Citrate Content of Liver and Kidney of Rat in Various Metabolic States and in Fluoroacetate Poisoning

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The citrate content of rat liver changes little when normal rats are starved, when starved rats are re-fed with various diets and when normal animals are made diabetic with alloxan. The citrate content of rat kidney changes little on starvation, but it doubles on induction of diabetes. Fluoroacetate poisoning has relatively little effect on the citrate content of liver under a variety of conditions except that normal female rats show a 2.4-fold increase. Fluoroacetate poisoning leads to increases in the citrate content of kidney under all conditions. The relevance of these observations to the regulation of fatty acid synthesis is discussed. The acetic anhydride-pyridine method and the pentabromoacetone method for the estimation of citrate are compared.

Citrate acts as a precursor of acetyl-CoA in the synthesis of fatty acids in various animal organs. The evidence for this is based on the incorporation of citrate carbon into fatty acids by cell-free extracts of lactating mammary gland and liver (Spencer & Lowenstein, 1962; Bhaduri & Srere, 1963). Supporting evidence rests on the demonstration that changes in the activity of ATP citrate lyase (citrate-cleavage enzyme) correlate with changes in the rate of fatty acid synthesis under various nutritional and hormonal conditions (Kornacker & Lowenstein, 1963, 1965*a,b*; Spencer & Lowenstein, 1966; Brown & McLean, 1965; Kornacker & Ball, 1965). The incorporation of radioactivity from α -oxo[5-¹⁴C]glutarate into fatty acids is most readily explained by its conversion into citrate and its subsequent cleavage to acetyl-CoA and oxaloacetate (D'Adamo & Haft, 1962, 1965; Madsen, Abraham & Chaikoff, 1964). In addition to supplying the acetyl group of acetyl-CoA, citrate activates acetyl-CoA carboxylase of vertebrates. The reaction catalysed by this enzyme is a key step in the synthesis of fatty acids (Wakil, 1962; Vagelos, 1964).

The citrate concentration at which the reaction velocity of citrate cleavage by high-speed supernatants of rat liver is one-half of maximum is about 0.3 mM. The citrate content of liver of normal rats is about 0.3 μ mole/g. fresh wt. The extent to which acetyl-CoA carboxylase of rat liver is activated to one-half of maximum activity *in vitro* depends on the concentration of citrate and on the time of contact with citrate, in an interdependent manner (M. Fang & J. M. Lowenstein, unpublished work).

Changes in citrate concentration of the liver may therefore influence the rate of fatty acid synthesis in this organ. These considerations led us to measure the citrate content of liver in various metabolic states. Similar measurements on kidney were made for comparison. A preliminary account of this work has appeared (Spencer, Kornacker & Lowenstein, 1964).

EXPERIMENTAL

Animals and diets. All rats were of the Sprague-Dawley strain. They were supplied by Charles River Breeding Laboratories, N. Wilmington, Mass., U.S.A., and were fed, unless otherwise stated, on a diet of Wayne Lab-Blox (Allied Mills Inc., Chicago, Ill., U.S.A.). The high-fat diet contained per 100 g. of diet: 60 g. of butter, 30 g. of vitamin-free casein and 10 g. of powdered cellulose. Other diets and treatment were as described by Kornacker & Lowenstein (1965*a,b*).

Preparation of deproteinized tissue extract. Rats were anaesthetized by intramuscular injection of sodium barbital (40 mg./kg. body wt.). In a few cases the animals were anaesthetized with ether. (No significant differences in citrate content of liver were observed with the two methods.) Liver was fixed by rapid freezing *in situ*, according to the technique of Wollenberger, Ristau & Schoffa (1960), and removed. A portion of the liver was weighed in the frozen state. Then 4 ml. of 5% (w/v) trichloroacetic acid/g. of liver was pipetted into a small porcelain mortar. The mortar was placed on powdered solid CO₂. When it had cooled to about -80° the frozen liver and trichloroacetic acid were ground together thoroughly with a porcelain pestle. The frozen powder was then poured into a beaker and allowed to melt with occasional stirring at 5°. After standing at this temperature for about 1 hr. the solution was centrifuged to remove precipitated protein and extracted three times with

2 vol. of ether to remove the trichloroacetic acid. The aqueous solution was then transferred to a 25 ml. conical flask and freeze-dried. The residue was dissolved in 1.0 ml. of 5% (w/v) trichloroacetic acid/3 g. of liver in the original sample. Duplicate analyses for citrate were performed on this solution.

