Studies of Fatty Acid Oxidation

1. THE OXIDATION OF THE ALKYLTHIO FATTY ACIDS

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Certain alkylthio fatty acids have been shown (Brown, Quastel & Scholefield, 1954) to influence the proliferation of nitrifying organisms and to inhibit the oxidation of ammonia to nitrate in soil. The effect of these substituted fatty acids on mammalian systems and their metabolism by such systems has now been investigated. It has already been suggested by du Vigneaud (1942) and Dubnoff & Borsook (1948) that methylthiopropionic acid ($\text{CH}_3\text{S}\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{COOH}$) and methylthioacetic acid ($\text{CH}_3\text{S}\cdot\text{CH}_2\text{COOH}$) are produced from the corresponding methyl-donating dimethylthetins. It was shown by Maw (1953) that those thetins which trimethylate also give rise to the largest quantities of extra urinary sulphate. Presumably therefore, the demethylated compounds, which are the alkylthio fatty acids, are further oxidized and eventually yield sulphate. Evidence is presented to prove that such compounds are not only oxidized by mammalian systems but that they may also influence the metabolism of mammalian tissues.

EXPERIMENTAL

Tissue slices were cut using a Stadie-Riggs tissue slicer (Arthur H. Thomas Co., Philadelphia, U.S.A.) and were immediately placed on top of a dry Petri dish containing cracked ice. They were then blotted, weighed on a torsion balance and placed directly into chilled Warburg vessels. All incubations, unless otherwise stated, were made for 1 hr. at 37° in an atmosphere of oxygen using the conventional Warburg apparatus. In the case of kidney, only cortex tissue was used.

The incubation medium was a calcium-free Krebs-Ringer phosphate medium, pH 7.4 (0.13M-NaCl, 0.005M-KCl, 0.0013M- KH_2PO_4 , 0.0013M- MgSO_4 , 0.01M phosphate buffer, pH 7.4). When the sodium salts of alkylthio fatty acids and other substrates were being studied the following technique was adopted in order to eliminate effects due to excess Na^+ . A calcium- and NaCl-free medium was made up to 6 times the normal strength and 0.5 ml. was used per vessel. After addition of substrates and inhibitors, sufficient 3M-NaCl was added to bring the total Na^+ concentration to 0.13M. The volume was then made up to 3 ml. with water. Q_{O_2} values were calculated from the wet weight assuming 80% moisture.

Homogenates of rat kidney were prepared as previously described (Brown & Scholefield, 1952). Rat-brain and rat-liver homogenates were prepared in a similar manner except

that one liver or two brains were used per 7 ml. homogenizing fluid.

Animals used were a laboratory strain of albino mice weighing 25–30 g. and hooded rats obtained locally weighing 150–200 g.

Substrates. All amino acids were obtained from Nutritional Biochemicals Corporation (Cleveland, Ohio, U.S.A.) and were used without further purification. The non-labelled alkylthio fatty acids were prepared as previously described (Brown *et al.* 1954). Dimethyl- and diethylthetin and propiothetin chlorides were synthesized by the method of Challenger & Simpson (1948).

Radioactive materials. All the carboxyl-labelled alkylthio substituted fatty acids were obtained from Dr D. E. Douglas of this Institute. Counting was carried out in a Geiger-Muller flow type counter with an Atomic Instrument Scaler, no. 101-A. The gas used was helium saturated with ethanol at 0°. During the incubation period CO_2 was absorbed by 0.25 ml. 20% NaOH placed in the centre well. 2N Sulphuric acid (0.3 ml.) was tipped into the main vessel from the side arm at the conclusion of the experiment to stop the enzymic reactions and to drive off any combined CO_2 . No acetoacetate determinations were made, since it has been shown (Avigan & Scholefield, 1954) that the alkylthio substituted acids inhibit acetoacetate formation. The collected CO_2 and carrier Na_2CO_3 were then converted into BaCO_3 with BaCl_2 and counted in the usual way. The amount (μmoles) of substrate oxidized to CO_2 was calculated from the original specific radioactivity and the total radioactivity recovered as CO_2 .

Mean values are quoted throughout. In general, the individual measurements did not deviate from the mean by more than $\pm 10\%$ in the case of Q_{O_2} values, or $\pm 15\%$ in the case of $^{14}\text{CO}_2$ or radioactive acetoacetate determinations.

