phospholipids by liver homogenates was stimulated by vanadium. On re-examination of these results we found that vanadium (10^{-3} M vanadate) had little effect when weak homogenates (0.5 %, w/v) were used, but that the main action was to overcome the inhibitory action of concentrated homogenates (Table I). Haemoglobin inhibition cannot normally be overcome by addition of vanadium.

TABLE I

EFFECT OF VANADIUM (10^{-3} M) AND LIVER HOMOGENATES ON THE RATE OF OXIDATION OF LINOLEIC ACID EMULSION

Liver homogenate concentration (%)	*Rate of oxidation of linoleic acid (µl O_2/min)*	
	No addition	*Vanadium added*
0.25	2.7	2.7
0.5	4.5	4.5
1.0	5.15	6.6
2.5	0.25	5.7
4.0	0.20	5.15

These findings may have important biological significance. Peroxides of unsaturated fatty acids are known to be toxic to animals[7], to enzymes[8] and to oxidise –SH-containing amino acids and proteins[5]. It is clear that wherever haematin proteins are present *in vivo* in relatively high concentration no peroxide will be formed, but at lower concentrations within a certain critical range rapid catalysis of the oxidation of unsaturated fatty acids and peroxide formation can occur.

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The effect of citrate on the metabolism of acetate by sheep tissues *in vitro*

Many workers have shown that low concentrations of [^{12}C]citrate stimulate the incorporation of [^{14}C]acetate into long-chain fatty acids *in vitro*[1,2] and that higher concentrations of citrate inhibit this incorporation[3–5]. Evidence has been produced

both for and against the stimulation being due to production of carbon dioxide or NADPH. MARTIN AND VAGELOS[4,5] have produced evidence that the stimulation is due to stimulation of acetyl-coenzyme A carboxylase (EC 6.4.1.2). SRERE AND BHADURI[3] suggest that the inhibition by higher concentrations of [^{12}C]citrate is due to production of [^{12}C]acetyl-coenzyme A. CHRIST AND HÜLSMANN[6] have recently shown that the mitochondria are the site of major incorporation of acetate into long-chain fatty acids by fractions derived from washed pigeon liver.

While investigating the more general problem of acetate metabolism in sheep[8], we have incubated homogenates of lamb liver and heart in media based on that of ABRAHAM *et al.*[2] containing [1-^{14}C]acetate. The reactions were stopped by the addition of $HClO_4$, and the mixtures were fractionated to give CO_2, long-chain fatty acids, organic acids and amino acids. As shown in Table I the long-chain fatty acids which were isolated contained much less of the label than did the combined organic acids and amino acids. The presence of citrate did result in increased incorporation into long-chain fatty acids; it resulted also in an increased incorporation into mixed organic and amino acids.

TABLE I

THE INCORPORATION OF [1-^{14}C]ACETATE BY HOMOGENATES OF SHEEP HEART

Sheep heart was minced into ice-cold 0.25 M sucrose, then homogenised in 3 vol. of 0.25 M sucrose made 0.03 M with respect to nicotinamide. The 2000 × *g* supernatant was used, 2.0 ml (31.6 mg protein) per vessel in a total volume 3.5 ml. The medium was that of ABRAHAM *et al.*[2] but with the citrate omitted; the medium was added in a concentrated form so that the final concentrations were those used by ABRAHAM *et al.* When citrate was added the final concentration was $1.25 \cdot 10^{-2}$ M. Each vessel was incubated aerobically and contained 6.0 μmoles sodium [1-^{14}C]-acetate (2.0 μC). Figures are mμatoms of acetate C incorporated per vessel per h. Each figure is the average of duplicate vessels incubated at 37° *minus* the average of identical duplicate vessels incubated at 0°.

Additions	*Carbon dioxide*	*Organic acids + amino acids*	*Long-chain fatty acids*
None	118	275	0.75
Citrate	26	401	1.45

The organic acids were separated from the amino acids on an ion-exchange resin. Paper chromatography of these two fractions showed that when citrate was not added to the incubation mixture, 75 % of the label (0.15 μC) in the organic acid–amino acid mixture was in glutamate, 25 % (0.05 μC) of the radioactivity being found in citrate, succinate, malate, fumarate, β-hydroxybutyrate and aspartate. When citrate had been added to the incubation mixture, only 33 % (0.092 μC) of the radioactivity was in glutamate, 67 % (0.186 μC) being in organic acids, mainly citric, succinic, malic acids.

Table II gives the results of experiments in which we incubated homogenates, or mitochondria prepared from them, in a medium based on that of ABRAHAM *et al.*[2], further modified so that the incubation mixture was 0.25 M with respect to sucrose. At the end of the incubation period, the incubation mixture was swiftly chilled to 0°, the mitochondria were centrifuged out as quickly as possible at 0°, the mitochondria and supernatants were treated with $HClO_4$ and fractionated as above. If one assumes

TABLE II

INCORPORATION OF [1-^{14}C]ACETATE BY HOMOGENATES AND MITOCHONDRIA OF SHEEP HEART

The homogenate was prepared in 0.35 M sucrose made 0.03 M with respect to nicotinamide. Mitochondria were prepared from this in the usual way, washed once with 0.35 M sucrose and resuspended in 0.35 M sucrose to give the volume of the original homogenate. Each vessel contained 5.0 ml homogenate or mitochondria (respectively, 103 and 14 mg protein) in a total volume of 7.0 ml. Each vessel contained 60 μmoles sodium [1-^{14}C]acetate (4.0 μC). For other details see Table I or text.