Assay of citric acid. The acetic anhydride-pyridine method was used essentially as described by Saffran & Denstedt (1948), except that one-fifth of the recommended volumes were used. Acetic anhydride (1.6 ml.) was added to 0.2 ml. of the extract prepared as above. The mixture was heated at 60° for 10 min. and cooled to room temperature by immersion in cold water. Pyridine (0.2 ml.) was added, the tube sealed with a glass stopper and heated at 60° for 40 min.

Table 1. *Specificity of acetic anhydride-pyridine method for determination of citrate*

The method used is described in the text. The final volume of the reaction mixture was 2.0 ml.

Compound	Amounts tested (μmoles/2.0 ml. of reaction mixture)	Molar extinction coefficient ($\epsilon_{425\text{m}\mu}$)
Citrate	0.05, 0.1, 0.2	3620
Isocitrate	0.2, 0.4, 1.0	900
<i>cis</i> -Aconitate	0.05, 0.1, 0.2	2360
<i>trans</i> -Aconitate	0.05, 0.1, 0.2	4160
Tricarballoylate	0.2, 2.0	0
Benzene-1,2,3- tricarboxylate	0.2, 2.0	0
Oxalate	5, 20	0
Malonate	100, 200	3
Succinate	5, 20	0
Glutarate	0.2, 2.0	0
β -Methylglutarate	0.2, 2.0	3
Oxaloacetate	1.25, 2.5*	90
α -Oxoglutarate	0.2, 2.0	0
Malate	5, 10, 20	25
Citramalate	0.2, 2.0	<1
β -Hydroxy- β -methyl- glutarate	2, 5, 10, 20	42
DL-Tartrate	0.25, 0.5, 1.0	286
Fumarate	1.25, 2.5, 5	132
Maleate	5, 10, 15	38
Citraconate	0.2, 2.0	2
Mesaconate	0.2, 2.0	0
Itaconate	10, 20, 50	3
Glutaconate	1.25, 2.5	310
Dihydroxymaleate	5, 20	0
Pyruvate	5, 20	0
Acetoacetate	5, 20	0
Lactate	5, 20	0
β -Hydroxybutyrate	5, 50	0
Crotonate	5, 50	0
Glyoxylate	5, 20	<1
Ascorbate	1.25, 3.5, 5.0	58†
Oxaloglycollate	0.2, 2.0	0

* Higher concentrations give anomalous results.

† From the lowest amount tested; larger amounts gave lower values.

The tube was then cooled in ice and the extinction of the solution determined at 425 m μ (light-path 1 cm.). This method is very reproducible; a number of attempts were made with different proportions of trichloroacetic acid, but it then yielded erratic results.

The specificity of the method was reinvestigated with the results shown in Table 1. Under the above conditions, *trans*-aconitate gives an extinction coefficient that is 15% higher, and *cis*-aconitate one that is 35% lower, than that for citrate. Isocitrate gives an extinction coefficient that is only 25% that of citrate. The aconitase equilibrium at pH 7.4 and 25° is 90.0% of citrate, 2.9% of *cis*-aconitate and 6.2% of isocitrate (Krebs, 1953). Under equilibrium conditions *cis*-aconitate will yield about 2% of the colour obtained with citrate, and isocitrate will contribute less than 2% of the colour. However, equilibrium conditions may not be attained in living cells. Unusual conditions can be guarded against by measuring the amount of isocitrate present with isocitrate dehydrogenase (Siebert, 1963); aconitate can be measured separately by carrying out the acetic anhydride-pyridine method at 0°. Under these conditions aconitate gives the colour reaction, whereas citrate gives little or no colour. Tartrate and glutaconate were the only other compounds found to yield significant extinctions at 425 m μ . Saffran & Denstedt (1948) state that itaconate also yields an appreciable colour in their method, but on a molar basis this is less than 0.1% of the extinction obtained with citrate (Table 1).

Fig. 1 shows absorption spectra of some of the coloured compounds formed in the acetic anhydride-pyridine method. Citrate, isocitrate, *cis*-aconitate, *trans*-aconitate and tartrate yield similar spectra with maxima at 425 m μ [the wavelength used by Saffran & Denstedt (1948) was

Fig. 1. Absorption spectra of compounds formed in the acetic anhydride-pyridine method. The spectra were obtained on a Cary recording spectrophotometer by using cuvettes with a light-path of 1 cm. The reference cuvettes contained water. The compounds used were as follows (in μmoles/ml. of solution placed in the cuvette): a, citrate (0.5); b, isocitrate (1.0); c, *cis*-aconitate (0.5); d, *trans*-aconitate (0.2); e, DL-tartrate (2.5); f, glutaconate (2.5); g, none.

