme. Obviously a method of elucidating this problem would be to investigate the activity of the enzyme in the presence of different oxidation-reduction systems which maintain the potential (r_H) at known levels but which do not react chemically in any other way with the constituents of the mixture—in other words to impose conditions similar in character to those employed to maintain a desired hydrogen ion concentration in a reaction mixture.

There is however a difficulty in that a very large number of different oxidation-reduction systems are necessary in order to cover the entire range of the r_H scale. Each of the common oxidation-reduction systems is suitable only for a range of about 4 r_H units, whereas the full range between the hydrogen and oxygen electrodes is about 40 units. For this reason approximately ten systems are necessary in order to obtain a complete curve covering relation between activity and r_H . However, the task can be restricted considerably by making a series of studies spaced over the whole range.

As a first example the activity of urease was investigated. The reason for this was that Quastel [1933] has recently found a number of substances belonging to the class of polyphenols (quinol, catechol, pyrogallol, adrenaline, *etc.*) all of which exerted a strong inhibitory action on urease. The corresponding quinones were found to be more toxic than the phenols. The inhibition could be counteracted by hydrogen sulphide and other thiol compounds. Quastel was of opinion that only the quinone actually inhibited the enzyme, and that such inhibition as was exhibited by the phenol was actually due to the presence of traces of quinone.

Since however these substances all form fairly strongly positive reversible oxidation-reduction systems and since other oxidising agents such as hydrogen peroxide are actually inhibitors it seems reasonable to suspect a relationship between the activity of the enzyme and the oxidation-reduction potential of the system.

In the present investigation the hydrolysis of urea by urease was studied in the presence of different types of reversible oxidation-reduction systems. In particular, it was important to study that part of the oxidation-reduction scale which is poised by the system quinol-quinone, that is to say the positive section. In addition a study was made of conditions at the negative end of the oxidation-reduction scale. The experimental difficulty of carrying out investigations with the very oxygen-sensitive negative systems, for example rosindulin or neutral red, is very great. Therefore the action of the enzyme was studied in a stream of hydrogen in the presence of an active catalyst in the form of palladised asbestos. Also, in the studies of the readily autoxidisable leucomethylene blue a stream of hydrogen was maintained through the apparatus while the hydrolysis was in progress. Other experiments were carried out in which a stream of air was employed and in which the reaction mixture was kept in a sealed vessel. The degree of hydrolysis of the urea was measured either by estimating the ammonia by aeration in the usual manner or by direct titration as follows: to a sample of the test solution an excess of standard trichloroacetic acid was added; the mixture was made up to a certain volume and filtered, and an aliquot part of the filtrate was titrated with $N/10$ NaOH to p_H 4.4 methyl orange being used as indicator. At this point the phosphoric acid used as a buffer had its first neutralisation point and consequently a minimum of buffering power. Free trichloroacetic acid being a strong acid can be titrated with this indicator without difficulty whereas ammonium salts do not give the acid colour.

The hydrogen employed was prepared in a Kipp's apparatus and purified by washing with potassium permanganate, mercuric chloride and alkaline pyrogallol. Air was purified by washing with acid in order to remove traces of ammonia. In all experiments in which a stream of gas was employed the outlet tube of the apparatus led into a vessel containing standard acid which was titrated for the estimation of the ammonia liberated. The enzyme preparation employed was either freshly ground soya bean or a 10 % aqueous extract thereof. The urea was purified by crystallisation. The palladised asbestos was prepared by carefully treating a warm suspension of finely divided asbestos in a very dilute solution of palladium chloride with glucose and sodium hydroxide.

Influence of air and hydrogen.

It was necessary to investigate whether any alteration of activity of urease could be detected in experiments in which air or hydrogen was bubbled through the solution, since Edlbacher *et al.* [1932; 1933, 1, 2] has detected a difference in the case of arginase. Similar reaction vessels containing 10 ml. of 6 % urea solution; 5 ml. M phosphate buffer p_H 7.4; 10 ml. 10 % aqueous soya bean extract; and 25 ml. distilled water, were set up. One was aerated at room temperature with air, whilst through another a stream of hydrogen was passed for the same time. In both cases the gas streams were regulated so that approximately one bubble per second passed through the solution, this being done in order to prevent frothing which might induce inactivation of the enzyme.

