CC. THE ACTION OF DYESTUFFS ON ENZYMES.

III. UREASE.

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THE action of dyestuffs on bacterial dehydrogenases and on an intracellular enzyme, fumarase, has been discussed in earlier communications [Quastel and Wheatley, 1931; Quastel, 1931]. It is now proposed to describe the effects of dyestuffs on another widely distributed intracellular enzyme, urease.

TECHNIQUE.

The method of investigation consisted in submitting a preparation of urease to the action of a dyestuff at p_H 7.4 and at 45° for a given time, usually 30 mins. or 1 hour, and in then determining the activity of the enzyme by estimating the amount of ammonia produced at 45° in a given time from a 0.3 % solution of urea. 1 cc. of the enzyme preparation was added to 2 cc. *M*/15 phosphate buffer solution p_H 7.4 and then the dyestuff and any other substance under investigation. The volume was made up to 9 cc. with distilled water and the mixture was incubated at 45° for the required interval of time (referred to as the initial incubation period). 1 cc. 3 % urea was then added and incubation was allowed to proceed at 45° for a further interval (referred to as the final incubation period). At the close of this interval the free ammonia was estimated on 1 cc. by a suitable aeration method and compared with that obtained from urease treated under similar conditions but in the absence of dyestuff. The amount of enzyme preparation taken was usually that required to decompose 1 cc. 3 % urea at 45° and at an initial p_H of 7.4 in one hour.

EFFECTS OF DYESTUFFS ON SOYA BEAN UREASE AND ON A PURIFIED UREASE PREPARATION.

A soya bean preparation of urease was made by shaking 10 g. soya bean meal with 90 cc. distilled water for 30 mins. in a shaking machine and centrifuging. The centrifugate was used as the enzyme preparation.

The purified urease preparation was made from a commercial sample of urease. The origin of the enzyme in this preparation was soya bean, purification having been effected by removal, to a considerable extent, of proteins by acid precipitation.

Representative results showing the effects of 23 dyestuffs, each at a concentration of 1/5000, on the activities of the two preparations of urease are shown in Table I. The percentage inhibitions of the activities of the enzymes are recorded.

Table I. *Percentage inhibitions of the activities of soya bean urease and a purified preparation of urease effected by dyestuffs (1/5000).*

Dyestuff	Nature of dyestuff	Soya bean preparation	Purified preparation
Congo red	Acid	0	0
Benzopurpurin	"	0	0
Orange G	"	0	0
Crystal scarlet	"	0	0
Acid fuchsin	"	0	0
Acid green	"	0	0
Soluble blue	"	0	0
Water blue	"	0	0
Brilliant green	Basic	90	3
Malachite green	"	95	86
Methyl violet	"	96	62
Ethyl violet	"	95	66
Crystal violet	"	93	50
Methylene blue	"	0	21
Toluidine blue	"	0	28
Neutral red	"	0	74
Janus green	"	5	53
Safranine	"	0	18
Methylene violet	"	0	10
Bismarck brown	"	0	0
Auramine O	"	0	0
Pyronin	"	0	0
Eosin	"	0	0

The following facts are of note.

1. The acid dyestuffs are entirely inert. This result may be contrasted with that found in the investigation of the action of dyestuffs on fumarase. Here Congo red, for instance, was toxic at a concentration of 1/100,000 [Quastel, 1931].

2. Most basic dyestuffs are toxic. The toxicity depends greatly on the nature of the preparation of the enzyme.

Basic dyes of the triphenylmethane series (brilliant green, malachite green, methyl violet, ethyl violet, crystal violet) show over 90 % inhibition of the activity of a soya bean preparation of urease. Yet this series proves to be less toxic with the purified preparation of the enzyme. Brilliant green, indeed, is almost inert with the purified enzyme at the concentration quoted. This finding with the triphenylmethane series may be contrasted with that obtained with neutral red or Janus green. These two dyes are highly toxic to the purified enzyme but they have no toxicity at the given concentration to the soya bean preparation. The same may be said of methylene blue or toluidine blue which have relatively feeble toxicities.

It was found from earlier work that the presence of proteins protected enzymes from dyestuffs (including the triphenylmethane series). The proteins combined with the dyes rendering them inert. This has been found to be the

case with neutral red, Janus green, *etc.*, no toxicity to urease being demonstrated using a soya bean preparation which contains much protein. It was very surprising therefore to find that the basic triphenylmethane series of dyes apparently lose their toxicity on purification of the enzyme preparation.