Table 2. *Comparison of acetic anhydride-pyridine method with pentabromoacetone method in determination of citrate content of liver*

The details of both methods are described in the text. Results obtained with the pyridine-acetic anhydride method are shown under (a), those obtained with the pentabromoacetone method are shown under (b). The animals were fed on a laboratory diet (Wayne Lab-Blox).

	No. of animals	Citrate content + s.e.m. (μ mole/g. fresh wt.)	
		(a)	(b)
Expt. 1	5	0.300 $\pm$ 0.022	0.300 $\pm$ 0.027
Expt. 2	5	0.29 $\pm$ 0.02	0.31 $\pm$ 0.02

440 μ]. Glutaconate yields an entirely different absorption spectrum with a maximum at 480 μ . Fürth & Herrmann (1935) considered the types of compound that might be responsible for the observed colours, as well as the effect of using bases other than pyridine.

The pentabromoacetone method for the determination of citrate was used according to the general procedure given by Stern (1957). We were unable to obtain easily reproducible results when using this method exactly as described by Stern (1957). The following modification of the method, devised and recommended by Dr R. J. Rubin, gave readily reproducible results. The unknown solution was deproteinized by addition of trichloroacetic acid to a final concentration of 5% (w/v). To 5.0 ml. of this solution was added 0.2 ml. of 18 N- H_2SO_4 and a boiling chip. The solution was heated in an oil bath at 120–130° until its volume was reduced to about 2 ml., and was then cooled to room temperature. Bromine water (0.8 ml.) was added, the solution was shaken vigorously, 2.0 ml. of a suspension of MnO_2 was added, and the mixture was again shaken vigorously and stood at room temperature for 15 min. (The suspension of MnO_2 was prepared by mixing together 1 vol. of m- MnCl_2 , 1 vol. of m- KMnO_4 and 0.8 vol. of 18 N- H_2SO_4). After cooling to 0° 6% (v/v) hydrogen peroxide was added dropwise until the suspension became colourless. Any slight excess of peroxide was destroyed by cautious addition of 0.05 M- KMnO_4 until a faint yellow colour appeared. Next 0.2 ml. of 0.05 M- KMnO_4 was added, the volume was adjusted to 5 ml., and 4 ml. of heptane was added. The tube was stoppered and shaken on a wrist-action shaker for 10–15 min. Part of the heptane layer (3.5 ml.) was withdrawn and added to 3.0 ml. of a solution containing 4% (w/v) of thiourea and 2% (w/v) of sodium borate. After shaking the mixture in a glass-stoppered tube for 10 min. the aqueous layer was withdrawn and its extinction at 430 μ measured. Under these conditions 0.1 μ mole of citrate yields an extinction at 430 μ of 0.126 (1 cm. light-path), corresponding to an extinction coefficient of 5040 mole⁻¹ cm.⁻¹. The corresponding extinction given by Stern (1957) for 0.1 μ mole of citrate, corrected for the use of slightly different proportions of heptane, was 0.063.

Identical results were obtained when the two methods were twice compared on five separate extracts prepared from rat liver (Table 2). As pointed out above, under conditions when the aconitase reaction is in equilibrium, the acetic

Table 3. *Citrate content of liver of rats in various nutritional states*

For conditions see the text. All rats used were males (wt. 180–435 g., the average about 300 g.).

Treatment	No. of rats	Citrate content $\pm$ s.e.m. (μ mole/g. fresh wt.)
Normal diet	20	0.30 $\pm$ 0.01
Starved for 1 day	5	0.33 $\pm$ 0.03
Starved for 2 days	7	0.33 $\pm$ 0.03
Starved for 3 days	4	0.32 $\pm$ 0.01
Starved for 2 days then re-fed for 2 days with:		
Normal diet	5	0.35 $\pm$ 0.03
High-glucose diet	5	0.29 $\pm$ 0.01
High-fat diet	5	0.29 $\pm$ 0.01
White bread	5	0.32 $\pm$ 0.02

anhydride-pyridine method might be expected to give results about 4% higher than those obtained by the pentabromoacetone method. In terms of the results in Table 2 this is equivalent to 0.012 μ mole of citrate/g. fresh wt., an amount that is about one-half of the standard error of the determination. In view of the excellent agreement between the two methods and the far greater simplicity of the acetic anhydride-pyridine method, this procedure was used in all measurements described below.