The following table gives the values of ammonia formed by fermentation in various experiments with different enzyme preparations (in ml. $N/10$ NH_4OH).

Exp. no.	Time min.	Closed vessel	Air stream	Hydrogen stream
1	85	4.65, 4.80	4.50, 4.65	—
2	205	9.35, 9.50	9.15, 9.05	—
3	95	4.30	—	4.40
4	220	9.15	—	9.05
5	95	—	9.80	10.00
6	40	—	5.20	5.40

This shows no significant difference between the tests.

Influence of active hydrogen.

In this experiment the reaction vessel contained 0.05 g. of palladised asbestos suspended with the enzyme preparation in the buffered medium. Hydrogen was passed through the mixture for 15 minutes after which the urea was added. In this manner it was ensured that the potential of the reaction mixture would be adjusted to the negative level of the hydrogen electrode at the p_H employed. At a temperature of 18° this is -427 mv. (referred to normal hydrogen electrode). The ammonia production was compared with that of two controls in closed vessels, one of which contained the same amount of catalyst.

The results of several experiments are:

Exp. no.	Time (min.)	Catalyst	Catalyst	Closed vessel
		In hydrogen stream	Closed vessel	
		Titrations in ml. $N/10$ NH_4OH		
7	85	—	7.45	7.75
8	205	—	13.70	15.30
9	85	—	7.05	7.45
10	205	—	13.95	14.70
1	85	4.45, 4.40	—	4.65, 4.80
2	205	8.85, 8.60	—	9.35, 9.50

This indicates a slight inhibition of the enzyme in the presence of the catalyst employed.

Influence of methylene blue.

Quastel [1932] has shown that methylene blue does not affect unpurified preparations of urease but that it exerts a strongly inhibiting action on the purified enzyme. Reaction vessels were set up, each containing 2 ml. 6 % urea solution, 30 ml. $M/5$ phosphate buffer p_H 7.4, 10 ml. 10 % soya bean meal extract. To one were added 10 ml. of 0.15 % methylene blue in $M/50$ phosphate buffer, to another the same solution reduced by hydrogen in the presence of palladised asbestos. The former experiment was aerated, whilst in the latter case a stream of hydrogen was employed. To a control 10 ml. of the buffer only were added. Hydrolysis was allowed to occur at room temperature.

Results of the ammonia estimations with different enzyme preparations in various times are given in the following table:

Exp. no.	Time min.	Methylene blue oxidised	Methylene blue reduced	Control
		Titrations in ml. $N/10$ NH_4OH		
11	90	17.2	16.9	—
12	80	4.55, 4.60	—	4.70, 4.60
13	120	7.30	—	7.30
14	60	13.70	—	13.10

If it be assumed that approximately 10 % of the methylene blue in the reduced preparation was present in the oxidised form the potential of this preparation can be regarded as -28 mv. (referred to the normal hydrogen electrode); that of the oxidised preparation (99 % oxidised) may be taken as

+58 mv. (The normal potential of the system methylene blue-leucomethylene blue at p_H 7.4 is zero.) It may be concluded, therefore, that the activity of the urease preparation employed is unaffected by potentials within the range -28 to +58 mv.

Ferro-ferricyanide system.

This system possesses a normal potential of approximately +450 mv. which is unaffected by hydrogen ion concentration. In the first place both extremes of the potential range were investigated. The potentials are not very sensitive to oxygen so that they can be measured electrometrically in the presence of air. As the substances are quite stable the hydrolyses were carried out in closed vessels kept in an incubator at 37°. Each vessel contained in a total vol. of 50 ml. 10 ml. 6 % urea solution; 5 ml. *M* phosphate buffer p_H 7.4; 5 ml. 10 % soya bean meal extract and 1 millimol of either ferro- or ferricyanide. Controls without addition were set up.

