SUMMARY

1. A closed-circuit respirometer has been devised for measurement of the respiratory exchange in mice. It is particularly suitable for ^{14}C-tracer experiments.

2. In the post-absorptive state the New Zealand strain of obese mice has a greater oxygen consumption and carbon dioxide production/g. body weight than a control strain of normal mice.

3. New Zealand obese mice oxidize [1-^{14}C]- or [2-^{14}C]-acetate at approximately the same rate as the control mice. The rates of oxidation of both [G-^{14}C]- and [1-^{14}C]-glucose are about 12% lower in the obese than in the normal strain.

4. The incorporation of ^{14}C derived from [G-^{14}C]-glucose into the glycogen and fatty acids of liver and carcass was determined in New Zealand obese and control mice. Incorporation of ^{14}C into the liver glycogen, but not the carcass glycogen, was considerably less in obese than in normal mice.

5. In several respects the metabolism of the New Zealand strain of obese mice differs from that of the obese strain of the Jackson Memorial Laboratory.

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A Protein Disulphide Reductase from Pea Seeds

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Sulphydryl groups play an important role in the reactions and properties of many proteins. The activity of many enzymes depends on the presence of sulphydryl groups; in addition to such functions as the binding of substrates or cofactors and participation as acyl acceptors, Swenson & Boyer (1957) and Boyer (1959) have suggested that sulphydryl groups may have a general role in maintaining proteins in a suitable configuration for enzyme activity. Oxidation of sulphydryl groups in proteins may explain oxygen-toxicity effects in whole tissues and extracts (Gerschman, Gilbert, Nye, Dyer & Fenn, 1954; Haugaard, Hess & Itskovitz, 1957; Turner & Mapson, 1958; Turner, Turner, Shortman & King, 1958; Hatch & Turner, 1959, 1960) and may be responsible in part for damage by ionizing radiations (Barron & Johnson, 1954; Ginzburg, Pandre & Binus, 1957; Hope, 1958; Clubb & Wills, 1959). There is evidence suggesting that the formation of protein-sulphydryl groups may precede cell division (Mazia, 1954; Nickerson & Falcone, 1956*b*; Kawamura & Dan, 1958; Sakai & Dan, 1959).

Nickerson & Falcone (1956*a*) reported that a particulate fraction from yeast reduced disulphide groups in a yeast cell-wall protein in the presence of a liver-coenzyme concentrate. Succinate and ethanol acted as hydrogen donors but the exact

nature of the reduction was not determined. During studies on the mechanism of the reversible aerobic inhibition of glycolysis in pea-seed extracts (Hatch & Turner, 1960) evidence was obtained for the reduction of protein-disulphide groups (especially those of glyceraldehyde 3-phosphate dehydrogenase) in the presence of reduced di- or tri-phosphopyridine nucleotide. A heat-labile component of the pea-seed extract was necessary for this reduction and the partial purification of this enzyme, protein disulphide reductase, and its properties are described in this paper.

EXPERIMENTAL

Preparation of protein disulphide reductase from pea seeds

Defatted pea-seed powder was prepared as described by Turner (1957), except that ether at 0° was used. The powder (60 g.) was extracted for 15 hr. with 200 ml. of 0·04 M-$NaHCO_3$ and 5 ml. of toluene. This and subsequent operations were carried out below 5°. The final pH of the extract was approx. 7·2. The extract was centrifuged at 1000 *g* for 10 min. and the supernatant further centrifuged at 20 000 *g* for 45 min. This preparation will be referred to as the crude supernatant. In the determination of protein disulphide (S.S) reductase activity in the crude supernatant, the protein was precipitated by treatment with saturated $(NH_4)_2SO_4$, pH 7·0, to 90% saturation, centrifuged at 20 000 *g*, dissolved in 0·01 M-KH_2PO_4–Na_2HPO_4 buffer, pH 7·0, and dialysed with stirring against 400 vol. of the same buffer. Cellulose dialysis tubing (Visking Corp., Chicago, Ill., U.S.A.) was used in all preparations.

