LXXXI. THE INFLUENCE OF THIOL-GROUPS IN THE ACTIVITY OF DEHYDROGENASES

BY FREDERICK GOWLAND HOPKINS AND EDWARD JAMES MORGAN

From the Biochemical Laboratory, Cambridge

(*Received 31 January 1938*)

THE work of Heffter [1907] in which the existence of labile thiol groups in tissues was demonstrated, and his enlightened prediction that they would be found to play a noteworthy part in tissue respiration, suffered neglect for several years. In the paper containing the first account of glutathione [Hopkins, 1921] it was pointed out that the presence of that substance in the oxidized no less than in the reduced form accelerated the reduction of methylene blue by partly washed tissues, and that this was because by its reduction and subsequent oxidation it acted as an intermediate transporter of hydrogen from tissue to dye. This seems to have given the first hint that intermediate H transport might be a process proper to living tissues. In this paper it was also shown that a tissue washed completely free from soluble H donators still reduced methylene blue in the presence of GSSG, though no attempt was made to decide on what is then the source of the H. In a later paper however [Hopkins & Dixon, 1922] the significance in this connexion of Heffter's thiol groups in proteins was made clear. It was shown that they are primarily responsible, in a thoroughly washed or heat-denatured tissue, for the reduction of GSSG; being themselves oxidized in the process. Later [Hopkins, 1925], in the course of a study of the influence of glutathione on the oxidation of fats and proteins, it was shown that GSH, in sufficiently concentrated solutions, actively re-reduced the oxidized protein groups, and further that these reversible relations resulted in the establishment of equilibria depending on the relative concentrations of GSH and GSSG in solution and the number and activity of similar groups in the tissue. These relations correspond in effect to an equilibrium reaction between heterologous thiols and disulphides.

$$R\mathrm{SS}R + 2R'\mathrm{SH} \rightleftharpoons R'\mathrm{SS}R' + R\mathrm{SH}.$$

Recently Bersin and Steudel [quoted Bersin, 1936] have studied such a reaction in the case of cystine and thiolacetic acid.

Grassmann *et al.* [1929], having the above-mentioned facts in mind, showed that papain and kathepsins can be activated by cysteine or by reduced glutathione; since then many researches have dealt with the influence of thiol compounds on the activity of hydrolysing enzymes. It has become clear, in many cases at least, that thiols can activate such enzymes, and this because of their reducing powers. It is true that the activation observed *in vitro* may in certain cases be due to the circumstance that during its preliminary treatment the enzyme preparation has been partly inactivated by oxidation, and full activity is then restored by contact with (say) GSH. The same enzyme, however, may on occasions be separated without loss of its full activity, and glutathione will then seem to have no functional relations with it. Yet, amid the diverse oxidations

proceeding within the living cell, the properties of glutathione may serve to preserve the activities of any such enzyme, and play such a part in the general equilibrium of the cell. Finally, evidence has accumulated to suggest, or perhaps to prove, that in many cases it is the fixed —SH groups in the protein element of each enzyme which suffer these reversible processes of oxidation and reduction. "The active enzyme is thus to be looked upon as a thiol compound, while its inactive form is a disulphide compound" [Bersin, 1936].

It has indeed been claimed that the activating powers of glutathione, in some cases at least, are due to its ability to form inactive associations with the ions of heavy metals of which the presence inhibits the activity of the enzyme. This view, we think, has been sometimes advanced without adequate evidence. In any case such inhibition may itself be due to the tendency of metals to block, by replacing the H, the —SH groups which are necessary for activity. A sufficient concentration of glutathione (GSH) will restore such thiol groups. If in certain cases GSH thus acts by preventing inhibition by metals, we think such cases will prove to be less common than those in which its reducing powers are concerned. Recent work has extended the field in which the phenomena under review are to be observed. In addition to the activity of proteinases those of lipases, phosphatases, a cerebrosidase and glucosidases have been shown to be related in one way or another to sulphur groupings. Urease and arginase are rather special cases, while the effect of glutathione on glyoxylase activity is, of course, known to depend on relations with the substrate rather than with the enzyme.