RESULTS

Citrate content of liver in various nutritional states and in diabetes. The rate of fatty acid synthesis is reduced greatly during starvation; it is increased greatly when starved rats are re-fed with a diet high in carbohydrate and low in fat. If citrate plays a role in regulating fatty acid synthesis *in vivo* by activating acetyl-CoA carboxylase, then the citrate content of liver would be expected to decrease in starvation, and increase on re-feeding starved animals with suitable diets. However, no change in citrate content of rat liver occurred under these conditions (Table 3). Moreover, there was no change in citrate content when animals that had been starved for 2 days were re-fed with a diet high in fat.

Fatty acid synthesis is depressed in alloxan-diabetes. The citrate content of liver of rats made diabetic with alloxan was slightly greater than that of normal rats (Table 4) ($P = 0.005$).

Effect of fluoroacetate poisoning on citrate content of rat liver. In view of the negligible changes in citrate content of liver under various dietary conditions and in diabetes, an attempt was made to produce such changes by administration of fluoroacetate. Results obtained with animals in various nutritional states are shown in Table 5. Although a 2.4-fold increase in citrate content was observed

Table 4. Citrate content of liver of diabetic rat

For conditions see the text. The animals were made diabetic with alloxan as described by Kornacker & Lowenstein (1965b). All animals were kept on the laboratory diet. Where shown fluoroacetate (4mg./kg.) was injected intraperitoneally 2hr. before death. M, Male; F, female. The citrate content of 21 normal animals (20 males and one female) was 0.30 ± 0.01 μ mole/g.

	No. and sex of rats	Citrate content $\pm$ s.e.m. (μ mole/g. fresh wt.)
No fluoroacetate	12M and 3F	0.41 ± 0.04
Fluoroacetate	3M and 3F	0.34 ± 0.04

Table 5. Effect of fluoroacetate poisoning on citrate content of liver of rat

Where indicated ('poisoned'), fluoroacetate (4mg./kg.) was administered intraperitoneally 2hr. before death. M, Male; F, Female. Comparison of the citrate content of liver from normal, unpoisoned animals with that from other animals by the *t* test showed the following: * $P < 0.001$; ** $0.005 > P > 0.001$; *** $P < 0.01$.

	Diet	No. and sex of rats	Citrate content $\pm$ s.e.m. (μ mole/g. fresh wt.)
Normal animals	Normal	20M	0.30 ± 0.01
Poisoned	Normal	6M	$0.45 \pm 0.08^{**}$
Poisoned	Starved for 2 days	10M	$0.48 \pm 0.06^*$
Poisoned	Starved for 2 days, then fed with diet high in glucose for 2 days	5M	0.27 ± 0.04
Poisoned	Starved for 2 days, then fed with diet high in fat for 2 days	6M	0.27 ± 0.03
Normal animals	Normal	4F	0.29 ± 0.02
Poisoned	Normal	5F	$0.72 \pm 0.10^*$
Poisoned	Starved for 2 days	5F	$0.45 \pm 0.06^{**}$
Poisoned	Starved for 2 days, then fed with diet high in glucose for 2 days	5F	$0.41 \pm 0.07^{***}$
Poisoned	Starved for 2 days, then fed with diet high in fat for 2 days	5F	0.24 ± 0.02

with female rats on a normal diet, most of the other increases amount to less than 50%. Tepperman & Tepperman (1955) reported that rats on a diet high in carbohydrate show a sixfold increase in the citrate content of liver after fluoroacetate poisoning,

Table 6. Citrate content of kidney of rat

Where indicated ('poisoned'), fluoroacetate (4mg./kg.) was given intraperitoneally 2hr. before death. M, Male; F, female. Comparison of the citrate content of kidney from normal, unpoisoned animals with that from other animals by the *t* test showed the following: * $P < 0.001$; ** $0.02 > P > 0.01$; *** $0.05 > P > 0.025$.