When greater concentrations of this series of dyes are used their toxicity towards the purified enzyme becomes evident. This is shown in Table II. It is clear that the triphenylmethane series of dyes possesses some property, not possessed by other basic dyes, which renders them more toxic to the soya bean preparation of urease than to the enzyme in the purified condition.

Table II. *Percentage inhibition of the activities of a soya bean preparation and a purified preparation of urease effected by varying concentrations of dyestuffs.*

Dyestuff	Concentration of dye		
	1/2000	1/3300	1/10,000
Purified urease preparation:			
Brilliant green	92	71	3
Methyl violet	92	88	35
Congo red	0	0	0
Neutral red	88	81	51
Acriflavine	72	69	3
Safranine	59	32	0
Methylene violet	54	19	0
Methylene blue	69	42	30
Bismarck brown	19	14	0
Orange G	0	0	0
Eosin	0	0	0
Soya bean preparation:			
Brilliant green	92	91	91
Methyl violet	94	94	93
Congo red	0	0	0
Neutral red	0	0	0
Acriflavine	46	37	6
Safranine	0	0	0
Methylene blue	0	0	0
Trypan blue	0	0	0

The relative inertness of these dyes to the purified enzyme is shown also to a preparation of urease obtained from soya bean by Zakowski's method [1931] in which purification is effected by precipitation of proteins *etc.* by CO_2 . If urease, purified in this way, is neutralised and examined in the usual manner, it is found that brilliant green at a concentration of 1/50,000 has lost its toxic activity. The addition to the purified preparation of the enzyme of a little of the material precipitated from soya bean by CO_2 , however, restores the toxicity of the brilliant green. The dye now effects a 90 % inhibition of activity. There is thus a co-toxic factor in the soya bean which is removed by acid or CO_2 precipitation. This phenomenon will be discussed in more detail later.

The inertness of all the acid dyestuffs investigated was to some extent unexpected. Urease has, according to Lövgren [1921] an optimum p_{H} between 7 and 8, which varies according to the substrate concentration. It

would have been supposed that the enzyme would have ampholytic properties and combine, as fumarase does, with both acidic and basic dyestuffs. The fact that the acidic dyestuffs investigated are inert would mean either (1) that an insufficient range of acid dyestuffs has been investigated, or (2) that it is incorrect to suppose that the enzyme centre, or the active portion constituting the enzyme, necessarily possesses both basic and acidic groups, even though the reaction is presumably a hydrating one and a symmetrical p_H -activity curve is evinced [see also Woolf, 1931]. It seems not unreasonable to suppose that the p_H -activity curve may be influenced by other factors, besides the enzyme proper, which are subject to change on variation of p_H . Such factors may, for example, be the constitution of the substrate molecule, or the colloidal carrier to which the active centre constituting the enzyme is attached.

INFLUENCE OF UNSATURATED GLYCERIDES ON THE TOXICITY OF
BASIC TRIPHENYLMETHANE DYES.

As stated earlier the addition of a substance, which is precipitated from a soya bean preparation by CO_2 , to the purified enzyme restores the susceptibility of the enzyme to the toxic action of brilliant green.

A preparation of urease was made from jack bean meal¹. 10 g. of this meal were shaken with 90 cc. distilled water for 30 mins. and centrifuged, and the centrifugate was used as the source of the urease preparation. This proved to be fifteen times more active than a soya bean preparation made in the same way. It was diluted with water twenty times, preparatory to use. This preparation of urease was found to resemble purified urease, obtained from soya bean, in so far as susceptibility to dyestuffs was concerned. Brilliant green and crystal violet had but little toxic action on the enzyme at low concentrations; the addition to it, however, of a little of the soya bean preparation brought about a great enhancement of the toxicities of these dyestuffs. The same phenomenon occurred with a preparation of crystalline urease, obtained from jack bean meal by Sumner's method [1926]. Here the addition of 0.25 cc. of a soya bean preparation of urease to a solution of crystalline urease caused brilliant green at a concentration of 1/50,000 to remove 90 % of its activity. This concentration of brilliant green had no effect on the activity of the solution of crystalline urease itself.