As this paper is concerned, not with hydrolytic enzymes, but with dehydrogenases, it does not seem necessary or justifiable to refer in detail to the now extensive literature dealing with the former which has been briefly reviewed above. Bersin, whose own work has contributed much to the subject, has more than once dealt fully and critically with the literature, and in his reviews full references are to be found [Bersin, 1935; 1936].

In the case of the dehydrogenases very little work seems to have been done in this particular field. Wagner-Jauregg & Möller [1935] studied, it is true, the activation of hexosephosphatase and alcoholdehydrogenase, and came to the conclusion that glutathione activates these enzymes by immobilizing inhibitory metallic ions.

A preliminary study, now to be described, of the behaviour of dehydrogenases has shown that, in one at least, it is exceptionally easy to demonstrate the apparent importance of the protein —SH groups for the enzymic activity, and, no less, the influence of glutathione in maintaining the integrity of these groups.

This is the succinic dehydrogenase, with which, and with the glycerophosphate dehydrogenase this paper is chiefly concerned. If an active preparation of this enzyme is first suspended anaerobically at 37° in a solution of GSSG buffered at *p*H 7·4, and if then after a suitable interval it is washed free from the glutathione, the SH groups are found to be oxidized and with their disappearance the activity of the preparation is found also to have completely disappeared. If then the inactivated preparation is treated as before, but now with a solution of GSH replacing the GSSG solution, the protein SH groups are restored and so is the whole original activity of the enzyme. This clear-cut response to the treatment is not displayed by all the enzymes so far studied; but the differences among them seem to be in themselves of no small interest.

Experimental

The experiments described in this paper were mostly made with the methylene blue technique of Thunberg. As they were chiefly concerned with the preliminary activation of substrate H, and not with H transport, the method is fully adequate. A few determinations of O_2 uptake, however, were also made.

When animal tissues were employed as a source of the enzymes they were, as is customary, first thoroughly washed with distilled water till free from H donators. In the case of muscle at least, it proved convenient and adequate to submit the tissue to no further disintegration than that obtained by the use of the Latapie mincer. It can then be filtered off and washed on a Büchner funnel at those stages in the subsequent treatment when previously added glutathione has to be removed (*infra*). If such tissues are more finely ground the washing must be by the centrifuge, which takes longer. With liver and kidney washing by the centrifuge usually proved necessary. When more soluble enzymes are under study (as those from yeast) the glutathione in either form must be added to their solutions and subsequently removed by dialysis. With these however the present paper is but little concerned.

Further details will receive mention in the description of individual experiments.

Succinic dehydrogenase

Preparation 1. In general the details described here apply to other experiments and need not afterwards be repeated. The thigh muscles of a rabbit were put through a Latapie mincer, and the mince washed thrice with successive quantities of distilled water, being squeezed out in linen at each stage. Of the washed tissue 3 g. were placed in a Thunberg tube, 50 ml. in capacity, with 10 ml. *M*/10 GSSG, 2 ml. phosphate buffer *p*H 7·4, together with a few drops of $CHCl_3$. The tube was evacuated and kept in the water bath at 37° for 3 hr. The tissue was then filtered off through linen on a Büchner funnel, and washed until completely free from glutathione, now in part reduced. It was then returned to the tube, which now contained 10 ml. *M*/20 GSSG with buffer, and, after evacuation, replaced in the bath for another 3 hr. The tissue was again washed free from glutathione, and, when sucked as dry as possible by the filter pump, it was removed and weighed. It was divided into 2 equal parts, one-half being reserved (*oxidized product*). The other half was replaced in the vacuum tube with buffer and 3 ml. *M*/10 GSH, placed in the bath for 3 hr. and then finally filtered, washed and well drained at the pump (*reduced product*). As equilibria are concerned it is necessary for satisfactory oxidation of the SH groups that the tissue should be treated more than once with the GSSG solution. It is to be noted that the oxidation of the protein SH is a slower process than the reduction of the protein SS. In this experiment the oxidized preparation showed a very faint nitroprusside reaction. In the reduced sample the reaction was as intense as in the original washed tissue.