	Diet	No. and sex of rats	Citrate content $\pm$ s.e.m. (μ mole/g. fresh wt.)
Normal animals	Normal	5M	0.45 ± 0.04
Normal animals	Normal	4F	0.35 ± 0.05
Normal animals	Starved for 2 days	5M	0.46 ± 0.04
Poisoned	Normal	5M	$3.31 \pm 0.44^*$
Poisoned	Normal	4F	$3.47 \pm 0.46^*$
Poisoned	Starved for 2 days	4M	$3.45 \pm 0.37^*$
Poisoned	Starved for 2 days, then fed with diet high in glucose for 3 days	5M	$1.77 \pm 0.43^{**}$
		6F	$2.12 \pm 0.18^*$
Poisoned	Starved for 2 days, then fed with diet high in fat for 3 days	6M	$2.32 \pm 0.11^*$
Alloxan-diabetic (not poisoned)	Normal	3M and 9F	$0.80 \pm 0.10^{***}$

whereas rats on a diet high in fat showed little or no increase under the same conditions. We failed to observe such a difference in the case of male rats, but observed a 37% increase in the citrate content of females given the diet high in carbohydrate and a 20% decrease in the citrate content of females given the diet high in fat. These differences are hardly significant (Table 5). There was little or no increase in the citrate content of liver from diabetic rats when these animals were poisoned with fluoroacetate (Table 4) ($P = 0.2$).

Citrate content of rat kidney. Since no appreciable changes in the citrate content of liver were obtained by nutritional means, or by the induction of diabetes with alloxan, and only slight changes were obtained with fluoroacetate, we also measured the citrate content of kidney. Starvation had no effect, but induction of diabetes with alloxan led to a doubling of the citrate content of kidney (Table 6).

Kidney accumulates large amounts of citrate in response to fluoroacetate poisoning (Buffa & Peters, 1950), a finding that has often been confirmed (see Tables 6 and 7). The increase in citrate content of kidney that occurs after fluoroacetate poisoning is

little affected by the nutritional state of the animal, although the increase was somewhat smaller in the starved and re-fed animals than in the others.

DISCUSSION

Acetyl-CoA carboxylase is activated by many polycarboxylic acids, of which citrate is by far the most effective (Lynen, Matsushashi, Numa & Schweizer, 1963). Under certain conditions, the reaction catalysed by acetyl-CoA carboxylase is the rate-limiting step of fatty acid synthesis by high-speed supernatants prepared from rat liver (Numa, Matsushashi & Lynen, 1961; Wieland, Neufeldt, Numa & Lynen, 1963). These observations have led to the suggestion that the rate of fatty acid synthesis is regulated by changes in the intracellular concentration of citrate (Vagelos, Alberts & Martin, 1963; Wieland & Neufeldt, 1963). Fatty acid synthesis in the liver undergoes large changes in rate when normal animals are starved, starved animals are re-fed with diets high in carbohydrate (but not diets high in fat) and normal animals are made diabetic with alloxan (Fritz, 1961; Spencer, Corman & Lowenstein, 1964; Howard & Lowenstein, 1965). Relatively large changes in citrate content of liver might therefore be expected in response to such changes if the citrate content is one of the factors that regulate fatty acid synthesis. The changes actually observed were negligible (Tables 3 and 4).

No method so far devised reveals the intracellular location of citrate, and therefore the possibility

remains that the activity of acetyl-CoA carboxylase is regulated by changes in the intracellular location, rather than the overall content, of citrate in the liver. This possibility was considered previously by Vagelos *et al.* (1963). The regulation of phosphofructokinase by citrate has been discussed in similar terms by Gevers & Krebs (1966). An energy-linked transport of citrate into isolated liver mitochondria has been demonstrated and studied by Max & Purvis (1965). The mitochondria lose the transported citrate passively. Also, the citrate that is synthesized in isolated liver mitochondria is, in part, lost into the incubating medium (Lowenstein, 1964).

The citrate content of liver increased 37% when rats were made diabetic with alloxan ($P=0.005$) (Table 4). Parmeggiani & Bowman (1963) found that it increased 2.5-fold under these conditions. In earlier reports, Frohman, Orten & Smith (1951) and Frohman & Orten (1955) found that the citrate content of liver decreased fourfold in diabetes. The concentrations of citrate reported by these authors were much lower than those found by us. Schneider, Striebig & Hogeboom (1956) presented data showing that the concentration of citrate is lower in mitochondria prepared from liver of diabetic rats than in those prepared from normal animals. In their work the preparation of the cell fractions involved relatively slow procedures, during which the citrate contents may well have changed.

A summary of previous determinations of citrate contents of liver and kidney is presented in Table 7.

Table 7. Comparison of citrate contents of liver and kidney from normal, diabetic and fluoroacetate-poisoned rats reported by various authors

Animals were fed on normal laboratory diets. Values are expressed as μ moles/g. fresh weight. Fluoroacetate was injected intraperitoneally at the dosages and times before death shown.