RESULTS

The influence of alkylthio fatty acids on mammalian enzyme systems

In soil-perfusion experiments (Brown *et al.* 1954) sodium ethylthiopropionate gave rise to ethyl mercaptan, but sodium ethylthioacetate did not. Injection of benzyl- and ethyl-thioacetate into mice produced no obvious effects at a level of 30 mg./mouse, whereas intraperitoneal injection of sodium benzyl- or ethyl-thiopropionate always resulted in the exhalation of the corresponding mercaptan. The propionate derivatives produced no ill effects at a level of 12 mg./25 g. mouse, but 18 mg./25 g.

mouse made all the animals appear moribund within 30 min., although all recovered overnight. At a dose level of 24 mg./25 g. mouse, all animals died within 6 hr. The influence of the alkylthio fatty acids on individual tissues was therefore investigated.

Typical Q_{O_2} values obtained on incubation of sodium methylthiopropionate with various tissues are given in Table 1. With three of the tissues examined the alkylthio fatty acid produced a decrease in the $-Q_{O_2}$, particularly in mouse liver where the effect appeared to increase with time. In the cases of rat liver, guinea pig liver and guinea pig kidney, an oxidation of the added substrate was obvious. These varying effects prompted an investigation into the influence of sodium methylthiopropionate on the oxidation of various common substrates. Rat-kidney slices were used, since the respiratory activity was highest in this tissue and since sodium methylthiopropionate had little effect on its endogenous activity. The results obtained are shown in Table 2. No inhibition of the oxidation of the acids of the tricarboxylic acid cycle occurred and the inhibition of the endogenous activity by sodium thiopropionate was reversed by these substrates. No significant effect on glucose or sodium acetate oxidation was obtained. The oxidation of sodium pyruvate, on the other hand, was greatly inhibited in the presence of the alkylthio substituted acid.

Pyruvate oxidation in rat-kidney slices was inhibited to the same extent by sodium methylthioacetate (Table 3). The latter substrate was not oxidized by this tissue, whereas in rat-liver slices it produced an increase in the $-Q_{O_2}$ value (Table 3). This increase was additive to that obtained on adding sodium pyruvate to rat-liver slices. Sodium methylthiopropionate was oxidized in rat-liver slices but the increase in respiratory activity due to this substrate was only partially additive to that due to the presence of sodium pyruvate. In contrast with the inhibition of sodium pyruvate oxidation in rat-liver and rat-kidney slices, no

effect of sodium methylthiopropionate on pyruvate oxidation in rat-brain homogenate could be obtained.

The nature and mechanism of the inhibition of pyruvate oxidation will be discussed in another communication.

Table 1. Q_{O_2} values obtained on incubation of sodium methylthiopropionate with various tissue slices

All measurements were made over a 60 min. incubation period except in the case of mouse liver. The values quoted are the means of at least three determinations.

	$-Q_{O_2}$	
	No addition	Methylthiopropionate (0.02 M)
Mouse kidney	15.3	12.4
Mouse liver	10.7*	9.9
	10.0†	5.5
Guinea pig kidney	9.0	12.1
Guinea pig liver	3.9	7.0
Rat kidney	17.0	14.7
Rat liver	8.8	12.0

* Measured over the period 0–30 min.

† Measured over the period 30–60 min.

Table 2. The effect of sodium methylthiopropionate on the oxidation of various substrates (0.02 M) in rat-kidney slices

All values are the means of at least two determinations.

	$-Q_{O_2}$	
	No addition	Methylthiopropionate (0.02 M)
Nil	14.5	10.0
Sodium citrate	21.0	19.0
Sodium α -oxoglutarate	23.8	22.4
Sodium succinate	30.4	29.5
Sodium fumarate	20.5	21.8
Sodium malate	18.9	17.0
Sodium oxaloacetate	21.7	19.7
Sodium pyruvate	26.9	17.1
Sodium acetate	15.3	10.9
Glucose	17.8	14.0

Table 3. The effect of sodium methylthio fatty acids on the oxidation of sodium pyruvate by tissue slices

Tissue	Methylthio fatty acid* (0.02 M)	No. of determinations	$-Q_{O_2}$ in presence of			
			No substrate	Sodium pyruvate (0.02 M)	Methylthio fatty acid	Methylthio fatty acid and pyruvate
Rat kidney	MA	4	14.8†	23.5	13.0	18.9
	MP	7	14.4	24.5	12.5	18.8
Rat liver	MA	6	9.0	11.7	11.6	13.7
	MP	3	8.7	12.0	14.2	14.8

* MA, sodium methylthioacetate; MP, sodium methylthiopropionate.