Extracts were made from a variety of other vegetable sources but soya bean seemed to be unique in its possession of a co-toxic factor for the basic triphenylmethane series of dyes.

It was then found that an ether extract of soya bean contained the co-toxic factor, soya bean carefully extracted with ether no longer being susceptible to the toxic action of brilliant green. On suspending a little soya bean oil in a purified urease preparation, the ability of a low concentration of brilliant green to inactivate 90 % of the enzyme was restored. Subsequent examination

¹ Kindly supplied me by Dr J. B. Sumner.

of very many oils showed that the faculty of restoring toxicity to brilliant green belonged apparently to unsaturated glycerides, linseed oil in particular being very efficient. Thus 1/50,000 crystal violet had no toxic action on a urease preparation from jack bean meal; on adding to the mixture of enzyme and dyestuff linseed oil to give a final concentration of 0.05 %, 91 % inhibition of the activity of the enzyme was effected. The linseed oil alone had no toxic action on the enzyme. The linseed oil was used as a 1 % alcoholic solution, 0.5 cc. of this being added to the solution containing enzyme and dyestuff.

Table III shows the effects of olive, linseed and cod-liver oils on the toxicity of brilliant green to jack bean urease.

Table III. *Percentage inhibition of the activity of jack bean urease by brilliant green (1/50,000) in the presence of various oils (0.05 %).*

Initial incubation period, 30 minutes.	
Oil	Percentage inhibition
Olive	10
Linseed	66
Cod-liver	28

The co-toxic action of linseed oil *etc.* applies apparently only to the basic triphenylmethane series of dyes. They have little or no effect on the toxicity of neutral red or methylene blue; nor do they enable acidic dyestuffs to become toxic. The mode of action of the unsaturated glycerides in promoting this toxicity is a problem for further investigation. They appear to behave as highly specific mordants between the enzymes and the dyestuff. They do not enhance the toxic effects of dyestuffs on another enzyme, fumarase—so that their specificity extends so far as is known at present to urease on the one hand and to the triphenylmethane series of basic dyes on the other.

It is probable that these results have a considerable bearing on the well-known differential antiseptic actions of the triphenylmethane series of basic dyes.

EFFECTS OF ADMIXTURE OF ACID AND BASIC DYESTUFFS.

It was shown in the earlier work on fumarase [Quastel, 1931] that addition of acid dyestuffs to toxic basic dyestuffs (and *vice versa*) led to the disappearance of toxicity. This was due, presumably, to mutual combination or adsorption between the oppositely charged dyes.

This phenomenon may be demonstrated also using urease. Owing to the fact that the dyes of the basic triphenylmethane series are extremely toxic at very low concentrations (1/100,000) to soya bean urease, it is difficult to show the reversing action of acid dyes with this preparation of enzyme. It is comparatively easy, however, to demonstrate the phenomenon with the purified enzyme to which the dyes are less toxic at low concentrations.

Ethyl violet (1/10,000) gave a 76 % inhibition of the activity of a preparation of jack bean urease. On adding to the ethyl violet trypan blue solution

(1/10,000), allowing to stand for 10 mins. and then adding the enzyme, the percentage inhibition was 18 %. The initial incubation period prior to addition of urea was 30 mins. Reversibilities of a similar kind were effected by benzopurpurin, orange G and crystal scarlet solutions at a concentration of 1/10,000.

There seems little doubt from these results that the basic dyes effect their toxicity by combining with an acidic or negatively charged group in the active centre constituting the enzyme. So far as is known at present the combination is irreversible.

Urea protects urease from the toxicity of the dyes. This is easily shown by adding urea at the same time as the dye to the enzyme. The ammonia liberation in such circumstances is considerably greater than when the urea is added after an initial incubation period of the enzyme with the dyestuff alone.

Thus the NH_3 liberation in 1 hour from urea (10 cc. 0.3 %) when this was added to urease + brilliant green (1/50,000) after 1 hour's initial incubation was 0.52 cc. $N/10$, whereas from the same quantity of urea when this was added to the enzyme and dyestuff without any initial incubation period it was 5.82 cc. $N/10$.

It has been found that many substances resemble urea in protecting urease from the toxic dyestuffs.

PROTECTION OF UREASE FROM THE TOXIC ACTION OF BRILLIANT GREEN.

