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OXIDATION OF SODIUM SULPHIDE BY RAT LIVER, LUNGS AND KIDNEY

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Abstract—Heparinized rat blood containing sodium [^{35}S]sulphide was perfused through isolated rat lungs, kidney or liver. The rate and extent of sulphide oxidation varied from one organ to another. In the isolated perfused lung system, [^{35}S]sulphide was oxidized slowly to [^{35}S]thiosulphate; only small amounts of [^{35}S]sulphate were detectable, possibly due to the absence of sulphide oxidase. In the isolated perfused kidney system, [^{35}S]sulphide was oxidized to [^{35}S]sulphate with [^{35}S]thiosulphate as a possible intermediate. In liver perfusion experiments [^{35}S]sulphide was oxidized rapidly and almost exclusively to [^{35}S]sulphate. The addition of unlabelled thiosulphate inhibited the formation of [^{35}S]sulphate and caused the release of [^{35}S]thiosulphate from the isolated liver. This suggests that thiosulphate is an intermediate in sulphide oxidation to sulphate. A mechanism for the rapid oxidation of sulphide to thiosulphate was shown to be present in rat liver mitochondria and, in the presence of glutathione, the thiosulphate was oxidized to sulphate. These results are discussed in relation to the contribution of lungs, kidney and liver to the oxidation of sulphide *in vivo*.

The detoxication capacity, with respect to sulphide, of both plasma and blood cells *in vitro* has been attributed to the binding of sulphide to proteins [1]. However, intravenously administered sulphide has only a transient existence in blood and is rapidly oxidized to sulphate in the whole animal [2] and by the isolated perfused rat liver [1]. The rapid rate of sulphide oxidation in the isolated liver suggested enzymic oxidation. Earlier work in the dog [3] also suggested that an enzyme, sulphide oxidase, was involved. In contrast, studies by Sorbo [4] do not support this view, although no unequivocal evidence has yet emerged which excludes the existence of sulphide oxidase [5].

Autoradiographic studies following intravenous administration of [^{35}S]sulphide [1] have shown the accumulation of isotope in areas corresponding to the lungs and kidneys. The present study attempts to measure the capacity of these organs to oxidize inorganic sulphide and to investigate further the subcellular site(s) and mechanism of sulphide oxidation in the liver.

MATERIALS AND METHODS

The materials, analytical and experimental techniques were as previously described [1], except for the following: glutathione (oxidized and reduced), lipoic acid and dihydrolipoic acid, were purchased from Sigma Chemical Co. Ltd., London, U.K. Sodium sulphide and all other reagents were AR grade. *N*-Ethyl [2, 3-^{14}C]maleimide (4mCi/mmole) was purchased from the Radiochemical Centre, Amersham, Bucks., U.K. Unlabelled *N*-ethyl maleimide was added to the labelled compound to give a final concentration of 0.1M in water. This solution was frozen until required.

Electrophoretic mobility of adducts formed between [^{14}C]*-labelled* N*-ethylmaleimide* ([^{14}C]*NEM*) *and sulphur compounds.* A number of workers have utilized the reaction of *N*-ethylmaleimide (NEM) with various sulphur compounds for qualitative analysis. Mudd [6] has shown that sulphite can be stabilized in this way and Ellis [1] has used NEM for the analysis of sulphide and sulphite.

Crystals of Na_2S, $Na_2S_2O_3$ and Na_2SO_3 were washed with water to remove surface impurities and, after blotting dry, the crystals were dissolved in 0.15 M phosphate buffer at pH 7.4. Each sulphur compound (0.08 ml of an 0.025 M solution) was mixed with 0.2 ml of 0.1 *M* aqueous [^{14}C]NEM and allowed to stand at room temperature for 30 min. Aliquots (10 μl) were subjected to paper electrophoresis in the presence and absence of authentic inorganic [^{35}S]sulphate. The mobilities of the [^{14}C]NEM adducts were measured relative to that of inorganic [^{35}S]sulphate.

Similar experiments were carried out in the presence of either rat plasma or whole blood. Reaction mixtures contained one of the sulphur compounds (0.08 ml), [^{14}C]NEM (0.02 ml) and either plasma or whole blood (0.2 ml). After mixing for 30 min at room temperature, aliquots (10μl) were subjected to paper electrophoresis in 0.1 M sodium acetate buffer at pH 4.5, using a potential gradient of 10V/cm for 1.5 hr.

Free thiosulphate was detected on electrophoretograms after spraying with starch–iodide reagent [8] or a fluorescein–silver nitrate reagent [9].

Experiments with isolated perfused rat liver. Rat livers were perfused with heparinized homologous blood [10].