Preparation	*Additions*	*Long-chain fatty acids*			*α-Keto acids**	*α-Amino acids**	*Organic acids**	*Total water soluble*
		Mitochondria	*Supernatant*	*Total*				
Homogenate	None	1.31	1.09	2.4	38.2	745.0	389.0	1170.0
Homogenate	Citrate	1.17	2.10	3.3	736.0	570.0	1860.0	3170.0
Mitochondria	None	0.15	0.045	0.2	None	150.0	330.0	480.0
Mitochondria	Citrate	0.99	0.135	1.1	11.3	180.0	3745.0	3940.0

* These substances were exclusively in the 15000 × *g* supernatant after incubation.

that the mitochondria had the same activity when they were incubated alone as they had when they were incubated with other components of the homogenate, these results indicate that all the labelled organic acids were formed by the mitochondria. The mitochondria synthesised between one third and one tenth of the long-chain fatty acids under these aerobic conditions. Much less radioactivity appeared in the amino acids and keto acids when the microsome-rich fraction was absent. Addition of citrate again resulted in increased incorporation of radioactivity into organic acids and keto acids with both homogenate and mitochondria. It will be noticed that when mitochondria incorporated acetate the keto, organic, and amino acids appeared exclusively in the supernatant fraction.

The amount of incorporation of acetate into long-chain fatty acids in these experiments was of the same order as that found by other workers, but it was very small, much smaller than the amount of incorporation into organic acids and amino acids. The same pattern of incorporation was found in the living sheep[8]. Although there is an increase in the total amount of radioactivity incorporated into long-chain fatty acids when citrate is present, the increase in the total amount of radioactivity incorporated into organic acids and amino acids is much larger. Our results suggest that the effect of addition of citrate on acetate incorporation into fatty acids could be the result of a general increase in the pool size of organic acids, *i.e.* citrate itself and intermediates of the tricarboxylic acid cycle which are derived from it. More of the radioactivity would then be held in these acids, mainly in citric acid, and this could make it more available for incorporation into fatty acids. It has been shown that carbon atoms of citrate are incorporated into long-chain fatty acids *in vitro*[3,6,7]. Formica[7] has in fact shown that the radioactivity of [1,5-^{14}C]citrate is incorporated more quickly than is that of [1-^{14}C]acetyl-CoA. As far as we know nobody has measured the specific activity of the long-chain fatty acids which have been synthesised in this kind of experiment. When citrate is added there is a small increase in the absolute amount of radioactivity in the fatty acids, but this is not necessarily accompanied by an increase in the specific activity of the fatty acids. There may be a drop in the specific activity of the fatty acids, just as there is a drop in the

specific activity of citrate as shown by the decrease in radioactivity in CO_2 (Table I). Addition of larger amounts of citrate would still further lower the specific activity of citrate, and, if citrate carbons were being incorporated into fatty acids, one would expect that a concentration of citrate could be reached at which the specific activity of citrate would be so low that there would be little incorporation of radioactivity from citrate into fatty acids. That is, high concentrations of [^{12}C]citrate would lower the incorporation of ^{14}C into fatty acids, a situation which has been shown to occur[3-5].

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A reciprocal relationship between fatty acid and cholesterol biosynthesis

The potential for the reciprocal regulation of fatty acid and cholesterol biosynthesis by competition for common precursors or cofactors has been investigated in several laboratories[1-4]. The yeast studies of KLEIN[1] clearly showed that when fatty acid synthesis was augmented by provision of a CO_2 source, the synthesis of lipids of the non-saponifiable fraction was depressed. Such an augmentation–depression phenomenon for fatty acid-non-saponifiable lipid synthesis has also been noted by BLOOMFIELD AND BLOCH[2] following addition of biotin to a strain of biotin-deficient yeast. Also, LAYNE *et al.*[3] found that when fatty acid synthesis was stimulated in pigeon-liver homogenates, resultant to increasing concentrations of glucose 6-phosphate, the synthesis of cholesterol was simultaneously depressed. FLETCHER AND MYANT[4], however, using rat-liver homogenates were unable to detect a consistent effect on cholesterol synthesis when fatty acid synthesis was stimulated by the addition of various cofactors.

In an endeavor further to clarify the potential of the fatty acid–cholesterol interregulation, the effect of potassium citrate on the synthesis of these lipids has been studied in both homogenates and slices prepared from rat liver. The results of these investigations indicate that under the conditions employed there exists a reciprocal relationship between fatty acid stimulation and cholesterol depression. It has further been demonstrated that citrate can augment fatty acid synthesis and depress cholesterol synthesis in the intact cell. The latter observation is of additional