The tissue used as a control in the determination of the reduction times was not the original washed tissue. In case the enzyme should suffer any spontaneous change of activity when alone, a portion of the original preparation was taken through all the stages of treatment side by side with the other samples but without added glutathione. This was the "original" in the methylene blue experiments. The same plan was adopted in all other experiments.

The reductions were carried out in smaller Thunberg tubes. As each preparation (original, oxidized and reduced) had its own control without donator,

6 tubes were employed. Each tube contained tissue to correspond with 0·5 g. washed muscle, together with 2 ml. phosphate buffer *p*H 7·4, 0·5 ml. *M*/2 succinate and 0·25 ml. water or (in the controls) 0·75 ml. water. To each was added 0·25 ml. *M*/1000 methylene blue. The tubes were evacuated as usual and placed in a bath at 37°. The observed reduction times are given in detail in Table I. The significant figures in this experiment were (with succinate) 11 min. for the original, > 5 hr. for the oxidized tissue, and 9 min. for the tissue when re-reduced.

Note. A small technical difficulty easily overcome may be mentioned. The three preparations, owing to the difference in their histories, may acquire slight differences in physical conditions, with the result that the imbibed water content when removed from the filter varies somewhat. The preparations were, however, so handled throughout as to avoid loss of the tissue. As in each case the original weight employed was known, the final weights before use measured the varying degree of moisture present, so that (in this and similar experiments) amounts equivalent to 0·5 g. of the original washed preparation could be employed in each tube. When disintegration is carried no further than by use of the Latapie mincer, the preparation after the exposure to GSSG when tested directly gives no trace of a nitroprusside reaction. Occasionally, though not usually, and only in the case of skeletal muscle, if the oxidized tissue be more finely ground before applying the test, a faint reaction may be obtained. This doubtless is due to a permeability factor. It does not happen with heart muscle. Presence or absence of this slight residuum of SH groups made no appreciable difference in the behaviour of a preparation in the reduction tests. It is likely that the concentration of the enzyme effective in these tests is on the more superficial parts of the relatively coarse particles. When, as in preparations 7 and 9 the original washed tissue was finely ground before being submitted to the influence of GSSG, the nitroprusside reaction was always found to be absent. In the tissue after re-reduction by GSH the reaction was always of an intensity comparable with that of the original washed tissue.

Preps. 2, 3 and 4. The details of the procedure used in making these, in each of which the muscles of different rabbits were used, were precisely the same as those of prep. 1, but a larger quantity (with correspondingly larger amounts of GSSG and GSH) was oxidized and reduced in each case, in order to test other enzymes on the same materials (for their behaviour see Table I).

Prep. 5. This was made from the liver of the animal of which the muscles yielded prep. 2. In general the treatment was the same as that used in the preparations from muscle. It was found best however to free the minced liver tissue from added glutathione by thorough washing at the centrifuge. It was so washed until the washings gave no more than a trace of reaction with nitroprusside, even in the presence of cyanide.

Prep. 6. This was made from a pig's heart obtained soon after death. The tissue was minced with the Latapie and in general received treatment similar to that applied to the skeletal muscle of the rabbit, as described for prep. 1.

Prep. 7. This was also from pig's heart. After washing and mincing however it received further treatment. This was exactly that described by Ogston & Green [1935] for the preparation of succinic dehydrogenase, and since used by Dr Green in other experiments. We owe to his kindness and to that of Dr Dewan a supply of preparations so made. In the table the preparations thus disintegrated are distinguished by a star.