† The $-Q_{O_2}$ values quoted are mean values.

The inhibition of the D-amino acid oxidase activity of rat-kidney homogenate

Methylthioacetate and methylthiopropionate inhibited the oxidation of D-amino acids in rat-liver and rat-kidney homogenates. Neither compound, however, inhibited any of the following enzyme systems: succinoxidase of rat-kidney homogenate, glutamate-pyruvate transaminase, amine oxidase and choline oxidase of rat-liver homogenate, octanoate oxidation by *Pseudomonas aeruginosa*, the Stickland reaction in *Clostridium sporogenes*, the carboxylase activity of, and glycolysis in, acetone powders of yeast, and sulphanilamide acetylation by acetate in pigeon-liver extracts. The effect on D-amino acid oxidase is therefore relatively specific.

Accurate results could not be obtained with rat-liver homogenates because of the high endogenous activity of these preparations. Rat-kidney homogenates have therefore been used as the source of D-amino acid oxidase. In general, the rate of oxidation of D-amino acids was not constant, tending to decrease with time. The rate of the reaction was, therefore, estimated manometrically from the increased rate of oxygen uptake measured over a 10–15 min. incubation time commencing 5 min. after tipping of the substrate and inhibitor. DL-Methionine was used as substrate since it is readily available in pure crystalline form and is rapidly oxidized by rat-kidney D-amino acid oxidase.

The inhibition by sodium methylthiopropionate of DL-methionine oxidation, on analysis of the experimental results by the method of Lineweaver &

Burk (1934), was found to be competitive (Fig. 1). From the results shown in Fig. 1 the K_m of D-methionine was estimated to be $9 \times 10^{-3} M$. Oxidation of DL-ethionine, DL-serine, DL-valine and DL-alanine in this preparation was also inhibited by methylthiopropionate. β -Alanine, DL-aspartate and L-glutamate are not oxidized by rat-kidney homogenate and were found not to inhibit the oxidation of the other DL-amino acids. All the methyl-, ethyl- and benzyl-thioacetates and thio-propionates are active inhibitors, but sodium methoxyacetate, ethoxyacetate and N-methyl-aminoacetate (sarcosine) have no effect on methionine oxidation.

Ethyl mercaptan and propionic acid were tested as inhibitors of DL-methionine oxidation in order to be certain that the effect of methylthiopropionate was not due to breakdown to such compounds. However, ethyl mercaptan is neither oxidized by rat-kidney homogenate nor does it inhibit the oxidation of D-amino acids. Furthermore, methylthiopropionic acid is a more effective inhibitor of DL-methionine oxidation than is propionic acid itself. The competitive inhibitory action of propionic and other fatty acids has already been reported (Brown & Scholefield, 1952), and the inhibition of D-amino acid oxidation by many other carboxylic compounds has recently been reviewed by Klein (1953). The action of the alkylthio fatty acids on D-amino acid oxidation is therefore not a specific one; it is rather an inhibition produced by most monocarboxylic compounds.

The oxidation of alkylthio fatty acids

It has already been inferred, from the fact that mercaptans are produced on injection of alkylthiopropionic acid derivatives into mice and from the increase of $-Q_{O_2}$ values on incubation of tissue slices with the alkylthio fatty acids, that these compounds are oxidized by mammalian tissues. The availability of certain alkylthio fatty acids labelled with ^{14}C in the carboxyl group has enabled us to make a more accurate investigation into the nature of this oxidation. The method of preparation of the labelled compounds favoured the synthesis of the ethylthio derivatives of acetic, propionic (β) and butyric (γ) acids rather than the methyl compounds used thus far. The ethylthio fatty acids have therefore been used throughout the remainder of the work reported here.