The protein S.S reductase was partially purified by treating the crude supernatant with neutralized, saturated $(NH_4)_2SO_4$. The fraction obtained between 43 and 50% saturation was dissolved in 30 ml. of 0·01 M-KH_2PO_4–Na_2HPO_4 buffer, pH 7·0. Acetone was added and the fraction precipitating between 0·85 and 1·4 vol. of acetone was separated by centrifuging at 5000 *g* for 20 min. During the acetone treatment the temperature was kept below −5°. The acetone fraction was taken up in 10 ml. of 0·01 M-KH_2PO_4–Na_2HPO_4 buffer, pH 7·0, and dialysed against 400 vol. of the same buffer for 3 hr.

Preparation of protein disulphide reductase from other plant tissues

Seeds of broad bean, pea, barley, maize and wheat were germinated and grown for 5–7 days in damp sand in a glass-house. The roots or shoots were excised and approx. 50 g. fresh wt. of the tissue was blended with 50 ml. of 0·05 M-$NaHCO_3$ and centrifuged at 20 000 *g* for 20 min. The supernatants were treated with saturated $(NH_4)_2SO_4$, pH 7·0, and the fraction precipitating between 38 and 55% saturation was dissolved in 0·01 M-KH_2PO_4–Na_2HPO_4 buffer, pH 7·0, and dialysed against the same buffer.

Soaked seeds of broad bean, wheat, barley, maize and oat, the laminae of leaves of silver beet (*Beta vulgaris* L. var. *cicla* Moq.) and potato tubers were also blended with 0·05 M-$NaHCO_3$ and treated as described above.

Materials

The following reagents were obtained from commercial sources: diphosphopyridine nucleotide (DPN), triphosphopyridine nucleotide (TPN), hexokinase preparation (Pabst Laboratories, Milwaukee, Wis., U.S.A.), reduced diphosphopyridine nucleotide (DPNH), oxidized glutathione (GSSG), crystalline preparations of muscle glyceraldehyde 3-phosphate dehydrogenase, glucose 6-phosphate dehydrogenase, aldolase, yeast alcohol dehydrogenase (C. F. Boehringer und Söhne, Mannheim, Germany), glucose 6-phosphate (G 6-P), *p*-chloromercuribenzoate (Sigma Chemical Co., St Louis, Mo., U.S.A.), cystine (L. Light and Co. Ltd.), cysteine (British Drug Houses Ltd.) and crystalline preparations of bovine-serum albumin, β-lactoglobulin, haemoglobin and ovalbumin (Nutritional Biochemicals Corp., Cleveland, Ohio, U.S.A.). Reduced triphosphopyridine nucleotide (TPNH) was prepared from TPN by the method of Hogeboom & Barry (1948), and was standardized spectrophotometrically at 340 mμ. Pea-seed glyceraldehyde 3-phosphate dehydrogenase was prepared as described by Hageman & Arnon (1955).

Preparation of pea-protein fraction for substrate

The pea-seed-protein fraction used as substrate for the assay of protein S.S reductase was prepared as follows. Defatted pea powder was extracted with a solution of $NaHCO_3$ as described for the preparation of protein S.S reductase. The supernatant after centrifuging at 20 000 *g* was treated with saturated $(NH_4)_2SO_4$, pH 7·0, and the fraction precipitated between 60 and 80% saturation was dissolved in water and dialysed against 200 vol. of mM-KH_2PO_4–Na_2HPO_4 buffer, pH 7·0, for 4 hr. at 5°. The extract was incubated in O_2 for 5 hr. at 20° to oxidize the remaining protein sulphydryl (SH) groups susceptible to aerobic oxidation. The preparation was free from protein S.S-reductase activity.

Analytical methods

Estimation of protein sulphydryl groups. The general procedure of Boyer (1954) was used for determination of protein SH groups. The increase in extinction at 255 mμ resulting from the reaction of SH groups with *p*-chloromercuribenzoate was determined in the presence of 0·05 M-KH_2PO_4–Na_2HPO_4 buffer, pH 6·3, and 0·25 M-Na_2SO_4. With concentrations of nucleotides greater than 15 μM the normal increase in extinction resulting from the reaction of protein SH groups and *p*-chloromercuribenzoate was not obtained, so that protein SH-group formation in the presence of substrate levels of DPNH or TPNH was determined by amperometric titration with $AgNO_3$ (Weissman, Schoenbach & Armistead, 1950).

