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

interest since earlier attempts to elicit such a citrate response with intact cells were unsuccessful[5,6].

Male Sprague Dawley rats fed laboratory chow were used in the experiments. Liver slices, approx. 0.5 mm thick, were prepared with a Stadie-Riggs microtome. Homogenates were prepared in a Dounce homogenizer using 1 ml of 0.1 M potassium phosphate buffer (pH 7.0) containing 10 μmoles of reduced glutathione/g of wet tissue. The homogenate was centrifuged at 10000 × *g* for 15 min at 0° and the supernatant fraction, containing the microsomes, was used without further treatment. Slices and homogenates were incubated with [2-^{14}C]acetate for 1 h at 37° with and without potassium citrate as detailed in Table I. At the end of the incubation the reactions were stopped with H_2SO_4, and CO_2, fatty acids, and cholesterol were isolated and assayed for radioactivity as previously described[7].

As can be seen from Table I, Expts. 1 and 2, the addition of citrate resulted in a 5–7-fold stimulation of fatty acid synthesis in the microsomal-supernatant system. Cholesterol synthesis fell simultaneously about 5-fold. In other experiments the citrate stimulation of fatty acid synthesis has been as high as 60-fold. Cholesterol synthesis, even with the highest stimulation, was never abolished.

Expts. 3 and 4 show the reciprocal relationship between fatty acid and cholesterol synthesis in rat-liver slices under the influence of potassium citrate. With a 2- to 3-fold stimulation of fatty acid synthesis, a concomitant fall in cholesterol of approximately the same magnitude occurred. The decrease in the CO_2 radioactivity doubtless represents dilution of the labeled acetate by unlabeled citrate in the reactions of the Krebs cycle.

In an attempt to show the reciprocal relationship by another approach, the

TABLE I

THE EFFECT OF CITRATE ON FATTY ACID AND CHOLESTEROL SYNTHESIS IN RAT-LIVER HOMOGENATES AND SLICES

Expts. 1 and 2 were performed using the microsomal-supernatant fraction described in the text. 1 ml of this fraction was incubated with 50 μmoles potassium phosphate buffer (pH 7.0), 20 μmoles ATP, 10 μmoles Mg $(Cl)_2$, 20 μmoles $KHCO_3$, 1.25 μmole TPN^+, and 0.13 μmole [2-^{14}C]acetate (1 μC) in a total volume of 1.5 ml. 20 μmoles of potassium citrate and 5 units of avidin were added where indicated. Expts. 3 and 4 were carried out with liver slices. 500 mg of slices were incubated in 3.0 ml of $KHCO_3$ buffer[11], (pH 7.4), containing 5 μmoles (1 μC) of [2-^{14}C]acetate and, when added, 100 μmoles potassium citrate. Incubations were for 1 h at 37°. Reactions were stopped by the addition of 0.1 ml of 10 N H_2SO_4. Results are tabulated as total counts/min incorporated into CO_2, cholesterol, and fatty acid for the 1-h incubation period.

Expt.	*System*	*Addition*	*Radioactivity incorporated*		
			CO_2 (counts/min)	*Fatty acid (counts/min)*	*Cholesterol (counts/min)*
1	Supernatant	None	—	8 742	699
	+	Citrate	—	47 757	140
	microsomes	Avidin	—	908	66
2	Supernatant	None	—	7 159	802
	+	Citrate	—	50 923	186
	microsomes	Avidin	—	897	84
3	Slices	None	11 854	10 726	3300
		Citrate	826	30 581	1952
4	Slices	None	8 893	11 144	4325
		Citrate	543	19 523	1047

microsomal-supernatant fraction was incubated with avidin. It was anticipated that inhibition of fatty acid synthesis would result in a stimulation of cholesterol synthesis. As can be seen, however, both cholesterol and fatty acid synthesis were inhibited. The inhibition of cholesterol synthesis has been regularly observed, though to varying degrees, with different batches of commercially prepared avidin (Nutritional Biochemicals Corp., Cleveland, Ohio, (U.S.A.)). Whether the effect represents inhibition of CO_2 fixation or whether it is non-specific, due perhaps to a contaminant in the avidin, is not known at present. It should be noted that FLETCHER AND MYANT[4] observed no inhibition of cholesterol synthesis by avidin in their studies.

The fact that the citrate effect on fatty acid biosynthesis was demonstrated in tissue slices in these experiments whereas MASORO *et al.*[5] found no such effect may be related to the much lower concentration of citrate used by the latter investigators. The existence of a citrate effect in the intact cell as well as in homogenates and purified enzyme systems does not, of course, imply physiological significance. While the concentration of citrate used here is comparable to that used in previous investigations[8–10] it is considerably greater than levels normally present in rat liver, where, per gram of tissue obtained from fed rats, values of 0.25–0.50 μmole citrate have been observed[12].

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