The rate of oxidation of sodium ethylthiopropionate, measured by the release of $^{14}CO_2$ from carboxyl labelled substrate, was greater in rat liver than in any other organ of this animal (Table 4). Subsequent experiments were, therefore, confined to studies of the oxidation of the alkylthio fatty acids in rat-liver slices. In no case could any acetoacetate formation from alkylthio fatty acids be

Fig. 1. The competitive nature of the inhibition by sodium methylthiopropionate of DL-methionine oxidation in rat-kidney homogenate. ●, no inhibitor present; ○, $6.7 \times 10^{-3} M$ sodium methylthiopropionate; ◐, $2 \times 10^{-2} M$ sodium methylthiopropionate; $[S]$ = molar substrate concentration; V = velocity expressed as l. of oxygen taken up in 15 min.

demonstrated. The rates of oxidation of the sodium salts of ethylthio-acetate, -propionate and -butyrate are all proportional to the weight of rat-liver slices present (Fig. 2) and the rate of production of $^{14}\text{CO}_2$ from carboxy-labelled substrates increased with increasing chain length of the fatty acid moiety. Anaerobiosis virtually eliminated oxidation of the ethylthiosubstituted acids to $^{14}\text{CO}_2$ (see Table 7). Substrate concentration also determined the rate of oxidation, and analysis of the experimental results by the method of Lineweaver & Burk (1934) gave K_m values of approximately $8 \times 10^{-4}\text{M}$ for sodium ethylthioacetate, $5 \times 10^{-4}\text{M}$ for the ethylthiopropionate and $7 \times 10^{-4}\text{M}$ for the ethylthiobutyrate (Fig. 3). The K_m value for butyrate oxidation in guinea pig-kidney slices, $3 \times 10^{-4}\text{M}$, obtained by Baxter & Quastel (1953), is of the same order.

Table 4. The rate of oxidation of sodium ethylthio-propionate in various organs of the rat

Each vessel contained 15 μmoles sodium ethylthio-propionate (specific radioactivity 1100 counts/min./ μmole) and approx. 100 mg. wet weight of tissue.

Tissue	Substrate* oxidized ($\mu\text{moles}/100\text{ mg. wet wt.}$ of tissue/hr.)
Liver	0.96
Kidney	0.62
Heart	0.23
Spleen	0.18
Lung	0.14
Brain	<0.01

* Calculated from the $^{14}\text{CO}_2$ produced.

Fig. 2. The relation between amount of tissue and rate of oxidation of alkylthio fatty acids. ●, $4 \times 10^{-3}\text{M}$ sodium ethylthioacetate; ○, $5 \times 10^{-3}\text{M}$ sodium ethylthiopropionate; ⊙, $3.2 \times 10^{-3}\text{M}$ sodium ethylthiobutyrate. All concentrations are equivalent to approximately five times the K_m value found for the alkylthio fatty acid.

These kinetic results substantiate the indications, previously obtained, that the alkylthio fatty acids are oxidized enzymically in rat-liver slices. On the other hand, the mechanism of the oxidation of these fatty acid derivatives was not certain. Preliminary experiments showed that after 1 hr. of incubation with liver slices in Warburg vessels the alkylthio-propionates gave rise to a strongly positive nitroprusside test for mercaptan on the alkaline filter paper of the centre well. The corresponding test with alkylthioacetates as substrates produced uniformly negative reactions, confirming the results of the *in vivo* experiments. To obtain further evidence concerning the mechanism of the oxidation of the alkylthio fatty acids, the influence of other substrates on the rate of oxidation of these compounds was investigated.

The influence of other substrates on the oxidation of alkylthio fatty acids

If the oxidation of the substituted fatty acids proceeds via the same sequence of enzymes as with the fatty acids themselves, then the addition of sodium acetate, propionate or butyrate should influence the rate of $^{14}\text{CO}_2$ production from carboxy-labelled ethylthio fatty acids. In fact, all three compounds inhibit the oxidation of the three ethylthio fatty acids to CO_2 (Table 5). Sodium butyrate proved to be the most effective inhibitor, but Baxter & Quastel (1953) reported even greater effects of sodium butyrate on (+)-biotin oxidation. In the case of (+)-biotin, however, the uninhibited rate of oxidation is much lower than the rates of oxidation of the ethylthio substituted acids quoted in Table 5 (approximately $0.03 \mu\text{mole}$ biotin/100 mg. wet wt./hr. compared with $0.19\text{--}1.15 \mu\text{moles}$ ethylthio acid/100 mg. wet wt./hr.).

