CXXI. GLUTATHIONE.

ITS INFLUENCE IN THE OXIDATION OF FATS AND PROTEINS.

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I. INTRODUCTORY.

THIS paper gives an account of observations which illustrate certain of the properties of glutathione as a promoter of oxidations. The fresh facts made available are as yet quite insufficient to explain its precise functions in living tissues, but they carry suggestions for future work. The experimental results touch at some points on phenomena concerning which basal chemical knowledge seems lacking. They cannot therefore be succinctly described¹.

Meyerhof has shown that the oxidation of certain unsaturated fatty acids is promoted by the presence of thiol compounds, and the results he obtained by the use of thioglycollic acid and cysteine (in particular the former) are easily reproduced with the dipeptide, though with certain differences that are significant. It will be shown in this paper that a curious limited oxidation of proteins is brought about by the agency of glutathione, exerted in quite peculiar relations. The observations of Miss Thurlow [1925] carried out in this laboratory have shown that in the progress of the oxidation of the SH group peroxide formation occurs, and that under such circumstances, in the presence of a peroxidase, certain secondary oxidations may be induced. On these lines it is probable that the functions of glutathione may come into relation with the events of carbohydrate metabolism.

In the experiments to be described, in so far as they have involved the use of tissue preparations, muscle tissue has been almost exclusively employed. In certain details, though not in fundamentals, the relations of muscle fibres with glutathione are special. The behaviour of cellular tissues requires separate treatment.

Since the publication of the paper by Dixon and myself [1922], which followed the original account of its isolation [Hopkins, 1921], the constitution

¹ Many of the observations described were made more than two years ago, but publication was unavoidably delayed. Unfortunately, more recent work—much limited by lack of leisure—still leaves the subject in a position which is curious and obscure. I think the fresh facts described are of real interest, however, and publication at the present time may stimulate the work of others. The paper should be looked upon, in part at least, as a prolegomenon to publications from this laboratory which will appear in the near future.

of glutathione as diglutaminyl-cysteine has been established on what seems to be conclusive evidence by the work of Quastel, Stewart and Tunnicliffe [1923], and by its synthesis in the hands of the latter two investigators [Stewart and Tunnicliffe, 1925]. The natural product has now been many times prepared (chiefly from yeast) by different individuals, using the method originally described. Szent-Györgyi [1925] has endeavoured to modify that method, but states that he was unable to improve upon it. With experience the original yields have been increased. Some 20 g. of the pure substance from 50 kg. of moist pressed yeast are obtained without difficulty. This falls much below the maximal figure given by direct titration, *circa* 0.2 % [Tunnicliffe, 1925]; but it is certain that serious losses occur during the process of isolation¹. Tunnicliffe [1925] finds that muscle contains about 0.035 % of which about one-third only has been actually isolated. It is in any case sure that Abderhalden and Wertheimer [1923] are mistaken in supposing that the concentration of free cysteine in the tissues is at all comparable with that of the dipeptide. I possess some evidence to show that the former amounts at the very most to one-twentieth of the latter and is probably much less. Free cysteine cannot play a part of quantitative importance in promoting tissue oxidation.

Any description of the properties of glutathione and any study of its functions must of course take due account of the important observation of Warburg and Sakuma [1923], showing that the SH group of thiol compounds is not strictly autoxidisable, but is oxidised by molecular oxygen only in the presence of minute traces of iron. That this holds in the case of glutathione (or, at least, that the autoxidation of its thiol form is relatively very slow in the absence of all but irremovable traces of iron) has been shown by Harrison [1924]. It would be desirable, therefore, to carry out all experiments with preparations containing a minimal, but exactly known, quantity of the metal. Owing however to the laborious nature of the process required for its complete removal and the waste of highly expensive material involved, either in the removal of the traces present in the preparations used, or in estimating these traces, this course is scarcely possible in practice. Since Warburg's discovery the use of specially pure reagents has yielded preparations containing, at most, infinitesimal quantities of the metal. The employment of these, instead of earlier, preparations has made surprisingly little difference to the experimental results. In any case, this relation with iron does not remove any significance from the presumed functions of the thiol group in respiration. The metal is only another link in the chain. None of the results to which significance is attached in this paper is due to the direct influence of iron alone. This question receives frequent reference; but, otherwise, when the addition of glutathione to a system is mentioned, the presence of such minute amounts of iron as the preparation might contain is presumed.

A statement has been made by Szent-Györgyi [1925] which, if correct,

¹ It is undesirable to attempt to isolate the substance from less than 20 kg. of moist yeast.

could not fail to affect profoundly any views concerning the biological functions of glutathione. This author states that the thiol group as it exists in the tissues (where it is certainly in contact with sufficient iron) is not oxidisable. He prepared an extract from the liver of a guinea-pig by grinding up the organ with specially prepared distilled water. The extract was boiled and filtered. The filtrate gave a strong nitroprusside reaction which did not (apparently) diminish on aeration. He does not mention the p_H of the preparation, and he aerated it for 15 minutes only. But such rapid oxidation is certainly not to be expected under such conditions. It would indeed be disconcerting to find that any factor supposed to be concerned with respiratory oxidations could so rapidly disappear from a tissue preparation of the kind. I have many times seen the nitroprusside reaction completely disappear from unwashed liver tissue suspended in distilled water at p_H 7.4 when shaken in a Barcroft apparatus, but only after 5–6 hours at 37°. For special purposes I have many times proceeded as follows. The liver (or other organ) after grinding (the pulp being first squeezed through coarse muslin) is mixed with twice its volume of distilled water, the mixture made neutral or slightly alkaline, placed in a large stoppered bottle and shaken vigorously in a mechanical shaker. I have several records showing that under these conditions the original intense nitroprusside reaction of an aseptic liver preparation may completely disappear within 8 hours at room temperature. When the preparation is first boiled and filtered the oxidation in the filtrate seems to be a little slower, but with efficient aeration the nitroprusside reaction always finally disappears. Since the publication of the above author Mr Albert Carruthers has repeated this procedure using, instead of ordinary distilled water, water twice distilled in quartz apparatus. This made no difference to the result.

In the discussions which follow, in order to save space and printing, the reduced, or thiol form of glutathione is generally referred to as GSH, and its disulphide form as G_2S_2 . It is often convenient to speak of "SH" and "S-S" as though they were independent chemical entities.

II. EXPERIMENTAL METHODS.

All the estimations of oxygen uptake were made in the Barcroft "differential manometer" apparatus of the type previously described in publications from this laboratory. The flasks are flat bottomed, shaped so as to provide a large surface for the suspension fluid and secure efficient aeration when the instrument is shaken. It was easy to observe that in none of the experiments described did the rate of oxygen supply become a limiting factor. The instruments were carefully calibrated and four could be shaken side by side by the same motor while immersed in the same bath. Different systems could therefore be compared under identical conditions. Unless otherwise stated, all the measurements were made at 37°. For a reason mentioned in the next section, the phosphate buffer solutions used as suspension fluids in earlier experiments [Hopkins and Dixon, 1922] were given up. As in the great majority of cases

the oxidations studied involved no change in p_H , Ringer solution or distilled water was used. It was found quite easy under these circumstances to adjust the p_H of the systems studied and in the experiments described it was found to be unchanged at the end. The dipeptide (partially or completely neutralised with soda) itself provides slight buffer action.

Many of the results are given in the form of curves relating the oxygen uptake with time. Meyerhof [1923] has correctly remarked that when tissue preparations are shaken in apparatus like the Barcroft manometer, the absolute velocity of uptake may be so greatly affected by accidental factors, such as the physical condition of the preparation, that its measurement has little significance. In certain cases it is doubtless of small value. Nevertheless, when relative, and not absolute, velocities are in question, and in some other cases, curves may give desirable information which is not supplied by a statement of total uptake. When oxidising systems are shaken side by side and when one factor only is varied as between a particular system and a control system, a comparison of the curves obtained is often of value. Those given in the figures are quite unsmoothened.

III. THE OXYGEN UPTAKE (WITH GLUTATHIONE) OF WASHED MUSCLE AND ITS THERMOSTABLE RESIDUE.

In the paper by Hopkins and Dixon [1922] it was shown that when washed muscle is extracted with boiling water, washed with alcohol, dried *in vacuo* and powdered, the dry powder, called in the above paper the thermostable residue, though showing no oxygen uptake by itself, displays a definite uptake in the presence of glutathione. A corresponding amount of the original washed muscle without further treatment is itself capable of the same quantitative uptake, but to establish a sufficiently active system within the swollen and somewhat impermeable fibres of the washed preparation a relatively high concentration of glutathione in the external fluid is necessary. A concentration of the order $M/200$ secures a brisk uptake with the powder. In the case of the washed muscle the uptake with $M/40$ is still relatively slow, but it reaches the same level as that of the powder within a reasonable experimental period (Table I).

Hopkins and Dixon [1922] using Clarke and Lub's phosphate buffer solutions obtained an oxygen uptake of about 400 mm³. per g. of dry powder. It was shortly afterwards found that the high concentrations of phosphate used limit the total oxygen to a remarkable extent, for reasons not yet fully explained. The uptake of an average preparation in Ringer's solution at p_H 7.6 is of the order of 2000 mm³. per g. A great number of such estimations were made at a time when more importance seemed to belong to the actual figures than is really the case. Unselected instances are given in Table I.

Table I.

Oxygen uptake of muscle preparations suspended in aqueous solutions (3 cc.) of glutathione (disulphide). Except where otherwise stated all preparations were in the form of the dry thermostable powder. p_H of system 7.6 in each case.