Much of the work on the protection of urease by various substances has been carried out with brilliant green as the toxic dyestuff. What applies to brilliant green, however, applies also qualitatively to the other toxic dyestuffs.

The enzyme was incubated at p_{H} 7.4 in the presence of brilliant green (usually at a concentration of 1/50,000) and the substance under investigation at 45° for an initial incubation period of 30 mins. or 1 hour. 1 cc. 3 % urea was then added to the mixture, which was finally incubated for 1 hour, after which the ammonia formed was estimated. This value was compared with that produced in the absence of the protective substance. If the inhibition was reduced from 90 % to 50 % by the addition of the substance, the protection was defined as $90 - 50 = 40$ %.

Table IV shows the percentage protections afforded by a number of substances towards soya bean urease against brilliant green.

It will be noted that the amino-acids and basic amines have considerable protective actions, whilst amides, or molecules in which the amino-group has been linked directly with the carbonyl group, are inert. Glycine is very active but hippuric acid is inert; aspartic acid is active but its near non-nitrogenous relatives, succinic, fumaric and malic acids are without action. This may be contrasted with the protective actions exhibited by fumaric and succinic acids towards fumarase against toxic dyestuffs [Quastel, 1931].

In considering, in particular, the protective action of glycine it seems possible that the protection may be due to combination between the amino-

Table IV. *Effects of various substances on the toxicity of brilliant green (1/50,000) towards urease (soya bean preparation).*

Initial incubation period, 30 mins. at 45°. All acids were neutralised with NaOH, the p_H of all solutions prior to addition of urea being 7.4.

Substance	Protection %	Substance	Protection %
Glycine 0.04 <i>M</i>	58	Hydroxylamine 0.04 <i>M</i>	23
<i>p</i> -Aminobenzoic acid "	29	Semicarbazide "	43
Aspartic acid "	49	Allantoin "	60
Glutamic acid "	60	Ethylurethane 0.08 <i>M</i>	0
Succinic acid "	0	Hippuric acid 0.04 <i>M</i>	0
Fumaric acid "	0	Oxamic acid "	0
Malic acid "	0	Betaine "	0
Ethylenediamine 0.08 <i>M</i>	92	Methylurea "	1
Hydrazine 0.04 <i>M</i>	47	Thio-urea "	5

acid and the dyestuff. This possibility may be dismissed, however, on the following evidence. Ethyl violet is toxic to urease (soya bean) at a concentration of 1/100,000. Glycine (0.04 *M*) protects the enzyme from the toxicity of the dye even when the concentration of the latter is increased to 1/10,000. Assuming that the protection is due to combination between the amino-acid and dyestuff, it must be concluded that one part of 0.04 *M* solution of glycine combines with one part of at least a 9/100,000 solution of ethyl violet. Now it may be shown that 1/50,000 ethyl violet solution has a very slight toxicity to fumarase and that 1/10,000 ethyl violet solution is completely toxic, no protection being afforded by the presence of 0.08 *M* glycine. If it is true however that one part of 0.04 *M* glycine combines with 9/100,000 ethyl violet solution, one part of 0.08 *M* glycine will combine with a larger quantity than one part of 1/10,000 ethyl violet solution and hence should completely protect fumarase. The reverse of this is true and hence it cannot be assumed that protection of the enzyme is due to combination between glycine and the dyestuff.

The protective action of glycine is greater, the smaller the quantity of dyestuff present. This is shown in Table V.

Table V. *Protection by glycine against toxic effects of brilliant green on urease (soya bean preparation).*

Initial incubation period of mixture of enzyme, dye and glycine at 45° was 30 mins.

Figures show cc. of $N/10$ NH_3 produced in 1 hour. Maximum obtainable = 10 cc.

Glycine concentration	Concentration of brilliant green		
	1/16,660	1/25,000	1/100,000
0	0.28	0.00	0.3
0.02 <i>M</i>	1.12	1.62	7.36
0.04 <i>M</i>	1.84	2.91	8.50
0.06 <i>M</i>	2.20	3.41	8.79

The protective effects of amino-acids and basic amines are shown equally well with a jack bean urease preparation or with a solution of the crystalline enzyme, 0.05 % linseed oil having been added to these preparations. Typical results are shown in Table VI where the figures quoted represent amounts of

ammonia produced after 1 hour's final incubation, given as percentages of the total quantity of ammonia obtainable (*viz.* 10 cc. $N/10$).