Fig. 3. The effect of substrate concentration on the rate of oxidation of alkylthio fatty acids. ○, ethylthioacetate; ●, ethylthiopropionate; [S] = substrate concentration in $\mu\text{moles/l.}$ V = velocity of oxidation expressed as $\mu\text{moles } ^{14}\text{CO}_2$ produced/hr./100 mg. wet wt. of tissue.

The common di- and tri-carboxylic acids affect the rate of oxidation of fatty acids (Geyer, Mathews & Stare, 1950) and of a substituted fatty acid (Baxter & Quastel, 1953). The conclusion is drawn, in both cases, that the results obtained implicate the participation of the tricarboxylic acid cycle in the complete oxidation of the fatty acid moiety, i.e. of the two-carbon fragment derived from the fatty acid. Similar increases in the rate of $^{14}\text{CO}_2$ production from the ethylthio fatty acids have been obtained (Table 6) through the addition of sodium succinate and sodium citrate.

The effect of metabolic inhibitors on ethylthio fatty acid oxidation

The effects of sodium malonate and fluoroacetate in inhibiting the oxidation of the three ethylthio fatty acids tested may be seen from Table 6, again indicating the participation of the tricarboxylic acid cycle in the oxidation of these substrates. Operation of this cycle, however, may only supply the energy required for the initial activation of the fatty acids and their derivatives. It is worth noting, in this connexion, that 2:4-dinitrophenol at a con-

Table 5. *The effect of sodium acetate, propionate and butyrate on the rate of oxidation of the corresponding carboxy-labelled ethylthio fatty acids by rat-liver slices*

All values refer to μmoles substrate oxidized/100 mg. wet wt. of tissue/hr. estimated from the $^{14}\text{CO}_2$ evolved from carboxy-labelled substrate and are the means of at least two determinations. The figures in parenthesis refer to the μmoles fatty acid added/3 ml. vessel contents.

Substrate	Concentration ($\mu\text{moles}/3\text{ ml.}$)	Additions			
		Nil	Na acetate	Na propionate	Na butyrate
Ethylthioacetate	7.5	0.193	0.183 (30)	0.082 (30)	0.049 (30)
Ethylthiopropionate	60	0.70	0.40 (60)	0.60 (60)	0.35 (60)
Ethylthiobutyrate	15	1.15	0.97 (15)	0.82 (15)	0.52 (15)

Table 6. *The effect of various carboxylic acids on the oxidation of ethylthio fatty acids in rat-liver slices*

Experimental conditions as in Table 5.

Substrate	Concentration ($\mu\text{moles}/3\text{ ml.}$)	Carboxylic acid added				
		Nil	Succinic	Malonic	Citric	Fluoroacetic
Ethylthioacetate	7.5	0.19	0.18 (30)	0.07 (30)	—	0.08 (30)
Ethylthiopropionate	60	0.92	1.15 (60)	0.25 (60)	1.25 (60)	0.40 (60)
Ethylthiobutyrate	15	1.17	2.13 (15)	0.34 (15)	1.94 (15)	0.51 (15)

Table 7. *The effect of anaerobiosis and 2:4-dinitrophenol on ethylthio fatty acid oxidation in rat-liver slices*

Experimental conditions as in Table 5.

Substrate	Concentration ($\mu\text{moles}/3\text{ ml.}$)	Gas phase		
		O_2	O_2^*	N_2
Ethylthioacetate	12	0.33	0.13	0.03
Ethylthiopropionate	15	0.56	0.16	0.03
Ethylthiobutyrate	9.5	1.09	0.49	0.02

* In presence of $3 \times 10^{-4}\text{ M}$ 2:4-dinitrophenol.

Table 8. *The effect of benzoic and phenylacetic acids on the oxidation of ethylthiopropionic and butyric acids in rat-liver slices*

All values refer to μmoles substrate oxidized/100 mg. wet wt. of tissue/hr. estimated from the $^{14}\text{CO}_2$ evolved from carboxy-labelled substrate. Values obtained from individual determinations are quoted.