No. of Exp.	Species of muscle	Weight employed g.	Concentration of G_2S_2	Time of observation hours	O_2 uptake mm. ³	Uptake mm. ³ per g.	Remarks
1	Cat	0.2	$M/200$	$7\frac{1}{2}$	385	1925	
2	Dog (1)	0.2	$M/200$	$7\frac{1}{2}$	395	1975	
3	Dog (2)	0.2	$M/200$	8	400	2000	
4	Rabbit	0.2	$M/200$	7	363	1815	
5	Rabbit	1.0	$M/50$	8	285	1425 (on dry weight)	Moist washed muscle—uptake not complete
6	Rat	0.2	$M/200$	$7\frac{1}{2}$	454	2270	
7	Frog	0.2	$M/200$	7	310	1550	
8	Frog	0.5	$M/50$	8	110	1100 (on dry weight)	Moist washed muscle—uptake not complete

It was shown in a previous paper that when the dipeptide forms an oxidising system with washed muscle it suffers itself no oxidation save the reversible change in which the sulphur group is alone concerned. The observations in question involved the use of phosphate buffers. It seemed worth while to demonstrate the fact again with the non-buffered media in which the uptake of the system is so much higher. The curves of Fig. 1 show that the total oxygen taken up reaches practically the same value, though of course at different velocities, with greatly varying concentrations of glutathione. These curves were obtained with the muscle powder; but since, as stated above, muscle when simply washed gives with high concentrations of glutathione the same uptake as a corresponding amount of powder with a lower concentration, it is sure that the oxidising agent in washed muscle which oxidises

Fig. 1. Oxygen uptake of thermostable powder from cat's muscle, 0.2 g. in 3 cc. Ringer at p_H 7.6. Of the three upper curves the lowest shows the effect of 4 mg., the middle of 8 mg., and the highest of 16 mg. glutathione (G_2S_2). The bottom curve shows the uptake of the powder alone.

free glutaminic acid [Thunberg, 1920] does not affect the glutaminy! moiety of the dipeptide.

Meyerhof [1923], in the important communication which appeared shortly after that of Dixon and myself, also describes observations upon the thermostable residue ("Alkoholpulver"). He reports uptakes (with thioglycollic acid) as high as 5000 mm.³ per g., though many of his data point to a lower figure than this. A comparison of his figures with my own is of small significance, however. Many of them were obtained under different conditions, especially in respect of the hydrogen ion concentration in the systems studied. Variations in this affect more than velocity of uptake. It will be shown, for instance, that in acid systems the oxidation of fat is favoured, whilst oxidation of quite other material proceeds best at the neutral point or in slightly alkaline solutions. A difference in hydrogen ion concentration will therefore affect the result both quantitatively and qualitatively (see Figs. 9, 10 and 11, p. 813, section vi). Meyerhof used thioglycollic acid. In the experiments of Table I glutathione was employed not as the thiol compound, but in its disulphide form.

As a matter of fact, the significance of the oxygen uptake of the thermostable residue is somewhat different from what may have been suggested or implied in previous publications. It will be convenient, before returning to it, first to consider the behaviour of systems containing unsaturated fats and glutathione alone, and afterwards that of fat-free tissue-systems. The former take up oxygen only when the thiol form of the dipeptide is present from the first, since the fats do not reduce the disulphide form. Oxidation in the latter proceeds with either form.

IV. THE OXIDATION OF UNSATURATED FATTY ACIDS AND FATS.

In aqueous systems containing GSH together with an emulsion of unsaturated fatty acids (linolenic, linoleic), so long as the p_H is of the order of 3.0 and 4.0, the concentration of the SH group is long (though not indefinitely) maintained during the aeration of the system. Oxygen many times in excess of what is required to oxidise the SH to S-S is taken up and a corresponding amount of fatty acid oxidised. When, however, the hydrogen ion concentration approaches the neutral point, or when it is near to this on the alkaline side (p_H 7.4–7.6), the behaviour of such systems is profoundly changed. The SH group (the presence of traces of iron being presupposed) is then so freely oxidisable that, unless its concentration in the system be relatively high, it rapidly disappears, even in the presence of the fatty acid. The system then becomes inert before more than relatively small amounts of fatty acid have been oxidised. The behaviour of systems composed of GSH and unsaturated fatty acids seems to be therefore essentially the same as that described by Meyerhof for the systems lecithin-thioglycollic acid and linolenic acid-thioglycollic acid. (See Table II and compare the results of Exps. 1–5 with those of Meyerhof [1923, Table XV, p. 548].)

In considering acid systems in which the concentration of the SH group

falls off very slowly during the aeration of the system, the view of Meyerhof [1923, p. 561] as to the mechanism of the oxidation seems adequate. It will be remembered that he postulates the preliminary formation of a peroxide by two thiol groups. This peroxide is assumed to decompose then in such a way that the oxygen is wholly transferred to the unsaturated linkages of the fatty acid, whilst the original SH groups are reconstituted. The oxidation of the former is what would usually be considered as a catalysed reaction and the thiol group is concerned essentially with oxygen transport.

Table II.

Ref. no.	Material employed 10 mg. as emulsion in distilled water	p_H	Time for uptake hours	Total uptake mm. ³	O ₂ equi- valent of SH present mm. ³	O ₂ to fat mm. ³	Remarks
1	Linolenic acid	3.5	6	210	32	178	Acid systems
2	"	3.8	6	225	41	184	In all cases the uptake was still progressing at end of observation
3	Linoleic acid	3.5	6	140	32	108	
4	Mixed acids from linseed oil	3.2	5½	710	200	510	
5	Mixed acids from linseed oil	3.5	8½	550	90	460	
6	Linolenic acid	7.6	6½	40	26	14	Neutral or alkaline systems
7	Mixed acids	7.4	7	62	35	27	
8	"	7.6	7	830	425	405	
9	"	7.6	4	180	90	90	Uptake complete in all cases
10	"	7.6	3	325	165	160	
11	Linoleic acid	7.4	5	250	132	118	
12	Lecithin	7.6	6½	732	390	342	

It is important, however, to consider in greater detail the behaviour of the system GSH + unsaturated fatty acid at p_H 7.4–7.6. Meyerhof states that at the neutral point the oxidation of fat under the influence of thio-glycollic acid is completely inhibited. With reduced glutathione this is not the case. I have found as the result of a number of observations that within a wide range of SH concentrations the oxygen uptake of the fatty acid, or its sodium salt, is equal, or approximately equal, to the amount of oxygen required to oxidise the SH. In other words, when the reaction ceases, the total uptake proves to be twice the oxygen equivalent of the SH present. To show this by the use of the Barcroft apparatus, it is necessary to work with relatively large concentrations of GSH. If small amounts are used, such as are ample for the demonstration of the more strictly catalytic effect at p_H 3.5, the total uptake at 7.6 becomes too small for accurate measurement, though the relation in question may nevertheless be indicated (Table II, Exps. 6 and 7). Moreover, unless the equilibration of the instrument at the beginning of the experiment is carried out with the flasks filled with nitrogen (oxygen being admitted only after equilibration), some oxidation of the SH group may occur before the system is, so to speak, properly established. This error does not count for much when the total amount of glutathione is sufficiently large. The figures of Exps. 6–12 of Table II show that the relation mentioned above holds within the limits of experimental error at p_H 7.4–7.6. It is the familiar relation found so often when the autoxidation of one molecule induces the oxidation of a

second non-autoxidisable molecule. The SH group is, in neutral or slightly alkaline systems, no longer an oxygen carrier in the sense that Meyerhof postulates for acid systems. It would seem as though its case becomes one represented by the first of the alternative schemata which have been so familiar from the publications of Engler and his colleagues; A being an Autoxidator, and B an Acceptor.

The first is an induced or coupled oxidation; the second is a catalysed oxidation. Whether it be justifiable or not to claim that the change in hydrogen ion concentration actually changes the type of reaction from (2) to (1), there is no doubt that in neutral or slightly alkaline solutions the oxidation of the SH group induces a limited oxidation of the unsaturated fatty acids, and, at the same time the figures obtained strongly suggest that equipartition of oxygen actually occurs. Such cases are at any rate common. The simple schema (1) above may not, of course, properly represent the details of the reaction. Without knowledge, however, as to what happens at the unsaturated linkages of the fatty acids as the result of the limited oxidation in question (it is not necessarily the same as what occurs in more acid systems), it is impossible to say whether or not there are chemical grounds against the above assumption.

It is at any rate of some interest to know that the oxidation of fats is influenced by glutathione at hydrogen ion concentrations near to that of the tissues as well as at others more remote.

The figures of Table II and the curves of Figs. 2 and 3 show plainly the effect of change of p_{H} on systems comprising GSH and fatty acids.

Fig. 2. Showing effect of p_{H} upon the oxygen uptake of mixed fatty acids from linseed oil in presence of GSH. Prolonged uptake at 3.8; short uptake at 7.6. Acids 10 mg. in emulsion. Oxygen equivalent of SH present 182 mm³. The fatty acids by themselves showed no uptake

Fig. 3. Oxygen uptake of fatty acids with varying amounts of GSH at p_H 7.6. The horizontal lines mark the oxygen equivalent of the SH present in each case.