The metabolic fate of $Na_2{}^{35}S$ in the isolated perfused rat liver was investigated under two sets of conditions:

(A) In the first experiment, $Na_2{}^{35}S$ (50 μmoles dissolved in 5 ml of 0.15 M phosphate buffer, pH 7.4) was added to the circulating perfusate (140 ml). To

minimize losses of ^{35}S as $H_2{}^{35}S$, the $Na_2{}^{35}S$ was added slowly (over 3 min) to the perfusate and the artificial lung was excluded from the circuit for the first 15 min. Samples of the perfusate were withdrawn after 15 min and the glass lung was then included in the circuit. Further samples of the perfusate were taken after 0.5, 1.0 and 2.0 hr.

(B) In a second set of experiments, the ability of the isolated rat liver to metabolize $Na_2{}^{35}S$ in the presence of unlabelled inorganic thiosulphate was investigated. Sodium thiosulphate (either 0.2 or 1 mmole dissolved in 5 ml of 0.15 M phosphate buffer, pH 7.4) was added to the circulating perfusate (140 ml). The blood/thiosulphate mixture was circulated through the liver for 10 min before adding $Na_2{}^{35}S$ (50 μmoles). Samples of the perfusate were removed at 15 min, 30 min, 1 hr and 2 hr and assayed for radioactive content by paper electrophoresis and scanning [1].

Perfusion of isolated lungs. The essential features of the perfusion apparatus are shown in Fig. 1. The heparinized blood [1], after filtration, enters the lungs from a reservoir, the height of which is adjustable and regulates the perfusion pressure. Lungs were perfused at a pressure of 8–9 cm of water. The lungs were suspended by a cannula inserted into the trachea and maintained in a humid atmosphere by enclosure in a glass chamber lined with moistened gauze. Lungs were inflated via the trachea using a Palmer respiratory pump. Expired gases from the lung were passed through two traps, in series, containing 3 ml of 1 M NaOH. Adequate deflation of the lungs was ensured by maintaining a slight negative pressure in the tracheal cannula.

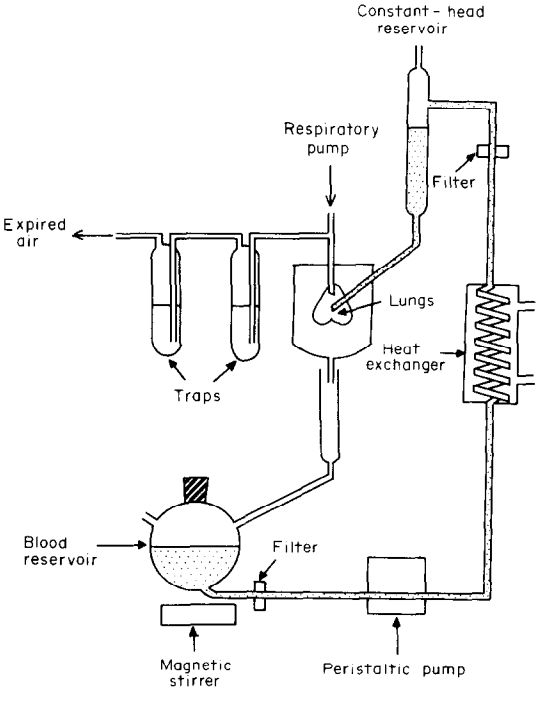

Fig. 1. Schematic representation of the apparatus for the perfusion of isolated rat lungs.

In preliminary experiments it was observed that the blood flow through the lungs dropped sharply within a short time (15 min) after the isolated lungs were connected to the perfusion apparatus. This was possibly due to the presence of vasoconstrictor substances in the blood and the problem was overcome in subsequent experiments by routinely perfusing an isolated rat liver for 1.5 hr before the lungs were attached to the apparatus. Lungs perfused using this protocol gave perfusion rates of 8–12 ml/min.

Operative procedure for the perfusion of isolated rat lungs. The donor rat was anaesthetized with ether and a glass cannula (with side arm) was inserted into the trachea. Anaesthesia was maintained with Nembutal (6 mg/200 g body wt in 0.2 ml of 0.15 M NaCl) administered via a cannula inserted into the right jugular vein.

The abdominal cavity was incised along the bloodless abdominal line and lateral incisions were made in the abdominal wall immediately distal to the diaphragm. The thoracic cavity was incised along the sternum and the ribs were removed to provide access to the lungs. The tracheal cannula was then attached to the respiratory pump. The animal was exsanguinated via the abdominal aorta (to prevent bleeding into the thoracic cavity when the heart was incised). The right ventricle of the heart was incised and a glass cannula (with an attached 10 ml syringe filled with oxygenated blood at 37°) inserted through the right ventricle into the pulmonary artery. The cannula was secured with ligatures. Oxygenated heparinized blood was slowly infused from the attached syringe and removal of the major portion of the left ventricle and the lower section of the left atrium allowed the efflux of venous blood. The lungs were dissected from the thorax, transferred to the perfusion apparatus and perfused with oxygenated heparinized blood at 8–12 ml/min.