Condition	Sex	Liver	Kidney	Reference
Normal	M	0.30	0.45	This paper
	F	0.29	0.35	This paper
Diabetic	M & F	0.41	0.80	This paper
Fluoroacetate (4 mg./kg., 2 hr.)	M	0.45	3.31	This paper
Fluoroacetate (4 mg./kg., 2 hr.)	F	0.72	3.47	This paper
Normal		0.24	0.29	Potter & Busch (1950)
Fluoroacetate (5 mg./kg., 1 hr.)		0.26	5.35	Potter & Busch (1950)
Normal		0.11		Tepperman & Tepperman (1955)
Normal		0.14*	0.19	Frohman <i>et al.</i> (1951)
Diabetic		0.034	0.050	Frohman <i>et al.</i> (1951)
Normal		0.15*		Parmeggiani & Bowman (1963)
Diabetic		0.38		Parmeggiani & Bowman (1963)
Normal		0.12	0.17	Gordon (1961)
Fluoroacetate (2.5 mg./kg., 3.5 hr.)	F	1.74	5.8	Gordon (1961)
Normal	M	0.27	0.23	Gal <i>et al.</i> (1956)
Fluoroacetate (5 mg./kg., 2 hr.)	M	0.18	5.4	Gal <i>et al.</i> (1956); Gal & Smith (1960)

* Recalculated assuming dry wt. is 30% of wet wt.

In kidney the citrate content did not change on starvation, but it doubled on induction of diabetes (Table 6). A similar difference in the citrate content of normal as compared with diabetic animals has been observed in heart and gastrocnemius (Parmeggiani & Bowman, 1963). These changes are not in the direction predicted by the hypothesis that citrate concentrations control fatty acid synthesis *in vivo*, since starvation and alloxan-diabetes are associated with a reduced rate of fatty acid synthesis. It must be pointed out, however, that fatty acid synthesis in kidney, heart and gastrocnemius is at best very slow compared with liver.

Citrate inhibits mammalian phosphofructokinase (Garland, Randle & Newsholme, 1963; Parmeggiani & Bowman, 1963; Passoneau & Lowry, 1963; Garland & Randle, 1964), which catalyses one of the key control reactions of glycolysis. The increase in citrate content of kidney may therefore be responsible, at least in part, for the reduced rate of glycolysis observed in diabetes. The addition of fatty acids or acetoacetate to kidney-cortex slices metabolizing glycerol or dihydroxyacetone leads to an increase in the rate of gluconeogenesis. This is associated with an increase in the citrate content of the slices. Since the concentrations of fructose diphosphate and AMP do not change, the increase in gluconeogenesis may in this case be solely due to the inhibition of phosphofructokinase by citrate (Newsholme & Underwood, 1966).

Fluoroacetate poisoning has little effect on the citrate content of rat liver, except for the 2.4-fold increase observed with female rats on a 'normal' diet. This may be compared with the seven- to eight-fold increases observed in kidney when normal or starved animals are given fluoroacetate. Animals receiving diets high in glucose or fat showed somewhat smaller increases in citrate content (Table 6). The different responses of liver and kidney to fluoroacetate poisoning (Tables 5 and 6) were reported previously by Potter and Busch (1950). They are consistent with the demonstration by Gal, Drewes & Taylor (1961) that administration to the rat of fluoro[¹⁴C]acetate leads to the accumulation of 15 times more labelled fluoroacetate in kidney than in liver. It is not easy to reconcile these results with those of Gal & Smith (1960), who showed that rat-liver mitochondria accumulate very large amounts of citrate (equivalent to 48 μ moles/g. fresh wt. of liver) when incubated with fluoroacetate.

Injection of fluorocitrate results in a large increase in the citrate content of the liver (Gal, Peters & Wakelin, 1956; Gordon, 1961). The lack of accumulation of citrate after injection of fluoroacetate cannot therefore be due to a rapid oxidation of citrate. Instead it may be a function of the different specificities of acetyl-CoA synthetase of liver and kidney (Peters, 1957). Acetyl-CoA

synthetase from pigeon liver activates acetate, but not fluoroacetate, whereas the enzyme from rabbit kidney activates both substances (Brady, 1955).

Gordon (1961) reported that intraperitoneal administration of fluoroacetate to female rats produced 15- and 30-fold increases in the citrate content of liver and kidney respectively. A similar observation was reported for rat liver, but not guinea-pig liver (Buffa & Peters, 1950; Peters, 1957). These results do not agree with those shown in Table 5 or those of Potter & Busch (1950). It is possible that the concentrations reported by Buffa & Peters (1950) were the result of metabolic changes after removing the liver, but these workers argue against this possibility.

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