Prep. 8. From the kidney of a pig brought while still warm from the slaughter-house. The general treatment of the tissue was again similar to that used for preps. 1–6, but the centrifuge was used for washing at each stage.

Prep. 9. From rabbit muscle. The tissue was used for experiment after receiving the treatment referred to under the head of prep. 7, and in the experiments the same technique was employed. In this case when, at the necessary stages, the tissue was being washed by the centrifuge to remove glutathione, the

material refused to settle satisfactorily from its suspension in the phosphate-buffered fluid. The addition of 5 or 6 drops of 10% $CaCl_2$ however induced separation.

All the results obtained for the succinic acid dehydrogenase are assembled in Table I.

Table I. *Succinic dehydrogenase*

Prep. no.	Animal	Tissue	Reduction times: Original min.	Oxidized hr.	Re-reduced min.
1	Rabbit	Muscle	11	3 + +	9½
2	,,	,,	18	,,	21
3	,,	,,	9	,,	9
4	,,	,,	11	,,	10
5	,,	Liver	16	,,	11
6	Pig	Heart	5	,,	5½
7*	,,	,,	10½	,,	12
8	,,	Kidney	34	,,	13
9*	Rabbit	Muscle	20½	,,	20½

It will be seen from the table that in every case so far studied, when succinate was the substrate, exposure to the influence of GSSG removed all dehydrogenase activity from the tissue. On the other hand, subsequent exposure to GSH re-established the activity in all cases, and, in the majority, to the same degree of activity as that displayed by the original tissue. In the case of the inactivated product it will be noticed that for convenience the reduction time is given in all cases as 3 hr. + +. It is difficult in the course of a working day to observe the tubes for longer than this, while to leave them in the bath overnight is undesirable. In every case of an oxidized preparation at the end of 3 hr. the dye remained apparently unaffected, the colour being still that of the control without added donator.

To the evidence yielded by the methylene blue technique may be added that obtained when a Keilin system is established with the tissue preparation, and the O_2 uptake observed in each case. In the first place, it proved easy to show that a tissue preparation oxidized by GSSG entirely fails in anaerobic conditions to reduce cytochrome, whereas after reduction by GSH the same tissue reduces it equally with the original preparation. It was not surprising therefore to find, when oxidized and reduced preparations were placed side by side in Barcroft manometers, each containing a buffered solution of succinate and a supply of cytochrome, that the latter showed a steady uptake of O_2 and the former no uptake at all. The necessary indophenol oxidase was of course contained in the tissue itself. This seems to undergo some loss of activity during the treatment of the preparation, and the O_2 uptake in the case of the re-reduced tissue was slower than in that by the original, but nevertheless quite active and steady.

The α-glycerophosphate dehydrogenase

Most of the preparations used in the study of the succinic system were used at the same time for testing the effect of the treatment they had received on the activity of the glycerophosphate enzyme. Thunberg tubes, otherwise quite identical in their contents except for the nature of the substrates (succinic acid and the glycerophosphate respectively), were placed in the water bath simultaneously, or observations on both were made with but a short interval between them. With this complete similarity in conditions it was striking to observe the difference in the two cases. Instead of the disappearance and reappearance of

activity so invariable in the succinate system, in the case of the glycerophosphate system oxidation and reduction produced no effect at all. As will be seen in Table II, the reduction times of the original, the oxidized and the re-reduced preparations were the same. The agreement indeed was striking and of course quite unexpected in advance. (The numbers attached to the preparations in Table II refer to the same preparations as do the similar numbers in Table I.)