In three separate experiments, $Na_2{}^{35}S$ (50 μmoles dissolved in 5 ml of 0.15 M phosphate buffer, pH 7.4) was added (over 2–3 min) to the circulating perfusate (120 ml). Lungs were perfused for 2 hr and samples of the perfusate were taken at 15 min, 30 min, 1 hr and 2 hr, and assayed quantitatively and qualitatively for ^{35}S-labelled materials.

Perfusion of the isolated kidney. Rat kidney was perfused with heparinized blood at 0.8–1.0 ml/min [11].

$Na_2{}^{35}S$ (50 μmoles dissolved in 5 ml of 0.15 M phosphate buffer, pH 7.4) was added to the perfusate (140 ml) at a point immediately before entry of the perfusate into the kidney. Samples of the perfusate were taken after 15 min, 30 min, 1 hr and 2 hr. In addition, a sample of blood was taken from the renal vein after 2 hr perfusion. The blood and urine samples were assayed quantitatively and qualitatively for ^{35}S-labelled materials.

The contribution of the perfusate to the conversion of [^{35}S]sulphide to [^{35}S]thiosulphate was evaluated in experiments in which $Na_2{}^{35}S$ was added to heparinized rat blood in the presence and absence of unlabelled thiosulphate. Oxygenated rat blood (10 ml) was mixed with $Na_2{}^{35}S$ (4 μmoles in 0.4 ml of 0.15 M phosphate buffer, pH 7.4) and 0.4 ml of 0.15 M phosphate buffer, pH 7.4 was added. Oxygenated rat blood (10 ml) was also mixed with sodium

thiosulphate (80 μmoles in 0.15 M phosphate buffer, pH 7.4) and after 10 min, 4 μmoles of $Na_2{}^{35}S$ was added. Both mixtures were incubated at 37°. Samples were removed from each flask at 15 min, 30 min and 1 hr after the addition of sulphide and assayed for ^{35}S-labelled materials.

Subcellular fractionation of rat liver. This was carried out by the method of De Duve *et al.* [12], as detailed by Milsom [13]. When the microsomal fraction only was required, conditions were 'biased' for the preparation of a 'pure' microsomal fraction [10].

Preparation of rat liver mitochondria. This was carried out by the method of Chappell and Hansford [14].

Marker enzymes. Rhodanese (thiosulphate–cyanide sulphotransferase, EC 2.8.1.1) was assayed as described by Rosenthal *et al.* [15], arylsulphatase C by the method of Dodgson *et al.* [16], as modified by Milsom *et al.* [17], and acid phosphatase by the method of Gianetto and De Duve [18]. These enzymes were assayed in sub-cellular fractions in order to establish the purity of the fractions.

Measurement of protein. Protein was determined by the method of Gornall *et al.* [19] using bovine serum albumin as standard.

Measurement of inorganic sulphide. Fresh solution of sulphide were prepared for each experiment and sulphide concentration was measured according to the method of Johnson and Nishita [20].

Measurement of oxygen consumption. This was carried out using an oxygen electrode as described by Chappell [21].

RESULTS

Electrophoretic mobility of [^{14}C] *NEM adducts*

The mobility of the adducts formed between [^{14}C]NEM and buffered solutions of sulphide, sulphite and thiosulphate are shown in Table 1. Na_2S and Na_2SO_3 adducts both gave single radioactive species which could be separated from each other and from $Na_2{}^{35}SO_4$ by paper electrophoresis. In contrast, the reaction between [^{14}C]NEM and thiosulphate resulted in the formation of two distinct radioactive species, having the same mobilities as [^{14}C]NEM–sulphide and [^{14}C]NEM–sulphite adducts.

When whole blood or plasma was included in the reaction mixtures the mobilities of the [^{14}C]NEM–sulphide and [^{14}C]NEM–sulphite complexes were not affected. However, the results obtained with thiosulphate were quite different. Only trace amounts of radioactivity corresponded in mobility with the NEM–sulphide and NEM–sulphite adducts. These results suggest that in the presence of either blood or plasma very little reaction occurs between NEM and thiosulphate. The minimum amount of blood or plasma required to inhibit the reaction between NEM and thiosulphate has not, as yet, been determined.

Table 1. Electrophoretic mobilities relative to inorganic [^{35}S]sulphate of the products formed between 2,3[^{14}C] *N*-ethyl maleimide and some sulphur compounds

NEM-adduct	Mobility (relative to $^{35}SO_4^{2-}$)
Sulphide	0.0
Sulphite	0.43–0.45
Thiosulphate	0.0
	0.43–0.45

The electrophoretic mobility of untreated thiosulphate in blood and plasma was 1.18–1.20 relative to inorganic [^{35}S]sulphate.