Table VI. *Effects of various substances on toxicity of brilliant green (1/50,000) towards urease (jack bean) in presence of 0.05 % linseed oil.*

Initial incubation period, 60 minutes.

Substance	Molar conc.	Percentage production of NH_3	Substance	Molar conc.	Percentage production of NH_3
Glycine	0.013	76	Hippuric acid	0.02	0
Sarcosine	0.011	71	Methylurea	0.027	7
Methylamine	0.03	40	Biuret	0.02	20
Dimethylamine	0.04	61	Thio-urea	0.027	7
Trimethylamine	0.04	0	Guanidine	0.033	6
Creatine	0.027	20	Diethylurea	0.033	0
Betaine	0.035	2	Formamide	0.2	0

REVERSIBILITY OF THE COMBINATION BETWEEN GLYCINE AND UREASE.

It appears that the union between urease and glycine is reversible, the law of mass action being followed. This may be shown as follows.

Let the initial concentration of urease be E and that of glycine be G . Let the concentration of the glycine-enzyme complex at equilibrium be EG .

Then the concentration of free enzyme at equilibrium = $E - EG$, and the concentration of glycine at equilibrium = $G - EG$. At equilibrium we have

$$K' = \frac{EG}{(E - EG)(G - EG)} = \frac{EG}{G(E - EG)} \quad .$$

since EG is taken as small compared with G .

Therefore
$$EG = \frac{K'E \cdot G}{1 + K'G}$$

where K' is a constant, the reciprocal of the dissociation constant of the glycine-enzyme complex.

Now the amount of urea-enzyme compound must be proportional to EG for that quantity of enzyme which has not combined with glycine has been almost entirely attacked and rendered inert by the dyestuff in the initial incubation period. Hence the amount of enzyme available to attack urea at the end of this period = constant $EG = \text{constant} \frac{E \cdot G}{1 + K'G}$.

The amount of ammonia produced will be proportional to the amount of enzyme available to attack the urea and hence

$$A = NH_3 \text{ produced} = \text{constant} \frac{E \cdot G}{1 + K'G}.$$

Keeping the initial quantities of enzyme and dyestuff constant and given an excess of urea, we should find that

$$A = \frac{\text{constant } G}{1 + K'G}$$

or

$$A = \frac{\text{constant } G}{K + G}$$

where K = dissociation constant of the urease-glycine complex.

Experimental results are given in Fig. 1, where the curve drawn is

$$A = \frac{0.086 G}{0.0153 + G}.$$

A being expressed as molar concentration of ammonia produced and G as the initial molar concentration of glycine present. In Fig. 1 A is plotted against $-\log G$. The experimental results are denoted by crosses and it will be seen

Fig. 1.

that the curve expresses, within the limits of experimental error, the observed variations in the concentrations of ammonia produced with the changes in initial glycine concentrations¹. The constants of the equation were obtained from two points on the curve. These give 0.0153 as the dissociation constant of the glycine-enzyme complex, but this must be considered as approximate (though of the correct order of magnitude) in view of the possibility that some further destruction of the enzyme occurs by means of the dyestuff during the final incubation period. The probability is that the correct dissociation constant of the glycine-enzyme complex is a little lower than 0.0153.

If the initial concentrations of glycine and dyestuff be kept constant A should vary directly with E . This is found to be true within the limits of experimental error.

NATURE OF THE GROUPINGS RESPONSIBLE FOR COMBINATION WITH UREASE.

It is evident from a study of Tables IV and VI that the groupings responsible for the attachment of a substrate molecule to urease consist of the primary amino-group and the secondary imino-group.

Molecules containing the primary amino-group which show protection are glycine, aspartic and glutamic acids, arginine and histidine (see Fig. 2), methylamine, ethylenediamine, hydrazine, hydroxylamine, semicarbazide and *p*-aminobenzoic acid.

Molecules containing the imino-group which show protection are sarcosine and dimethylamine.

Molecules possessing the $N(\text{CH}_3)_3$ group which have no protective effects are betaine and trimethylamine.

¹ Brilliant green, 1/50,000, was used as the toxic dyestuff.

Molecules containing a —CO—NH_2 or a —CO—NH— linkage which have no protective action are oxamic acid, hippuric acid, ethylurethane, diethylurea and formamide.