It is perhaps desirable to refer here to a point in the technique used for these experiments which might seem to make it inadequate. When, in any combination, fat emulsions are shaken with air, the degree of dispersion of the fat phase might be expected to prove a factor in the velocity of oxygen uptake so dominant as to obscure the effect of other variants. This is not the case. In practice it has proved easy to reproduce any given experimental result, including even a velocity curve, without any special effort to standardise the emulsion, save in respect of the amount of fat present in it and the p_H of the system. Once the preparation is measured into the flask of the Barcroft apparatus, the shaking of the instrument maintains dispersion of the fat, and when other conditions have been kept constant, it has proved easy to obtain accurate reproductions of a given result on successive occasions, without giving more than ordinary care to the rate of shaking. When in the experiments described emulsions at p_H 7.4–7.6 were under study, small quantities of sodium carbonate were used to adjust the reaction and sodium salts were therefore present from the first. In this case also smooth and reproducible curves of uptake were obtained. It is remarkable that in such systems the surface relations should appear to play a relatively minor part in determining the velocity of oxidation.

It is clear that the significance of the results so far described is in any case quite independent of the absolute velocity of oxygen uptake. The curves of Figs. 2 and 3 satisfactorily illustrate however the complete change of type which the oxidising system undergoes on change of p_H .

In observations dealing with the oxidation of linseed oil emulsions, to which reference will be made immediately, changes of velocity during the course of uptake are assumed to be of chemical significance. I could find indeed no evidence whatever to suggest that changes in the grade of dispersion or other non-significant factors played any part in them.

In parenthesis, it may be here remarked that although minute traces of iron (such as were doubtless present in the preparations used) may be necessary for the autoxidation of the SH group of glutathione, none of the results described is brought about by the direct effects of the iron as such. If (as I have repeatedly found) any previously active preparation of the reduced dipeptide

be oxidised by aeration to the disulphide form, it then induces no observable oxygen uptake by the fatty acids, although of course its iron content is intact. An ionised salt of iron will doubtless in weakly acid systems catalyse the oxidation of linolenic acid at concentrations which, in an absolute sense, are low; but to produce an observable effect the concentration must be much larger than that introduced with the glutathione into the systems studied. At p_H 7.4–7.6 iron by itself fails to promote oxidation of the fatty acids, or their alkali salts, and the point does not arise.

It is a remarkable fact that the course of oxygen uptake by the glycerides of linoleic and linolenic acids, under the influence of GSH, differs in several particulars from that exhibited by the free fatty acids or their sodium salts. This statement is based upon experiments done with the purest obtainable specimens of linseed oil.

Fig. 4. Uptake of linseed oil (20 mg. in emulsion) with GSH at p_H 3.5. Left-hand curve with 12 g., middle curve with 8 mg. GSH. Right-hand curve 8 mg., but with oil first boiled *in vacuo*.

In acid systems (p_H 3.5–4.0) the curve of uptake of the system (GSH + linseed oil emulsion) shows a marked "period of induction," during which the system is almost completely inert. This period generally lasts from 1–3 hours, being diminished, but never abolished, by increasing the concentration of GSH. It is diminished by previous aeration of the oil, and lengthened if the oil be first boiled *in vacuo* (see curves of Fig. 4). These phenomena are of course familiar from studies concerned with inorganic "driers," or "siccatives," used technically to accelerate the drying of linseed oil in paints and varnishes. The fact that the progress of oxygen uptake during the drying of the oil yields the sinuous time curve (with its period of induction) characteristic of an auto-catalytic reaction has been established by many observers. Evidence suggests

that the autocatalyst involved is an autogenous peroxide which when the fat is aerated alone only very slowly reaches an effective concentration. Rideal [1919], Coffey [1921], and others have shown that the period of induction persists, though much shortened, in the presence of driers. Agents which promote the oxidation of linseed oil may therefore be "pseudo-catalysts" serving to assist in the formation of (or in maintaining the concentration of) the autocatalytic peroxide. The curves of Fig. 4 obtained at p_H 3.5 suggest that at least when glycerides are concerned the thiol compounds come into the same category¹. I have never succeeded in obtaining evidence of an induction period in the case of the free fatty acids, however treated, or at any p_H . If Meyerhof's view, as stated above, concerning the oxidation of the free acids by SH compounds be correct, and in acid systems it seems fully justified, it would seem that the mechanism of oxidation is actually different in the case of the fatty acids, or their sodium salts, and that of their glycerides, respectively. I cannot find from the chemical literature that any study of the effect of ordinary driers upon the free acids has been made².

At p_H 7.4–7.6 linseed oil again shows a behaviour which is strikingly different from that of free linoleic and linolenic acids.

When an emulsion of the oil is shaken in the presence of relatively high concentrations ($M/100$ – $M/50$) of GSH, in a neutral or weakly alkaline medium, the equipartition of oxygen so characteristic of systems containing the free fatty acids is not seen. The curve of oxygen uptake assumes a characteristic form, which has shown itself constant in a great number of observations (Fig. 5). There is no period of induction. The high initial velocity falls off somewhat rapidly and gives place to an uptake with a lower velocity which remains exactly linear for several hours and until the fat is nearly all oxidised. The inflexion in the curve occurs when sufficient oxygen has been absorbed to correspond nearly (but not quite) with that required to oxidise the SH present. During the linear period the concentration of SH is therefore very low, a circumstance which has been repeatedly confirmed by the use of the nitroprusside reaction. It would seem that during the first stage of uptake the SH group of the glutathione is oxidised without any associated effect upon the fat. The impression is nevertheless given (though it is not necessarily correct) that an efficient concentration of the thiol group must survive long enough to secure the establishment of some condition in the system which is not found initially. When quite small concentrations (less, say, than $M/200$)

¹ If so their influence upon the unsaturated linkages must be somewhat different from that exerted when they are oxidising fatty acids. In spite of the general assumption it is not proven that the autocatalyst is necessarily a peroxide. The following observation has some interest. One of the samples of linseed oil used in my experiments was aerated alone in the form of an emulsion for long periods. After 12 hours it showed no tendency to autoxidise. After 20 hours however when a sample of the emulsion was transferred to a Barcroft apparatus it displayed straightway without any addition a slow but maintained uptake of oxygen. This was at once greatly accelerated on the addition of a little GSH.

² Armstrong and Hilditch [1925] have shown that the simple esters (of oleic acid) are much more resistant to the action of oxidising agents than is the free acid.

are present initially, the oxidising system may not be established. The SH is rapidly oxidised and all uptake may then cease.

The longer the induction period displayed by a sample of oil at p_H 3 to 4, the higher the concentration of SH required to establish an active system at p_H 7.6.

What then is the actual state of affairs during the highly characteristic linear uptake? Is the oil at this stage undergoing autoxidation, the period of induction having been much shortened by the conditions, and obscured by the preliminary oxygen uptake of the SH group? Or, is the efficient promoter of oxidation during the linear period a highly active compound formed by the traces of iron present with that very small residual amount of glutathione which is found to exist in the system during this later stage of the oxygen uptake?

The direct promotion of oxidation by iron cannot be ignored here, because, unlike that of fatty acids, the oxidation of linseed oil is definitely promoted by moderate concentrations of iron in neutral or slightly alkaline systems. It is sure, however, that the minute amounts present in the glutathione preparations used are not by themselves responsible for the phenomena under discussion, since when preparations of the dipeptide are oxidised by aeration they have no effect upon the system when added during any stage of the course of uptake, though the iron content is not altered.

In oil emulsion systems the use of cyanide to inhibit any possible influence of iron offers certain difficulties. The perfectly smooth curves obtained in its absence are apt to become irregular. It is certain, however, that cyanide does not prevent the oxidation of linseed oil under the influence of glutathione preparations at p_H 7.6. When special care has been taken to avoid any change in the p_H as the result of its addition, I have found that even with concentrations of cyanide so high as $M/12$ the effect has been relatively slight. Curves have been obtained precisely similar in form to those seen without cyanide, though usually the velocity of the linear period is somewhat lowered (Fig. 5).

I have obtained no evidence upon which to base any clear explanation of the behaviour of these glyceride systems when neutral or slightly alkaline; but the facts which indicate such marked differences in the behaviour of free fatty acids, or their sodium salts, and their glycerides, respectively, when undergoing oxidation (as emulsions) under the influence of an agent present in living cells, seemed to deserve record. It is of some biological interest to know that lecithin behaves like the former rather than like the latter.

(The data were obtained by the use of three different samples of linseed oil, a sample of pure linolenic acid from Kahlbaum—of which I possessed only a small quantity and could obtain no more—a supply of pure linoleic acid (Kahlbaum) and one of mixed acids, of iodine value 162, carefully prepared from linseed oil¹ and given me by Mr N. K. Adam.)

¹ It is clear that the observations upon linseed oil described in this section should be repeated upon purified individual glycerides.

V. OXYGEN UPTAKE (WITH GLUTATHIONE) OF LIPOID-FREE PREPARATIONS.

As the result of his studies with thioglycollic acid and cysteine, Meyerhof held that the induced oxygen uptake of a thermostable preparation of muscle after thorough extraction with alcohol and ether is so small as to lack significance. I have no doubt that this was mainly due to the hydrogen ion concentration of the systems he studied¹.

Fig. 5. Uptake of linseed oil with GSH at p_H 7.6 (upper curve). Lower curve the same in presence of $M/12$ KCN. The horizontal line indicates the oxygen equivalent of the SH present. The uptake remained strictly linear till the 15th hour when the observation ceased.

A preparation from washed muscle after very thorough extraction gives in the presence of glutathione at p_H 3.5 no uptake at all. At p_H 7.6, however, it displays an uptake which is never less than one-third of that of an unextracted preparation. It may, indeed, constitute a considerably larger fraction of the latter.

This noteworthy effect of change in p_H shows that the material oxidised in the extracted material is not residual lipoids. It bears also on the nature of the mechanism involved.