Cystine reductase, glutathione reductase and glucose 6-phosphate dehydrogenase activity. Cystine reductase and GSSG reductase were assayed as described by Romano & Nickerson (1954) and Racker (1955) respectively. G 6-P-dehydrogenase activity was determined by measuring TPNH formation spectrophotometrically at 340 mμ in a reaction mixture containing: G 6-P, 30 μmoles; TPN, 0·9 μmole; KH_2PO_4–Na_2HPO_4 buffer, pH 7·0, 45 μmoles; total vol. 3 ml.

Determination of protein. The protein content of enzyme and substrate preparations from pea seeds was estimated by the method of Warburg & Christian (1941).

Protein disulphide-reductase activity. Enzyme studies were carried out at 30° in Thunberg tubes gassed with O_2-free N_2. Protein S.S-reductase activity was determined from the increase in protein SH-group concentration in reaction mixtures of the following composition; enzyme and substrate as indicated in individual experiments; KH_2PO_4–Na_2HPO_4 buffer, pH 7·0, 15 μmoles; G 6-P, 10 μmoles; G 6-P dehydrogenase, 18 μg.; TPN, 0·1 μmole; total vol., 1·1 ml. Modifications of this general procedure are indicated in the relevant tables or figures. The rate of reaction was linear until approx. 35 μequiv. of SH groups/g. of substrate protein were formed. For the assay of protein S.S-reductase activity, the reaction was allowed to proceed until approx. 10–15 μequiv. of SH groups/g. of substrate protein were produced.

RESULTS

Purification of protein disulphide reductase. Fractionation of a pea-seed extract with ammonium sulphate and acetone (see Experimental section) resulted in a more than 40-fold purification of protein S.S reductase. The results of a typical purification are shown in Table 1. Removal of protein, which was precipitated during storage of the acetone fraction at − 15°, often increased the specific activity of the partially purified preparation to more than 60 times that of unfractionated extracts. Except where otherwise indicated the partially purified enzyme was used in subsequent studies.

Reduction of disulphide groups of a pea-protein fraction. In the presence of the partially purified enzyme and TPNH (generated by G 6-P dehydrogenase), S.S groups of the pea-seed-protein extract were reduced (Fig. 1). The rate fell to zero when the amount of protein-SH group increased by approx. 48 μequiv./g. of substrate protein. For more than 65 % of the observed SH-group formation the rate of reaction was approximately linear. Whether the reaction proceeded to completion was not determined as the initial concentration of protein S.S groups and their susceptibility to reduction were not known.

Table 1. *Purification of protein disulphide reductase*

The reaction mixtures were of the composition described for the assay of protein S.S reductase and contained 8 mg. of substrate protein. The amount of enzyme preparation added was adjusted to give not more than 10–15 μequiv. of SH groups/g. of substrate protein/hr. Temperature 30°.

Fraction precipitated by	Total units*	Protein concn. (mg./ml.)	Specific activity (units/mg. of protein)
0–90 % $(NH_4)_2SO_4$	95	35	0·09
43–50 % $(NH_4)_2SO_4$	83	7·3	0·99
0·85–1·4 vol. of acetone	43	2·3	3·7

* A unit of protein disulphide reductase was defined as the amount of enzyme which catalysed the formation of 0·1 μequiv. of SH group/hr.

Requirement for reduced di- and tri-phosphopyridine nucleotide. In the presence of systems which produced either DPNH or TPNH, protein S.S groups of unfractionated pea-seed extracts were reduced (Hatch & Turner, 1960). The partially purified protein S.S reductase also utilized both DPNH and TPNH (Table 2). It was reported previously that pyridine nucleotide transhydrogenase was not detected in the pea-seed extracts (Hatch & Turner, 1960). With TPNH as hydrogen donor the rate of protein S.S reduction was approximately twice that with DPNH. A similar ratio was

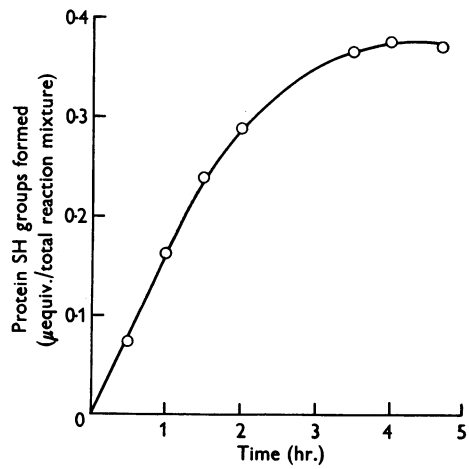

Fig. 1. Reduction of protein S.S groups by TPNH. The reaction mixtures (containing 0·38 mg. of enzyme protein and 8 mg. of substrate protein) were of the composition described for the assay of protein S.S reductase. Temperature 30°.