Table II. *α-Glycerophosphate dehydrogenase*

Prep. no.	Animal	Tissue	Reduction times (min.) Original	Oxidized	Re-reduced
2	Rabbit	Muscle	5	6	6
3	,,	,,	8	7½	8
9*	,,	,,	7	7	7
6	Pig	Heart	32	32	32
8	,,	Kidney	20	22	20

The agreement in the results yielded by each enzyme individually, and the sharp difference shown when the collective results are compared, add to confidence in the technique employed. The results seem also to establish as a fact the existence of a real difference in the configuration of two enzymes which otherwise have properties so similar that it was at one time thought they might even be identical. Their specificity in relation with their substrates would seem, however, to be based on some fundamental differences in their constitution, or in the mechanism of their action. The presence of a thiol group is apparently necessary for the activity of the one while having no apparent function in the case of the other. This point receives some further notice later. It may be added that the results got by the methylene blue technique with the glycerophosphate enzyme were, as with the succinate, confirmed by constituting cytochrome-indophenol-systems with the oxidized and reduced forms. In the case of the former enzyme both showed a notable O_2 uptake.

Other enzymes

No such clear-cut results were obtained in preliminary studies of two other enzymes which function in the activation of substrate hydrogen. These were the lactic acid and the hexosediphosphate enzymes which as dehydrogenases require of course the presence of a coenzyme for their activity. When the various preparations of muscle tissue used in the experiments already described were employed in the same way as before but with lactic acid as the hydrogen donator the results, though irregular, suggested that the enzyme behaved similarly to the succinic dehydrogenase, but was more resistant to the influence of glutathione. The reduction time of the tissue, after exposure to GSSG for periods similar to those otherwise employed, was slower than in the case of the original tissue, but it still reduced methylene blue; while after treatment with GSH reduction was quickened, but the time taken did not fall to that of the original, occasionally the effects were small. When hexosediphosphate was the donator similar behaviour was observed. Further work is necessary before definite statements can be made concerning these two enzymes, but the results again illustrate the existence of fundamental differences in the specific make-up of different dehydrogenases.

It became clearly desirable to test the behaviour of yeast dehydrogenases when treated on similar lines. In the case of soluble enzymes however, the technical difficulties proved greater than when washed tissues were employed. The glutathione in both forms had of course to be added to the enzyme solutions,

and its subsequent removal by dialysis proved at each stage to be a slow process. The whole treatment occupied therefore a long time during which the activity of the enzymes became much reduced. In further experiments an endeavour will be made to employ a different technique for the removal of glutathione, possibly with the use of ultrafiltration.

Oxidation and reduction by cystine and cysteine

The question might arise as to whether the influence of glutathione on an enzyme such as the succinoxidase depends on some relation between the structure of the tripeptide as a whole and the structure of the enzyme, or whether the S groups alone are directly concerned. It seemed therefore desirable to ascertain if cystine and cysteine could act respectively as oxidant and reductant. The slight solubility of the former was expected to involve difficulties, but success was easily reached in the following way. Of pure cystine 0·12 g. was dissolved by boiling it in 30 ml. phosphate buffer at *p*H 7·6. It was then placed in the water bath until it had cooled to 37°, no separation occurring. It was then poured straightway into a large Thunberg tube already in the water bath containing 6 g. minced and washed pig's heart with a few drops of $CHCl_3$. The tube was then evacuated and left in the bath for 4 hr. No visible separation of cystine occurred; it remained apparently in supersaturated solution. A portion of this product after washing at the pump was then reduced on the lines of previous experiments using *M*/10 cysteine as the reductant. Tested for their reduction times the preparations gave the following data: original $4\frac{1}{2}$ min., oxidized 3 hr. + +, reduced 5 min. The cystine and cysteine adequately replaced glutathione.

Reaction with iodoacetate

Since the observations of Quastel & Wheatley [1932] and Quastel [1933] on the glyoxalase system and the very complete study published almost simultaneously by Dickens [1933; 1, 2], it has been generally believed that when the activities of tissues *in vitro* prove to be inhibited by iodoacetic acid, it is because the acid interacts with SH groups, be they in the substrate or the enzyme.

In a study of the action of the halogen-acid on various dehydrogenases Dixon [1937] found that in the conditions of his experiments the succinic dehydrogenase was relatively insensitive to its action. Incubation of a preparation from muscle with a *M*/100 solution for 10 min. reduced its activity by 11 % only. If therefore the acid interacts with no groups in proteins other than thiol groups, it might seem that Dixon's results are not to be easily reconciled with ours. This, we think, does not follow.