Two precautions in technique increase the uptake of such preparations. The first relates to the method of washing the original muscle. If, as recommended by Thunberg in his classical paper on hydrogen donors [1920], the chopped muscle be shaken with water in stoppered cylinders, it is essential that the first washing should be with as large a bulk of fluid as possible and that this should be quickly renewed. So long as the concentration of glutathione in contact with the muscle is appreciable (and most of the substance comes out in the first washings) shaking with air involves an oxidation² which appreciably reduces the uptake of a final preparation. Later washings than the first have

¹ Meyerhof [1923, p. 542] does not mention the p_H of his media in this section of his paper, but it is likely from internal evidence that the observations were made on the acid side of neutrality.

² The "fixed SH" group is partly oxidised.

much less effect. The second point in technique is to avoid oxidation due to peroxide formation in the ether in the residue undergoing extraction. In my own experiments thermostable muscle powders were extracted in a Soxhlet apparatus, first for 8 hours with absolute alcohol; then for a similar period with thoroughly dry ether, and finally for a few hours with alcohol again. The ether is thus removed before the preparation is taken out of the Soxhlet tube. The alcohol is removed in a vacuum desiccator.

In Fig. 6 will be found typical illustrations of the oxygen uptake at p_H 7.6 of lipid-free preparations obtained as above. None of them showed any appreciable uptake in the absence of glutathione. It should be observed that all the curves of Fig. 6 were obtained by adding the glutathione in its disulphide form. This means, as will be later more fully demonstrated, that the extracted, lipid-free tissue retains intact the ability to reduce the disulphide, a circumstance which together with the special influence of the hydrogen ion concentration already mentioned becomes of importance when the nature and mechanism of oxidation in the muscle residue are being considered.

Fig. 6. Uptake of lipid-free muscle preparations (0.2 g.) at p_H 7.6 with G_2S_2 ($M/200$). The dotted curve was obtained from the preparation which originally yielded the middle curve. It had been kept at room temperature for 3 weeks.

It will be later shown that the oxidation involves the proteins of the tissue. As however most of the observations were made upon muscle before this fact was known, it will be convenient to ignore it for a moment, while some experimental results are discussed in terms of the muscle residue as such.

The oxidation of the fat-free material which is induced by contact with glutathione depends in a remarkable way upon the existence in the former of what was described by Dixon and myself [1922] as the "fixed SH group." It is especially characteristic of muscle as a tissue that after the whole of the soluble glutathione has been removed from it by thorough washing, the insoluble residue still gives a strong nitroprusside reaction. It may perhaps be said that no absolute proof has been given that this reaction is, as a matter of fact, due

to an SH group. By a process of exclusion, however, and more particularly by consideration of certain facts dealt with in this paper, the assumption will be found to be justified. According to Abderhalden and Wertheimer [1923] the intensity of the reaction is increased in rigor mortis and diminished by denervation of the muscle during life.

It is essential to any understanding of the oxidations controlled by glutathione, that certain facts concerning the fixed SH group¹ should be fully described. They are curious and necessitate a somewhat detailed discussion.

The fixed SH group is resistant to oxidation by molecular oxygen. Washed muscle or a powdered thermostable residue giving a strong nitroprusside reaction may be suspended in water, or a buffer solution, at p_H 7.6 and aerated for many hours without any appreciable change in the intensity of the reaction. When aerated, however, under conditions otherwise similar but in the presence of glutathione (GSH, or G_2S_2 ; 50–100 mg. per g. of tissue) the colour reaction progressively diminishes, till after 5 or 6 hours at 37° no reaction is given either by the tissue or the fluid in which it is suspended.

Moreover, the tissue, even when under strictly anaerobic conditions, can with equal ease be deprived of its power to yield the nitroprusside reaction. If it be suspended in an aqueous solution of oxidised glutathione at p_H 7.6 in a fully evacuated vessel the reaction is found progressively to disappear from the tissue, while, under these anaerobic conditions, the solution, which of course gives none at the outset, comes to display an intense reaction.

It is important, however, to make it clear that in this phenomenon equilibrium relations are displayed. The transference of the colour reaction (to use for the moment a non-committal phrase) from tissue to solution may cease while it is incomplete. Before the tissue ceases to give any observable reaction, it must have been exposed to G_2S_2 not only for a sufficient time, but to a solution of sufficient volume and concentration.

The following describes an observation, typical of many, illustrating the above-mentioned facts. 1 g. of thermostable muscle powder giving a strong nitroprusside reaction was placed in a vacuum tube with 20 cc. of $M/100$ G_2S_2 . The tube was evacuated and allowed to stand, with occasional shaking, for 3 hours at 37°. On opening the tube the fluid was found to give an intense reaction, whilst a sample of the tissue after thorough washing yielded one which was still definite, though relatively slight. The tissue and fluid were then returned to the vacuum tube which was again exhausted. No further change occurred; the system was already in equilibrium. The original fluid was then replaced by a second quantity (10 cc.) of G_2S_2 solution. After 2 hours the solution gave a slight reaction, whilst the washed tissue now gave no reaction strong enough for observation.

A tissue preparation which, as the result of the treatment described, gives no observable nitroprusside reaction again yields an intense reaction after contact with solutions of thiol compounds in sufficient concentration. This restored colour reaction, like the original, is still displayed with undiminished intensity after the most thorough washing of the tissue with water.

¹ The term is not a particularly happy one, but it is convenient in discussion. It has proved necessary to use it very frequently in this paper.

Effective in producing the above result are solutions (*circa* $M/10$) of reduced glutathione, cysteine, and thioglycollic acid. As will be later shown, the intensity of the reaction as thus restored increases up to a limit with the concentration of the SH solution to which the tissue has been exposed. There is no doubt that a "fixed" SH group is in this way restored to any tissue preparation which by contact with the disulphide form of glutathione has previously lost this factor. Like that in the original tissue, the restored group is, as already stated, quite irremovable by washing. It is resistant, like the former, to direct oxidation, but disappears in like manner as the result of contact with solutions of G_2S_2 . By repeating the treatment just described it may be again restored. A fact of special note is that a muscle preparation which on the loss of its original fixed thiol group ceases to take up oxygen in the presence of glutathione is, after the restoration, capable of a renewed uptake greatly in excess of the oxygen equivalent of the SH restored. This matter is fully dealt with later.

An endeavour has been made to throw further light on the nature of the phenomena just described. On reading the above description, or on observing the phenomena for the first time the impression might be left that the thiol compound used to restore the SH group is merely adsorbed by the tissue residue; or—since the "restored" group in the latter withstands all washing—that it has become fixed at some special surface in the muscle. If this view were justified, it might be further assumed that the original nitroprusside reaction of washed muscle is due to "fixed" glutathione. The distinction between the soluble dipeptide, which can be so readily washed out, and the molecule, or structure, carrying the SH group, which no amount of washing affects, is doubtless sharp. It must be remembered, however, that the muscle fibre is heterogeneous. While the greater part of the glutathione may be simply dissolved in the fibres, a part might be fixed owing to the properties of a special surface. When by contact with solutions of G_2S_2 the nitroprusside reaction disappears from the tissue and reappears in the solution, it might be because the disulphide form, when in sufficiently high concentration, is able to replace the thiol form (or another thiol compound) at the surface in question. Such a view is perhaps unlikely, but I find as a result of discussing the facts with others that it is necessary to consider it. It can easily be disproved, however.

A tissue preparation which has lost its nitroprusside reaction as the result of contact with a solution of G_2S_2 will again show a reaction upon treatment with sulphites. The best result is got by warming it with sodium hydrosulphite. The reaction is never so intense as when it is restored by exposure to solutions of GSH, etc., but it is similarly removed by fresh contact with a G_2S_2 solution which then itself shows a typical reaction. Hydrosulphites induce colour changes in nitroprusside solutions, but give no reaction which could possibly be confused with that of the SH group. When a nitroprusside reaction has been restored to the tissue by exposure to a thiol compound and when the

reaction is found to be transferable to a solution of G_2S_2 , it is easy to show that this is not because the latter liberates the thiol compound from some previously established association with the tissue. Clear proof can be obtained by the use of thioglycollic acid. This substance is extracted from acid aqueous solution by ether, but can be easily removed from the ether by shaking with a small volume of alkaline water. In the circumstances of the experiments under description, it is therefore easy to decide whether it be or be not present in a G_2S_2 solution. Neither form of glutathione is soluble in ether.

The nitroprusside reaction was restored to a muscle preparation (2.0 g.) by exposure to a thioglycollic acid solution ($M/10$) at p_H 6.8 for 3 hours. After the usual thorough washing, it was suspended anaerobically in 10 cc. of G_2S_2 solution ($M/50$, p_H 7.4) for 3 hours at 37° . The solution, which then showed an intense reaction, was made slightly acid with sulphuric acid and shaken with 10 times its volume of ether. The ether after filtration through three dry filter papers, to remove traces of the aqueous phase, was then shaken with 10 cc. of water made slightly alkaline. The extract, after filtration through two wet filter papers to remove traces of the ether phase, was tested for the nitroprusside reaction. In spite of its extreme delicacy the reaction was entirely absent. At least 8 mg. of thioglycollic acid must have been present, were this substance responsible for the amount of SH in the solution; whereas in a control experiment, when 3 mg. only were added to the same bulk of G_2S_2 solution and the above processes applied, the aqueous extract from the ether gave an intense nitroprusside reaction. This experiment has been many times repeated; always with the same result¹.

It is clear that in the experiments just described the observed transference of the nitroprusside reaction from tissue to solution could not have been due to the replacement of adsorbed thioglycollic acid. Now the general properties of the fixed SH group are the same in the original washed muscle, and in preparations to which it has been restored by contact with solutions of thioglycollic acid, cysteine, or reduced glutathione.