Additions	Concentration ($\mu\text{moles}/3\text{ ml.}$)	Substrate	
		Sodium ethylthiopropionate (15 μmoles)	Sodium ethylthiobutyrate (15 μmoles)
Nil	—	0.87	1.26, 1.05
Sodium benzoate	15	0.43	0.93, 0.83
Sodium phenylacetate	15	0.84	1.50, —

centration of $3 \times 10^{-4} M$ inhibited the oxidation of ethylthio substituted acids to CO_2 by 70 % (Table 7). This concentration of 2:4-dinitrophenol had no appreciable effect on the Q_{O_2} obtained with ethylthio substituted acids as substrate.

Certain aromatic carboxylic acids inhibit acetoacetate production from butyrate and crotonate (Jowett & Quastel, 1935a), but have no effect on the increased rate of oxygen uptake obtained on addition of these two acids to liver slices. The effect of benzoic and phenylacetic acids on the oxidation of ethylthio-propionic and -butyric acids was therefore examined, and the results obtained are quoted in Table 8. At the concentration used ($0.005 M$) phenylacetic acid had no significant effect, but benzoic acid inhibited the $^{14}CO_2$ production from ethylthiopropionate by 50 % and from ethylthiobutyrate by 20 %. This is in agreement with the observation of Jowett & Quastel (1935b) that the effect of benzoate on acetoacetate formation is greatest with the short-chain fatty acids.

Some effect of individual alkylthio fatty acids on the oxidation of other members of the series could be demonstrated. However, the effect was small; $12.5 \times 10^{-3} M$ sodium ethylthioacetate inhibited the oxidation of $5 \times 10^{-3} M$ sodium ethylthiopropionate by only 25 % and no significant effect was observed with $7.5 \times 10^{-3} M$ sodium ethylthioacetate.

Oxidation of sodium ethylthiopropionate derivatives

The evidence presented by Maw (1953) indicates that the alkylthio fatty acids are oxidized *in vivo* to sulphate. This oxidation might proceed by direct loss of two-carbon fragments from the fatty acid moiety or after initial oxidation of the sulphur to a sulfoxide or sulphone. Experiments with carboxy-labelled sodium ethylthiopropionate sulphone showed that it was oxidized at about one-third the rate of the corresponding sulphide compound, 0.26 compared with $0.72 \mu\text{mole/hr./100 mg. wet wt. rat liver}$.

In preliminary experiments with non-labelled dimethyl- and diethyl-propiothetins we have been unable to demonstrate the oxidation of these compounds or any inhibitory action by them on the oxidation of labelled ethylthiopropionate.

DISCUSSION

The alkylthio fatty acids are oxidized by several rat tissues, the most active being the liver. The rate of oxidation is greatest with ethylthiobutyric acid, less with the propionic derivative and smallest with ethylthioacetate as substrate. Of these three compounds, only the propionic acid derivative gave rise to mercaptan on injection or on incubation with

tissue slices. The following scheme for the oxidation of these compounds is therefore proposed:

According to this scheme, the derivatives of the even-numbered fatty acids would give rise, on continued oxidation, to the corresponding acetic acid derivative. This derivative, slowly oxidized by liver, would eventually yield sulphate. The derivatives of the odd-numbered fatty acids would give rise to the corresponding formic acid derivatives (cf. benzoic) which, in this case, are the alkyl thiolcarbonates. On hydrolysis of these compounds, free mercaptans and CO_2 would be formed.

The effects of sodium acetate, propionate and butyrate in diminishing the rate of $^{14}CO_2$ production from sodium ethylthiopropionate and ethylthiobutyrate may be due to dilution or to a direct inhibition, e.g. by competition. In either case the same or similar pathways for the oxidation of the fatty acids and their ethylthio derivatives are indicated.

There seems to be, at present, no satisfactory explanation for the oxidation of the ethylthioacetate. It should be noted that sodium acetate has no effect on ethylthioacetate oxidation so that dilution by inactive sodium acetate or its oxidation products does not occur. The inhibition by propionate and butyrate (Table 5) cannot therefore be explained in terms of a dilution effect. Furthermore, no stimulation of ethylthioacetate oxidation by succinate or citrate is observed (Table 6), whereas the rates of oxidation of the other alkylthioacids are greatly increased by these substrates. Malonate and fluoroacetate, on the other hand, both inhibit the oxidation of ethylthioacetate. This inhibition may be explained by a limitation of adenosine triphosphate production required for initial activation of ethylthioacetate rather than by a direct inhibition of the pathway for $^{14}CO_2$ release.