Table 2. *Requirement of reduced di- and tri-phosphopyridine nucleotides for protein disulphide-reductase activity*

The reaction mixtures of Expt. 1 contained 0·24 mg. of enzyme protein and 11 mg. of substrate protein and were of the composition described for the assay of protein S.S reductase. The reaction mixtures of Expt. 2 contained: enzyme protein, 1·3 mg.; substrate protein, 18 mg.; KH_2PO_4–Na_2HPO_4 buffer (pH 7·0), 25 μmoles; DPNH, 4·8 μmoles, or TPNH, 4·0 μmoles, added as shown; total vol. was 2·2 ml. Incubation time, 1 hr. Temperature 30°.

Expt. no.	Reaction-mixture composition	Protein SH groups formed (μequiv./reaction mixture)
1	Complete system	0·11
	No G 6-P dehydrogenase	0·12
	No G 6-P	0
	No TPN	0
2	Complete system with DPNH	0·18
	Complete system with TPNH	0·32
	No DPNH or TPNH	0

obtained with crude enzyme extracts, which suggested that the activity of crude and partially purified preparations was due to a single enzyme. Protein S.S groups were also reduced by TPNH generated by G 6-P dehydrogenase (Table 2). Although the partially purified protein S.S-reductase preparation generally contained sufficient G 6-P dehydrogenase, additional enzyme was added to ensure that TPNH supply was not rate-limiting. In separate experiments no oxidation of protein SH groups was observed in reaction mixtures containing enzyme, substrate protein not pretreated in oxygen and substrate amounts of DPN or TPN.

The K_m for TPNH was 2 μM. This value was calculated from the Lineweaver–Burk plot (Lineweaver & Burk, 1934) of the rates of protein S.S reduction in the presence of various concentrations of added TPN and an excess of G 6-P and G 6-P dehydrogenase (Fig. 2). It was assumed that the concentration of TPNH in the reaction mixtures approximated to the concentration of added TPN. A dehydrogenase with a suitable equilibrium was not available for determining the K_m for DPNH.

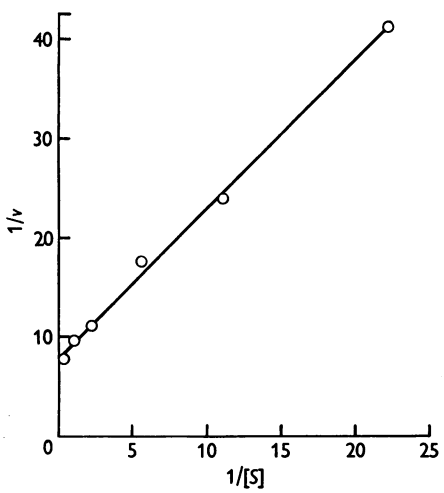

Fig. 2. Lineweaver–Burk plot of $1/v$ against $1/[S]$ for protein S.S reductase. v is the initial velocity (μequiv. of SH groups formed/reaction mixture/hr.); $[S]$ is 10^5 × concn. (M) of TPNH. The reaction mixtures (containing 0·6 mg. of enzyme protein, 11 mg. of substrate protein and varying amounts of TPN) were of the composition described for the assay of protein S.S reductase. Incubation time, 1 hr. Temperature 30°.

Relationship of protein disulphide-reductase activity to cystine reductase and glutathione reductase

Previous observations suggested that neither cystine reductase nor GSSG reductase was responsible for the reduction of protein S.S groups by crude pea extracts (Hatch & Turner, 1960). This conclusion was supported by the observation that during the purification of protein S.S reductase, comparable purification of cystine reductase and GSSG reductase was not obtained (Table 3). Protein S.S reductase was purified more than 40-fold compared with a twofold purification of GSSG reductase. Cystine reductase was completely removed by the fractionation procedure.