It is sure, therefore, that the phenomena under discussion are not due to interchange between molecules in solution and molecules fixed under reversible conditions in the tissue. The only alternative is to recognise that they involve actual oxidation and reduction. The carrier of the fixed SH group in the tissue must be able to reduce glutathione when, as a disulphide, it is present in solution, and itself undergo oxidation by the latter. On the other hand, the tissue factor when oxidised (presumably to a disulphide form) can upon change of concentrations be reduced by thiol compounds in solution. It must, of course, be remembered that the molecular associations of the reacting sulphur groups are not the same. The result of such reversible relations must be the establishment of equilibria depending upon the relative concentrations of SH and S-S in solution and the activity of similar groups in the solid phase.

With reversible reactions occurring in a heterogeneous system of this kind equilibrium conditions must be complex, and their quantitative study (especially having regard to the nature of the materials) by no means easy. Some estimations dealing with the exchange of the SH group between the solution and the tissue have been made, however.

¹ I owe to Mr D. C. Harrison the suggestion that the properties of thioglycollic acid would make the above test available. When it is used as above to reduce a tissue preparation, prolonged washing is required for the removal of adsorbed traces.

Oxidised tissue preparations giving no nitroprusside reaction were exposed to solutions of cysteine, reduced glutathione or thioglycollic acid in varying concentrations. By means of titrations with iodine ($N/100$) the diminution in the SH group of the solutions was determined. Since the concentrations were sometimes high and the change relatively small, estimations by difference were not satisfactory. Moreover, when the observations are made at p_H 7.6, the thiol compounds are apt to oxidise during the processes of measurement, etc. The tissue preparations were therefore first reduced by anaerobic exposure to a solution of the thiol compound and after thorough washing were suspended, also under anaerobic conditions, in a solution of the disulphide form of glutathione, sufficient in amount to secure complete oxidation of the SH group in the tissue. As the amount of SH which then appears in the solution is proportionate to, and, as will later be pointed out, probably identical with that previously in the tissue, direct titration of the solution gives the information required. The following are typical observations.

A thermostable muscle powder in quantity was oxidised until it gave no trace of a nitroprusside reaction, and was then washed, dehydrated with alcohol, and re-dried in a desiccator. 1 g. was placed in each of a series of vacuum tubes containing 5 cc. of aqueous solutions of thioglycollic acid of varying concentrations (see below). The acid was neutralised with sodium carbonate (p_H 7.6). After 3 hours in evacuated tubes at 37° , the tissue residues were filtered off and very thoroughly washed, until the washing gave no trace of a nitroprusside reaction. Each lot was then placed in a fresh vacuum vessel and covered with 20 cc. of G_4S_2 solution ($M/50$). The tubes were evacuated and stood with occasional shaking for 3 hours at 37° . They were then opened and their contents immediately made slightly acid with sulphuric acid to stabilise the SH group. The tissue residues were then filtered off and washed, and the filtrates titrated with $N/100$ iodine; starch being used as an indicator. The results were as follows:

Concentration of the thioglycollic acid solutions	$M/100$	$M/50$	$M/20$	$M/15$	$M/10$
cc. of $N/100$ I_2 required ...	0.95	1.90	3.3	3.7	4.7

Although the titration figures in this experiment are small, they are reliable and consistent with the results of other experiments of the same type. They lie on a smooth curve and when cysteine or glutathione was used to reduce the oxidised tissue similar curves were obtained.

In one experiment G_2S_2 solutions of different concentrations were placed in contact with equal weights of muscle powder (containing its original fixed SH) and, after equilibrium was established anaerobically, the GSH in solution was determined, as before, by iodine titration.

	$M/100$	$M/50$	$M/50$	$M/10$
Volume and concentration of G_2S_2 in contact with 1 g. muscle residue	5 cc.	5 cc.	10 cc.	5 cc.
cc. of $N/100$ I_2 required ...	2.4	3.1	3.4	4.2

The figures of these experiments are reproduced as they demonstrate clearly the existence of equilibria between glutathione in solution and sulphur groups in the insoluble muscle residue. No more than this is claimed for them.

The important influence of the fixed SH group upon the oxygen uptake of the lipid-free muscle residue now calls for discussion. There is no uptake

without contact with glutathione in solution; there is also no uptake without the existence of the SH group in the tissue itself. Seeing then that the disappearance of the latter is so characteristic an indicator of oxidation by G_2S_2 , the suggestion might arise that its oxidation is responsible for the whole oxygen uptake. This however as will be shown immediately is certainly not the case.

On the other hand, it seems likely that the SH group is the only reducing agency left in the thermostable residue, and probably the only factor, even in muscle which has been simply washed, which reduces glutathione. Tunncliffe [1925] found that the oxygen equivalent of the SH which appears in G_2S_2 solutions after reduction by 1 g. of thermostable muscle powder is of the order of 140–150 mm.³. Such figures I have frequently obtained. When, however, the original muscle has been washed with the precautions mentioned at the beginning of this section, and when (remembering the equilibrium relations) care is taken to see, by obtaining a negative nitroprusside test, that the tissue is completely oxidised¹, the above value comes nearer to 200 mm.³. I do not find that it is altered by removal of the lipoids if care be taken to avoid oxidation during extraction by ether. Now, if one estimates by iodine titration, with such accuracy as is possible, the oxygen equivalent of the SH which disappears from a strong GSH solution ($M/10$, or higher) when employed to restore the SH group to 1 g. of an oxidised preparation, it again proves to be of the same order. That the SH group alone is the essential reducing factor in the thermostable muscle residue, and that the above data give therefore a measure of its influence become nearly certain when the phenomena under discussion are found as will be immediately shown to depend upon the properties of protein.

If the thiol group be the sole reducing factor acting in the system constituted by the muscle residue and glutathione, it is certain that the capacity for oxygen uptake of the system is greatly in excess of the 200 mm.³ per g. dry tissue which represents the oxygen equivalent of that group. The former, as we have seen, is of the order of 800–1000 mm.³ per g. or even more. Moreover, it is limited to this amount solely because during the oxygenation of the system under the experimental conditions the fixed SH becomes itself oxidised long before other processes of oxidation which it promotes, and for which its presence is necessary, are complete. If a muscle residue, which has been oxidised by G_2S_2 on lines which have been fully discussed, so that it no longer gives a nitroprusside reaction, and, in consequence, has ceased to take up oxygen, be again completely reduced by contact with a strong solution of GSH, etc., and thoroughly washed, it is found to display a fresh uptake which may be of the order of 2000 mm.³ (in the case of lipoid-free preparations) per gram. The amount of this uptake depends upon the concentration of the thiol solution to which the preparation has been exposed; that is, upon the

¹ Szent-Györgyi [1925] assumes that the fixed SH is not oxidised by G_2S_2 . He evidently did not observe that the reaction comes to an equilibrium and in any case takes time to complete.

extent to which it has been reduced and its fixed SH group restored. The above figure is obtained after contact with GSH in solutions of $M/10$, or upwards. After this second uptake has ceased, fresh reduction again induces a fresh capacity for the absorption of oxygen (always necessitating the presence of glutathione) which may again amount to a figure of the above order! It will now be understood that the actual uptake of a lipoid-free muscle residue as first prepared will depend upon whether its fixed SH group is at a maximum or not.

The following table illustrates the nature of the quantitative data which have been obtained during a study of the phenomena in question. Preparation A was a fat-free thermostable powder, prepared without special precautions and used some weeks after its preparation. In the preparation of B, an otherwise similar powder, care was taken at each step to avoid oxidation, and it was used immediately.

Table III.

Thermostable preparations from rabbit's muscle, extracted with alcohol followed by ether. "A" a stock preparation, "B" a preparation made with careful avoidance of oxidation (see text) and used when fresh.

Treatment of material before determination of uptake	Total O_2 uptake when shaken in G_2S_2 solution ($M/200$) at p_H 7.6; mm. ³ per g.	
	A	B
Original preparation before removal of lipoids	2100	—
Extracted (alcohol-ether-alcohol)	710	1020
Oxidised aerobically with G_2S_2 till free from nitroprusside reaction	± 40	0
Reduced in GSH solution ($M/10$)	1010 +	1850
Oxidised anaerobically with G_2S_2	0	—
Reduced in cysteine solution ($M/10$)	1810	—
Oxidised aerobically and then reduced by thioglycollic acid ($M/10$)	—	2010

V a. UPTAKE OF PURE PROTEINS.

It will now be shown that the special characters of the fat-free muscle residue which have just been discussed are due to the properties of its proteins, and that the oxidations which occur in it involve apparently no other materials.

Earlier observations bear upon the matter, but I think the significance of the known facts is much increased in the light of the observations to be described.

Arnold [1914] observed that muscle proteins give a nitroprusside reaction, though at the time it was somewhat of an assumption that this was due to the proteins as such. Especially intense, he found, was the reaction in the case of the least soluble proteins (the "myostromin" of Danilewski). The proteins of blood plasma and those of "excretions and secretions" gave no reaction. Heffter [1907] had previously shown that blood proteins, serum globulin at

any rate, can be made to yield a reaction on treatment with sodium sulphite. Gelatin, caseinogen, egg-globulin and fibrin did not display this property.

Heffter showed that egg-albumin, which gives no reaction in its natural state, acquires the power to do so after denaturation. This remarkable accompaniment of denaturation formed the subject of an extensive study by L. J. Harris [1923] in this laboratory. He advanced some suggestive hypotheses in explanation.