Liver perfusion. (A) After perfusion for 15 min, 94 per cent of the total radioactivity in the perfusate was associated with the plasma. Subsequently, the distribution of ^{35}S between plasma and cells remained relatively constant up to 2 hr, although the TCA-insoluble ^{35}S in the plasma decreased from 25 per cent (of the total ^{35}S in plasma) after 15 min to 14 per cent after 2 hr. Only small amounts of radioactivity (6–10 per cent of the ^{35}S in the perfusate) were associated with the cells. Scans of electrophoretograms of the NEM-perfusate adducts (Fig. 2, panel a) showed that after 15 min the major radioactive component in the perfusate (70 per cent of total ^{35}S) was identical in mobility with inorganic [^{35}S]sulphate. This value rose to 82 per cent after 2 hr perfusion (Fig. 2, panel b).

(B) When unlabelled thiosulphate (200 μmoles) was added to the perfusate, electrophoretograms of the perfusate showed that [^{35}S]thiosulphate accounted for 54 per cent of the radioactivity present after 15 min perfusion. Inorganic [^{35}S] sulphate accounted for 22 per cent of the total [^{35}S] in the perfusate (Fig. 2, panel c) at this time. After perfusion for 30 min the relative proportions of [^{35}S]thiosulphate and [^{35}S]sulphate were reversed, the inorganic [^{35}S]sulphate (approx. 46 per cent) predominating. The amount of radioactivity remaining at the origin decreased during the perfusion and accounted for approximately 13 per cent after 2 hr perfusion (Fig. 2, panel d). At this time [^{35}S]thiosulphate could not be detected.

In contrast, when 1 mmole of unlabelled thiosulphate was added to the perfusate the major radioactive peak in all samples of the perfusate corresponded to inorganic [^{35}S]thiosulphate (Fig. 2, panels e and f). Between 15 min and 2 hr perfusion, the percentage of total radioactivity present in the purfusate as [^{35}S]thiosulphate remained relatively constant (approx. 75 per cent). A radioactive peak corresponding to inorganic [^{35}S]sulphate was detected after 15 min but this represented only 6.3 per cent of the total radioactivity. The proportion of inorganic [^{35}S]sulphate increased to approximately 19 per cent after 2 hr and this coincided with a proportional decrease in the radioactivity remaining at the orgin. The relative distribution of total ^{35}S-labelled material between the plasma and cells was very similar to that obtained in the absence of any added thiosulphate.

Lung perfusion. During the first 15 min perfusion, 32 per cent of the administered dose was lost from the blood as volatile ^{35}S but after 15 min no further losses were observed. The distribution of ^{35}S between plasma and cells remained relatively constant over the 2 hr perfusion period with approximately 90 per cent of the total radioactivity associated with the plasma. At 15 min, 61 per cent of ^{35}S in plasma was TCA-insoluble. This value decreased to 54 per cent after 2 hr. In the cells all the ^{35}S-labelled material