Reduction of disulphide groups of other protein preparations. The enzyme showed some specificity towards different proteins, the rate of reduction of their S.S groups varying considerably (Table 4). Some proteins were not attacked whereas with others only a small fraction of the S.S groups in the protein preparations was reduced. The concentration of substrate protein may have influenced the activity observed.

Effect of pH. Protein S.S-reductase activity was optimum between pH 6·9 and 7·4 and declined rapidly on either side of this range (Fig. 3). Some precipitation of protein from the reaction mixtures occurred at pH 6·2.

Effect of inhibitors. The effect of several enzyme inhibitors on protein S.S-reductase activity was examined (Table 5). *p*-Chloromercuribenzoate, iodoacetate, Hg^{2+} ions, Ag^{+} ions and Cu^{2+} ions, which are known to affect SH groups, inhibited activity. The enzyme was also inactivated by

Table 3. *Effect of fractionation procedure for protein disulphide reductase on the activity of glutathione reductase and cystine reductase*

The various enzyme activities were assayed as described in the Experimental section. The reaction mixtures for the assay of protein S.S-reductase activity contained 8 mg. of substrate protein and the amount of enzyme fraction added was adjusted to give not more than 10–15 μequiv. of SH groups/g./hr. Temperature 30°.

Fraction precipitated by	Protein S.S-reductase activity (units/mg. of enzyme protein)*	GSSG-reductase activity (extinction change/mg. of enzyme/min.)	Cystine-reductase activity (extinction change/mg. of enzyme/min.)
0–90% $(NH_4)_2SO_4$	0·065	0·025	0·003
43–50% $(NH_4)_2SO_4$	0·70	0·184	0
0·85–1·4 vol. of acetone	2·8	0·053	0

* Units as defined in Table 1.

Table 4. *Reduction of disulphide groups of various protein preparations*

The reaction mixtures were of the composition described for the assay of protein S.S reductase. Expts. 1–3 contained 0·38, 0·40 and 0·34 mg. of enzyme protein respectively; amounts of substrate protein are shown in the table. The substrate-protein solutions were incubated in O_2 for 5 hr. at 20° to oxidize SH groups. Reaction time, 1 hr.

Expt. no.	Substrate protein	Amount (mg./total reaction mixture)	Protein SH groups formed (μequiv./reaction mixture)*
1	Cryst. glyceraldehyde 3-phosphate dehydrogenase	5	0·025
	Cryst. alcohol dehydrogenase	3	0·086
	Cryst. aldolase	2·5	0
	Hexokinase preparation	4	0·038
2	Cryst. ovalbumin	3·5	0
	Cryst. serum albumin	10	0·024
	Cryst. haemoglobin	6	0
	β-Lactoglobulin	6	0
3	Pea-seed glyceraldehyde 3-phosphate-dehydrogenase preparation	12	0·092

* No activity was found in the absence of the enzyme preparation.

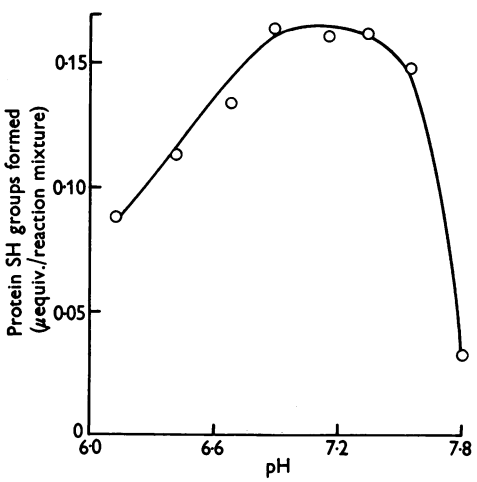

Fig. 3. Effect of pH on protein S.S reductase activity. Reaction mixtures (containing 0·48 mg. of enzyme protein and 8 mg. of substrate protein) were of the composition used for the assay of protein S.S reductase. The reaction was studied in the presence of 15 μmoles of KH_2PO_4–Na_2HPO_4 buffer of the pH indicated. Incubation time was 1 hr. and the temperature was 30°.