Abderhalden and Wertheimer [1923] have made the interesting observation that when egg-albumin is coagulated by heat, and shows in consequence a nitroprusside reaction, it can form (with cystine) a system which takes up an appreciable amount of oxygen (45 mm.³ per g. per hour). The total uptake, however, is, I find, very small.

The nitroprusside reaction as conferred upon certain pure proteins when, as coagula, they are brought into contact with strong solutions of thiol compounds is very much more intense than can be produced by denaturation processes alone, even in the case of ovalbumin, which among native proteins offers the only case of a marked reaction developing on simple coagulation by heat. The proteins of blood serum which develop no trace of a reaction on denaturation, acquire one of peculiar intensity when afterwards treated with GSH in high concentrations. The amount of the reactive SH group thus established in the protein complex varies with the nature of the protein as will be immediately shown. It seems to be a definite characteristic of each individual protein.

In every case the group when established has the properties described for it in the case of the muscle proteins. That its appearance is due to a reduction in the protein complex, and not to any form of adsorption of the thiol compounds used to produce it, can be demonstrated on the lines already described. It is highly resistant to oxidation by molecular oxygen in the absence of glutathione or its equivalent, whilst it is oxidised by G₂S₂ (and of course reduces the latter) under the conditions defined above.

The establishment of the SH group in its molecule determines in every susceptible protein a capacity to take up oxygen in amount which, if not high in a chemical sense, is high in relation to the phenomena which are under review. It is indeed not yet sure what are the limits of the oxidation involved.

Experiment has shown that, as already stated, the maximal production of the SH group on reduction varies from protein to protein, but the data obtained have not yet been sufficiently controlled to give them a strictly quantitative value. The matter is under study. To take one example: after treating coagula from (a) mixed serum proteins wholly free from lipoids, (b) pure crystalline egg-albumin, and (c) crude fibrin, with *M*/10 solutions of GSH or thioglycollic acid, I found that the oxygen equivalent of the SH group established in (a) (determined as described above) was of the order of 300 mm.³ per g. protein, of that in (b) 160 mm.³, and of that in (c) at most 60 mm.³. It is perhaps not sure that really pure fibrin displays the phenomenon at all.

Gelatin is incapable of developing an SH group. No protein or protein preparation which fails to exhibit a nitroprusside reaction shows any tendency to reduce solutions of G_2S_2 .

The oxygen uptake of the reduced proteins in the presence of glutathione at p_H 7.6, while more or less proportionate to the amount of SH established, is, as already stated, greatly in excess of the actual oxygen equivalent of this. The specimen of mixed serum proteins just referred to, prepared free from lipoids by the ice-cold alcohol method of Hardy and Gardiner [1910] was exposed in powdered form to strong solutions of reduced glutathione and thioglycollic acid, respectively ($M/5$ in each case). Each portion was then washed with water till no trace of nitroprusside reaction was given by the washings (whenever thioglycollic acid is used this process is prolonged) and dried in a vacuum desiccator.

The oxygen uptake of each portion in the presence of G_2S_2 was then determined at p_H 7.6 in a Barcroft manometer; the two instruments being shaken side by side. (There was no uptake alone, and none with glutathione at p_H 3.5–4.5.) The uptake at p_H 7.6 was exactly the same in each case, suggesting that the maximal reduction had been produced. The figure obtained was 2350 mm.³ per g. protein. In Fig. 7 the curves of uptake in this experiment are given to show how definite is the condition established, and also to show that, when properly standardised, the Barcroft apparatus gives highly consistent results. The two curves are practically superimposable.

Fig. 7. Uptake of lipid-free serum proteins (0.2 g.) after coagulation and reduction with GSH and thioglycollic acid; suspended in G_2S_2 $M/200$; p_H 7.6.

But it was easy, as in the case of the lipid-free muscle residue, to show that the oxidation of the protein stopped at the above figure only because the fixed SH group was exhausted. Freshly reduced by GSH it showed a second uptake of nearly 2000 mm.³. Reduced a third time it showed a further uptake

nearly as large, each oxidation involving, of course, the simultaneous influence of glutathione (G_2S_2) in solution. I have not yet determined what is the limit to these successive processes. If each successive uptake corresponded only to the oxygen equivalent of the SH group as originally present, or as re-constituted, the significance of the facts would be less. It is, as a matter of fact, at least ten times as great. If therefore it should prove that the processes of alternate reduction and oxidation can be yet further extended, the final amount of oxidation involved must be great enough to produce an effect upon the protein such as should be easily detected. This matter is being fully investigated¹.

One other aspect of these curious relations must be emphasised. In order that the various proteins should be reduced by contact with thiol solutions they must be in the form of a solid phase. If, *e.g.*, native serum be mixed with a high concentration of GSH and allowed to stand anaerobically, and if after some hours the mixture be diluted, the proteins being then coagulated by heat, filtered off, and quickly washed, the coagulum shows no trace of a nitroprusside reaction. None, moreover, is shown by the precipitate obtained by saturation of the mixture with ammonium sulphate, if it be first thoroughly washed by a saturated solution of the salt. The most probable explanation of this circumstance is that in the process of coagulation a molecular change occurs which is necessary to establish the group which is susceptible of reduction. If this be so, it may appear to reduce the biological significance of the facts. It must be remembered, however, that the proteins which are left in muscle after thorough washing and which constitute an important part of the permanent structure of the fibres, are susceptible of reduction in their native state. If muscle after simple extraction with water be aerated in the presence of G_2S_2 at p_H 7.4 it may be completely oxidised and deprived of its nitroprusside reaction. This is then normally restored by exposure to GSH solutions. It would be interesting to find that the less soluble among tissue proteins share some special character with blood-proteins rendered insoluble by denaturation.

VI. FACTORS CONCERNED IN THE OXIDATIONS DESCRIBED.

In Section III evidence was advanced to show that in the oxidation of fatty acids by glutathione the reaction changed in type with change of hydrogen ion concentration. In acid systems the thiol group acts essentially as an oxygen carrier, in neutral or alkaline systems its own oxidation involves the fatty acids in a coupled or induced reaction. Does the oxidation of protein conform to either of these types, or is it rather a process to be conveniently viewed from the standpoint of Wieland as one of hydrogen activation and transport? Some facts seemed to suggest this, but others offer difficulties. It must indeed be admitted that the ascertained facts are insufficient to make clear the nature of the phenomena.

¹ Mr A. Carruthers has found that as the result of four successive reductions and oxidations a sample of lipid-free serum proteins took up 10 cc. per g. The limit was not then reached however,

In discussing the evidence it will be convenient to deal first with systems consisting solely of protein and the oxidised or disulphide form of glutathione. Only proteins such as those of washed muscle which naturally contain an SH group, or denaturated proteins upon which an SH group has been conferred, suffer oxidation in such systems. It will be convenient to speak of these—for lack of a better term—as “reduced” proteins.

For a sustained oxygen uptake by unsaturated fatty acids, the conditions in the system must, as we have seen, be such as secure stability of the SH group (Section III). For the oxidation of protein, on the other hand, the conditions must favour the autoxidation of SH (Section IV). This distinction is fundamental. In each case the hydrogen ion concentration exerts a dominant action in determining these requisite conditions. In the latter case the presence of dissolved iron in minute amounts may be a necessary factor.

As the disulphide form of glutathione is reducible by the “fixed SH” throughout a wide range of hydrogen ion concentration, fat is oxidised in the presence of “reduced” protein at p_H 3·5, even though G_2S_2 , and not GSH, is initially supplied to the system. Experiments in proof of this will be described immediately. On the other hand, in systems containing reduced protein and G_2S_2 alone, there is no oxygen uptake at such a grade of acidity. The disulphide in solution is reduced until equilibrium is established with the SH in the solid protein, and the system then becomes inactive, exactly as though it were under anaerobic conditions. When the p_H is changed to 7·4–7·6, the oxygen uptake already described actively proceeds. It has been shown that this uptake depends upon the existence of the fixed SH group, and also that the total uptake is much in excess of the oxygen equivalent of this.

A significant circumstance in the behaviour of a system consisting of reduced protein (or a thermostable muscle residue) and G_2S_2 is that throughout the course of its oxygen uptake at p_H 7·6 the concentration of SH in solution remains very low. If the process be arrested at successive periods during the course of the uptake and a nitroprusside test applied to the contents of the flask of the Barcroft apparatus, the colour reaction in the protein is found to diminish progressively, whilst that in the solution is very faint throughout, even when the oxygen uptake is proceeding at its highest velocity. By the time, however, the nitroprusside reaction has become negative in both, the uptake ceases. The reason for the low concentration of SH in solution is clear. Compared with the rate of the oxidation of GSH at p_H 7·6 the reduction of G_2S_2 is a slow reaction. Conditions in the system adjust therefore to this relation. Oxidation follows reduction and the process is continuous, but because of the velocity relations the intermediate reduction product never reaches more than a very low concentration. The reduction process is much less affected by p_H than is the oxidation [Tunncliffe, 1925].

Now although the oxidation of protein by glutathione is so entirely inhibited at grades of acidity such as correspond to a p_H of 3·5–4·0, in the region of p_H 6·5–6·8 it proceeds, though at a velocity somewhat less than at 7·6.

In accordance with the relations indicated the SH concentration in solution is higher during the course of the oxygen uptake in the slightly acid system. At such a grade of acidity the rate of reduction is scarcely affected; that of oxidation is considerably slower.