A tissue preparation as generally employed contains, of course, in addition to the specific protein of the enzyme studied, a large proportion of other protein material carrying thiol groups. If such a preparation be treated exactly on the lines described by Dixon, it will be found afterwards to give still a well-marked nitroprusside reaction.

Rapkine [1933] has shown that the reaction of the acid with the SH groups of proteins when in the physical condition of those in a washed tissue preparation is a slower process than the action with soluble thiol compounds, and it is clear that if it is to react with every thiol group present during any given incubation time, there must be some definite relation between an effective concentration of the acid and the total amount of thiol proteins present. Smythe [1936] has shown, it is true, that in the case of urease (using iodoacetamide instead of the acid) for the complete inactivation of the enzyme it is apparently not necessary

to destroy more than, at most, half of the SH groups present. We do not, of course, yet know whether these groups in different enzymes, or in other proteins, do or do not vary in their rate of reaction with iodoacetic acid.

In any case it is clear from the following experiments that our results do not really conflict with those of Dixon.

A preparation from pig's heart had been minced and washed as in the other experiments. A portion was incubated with $M/100$ iodoacetic acid for 10 min. as in the experiments of Dixon [1937], and a second portion was similarly incubated for 30 min. The reduction times with methylene blue and succinate (0·5 g. tissue in each case) were then determined, using the same technique as that employed by Dixon. They were as follows: *untreated*, 4 min.; *incubated 10 min.*, 7 min.; *incubated 30 min.*, 40 min. A preparation which had been oxidised and re-reduced gave the following reaction times: *untreated*, 7 min.; *incubated 10 min.*, 27 min.; *incubated 30 min.*, >2 hr. The effect of incubation time is therefore very great.

A second preparation was made from rabbits' heart muscle by the method of Ogston & Green and used as a suspension. It retained an adequate amount of cytochrome for estimations of O_2 uptake. When 0·5 ml. was shaken in a Barcroft manometer together with 0·2 ml. $M/10$ succinate, the system took up 215 μl. O_2 in 15 min. When the same amount of enzyme was first incubated with $M/300$ iodoacetic acid for 30 min., its activity judged by the induced O_2 uptake was reduced by some 20 % only. When however the same amount of enzyme was incubated with $M/100$ acid for 30 min. and used as before, the O_2 uptake was reduced almost to zero. On the other hand, when the amount of enzyme was doubled (1 ml.) and incubated with the $M/100$ solution for 15 min., its activity was still nearly half that of the original. The O_2 uptake experiments are perhaps less significant than the reduction experiments though they certainly support them. In the former it might be said that the effect is due or partly due to the effect of iodoacetic acid on the indophenol oxidase.

It seems sure that in Dixon's experiments the ratio of the amount of enzyme preparation to the concentration of iodoacetic acid was probably too high, and the incubation time certainly too short, for the effect on activity to be considerable.

On the other hand, an explanation of the behaviour of the α-glycerophosphatase is more difficult to find. Our experiments seem to prove that it is wholly independent of SH groups whereas in Dixon's its activity showed 60 % reduction on incubation with $M/100$ iodoacetic acid. This case seems to present an interesting problem for future study.