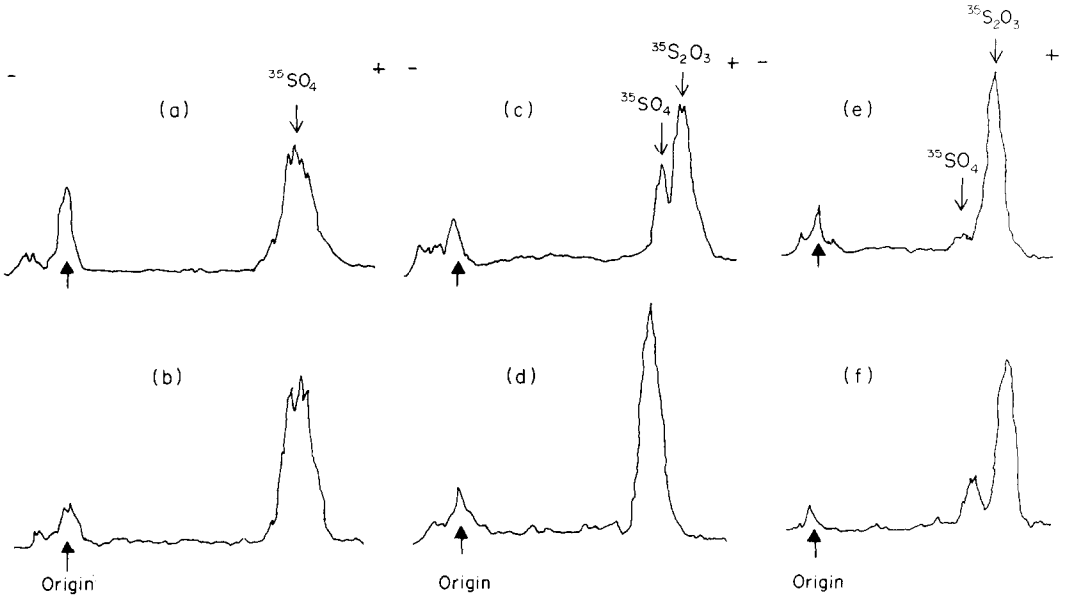

Fig. 2. Effect of thiosulphate on the oxidation of $Na_2{}^{35}S$ by the isolated perfused rat liver. Distribution of ^{35}S on electrophoretograms obtained following the perfusion of rat liver with heparinized blood (140 ml) containing $Na_2{}^{35}S$ (50 μmoles). Scans (a) and (b) are the controls and were obtained 15 min and 2 hr after the addition of $Na_2{}^{35}S$ to perfused rat liver; (c) and (d) show the distribution of ^{35}S after 15 min and 2 hr, respectively, when $Na_2S_2O_3$ (0.2 mmole) was added 10 min before the addition of $Na_2{}^{35}S$; (e) and (f) were also obtained after 15 min and 2 hr perfusion when $Na_2S_2O_3$ (1.0 mmole) was added 10 min prior to the addition of $Na_2{}^{35}S$.

was TCA-insoluble. Scans of electrophoretograms of the *N*-ethylmaleimide adducts (Fig. 3, panels a and b) showed that the [^{35}S]sulphide was slowly oxidized to [^{35}S] thiosulphate. Trace amounts only of [^{35}S]sulphate were detectable after 2 hr perfusion.

Kidney perfusion. After 15 min perfusion, 70 per cent of the radioactivity was associated with the plasma fraction and this value increased to 84 per cent after 2 hr. The amount of TCA-insoluble ^{35}S in the plasma remained relatively constant at approximately 30 per cent of plasma radioactivity. However, in the whole perfusate, the total TCA-insoluble ^{35}S decreased from 52 per cent after 15 min to 42 per cent after 2 hr. Urine flow was considerably reduced following the addition of $Na_2{}^{35}S$ to the perfusate and only 2.9 per cent of the administered ^{35}S appeared in the urine over 2 hr.

Scans of electrophoretograms of the NEM–perfusate mixture after 15 min (Fig. 3, panel c) showed two major radioactive peaks. The larger peak (65

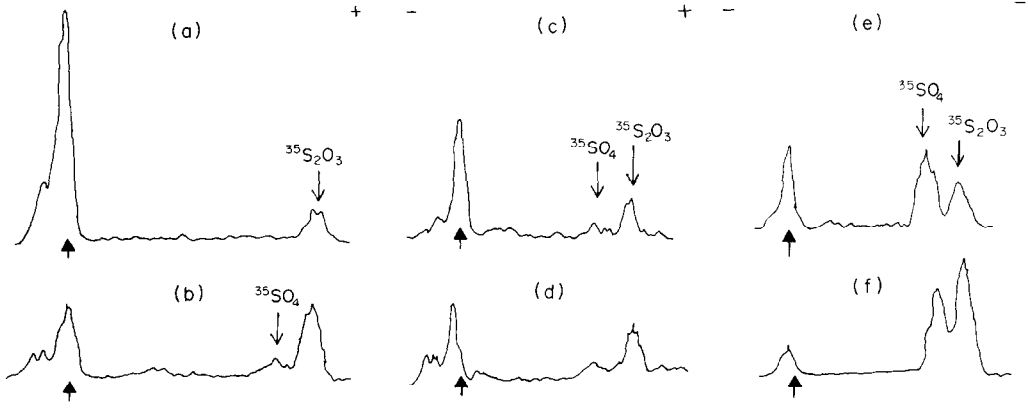

Fig. 3. Oxidation of $Na_2{}^{35}S$ by isolated perfused rat lungs or kidney. Distribution of ^{35}S on electrophoretograms obtained following perfusion of rat lungs or kidney with heparinized blood (120 and 140 ml, respectively) containing $Na_2{}^{35}S$ (50 μmoles). Scans (a) and (b) were obtained 15 min and 2 hr after addition of $Na_2{}^{35}S$ to isolated perfused lungs; (c) and (d) were obtained 15 min and 2 hr after addition of $Na_2{}^{35}S$ to perfused kidney; (e) and (f) show distribution of ^{35}S in renal vein blood after 2 hr perfusion and in 0–2 hr urine, respectively.

per cent of the radioactivity) remained at the origin, while the other peak was identical in mobility with [^{35}S]thiosulphate. The distributions of radioactivity in samples obtained between 30 min and 2 hr (Fig. 3, panel d) were essentially the same except for a small but clearly distinguishable peak of [^{35}S]sulphate. By contrast, blood obtained from the renal vein after 2 hr showed radioactive peaks corresponding to [^{35}S]sulphate (40 per cent) and [^{35}S]thiosulphate (29 per cent) (Fig. 3, panel e). In the urine collected over 2 hr, [^{35}S]thiosulphate and [^{35}S]sulphate were the major components (Fig. 3, panel f).