All these facts seemed to justify the view that in the system now under discussion glutathione is an intermediate hydrogen acceptor. It would seem to share in a process of hydrogen transport of the kind pictured by Wieland and Thunberg. On these lines the smallness of its concentration in an active system offers no difficulties. It is indeed, as was indicated above, to be expected. This was the view put forward in previous communications in respect of the oxidation of washed muscle, or its thermostable residue, by glutathione. I had myself always pictured that there were constituents of the washed muscle oxidised on such lines; constituents, namely, which are capable of reducing the disulphide form; an essential for their oxidation on the lines conceived. It would now seem from evidence discussed in the last section, that the one essential reducing factor is the SH group present in the insoluble muscle proteins. When limited to this factor the above view certainly holds. But what is the mechanism of the oxidation in protein which, while certainly depending upon the presence of the fixed SH group, involves an uptake of oxygen ten times as great as that required to oxidise the SH group itself? It seemed during earlier stages of this enquiry as though the behaviour of the SH group in the muscle residue might only be, so to speak, an indicator, and that other agencies in the tissue also possessed reducing power. But when it was realised that the material oxidised was protein, it was further found that no protein reduces G_2S_2 unless it contains an SH group. Moreover, as was stated in the last section, Tunncliffe's quantitative studies, and data which I have since repeatedly obtained myself, show that when the muscle residue (or a "reduced" protein) is in contact under anaerobic conditions with solutions of G_2S_2 , the maximal reduction of the latter corresponds quantitatively with the reducing capacity of the fixed SH group. Nevertheless, aerobically, the oxygen uptake is ten-fold the oxygen equivalent of this. These results seem to preclude the possibility that the whole process of oxidation is one of hydrogen transport. Yet if oxidation, say at unsaturated linkages in the protein molecule, or the oxidation of structures other than the SH group, depends upon a function of SH in solution (akin, say, to that displayed in the oxidation of fatty acids) it is difficult to understand how it actively proceeds when the concentration of soluble SH in the system is so exceedingly low.

Faced with this difficulty it is perhaps legitimate to consider the following possibility, even if it be unlikely. In the process which results in the oxidation of protein by glutathione, the fixed sulphur group might be an additional intermediate hydrogen acceptor. While the SH in the protein reduces G_2S_2 in solution, it might be necessary, in order that the process should continue, that the fixed SH group should itself be again reduced by other factors in the protein which, while ultimately susceptible of oxidation, cannot directly reduce

G_2S_2 . Now, the latter reduction may be a much slower process than the former, and may not have been given time to display its effects appreciably in the anaerobic experiments described in the last section. Some of these were prolonged, however, and the suggestion is unlikely. Yet the following facts should be considered here. If washed muscle be thoroughly aerated in the presence of glutathione until it gives no trace of a nitroprusside reaction, it will upon heating in water to 100° again give a reaction which is quite intense, and it will now again reduce G_2S_2 though, quantitatively, much less effectively than when it has itself been reduced by contact with a thiol compound. The proteins behave like egg-albumin. This shows at least that the reconstitution of the fixed SH group may in some sense result from changes occurring within the solid (protein) phase of the system. But that this reappearance of the SH group does not strictly represent the process postulated above (accelerated by the higher temperature) is indicated by the fact that though it occurs immediately at 100° , and more slowly at 60° , it does not seem to occur, even slowly, at temperatures below the coagulation point of the muscle proteins. It involves denaturation, therefore. But, as was made clear at the close of the last section, the proteins of washed muscle do not need to be denaturated in order to show their normal large oxygen uptake. It is difficult therefore to see how the whole process of protein oxidation by glutathione can be entirely one of hydrogen transport. Provisionally at least, it seems we must look to the SH group in solution as the promoter of oxidation, maintained, as it is, in very low concentration, by the slow reducing action of the fixed SH group.

If, however, GSH be supplied to the system initially (instead of G_2S_2) there is no increase in the oxidation of the protein. What happens is noteworthy. If two Barcroft manometers be shaken side by side, each containing muscle powder and glutathione in Ringer's solution, but with GSH in one and G_2S_2 in the other (all concentrations and conditions being otherwise the same) curves of uptake such as those in Fig. 8 are (at p_H 7.6) consistently obtained.

The system containing GSH shows the higher initial velocity, but this must be due in the main to the independent oxidation of SH. For at a point when the excess of oxygen taken up by this system has become nearly (but not quite) equal to that required for the oxidation of all the GSH added, the two curves become parallel for the rest of the period of uptake. During this later period, which may be prolonged, each system contains the same exceedingly low concentration of SH in solution. This means that in both cases the oxidation of the protein is essentially dependent upon the fixed SH group as the agent which reduces glutathione. The reduction of the S-S form is, as we have seen, a slower reaction than the oxidation of the SH form and therefore finally determines the velocity of oxygen uptake.

Having dealt with the oxidation of fats and proteins separately, a few words may now be said concerning the behaviour of the original thermostable residue from muscle as used by Dixon and myself and afterwards by Meyerhof. Its oxygen uptake has received preliminary discussion in Section III. It is chiefly of interest from the fact that it contains both protein and fat, so that

Fig. 8. Uptake of lipid-free muscle powder at p_H 7.6. Upper curve with GSH, lower curve with G_2S_2 . The horizontal line by its position on the scale indicates the oxygen equivalent of the SH initially present in the former system, and the length of the vertical lines between the curves corresponds with this value.

Fig. 9. Uptake of thermostable muscle powder (0.2 g.) at p_H 7.6. Upper curve with GSH, lower curve with G_2S_2 . Indications as in Fig. 8.

Fig. 10. Thermostable muscle powder (0.2 g.) at p_H 6.0. Upper curve GSH, lower curve G_2S_2 . Horizontal and vertical lines show oxygen equivalent of GSH. See Fig. 8.

Fig. 11. Thermostable muscle powder (0.2 g.) at p_H 3.8. Upper curve GSH, lower curve G_2S_2 . Horizontal and vertical lines show oxygen equivalent of SH.

its uptake varies with changes in hydrogen ion concentration on lines that are instructive. It has been shown in previous sections that at p_H 3.5–4.0 fatty acids are continuously oxidised during the survival of the SH group, while no oxidation occurs in protein. At p_H 7.4–7.6 conditions present secure the peculiar oxidation of protein which has been so fully discussed, while fatty acids undergo only a limited oxidation. With change of p_H therefore different materials predominate in the oxidation and the total uptake may not be the same even with the same preparation. Variation in the fat content of preparations will also have a different influence at different hydrogen ion concentrations. These relations will be sufficiently illustrated by the curves of Figs. 9, 10 and 11, which show the course of oxygen uptake by a muscle powder at p_H 3.8, 6.0 and 7.6 respectively. In each case, the effects of adding GSH and G_2S_2 initially are compared.

The total concentration of glutathione was the same in each system. In one of each pair as compared the dipeptide supplied was partially (but not completely) reduced. The actual oxygen equivalent of the SH present initially is indicated in the figures.

At p_H 7.6 (Fig. 9) the curves are not different in type from those yielded by a lipid-free preparation (Fig. 8) though the total uptake may be higher. It will be seen that after showing a more rapid initial rise, the curve of uptake with GSH becomes parallel to that with G_2S_2 . The oxidation mainly concerns protein, and its velocity in each case is ultimately controlled by the rate of reduction of G_2S_2 by the fixed SH group (see earlier discussion). Hence the remarkably accurate parallelism which is established after the excess of GSH has been oxidised in the system which contained it initially.

At p_H 6.0 (Fig. 10) both fat and protein are oxidised in each system. The curves do not become parallel. In the system which initially contained GSH, an efficient concentration of the SH group is longer maintained and therefore more fat is oxidised than at 7.6. In that supplied with G_2S_2 the uptake depends as before upon the velocity of reduction, but in this more acid medium the SH concentration is again maintained at a higher level, and more fat is oxidised than in the alkaline system.

At p_H 3.8 the curves diverge still more widely. Fat alone is oxidised in either system. In the GSH system the thiol group is stable and fat is more efficiently oxidised than at p_H 6.0. In the G_2S_2 system the oxidation of the fat by SH is controlled by the slow reduction process¹.

These brief references to the curves will be sufficient to indicate the behaviour of systems which contain both "reduced" proteins and fats. The most significant aspect of their behaviour is seen in the influence of protein in determining the oxidation of fat in circumstances which do not secure the oxidation of the latter when it is alone; for instance, when the disulphide is

¹ The experiments yielding these curves have shown how entirely the total oxygen uptake of the thermostable residue (or of water-extracted muscle) depends upon circumstances (see Section III).

supplied to the system. Without reduction of this there is no oxygen uptake; and fats do not reduce it. But if the system, in addition to the disulphide, contains a muscle residue, or any protein containing the SH group, fat is freely oxidised. This has just been shown in the case of the thermostable muscle residue (Fig. 11, lower curve). The effect is more strikingly demonstrated when excess of fat, in the form of an emulsion, is added to a system consisting of the muscle residue, or a "reduced protein," together with G_2S_2 . At p_H 3.0-4.0 a large excess of oxygen is taken up by such a system as the result of adding free linolenic acid, linoleic acid or lecithin, the curve of uptake being linear if the lipid be in sufficient excess. The velocity, however, is controlled by the relatively slow reduction process. At p_H 7.6 when lecithin or a fatty acid is added to the same system there is an uptake greater than that displayed without them; but the excess is relatively small. This would be expected if the results of Section III are taken into consideration. On the other hand, the addition of glycerides (linseed oil) to the alkaline system results in that linear uptake which results when GSH alone is added to an emulsion. It continues, as in the latter case, with an existing concentration of SH which is exceedingly small (see p. 797). Fig. 12 shows the relation just discussed.