The phenyl fatty acids, which are effective inhibitors of fatty acid oxidation (Jowett & Quastel, 1935a, b), also inhibit oxidation of the alkylthio fatty acids. The inhibitory effect with fatty acids is on acetoacetate formation, but with the alkylthio fatty acids, which do not yield acetoacetate (Avigan & Scholefield, 1954), benzoic acid inhibits the oxidation to CO_2 . Phenylacetic acid, on the other hand, had no effect on alkylthio fatty acid oxidation.

SUMMARY

1. The addition of alkylthio fatty acids to slices of mouse kidney, mouse liver and rat kidney depressed the respiratory activity of these tissues but when added to slices of rat liver, guinea pig kidney and guinea pig liver they produced a marked increase in $-Q_{O_2}$ values.

2. Sodium methylthiopropionate (0.02M) and methylthioacetate (0.02M) both inhibited the oxidation of sodium pyruvate (0.02M) in rat-kidney slices, but had no effect on the oxidation of the sodium salts of carboxylic acids that were tested. Pyruvate oxidation in rat-brain homogenate was not effected.

3. All the alkylthio fatty acids examined inhibited the D-amino acid oxidase of rat-kidney homogenate but had no effect on several other enzyme systems that were examined.

4. The oxidation of ethylthio-acetate, -propionate and -butyrate was studied using [*carboxy*- ^{14}C]-labelled substrates. Evidence is presented which indicates that these compounds are oxidized in a manner analogous to the oxidation of the unsubstituted fatty acids.

5. A scheme has been outlined to explain the metabolism of the alkylthio fatty acids and the production of mercaptans.

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Studies of Fatty Acid Oxidation

2. THE EFFECT OF ALKYLTHIO FATTY ACIDS ON ACETYLATION REACTIONS

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It has been shown (Brown & Scholefield, 1954) that ethylthioacetate and ethylthiopropionate inhibit the oxidation of pyruvate in rat-kidney and rat-liver slices but not in rat-brain homogenate. Subsequent work has shown that the alkylthio fatty acids also affect the metabolism of fatty acids. The purpose of the present work was to investigate the nature of these phenomena. Alkylthioacetic acid derivatives were used throughout whenever possible because of the relatively low rate at which they are oxidized in mammalian tissues.

METHODS AND MATERIALS

The preparation of tissue slices, substrates, radioactive materials and the incubation medium were as previously described (Brown & Scholefield, 1954). We are indebted to Dr D. E. Douglas of this Institute for supplying all the radioactive compounds used in the present work.

Pigeon-liver acetone powders. These were obtained by standard procedures and stored in a vacuum desiccator at 0°.

Determinations of sulphanilamide. These were made by the method of Bratton & Marshall (1939).

Mitochondria. These were prepared by the method of Schneider (1948) using 0.25M sucrose. Acetone powders were obtained by a method that was essentially that of Drysdale & Lardy (1953). Excess acetone was added to the packed mitochondria after two washings with isotonic sucrose, the mixture was stirred and, after all lumps were broken, the powder was isolated by centrifuging or filtration. After several washings with acetone the powder was dried in a vacuum desiccator and stored at 0°.

Acetoacetate determinations. These were made on the contents of Warburg flasks (slice and medium) after displacement of $^{14}\text{CO}_2$ with HCl. Aniline hydrochloride (45% (w/v); 0.2 ml.) was tipped into the vessels from the side arms (Jowett & Quastel, 1935) and incubation at 37° maintained for 2 hr. Evolved $^{14}\text{CO}_2$ was collected in 0.2 ml. 20% NaOH in the centre well. Carrier Na_2CO_3 was then added, BaCO_3 formed and counted in the usual way.

RESULTS

The effects of alkylthio substituted acids on the oxidation of sodium pyruvate, acetate and butyrate

In preliminary experiments with [*carboxy*- ^{14}C]-pyruvate, there was no inhibitory effect of ethylthioacetate on $^{14}\text{CO}_2$ production in the presence of