Fig. 2. Effects of amino-acids, potassium cyanate and ammonium carbamate on NH_3 production by urease in presence of brilliant green (1/50,000).

Thio-urea, guanidine and methylurea have very little protective action at the concentrations tested, whereas creatine, biuret and allantoin have decided effects. It is evident that protective effects with substituted ureas depend greatly on the chemical nature of the substituents.

It is necessary at this juncture to mention that in working with crystalline or highly purified preparations of urease sometimes the reverse of protective effects are obtained—particularly with hydrazine and hydroxylamine. These substances may themselves show considerable toxicity towards the enzyme. This effect is in all probability due to the presence of metals (? Cu), for their toxicity disappears entirely in the presence of a trace of potassium cyanide. Potassium cyanide itself shows no protective action towards urease from brilliant green, but in its presence both hydroxylamine and hydrazine show powerful protective effects to the purified enzyme.

It seems clear from the results as a whole that protective action is associated with the presence of basic amino- or imino-groups. These allow the protective molecules to combine reversibly with the acidic or negatively charged groups which form, either wholly or partially, the active centre of urease. With these groups basic dyes appear to combine irreversibly. Presumably urea is combined also with these groups, activation occurring after combination has taken place.

PROTECTIVE ACTION OF POTASSIUM CYANATE.

It is taken for granted that since an enzyme catalyses a reversible reaction in both directions, the end products, as well as the initial substances, have some affinity for the enzyme. In view of the well known controversy as to whether cyanic acid or carbamic acid is formed from urea by the action of urease it was of interest to determine whether either or both these substances

combined with the enzyme. If combination occurred, clearly some protection would be afforded against a toxic dyestuff.

Experiment showed that ammonium carbamate had little or no protective action, whilst potassium cyanate had a very definite protective effect. Control experiments were carried out to take into account any spontaneous breakdown of cyanate itself into ammonia. The results are shown in Fig. 2 where comparative results with glycine, histidine and arginine are also given. It will be seen that the affinity of cyanate is less than that of glycine but out of all proportion greater than that of ammonium carbamate. The fact that carbamic acid has no protective action is in accord with results already recorded which show that molecules containing the carbamide linkage are usually inert. The definite protective action of cyanate shows that combination with the enzyme does occur with this substance and is evidence in favour of the view that cyanic acid, rather than carbamic acid, is formed from urea by urease. Sodium thiocyanate, it is worthy of note, is entirely without protective action.

SUMMARY.

1. The action of 23 dyestuffs on urease, prepared from soya bean and jack bean, has been investigated. Acidic dyes are entirely inert. Most basic dyes are toxic.

2. The triphenylmethane series of basic dyes is highly toxic to a soya bean preparation of urease, brilliant green being toxic at a concentration of 1/100,000. This toxicity diminishes and may disappear at low concentrations on purifying the urease preparation. The toxicity of other dyestuffs such as Janus green or neutral red increases with increased purification of the enzyme.

3. A substance is present in soya bean which enhances greatly the toxicity of the basic triphenylmethane dyes towards purified urease. This is found to be present in soya bean oil. The property of enhancing the toxicity of brilliant green *etc.* to urease appears to be confined to the unsaturated glycerides. Linseed oil is particularly effective. It seems that the unsaturated glycerides act as highly specific mordants between urease and the basic triphenylmethane dyes.

4. Many substances protect urease from the toxic action of dyes. Such substances are urea, α -amino-acids, sarcosine, ethylenediamine, methylamine, dimethylamine, hydrazine and hydroxylamine.

Trimethylamine, betaine, urethane, methylurea, diethylurea, oxamic acid *etc.* are without effect.

5. Glycine combines reversibly with urease, the dissociation constant of the urease-glycine complex having been determined.

6. The combining group of the protective substances is either a basic amino-group or imino-group. Combination of α -amino-acids, basic amines *etc.* takes place reversibly at acidic or negatively charged groups which constitute either wholly or partially the active centre of urease. Basic dyes appear

to combine with these groups irreversibly. Urea is combined at these groups, activation of the molecule then occurring.

7. Potassium cyanate protects urease from the action of brilliant green; ammonium carbamate has little or no effect. This is evidence for combination between cyanic acid (but not carbamic acid) and the enzyme, and supports the contention that cyanic acid is produced from urea by urease.

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