Discussion

The experiments described show unequivocally in the case of the succinic dehydrogenase that when the enzyme is exposed to the oxidizing influence of GSSG its activity disappears simultaneously with the disappearance of the tissue-thiol groups, while both reappear together under the reducing influence of GSH. It might perhaps be suggested that this does not furnish a complete proof that the thiol groups are essential for the activity of the enzyme. Might not the protein-thiol groups act merely as indicators of the occurrence of the oxidation and reduction which is shared by other and actually essential groupings on the enzyme surface? This however is exceedingly unlikely. GSSG is a very mild oxidizing agent. The redox pair, —SS— $\rightleftarrows$ 2SH—, is a relatively negative system. There is indeed evidence to show that as an oxidant for proteins GSSG is specific for the thiol groups. Work by Mirsky & Anson [1935] for instance (though it is true they were studying denatured proteins, and using cystine as the oxidant)

seems to show that the total amount of hydrogen transferred to the oxidant is at any rate an approximate measure of the number of "fixed" thiol groups in a protein. Similar results have been obtained in this laboratory. It seems sure that the integrity of a thiol group (or groups) in its structure is essential for the activity of the succinic dehydrogenase. This assumption receives indirect support at least from the cumulative evidence derived from the study of hydrolysing enzymes.

Equally unequivocal evidence is given by our experiments that the activity of the α-glycerophosphatase is not based on the influence of sulphur groupings, or, at least, that it does not require the integrity of thiol groups. Oxidation of these has not the slightest effect upon the activity. It is remarkable that so sharp a distinction should be found between two dehydrogenases which in most respects—including their independence of a coenzyme—show very similar qualities.

The experiments described in this paper have proved the unmistakable importance of thiol groups only in the case of the succinic enzyme. But this is perhaps the most typical among dehydrogenases; it is widely distributed among animal tissues, and the succinate system seems to hold a prominent position in the chemical economy of living cells. If thiol groups play a leading part in the mechanism of its action their influence will yield an objective illustration of an aspect of specific structure in correlation with specific functions.

It is of course possible that other dehydrogenases may depend for their activity on the integrity of thiol groups. This however, is not yet proved in the case either of the lactic acid or of the hexosediphosphate dehydrogenase. These do not show that complete indifference to the effect of oxidation and reduction which is displayed by the α-glycerophosphate enzyme, but the effects observed in their cases were not clear-cut, and for some reason varied with different preparations. Further studies of these and other dehydrogenases are in progress.

Summary

The activity of the succinic dehydrogenase is completely abolished when a preparation containing it is placed in a solution of GSSG at *p*H 7·6 and incubated. When, after filtering off and washing, the preparation is next placed in a similar solution of GSH the activity of the enzyme is completely restored. The first treatment involves the removal of the protein SH groups by oxidation and the second their restoration by reduction.

When, on the other hand, similar preparations containing the α-glycerophosphate dehydrogenase are treated in the same way there is no effect whatever on the activity of this enzyme.

These facts and the probable indispensability of the SH group for the activity of the former enzyme are discussed.

The behaviour of certain other dehydrogenases was studied, but requires further elucidation.

REFERENCES

Bersin (1935). *Ergebn. Enzymforsch.* **4**, 68.
—— (1936). *S.B. Ges. ges. Naturw. Marburg.*
Dickens (1933). *Nature, Lond.*, **131**, 130.
—— (1933). *Biochem. J.* **27**, 1141.
Dixon (1937). *Nature, Lond.*, **140**, 806.
Grassmann, Dyckerhoff & v. Schoenebeck (1929). *Hoppe-Seyl. Z.* **186**, 183.
Heffter (1907). *Med. Naturwiss. Arch.* **1**, 81.
Hopkins (1921). *Biochem. J.* **15**, 286.
—— (1925). *Biochem. J.* **19**, 987.
—— & Dixon (1922). *J. biol. Chem.* **54**, 527.
Mirsky & Anson (1935). *J. gen. Physiol.* **18**, 307.
Ogston & Green (1935). *Biochem. J.* **29**, 1893.
Quastel (1933). *Nature, Lond.*, **131**, 206.
—— & Wheatley (1932). *Biochem. J.* **26**, 2169.
Rapkine (1933). *C.R. Soc. Biol., Paris*, **112**, 790, 1030.
Smythe (1936). *J. biol. Chem.* **114**, 601.
Wagner-Jauregg & Möller (1935). *Hoppe-Seyl. Z.* **236**, 222.