In control experiments in which $Na_2{}^{35}S$ was incubated with the perfusate alone, the scans of electrophoretograms of the NEM adducts showed that similar small amounts of [^{35}S]thiosulphate were produced in the presence and absence of added unlabelled thiosulphate.

Oxygen consumption by mitochondria in the presence of sodium sulphide. Rat liver mitochondria were prepared by the method of Chappell and Hansford [14] and the rate of oxygen consumption was measured in the presence and absence of sodium sulphide.

A typical trace of the oxygen utilization obtained following the addition of sodium sulphide to a suspension of rat liver mitochondria is shown in Fig. 4. Each addition of sulphide (32 nmoles) was characterized by an immediate increase in the rate of oxygen utilization lasting approximately 8 sec. This was followed by a much slower rate of oxygen consumption, although this rate was slightly higher than the basal level obtained in the absence of sulphide. This biphasic response was reproducible with each new addition of sulphide and was not recorded when mitochondrial protein was omitted or when heat denatured mitochondrial protein was used. Increasing the sulphide concentration to 30 and 60 μM (Fig. 5) extended the duration of the rapid phase of oxygen uptake to 18 and 86 sec, respectively, while the secondary phase remained relatively unaffected. However, increasing the sulphide concentration to 60 μM resulted in a dual effect. The duration of the rapid phase was increased, but the rate of oxygen uptake was reduced.

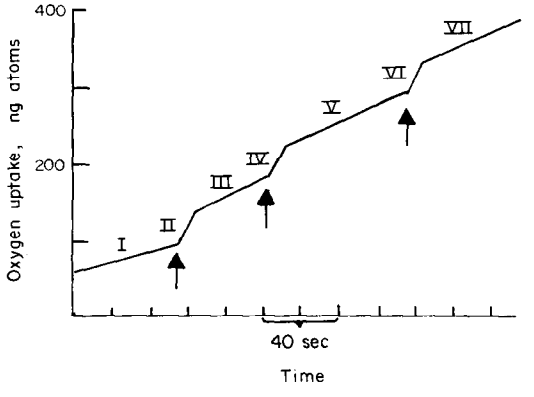

Fig. 4. Mitochondrial oxidation of Na_2S. Oxygen utilization following the addition of Na_2S (5 μl samples of a 6.4 mM solution) to a suspension of rat liver mitochondria (5.2 mg of protein in 2.1 ml of 0.15 M KCl, 10 mM Tris–HCl, pH 7.4 at 30°). The arrows indicate each addition of sulphide and the rates of oxygen utilization (ng atom/min) were: I, 50; II, 334; III, 84; IV, 301; V, 74; VI, 403 and VII, 74.