The respective ammonia productions in various experiments were:

Exp. no.	Time min.	Ferro-cyanide	Ferri-cyanide	Controls without addition
Titrations in ml. <i>N</i> /10 NH_4OH				
7	85	7.70	7.15	7.75
8	205	15.20	13.90	15.30
9	85	7.45	6.80	7.45
10	205	14.75	13.65	14.70

The oxidation-reduction potentials were measured with gilded platinum electrodes against a standard calomel electrode. Owing to inadequate poisoning the readings were not quite steady; the values for ferrocyanide ranging between -22 and +10 mv., those for ferricyanide between +264 and +336 mv. Referred to the normal hydrogen electrode these values become +228 to +260 mv. (ferrocyanide) and +514 to +586 mv. (ferricyanide). The rate of hydrolysis of urea was unaffected by these substances, apart from a very slight inhibition exerted by the ferricyanide.

Influence of quinol.

The poisoning of urease by quinol which was observed by Quastel, and which can be demonstrated both with the crude soya bean meal and with extracts, can, as he also showed, be prevented by the addition of substances containing the SH group. The inhibition thus removed could be prevented likewise by treatment with active hydrogen. Three experiments were set up. In each the reaction vessel contained in a total volume of 50 ml. 10 ml. 6 % urea solution; 5 ml. *M* phosphate buffer solution p_H 7.4; 10 ml. of a 10 % aqueous soya bean meal extract or the corresponding quantity of soya bean meal. To the contents of two vessels 0.1 g. quinol was added, the third serving as a control. One of the vessels containing the quinol was exposed to a stream of hydrogen in contact with 0.05 g. palladised asbestos for 15 minutes before the enzyme preparation was added, and for a further 15 minutes afterwards. The urea solution was then added and the stream of hydrogen maintained during the hydrolysis.

Results of ammonia estimations in various experiments are shown in the following table:

ing table:				
Exp. no.	Time min.	No. 1 quinol Pd-catalyst in hydrogen stream	No. 2 quinol (closed vessel)	No. 3 control
		Titrations in ml. <i>N</i> /10 NH ₄ OH		
15	125	4.30	0	4.55
16	210	8.80	0	9.15
17	120	6.50	0.90	7.05
				}soya bean extract meal

The oxidation-reduction potential in No. 2 was between -53 and -34 mv. measured against the standard calomel electrode (*i.e.* $+197$ to $+216$ mv., referred to the normal hydrogen electrode).

It is apparent therefore that there is almost complete inhibition of urease in the presence of quinol and that this is prevented by reduction with hydrogen in the presence of catalytic palladium.

DISCUSSION.

The activity of urease was found to be unaffected by the oxidation-reduction potential of the medium within the range investigated—namely, that covered by the systems palladium-hydrogen, methylene blue-leucomethylene blue, and ferri-ferrocyanide. Inhibition by quinol is apparently a specific action which is not related to the oxidation-reduction potential. It can probably be ascribed, as Quastel has suggested, to the action of traces of quinone rather than to the quinol itself, because no inhibition occurs when a stream of hydrogen is passed in the presence of palladium. It can be concluded that the reaction of the enzyme with quinone, if it is oxidative in nature, is not reversible in the thermodynamic sense; otherwise ferricyanide with a potential of more than $+500$ mv. would exert a stronger action than that of quinol with only $+200$ mv.

SUMMARY.

1. A stream of hydrogen or air did not affect the activity of urease. Moreover, activation of the hydrogen by palladium-asbestos had no appreciable influence on the rate of hydrolysis of urea.
2. The reversible oxidation-reduction systems, methylene blue and ferro-ferricyanide, did not influence the activity of the enzyme.
3. Quinol inhibits urease action, but on reduction with active hydrogen this effect disappears. It is believed that the original inhibition is due to a chemical reaction with the enzyme rather than to an influence on the oxidation-reduction potential.

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