Fig. 12. Curves with continuous line show uptake of a thermostable muscle powder (0.2 g.) plus G_2S_2 with and without the addition of linseed oil; p_H 7.6. Dotted line curves, the uptake of a similar system with and without added lecithin but at p_H 3.5. Each pair of curves was obtained by the use of two Barcroft manometers shaken side by side.

GENERAL DISCUSSION.

The experimental studies described in this paper have yielded certain results which were unexpected. Some of the phenomena indeed seem to find no close analogy in the behaviour of chemical systems which are more familiar. It is somewhat surprising, moreover, to find, when a substance presumed to promote biological oxidations in muscle is in question, that its activity should

be more easily demonstrable in the case of fats and proteins than in that of carbohydrates or their derivatives. This may be of physiological significance; but it may be rather an accidental result of the particular experimental conditions which have been empirically chosen. The influence of glutathione in promoting oxidations, has, even in cases where its potency has been already demonstrated, shown itself highly sensitive to conditions, and the effect of these has not been by any means fully explored. The highly artificial preparations of the tissue which, in pioneer studies, have been perforce and justifiably employed, are, of course, without many factors which, in the living tissue, may exert a directive influence. One illustration may be mentioned. Miss Thurlow [1925] has shown that the oxidation of the SH group when it occurs in the presence of a peroxidase may induce other oxidations (*e.g.* of nitrites) which do not occur in the absence of this latter factor. The possibilities suggested by this observation are being explored in this laboratory but as the work is being done by others the results are not discussed in this paper.

It will serve the purpose of a brief general discussion of the results obtained in the present research if a comparison be made with those described by Meyerhof in his classical paper on the subject [1923].

Meyerhof was the first to observe the oxidation of lipoids (lecithin, linolenic acid) under the influence of a thiol compound (thioglycollic acid). He was inclined to think that this oxidation might be the only one induced by glutathione in muscle, though in the closing words of the paper just referred to he disavows any finality in that opinion.

He found that the oxidation of the lipoids occurred in acid systems (most effectively at p_H 3.0–4.0) and upon the behaviour of such systems he based his view as to the mechanism of the oxidation by SH. He did not observe the oxidation of fats (limited but quite significant) that occurs at hydrogen ion concentrations nearer to that of living tissues (Section IV). Nor did he observe the oxidation of protein which has been described in this paper. In an earlier publication [1918] he was inclined, it is true, to attribute to the oxidation of protein part of the uptake of oxygen displayed by bouillon to which thioglycollic acid had been added. In his later work [1923], however, he rejected this view on finding that serum proteins when thoroughly freed from lipoids showed no uptake. This observation was undoubtedly correct. The native blood proteins show no uptake whatever under the influence of glutathione; but this, as will be understood from facts advanced in previous sections, is because they lack what has been termed throughout this paper "a fixed SH" group. It is of interest indeed to note that the proteins of the fluids of the body, and the albuminoids of connective tissues generally, are immune from the type of oxidation suffered by the proteins of such an active tissue as muscle. They are not exposed to the influence of glutathione, which appears to be absent from the plasma, nor are they exposed to influences which establish in them the second factor necessary for the oxidation in question, namely, the fixed SH group.

But Meyerhof also found that muscle preparations when deprived of all lipoids showed no more than a slight and non-significant oxygen uptake with thioglycollic acid or cysteine. He could not therefore attribute an oxidation to muscle proteins, whereas, as has been shown in previous sections, their oxidation by glutathione is a definite and quantitatively important phenomenon.

The difference between Meyerhof's results and my own is not difficult to explain, however. In the first place the proteins are not oxidised at all in acid systems. Now although it happens that in reporting the results obtained with alcohol-ether extracted preparations [1923, Table IX, p. 542] he does not specify the p_H employed, it is probable, from what comes earlier in his paper, that the systems studied by him were acid. If so, his negative results are fully explained. There is probably a further reason for them, however. If the facts have been made clear in the discussion of Sections Va and VI it will be understood that the oxidation of proteins by glutathione unlike that of fats in acid systems, depends upon a succession of oxidation and reduction. That the SH group in solution should be progressively oxidised is essential; but when in any system containing a fat-free muscle residue (or any "reduced" protein) is supplied with SH in excess of a certain low concentration, the excess is (at p_H 7.6) rapidly and (with respect to the protein) ineffectively oxidised. Only when the oxidation adjusts to a slower reduction process; only that is when the properties of the fixed SH group control the velocity of change (Section VI, p. 809) is the effective mechanism for protein oxidation established. Now I have found that neither thioglycollic acid (iron-free) nor cysteine is so efficient as glutathione in establishing such a system. Those who are familiar with Meyerhof's paper will remember that his own experimental studies finally led him to attach a predominant importance to the function of the SH group as an oxygen carrier (being especially active therefore in systems where its concentration is maintained) rather than to the circumstance that it is a reducing agent easily oxidisable to an S-S group, from which it is again easily reproduced by reduction. It was these properties which determined my own previously published views as to the importance of glutathione as a carrier of hydrogen. Nothing, I think, is now more sure than that both views may be correct. With change of conditions glutathione from being an oxygen carrier (oxidation of fatty acids in acid systems) may become, to use a term which is at least convenient, a carrier of hydrogen (oxidation of proteins in neutral or slightly alkaline systems). It is true that, in protein, only the fixed SH group has so far been proven to be a direct source of this transportable hydrogen. The large uptake of oxygen (many times the oxygen equivalent of the above group) which a "reduced" protein displays occurs on lines which are not yet clear. It is at any rate certain that it proceeds only for so long as the fixed SH group survives. Moreover, in any case the influence of this simple reducing factor by itself secures exactly the result which has been pictured [Hopkins, 1923] as following from the ability of tissue agencies to reduce glutathione in

solution. It links one form of oxidation to another; *e.g.* that of proteins to that of fats. It is an essential controlling agency. In neutral or alkaline systems the thiol form of glutathione is so rapidly oxidised that, in the absence of a reducing factor, its disappearance quickly renders the system inert. The slow reducing action, for which the fixed SH group is responsible, maintains a concentration of SH in solution, and determines the whole velocity of oxidation. The process continues so long as (and only so long as) the SH in the protein survives. In acid systems its reducing power remains, and, by again securing a continued concentration of SH, determines a prolongation of the oxidising capacity of the system (Section VI). In the cell reduced proteins always co-exist with glutathione. Their coexistence and their mutual influence may well prove to be a biological essential. It must only be remembered that experimental studies have so far been confined to a preliminary analysis of oxidising mechanisms. We have no knowledge of their complex relations in the tissues. In a tissue, for instance, at least after it has been removed from the body and prepared for examination (by which time it has, of course, been in an essentially anaerobic condition for an appreciable interval), the whole of the glutathione is in the reduced form [Tunncliffe, 1925]. I have indeed found that a tissue in such circumstances—the liver at any rate—will rapidly reduce considerable quantities of additional glutathione when it is added in the disulphide form.

I am well aware indeed that on first acquaintance the curious and at many points obscure phenomena described in this paper may seem to lack biological reality. The experimental results described, however, are constant and easily reproducible. They are yielded by systems which contain actual cell constituents in contact with one another, and depend upon actual properties of these constituents. Such studies would seem to be a necessary, if remote, antecedent to a fuller understanding of the behaviour of the same constituents in the organised phenomena of the living cell itself.

SUMMARY.

In acid systems (p_H 3·0–4·5) glutathione (GSH) promotes the oxidation of certain unsaturated fatty acids and of lecithin. The mechanism of oxidation is, in this case, probably that postulated by Meyerhof [1923].

In neutral or alkaline systems (p_H 7·4–7·6) the nature of the process is different. During the oxidation of the SH group fatty acids are simultaneously oxidised in such a way that equipartition of oxygen occurs.

The oxidation of the glycerides of the above fatty acids proceeds under the influence of GSH, but in detail the process displays remarkable differences from the course of oxidation in the case of the free acids.

Glutathione (GSH or G_2S_2) promotes an oxidation of certain pure proteins on peculiar lines. The oxidation proceeds only when and while the protein itself displays (as shown by the nitroprusside reaction) an SH group. The

proteins of water-extracted muscle contain this group, and suffer the oxidation in question in neutral or faintly alkaline solutions. In acid systems (p_H 3.0–4.5) no oxidation of protein occurs.

In this oxidation the protein SH is oxidised and the nitroprusside reaction disappears, while the total oxygen taken up amounts to ten times the oxygen equivalent of the SH. The protein can then be again reduced; the SH group reappears and (with glutathione) a further uptake of oxygen becomes possible, which is again greatly in excess of the oxygen equivalent of the SH. Successive reductions and oxidations of this kind can be carried out, till finally the protein takes up at least 10 cc. per g. and possibly much more.

At any stage the "oxidised" protein (showing no nitroprusside reaction) can be reduced by contact (as a solid phase) with strong solutions of glutathione (GSH), cysteine, or thioglycollic acid, so that it again displays an intense nitroprusside reaction. Such a "reduced" protein then again takes up oxygen on the lines already described. When insoluble protein is in contact with solutions of glutathione, or other thiol compounds, equilibria are established between sulphur groupings in the solid (protein) phase and sulphur groups in solution. Mutual oxidation and reduction occur as reversible processes.

Whilst the proteins of water-extracted muscle undergo oxidation and reduction, on the lines described, in their native state, certain other proteins (those of blood serum, for example) need to be first denaturated. In an insoluble form the proteins of serum can be so reduced by contact with strong solutions of thiol compounds as to display an intense nitroprusside reaction. They then behave like the muscle proteins.

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