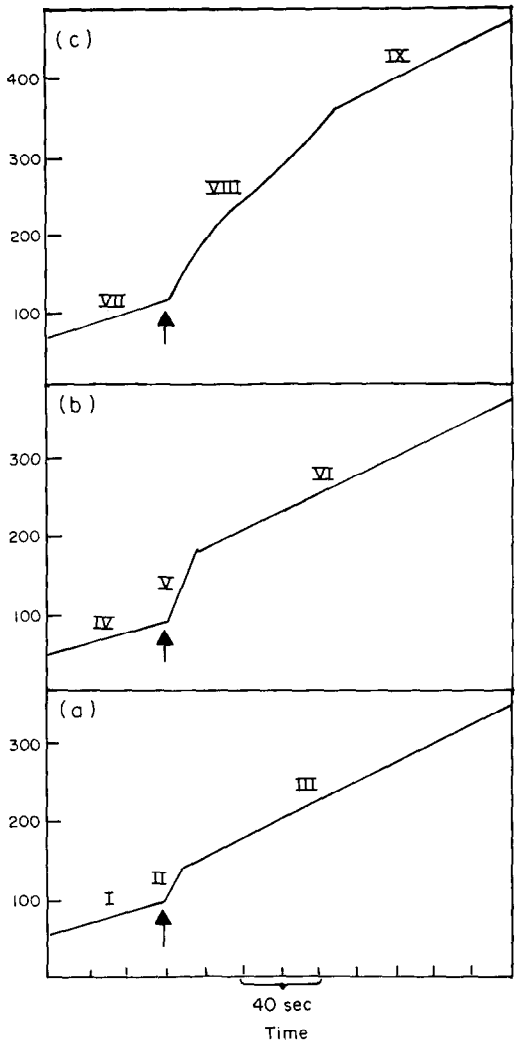

Fig. 5. Effect of sulphide concentration on mitochondrial oxidation of Na_2S. To a suspension of rat liver mitochondria (5.2 mg protein in 2.1 ml of 0.15 M KCl, 10 mM Tris–HCl, pH 7.4 at 30°) was added (a) 5μl; (b) 10 μl and (c) 20 μl aliquots of 6.4 mM Na_2S (in 0.2 M KCl, 10 mM Tris–HCl, pH 7.4). Rates of oxygen utilization (ng atom/min) were I, 50; II, 334; III, 84; IV, 50; V, 352; VI, 84; VII, 50; VIII, 142 and IX, 83.

Oxygen utilization during the rapid phase was directly proportional to the amount of sulphide added. In each case, at the end of the rapid phase the ratio of oxygen used to sulphide added was approximately 1:5:1. This would imply mitochondrial oxidation of sulphide to thiosulphate ($S_2O_3^{2-}$), although further oxidation (at a much reduced rate) could occur. In a similar series of experiments, the rate of oxygen consumption was measured following the addition of sodium sulphide (30–200 μM) to either rat liver microsomes or supernatant fractions (2–10 mg protein). With these fractions there was no significant change in the rate of oxygen consumption.

The products of ^{35}S *sulphide oxidation by rat liver mitochondria* in vitro. Mitochondria preparations (2–10 mg protein) were incubated (10 min at 30°) with $Na_2{}^{35}S$ (0.1 mM) and the volumes of the incubation mixtures were adjusted to 1 ml with 0.12 M KCl : 10 mM Tris–HCl, pH 7.4. Controls contained mitochondria denatured by boiling for 2 min. The reaction was stopped by adding 0.2 ml of 1 M cadmium acetate which precipitates the protein and free sulphide. After centrifuging, 0.2 ml samples of the supernatants were mixed with 0.025 ml of 0.1 M NEM and stored at room temperature for 30 min prior to electrophoresis and scanning.

Electrophoretograms (Fig. 6, panel a) showed three major radioactive components corresponding to inorganic [^{35}S]sulphate, [^{35}S]thiosulphate and the NEM adduct of [^{35}S]sulphite. The NEM[^{35}S]sulphite adduct was the major component. However, it was subsequently shown that in the absence of protein, NEM at a concentration of 0.011 M reacts with [^{35}S]thiosulphate yielding the NEM adducts of sulphite and sulphide. The sulphide is then precipitated by the cadmium ions present. Therefore, in subsequent experiments, *N*-ethyl maleimide was not added to the supernatants and under these conditions only [^{35}S]thiosulphate and [^{35}S]sulphate were detected on the electrophoretograms (Fig. 6, panel b). With heat-denatured mitochondria ^{35}S-labelled oxidation products could not be detected.