arsenite and Cd^{2+} ions; at low concentrations these reagents are considered to be specific for reactions involving a dithiol grouping (Sanadi, Langley & White, 1959). Zn^{2+} ions (3 μM) strongly inhibited the enzyme. At the concentrations used in these experiments the SH and dithiol reagents did not interfere with the estimation of protein SH groups. When G 6-P-dehydrogenase activity was examined under the same conditions as those used for the assay of protein S.S reductase, Ag^{+} ions, Hg^{2+} ions and *p*-chloromercuribenzoate inhibited the enzyme by 37, 48 and 15% respectively; Zn^{2+} ions, Cu^{2+} ions, Cd^{2+} ions, arsenite and iodoacetate did not affect the activity. The results obtained for protein S.S-reductase inhibition were therefore not due to the inhibition of G 6-P dehydrogenase as reaction mixtures for the assay of protein S.S reductase contained excess of this enzyme.

Table 5. *Effect of enzyme inhibitors on protein disulphide-reductase activity*

The reaction mixtures, which contained 0·30–0·40 mg. of enzyme protein and 9 mg. of substrate protein, were of the composition used for the assay of protein S.S reductase. Incubation time, 1 hr. Temperature 30°.

Inhibitor	Concn. (mM)	Inhibition (%)
Sodium molybdate	0·1	0
Sodium cyanide	10	0
Ethylenediaminetetra-acetate	0·045	0
Sodium fluoride	10	0
p-Chloromercuribenzoate	0·054	88
Iodoacetate	0·054	57
Sodium arsenite	0·15	100
Sodium arsenite	0·045	89
Cadmium sulphate	0·005	91
Zinc sulphate	0·003	82
Mercuric chloride	0·002	92
Silver nitrate	0·009	66
Cupric sulphate	0·015	100

Enzyme stability. Protein S.S-reductase preparations could be stored at −15° for at least 8 weeks without loss of activity. Heating at 60° for 10 min. had no effect, but at 70° the enzyme was completely inactivated. Adjustment of the pH to 5·5 for 5 min. at room temperature or at 5° irreversibly inactivated the enzyme.

Distribution in plant tissues

Protein S.S-reductase activity was detected in extracts from the following tissues: broad bean, wheat, barley, maize and oat seeds; shoots of pea, broad bean, maize, wheat and barley seedlings and roots of pea, barley and maize seedlings. With extracts from barley and oat seeds, and pea, wheat and barley shoots, activity was increased by the addition of 0·035 mM-ethylenediaminetetra-acetate (EDTA). In these extracts heavy-metal ions probably affected activity. However, extracts from broad-bean roots, silver-beet leaves and potato tubers were inactive in the presence or absence of EDTA. In all cases activity was completely inhibited by 0·2 mM-sodium arsenite, indicating that neither cystine reductase nor GSSG reductase was involved. In separate experiments it was found that pea GSSG reductase was only slightly inhibited by 0·5 mM-arsenite whereas cystine reductase was not affected.

DISCUSSION

The present investigation has shown that active preparations of an enzyme catalysing the reduction of protein S.S groups by reduced pyridine nucleotides may be readily obtained from pea seeds. This enzyme, protein disulphide reductase, acted on the S.S groups of several enzyme and other protein preparations and may thus be of considerable importance in cell metabolism in reducing the S.S groups of these (and probably other) proteins.

The purification data and the effect of arsenite as an inhibitor indicated that neither cystine reductase (Romano & Nickerson, 1954) nor GSSG reductase (Mapson & Goddard, 1951; Conn & Vennesland, 1951) was involved in the reduction of protein S.S groups. Protein S.S reductase was irreversibly inactivated at pH 5·5, whereas Moustafa (1955) found that pea GSSG reductase was stable at this pH. Boyer (1959) suggested that GSSG reductase or cystine reductase could reduce protein S.S groups in the presence of catalytic amounts of substrate. Pihl, Eldjarn & Bremer (1957) found that liver GSSG reductase catalysed the reduction of a number of non-protein S.S compounds if a catalytic amount of GSH was added, electrons being transferred non-enzymically from GSH to the S.S compounds.