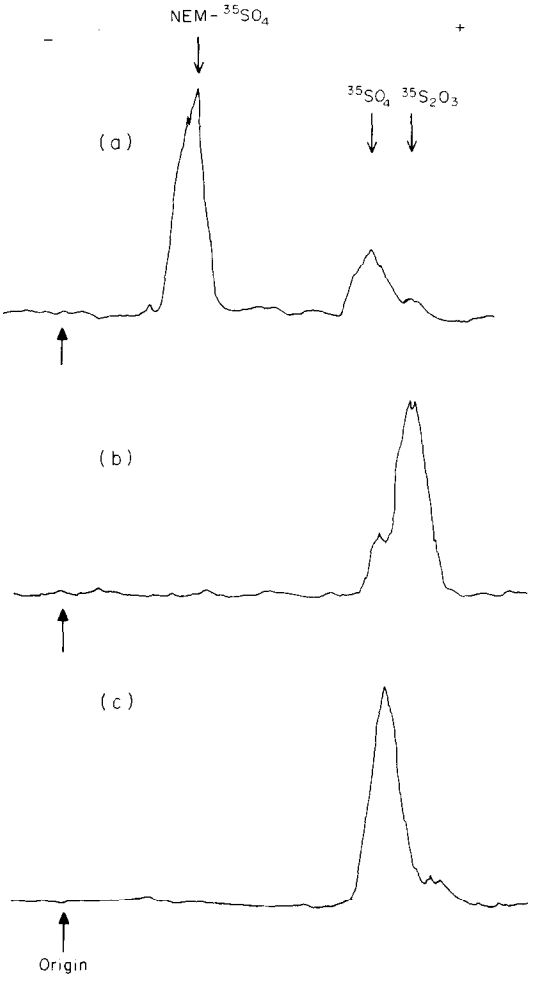

Fig. 6. Distribution of radioactivity on electrophoretograms following incubation of rat liver mitochondria with $Na_2{}^{35}S$. Incubations contained mitochondrial protein (5 mg) and $Na_2{}^{35}S$ (0.1 μmole) in 1 ml of 0.12 M KCl, 10 mM Tris–HCl, pH 7.4. Reactions were stopped after 10 min at 30° by the addition of 0.2 ml of 1 M cadmium acetate. After centrifuging (2000 *g* for 10 min) aliquots of the supernatant fraction were subjected to electrophoresis with (a) and without (b) the addition of *N*-ethylmaleimide (25 μl of a 10 mM solution of NEM per 0.2 ml of reaction mixture supernatant fraction). Sample (c) shows the oxidation products obtained under the same conditions except for the inclusion of reduced glutathione (1 mM) in the reaction mixture.

When glutathione (oxidized or reduced), lipoic or dihydrolipoic acids were included at a final concentration of 1 mM, the pattern of oxidation was altered (Fig. 6, panel c), the main product being [^{35}S]sulphate with only small amounts of [^{35}S]thiosulphate. Neither unlabelled thiosulphate nor sulphite up to concentrations of 4 mM had any apparent effects on the mitochondrial oxidation of $Na_2{}^{35}S$.

DISCUSSION

When blood containing $Na_2{}^{35}S$ was perfused through either isolated rat lungs, or kidney or liver, oxidation products were formed. However, the rate and extent of oxidation varied with each organ. With the isolated lung, the release of volatile ^{35}S in the expired air provides an explanation for the apparent accummulation of ^{35}S in this organ [1]. The [^{35}S]sulphide remaining in the blood was oxidized, albeit slowly, to [^{35}S]thiosulphate and it is therefore unlikely that the lungs make a major contribution to the oxidation of exogenous sulphide to sulphate. Small amounts only of [^{35}S]sulphate were detected in the perfusate and this may be due to the absence of sulphite oxidase in lungs [22].

In the isolated kidney, $Na_2{}^{35}S$ was also oxidized slowly, the major product in the perfusate after 2 hr being [^{35}S]thiosulphate. However, it is not possible from these studies to assess the contribution made by the kidneys in the intact animals to the oxidation of $Na_2{}^{35}S$. This is due to the relatively poor perfusion rate (0.8–1.0 ml/min) obtained with the kidney compared with the lungs and liver (8–12 ml/min and 30–35 ml/min, respectively). Since the total volume of the perfusate was approximately 140 ml, it is clear that oxidation products produced in the blood itself could mask the contribution made by the kidney. Nevertheless, the amount of [^{35}S]thiosulphate in the perfusate was greater than that formed in blood alone. The appearance of [^{35}S]sulphate as the major radioactive component in renal vein blood and the appearance of both [^{35}S]thiosulphate and [^{35}S]sulphate in the urine demonstrates the capacity of rat kidney to oxidize [^{35}S]sulphide to [^{35}S]sulphate with [^{35}S]thiosulphate as a possible intermediate.

In liver perfusion experiments $Na_2{}^{35}S$ is oxidized rapidly and almost exclusively to [^{35}S]sulphate; no [^{35}S]thiosulphate was detected in the perfusate. However, the ability of unlabelled thiosulphate to prevent the formation of [^{35}S]sulphate from [^{35}S]sulphide and

cause the release of [^{35}S]thiosulphate from the isolated liver into the perfusate suggests that [^{35}S]thiosulphate is an obligatory intermediate. In the present study, a mechanism for the rapid oxidation of sulphide to thiosulphate was shown to exist in the liver mitochondria. However, this mechanism operates only at low concentrations of sulphide. At sulphide concentrations of 60 μM and above, oxygen consumption by the mitochondria is reduced due to the inhibition of the cytochrome oxidase system (Dr. R. G. Hansford, personal communication). Oxygen consumption by mitochondria in the presence of sulphide is a biphasic process consisting of an initial rapid phase followed by a slower phase. The ratio of oxygen consumed to sulphur added is approximately 1.5 at the end of the rapid phase, which suggests that thiosulphate is the major oxidation product. This was confirmed by electrophoretic analysis of the oxidation products using $Na_2{}^{35}S$ as substrate even at the relatively high concentration of 0.1 mM.

The subcellular site(s) for the subsequent oxidation of thiosulphate generated by liver mitochondria is not known. However, in the presence of glutathione the major product of [^{35}S]sulphide oxidation by the mitochondria is [^{35}S]sulphate. This has also been demonstrated by Koj *et al.* [23]. The glutathione is involved in the reductive cleavage of thiosulphate to give sulphite, which is then oxidized to sulphate, and some form of sulphide, which is recycled to give more thiosulphate. Thus, liver mitochondria have the ability to bring about the complete oxidation of sulphide to sulphate, with thiosulphate and sulphite as intermediates.

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