It was not possible to determine the equilibrium constant for the reaction between reduced pyridine nucleotides and protein S.S groups. No oxidation of protein SH groups was observed in reaction mixtures containing substrate levels of DPN or TPN and from this it appeared that the reaction equilibrium for protein S.S reductase favours the reduction of protein S.S groups. This conclusion is supported by the experiments of Rall & Lehninger (1952), who found that GSSG was almost completely reduced by TPNH in the presence of GSSG reductase. Boyer (1959) has pointed out that at least some of the SH–S.S groups in proteins have electrode potentials close to those for low-molecular-weight thiols such as GSH. The values of the electrode potentials of SH–S.S systems (and therefore the expected degree of reduction of S.S groups by TPNH) are in doubt (Calvin, 1954; Boyer, 1959).

Inhibition of protein S.S reductase by SH-group reagents and by low concentrations of the dithiol reagents, arsenite and Cd^{2+} ions, indicated that SH groups have a primary role in the activity of the enzyme. Arsenite and Cd^{2+} ions are specific inhibitors of reactions involving the dithiol grouping (Sanadi *et al.* 1959). A number of enzymes are inhibited by reagents which combine with monothiols but are not affected by low concentrations of arsenite (James, 1953). Arsenite forms only a weak complex with monothiols but a stable ring structure with dithiol compounds, provided that the SH groups are spatially adjacent (Stocken & Thompson, 1946; James, 1953). Inhibition of protein S.S reductase by arsenite and Cd^{2+} ions suggests that the enzyme contains spatially adjacent SH groups at the catalytically active site. Although it might be anticipated that protein SH groups in this configuration would be susceptible to oxidation to the S.S form in the presence of molecular oxygen (Boyer, 1959), the enzyme remained active even after prolonged pretreatment in air or oxygen. A mechanism of action for the enzyme involving the alternate oxidation and reduction of the catalytically active dithiol grouping would be consistent with this observation and with the inhibition by arsenite and Cd^{2+} ions. If such a mechanism operates, the steps leading to the reduction of substrate–protein S.S groups may involve: (*a*) reduction of the S.S groups at the enzyme surface to a dithiol by DPNH or TPNH, (*b*) the transfer of electrons from the dithiol group to the S.S group of the substrate protein, leaving the enzyme in the S.S form.

Liver GSSG reductase and the pea-protein S.S reductase have some properties in common. Langdon (1958) showed that GSSG reductase was inhibited by low concentrations of Zn^{2+} ions and *p*-chloromercuribenzoate. Inhibition by Zn^{2+} ions was observed only if the enzyme was pretreated with the reagent, and TPNH and GSSG protected the enzyme. The results suggested that Zn^{2+} ions were chelated at the catalytically active site by groups responsible for the binding of GSSG and TPNH to the enzyme. Langdon proposed a two-step reduction of GSSG by TPNH. The scheme involved the transfer of an electron from an enzyme SH group to give GSH and an enzyme–SSG

complex which was then reduced by TPNH to give enzyme-SH plus GSH. With protein S.S reductase, TPNH and substrate protein did not prevent inhibition by Zn^{2+} ions, which suggested that the enzymes operated by different mechanisms.

SUMMARY

1. Protein disulphide reductase, which catalyses the reduction of protein disulphide groups, was purified approximately 40-fold from pea-seed extracts.

2. The enzyme utilized reduced di- and triphosphopyridine nucleotide to reduce protein-disulphide groups. The rate of reaction with reduced triphosphopyridine nucleotide was approximately twice that with reduced diphosphopyridine nucleotide.

3. A protein fraction from pea seeds was used as substrate in most experiments. The enzyme also reduced disulphide groups of a pea-seed glyceraldehyde 3-phosphate-dehydrogenase preparation and of some other enzymes and proteins.

4. Cystine reductase and glutathione reductase, both of which were present in crude pea-seed extracts, were not involved in the reduction of protein-disulphide groups.

5. The effects of pH and concentration of reduced triphosphopyridine nucleotide on the enzyme activity were studied.

6. Protein disulphide-reductase activity was strongly inhibited by low concentrations of *p*-chloromercuribenzoate, iodoacetate, Hg^{2+} ions, Ag^{+} ions, Cu^{2+} ions, Cd^{2+} ions, Zn^{2+} ions and arsenite.

7. Active preparations of the enzyme were obtained from a number of plant tissues.

8. A possible mechanism of action of protein disulphide reductase is presented.

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