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Triacylglycerol Metabolism in Isolated Rat Kidney Cortex Tubules

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Triacylglycerol metabolism has been studied in kidney cortex tubules from starved rats, prepared by collagenase treatment. Triacylglycerol was determined by a newly developed fully enzymic method. Incubation of tubules in the absence of fatty acids led to a decrease of endogenous triacylglycerol by about 50% in 1 h. Addition of albumin-bound oleate or palmitate resulted in a steady increase of tissue triacylglycerol over 2 h. The rate of triacylglycerol synthesis was linearly dependent on oleate concentration up to 0.8 mM, reaching a saturation at higher concentrations. Triacylglycerol formation from palmitate was less than that from oleate. This difference was qualitatively the same when net synthesis was compared with incorporation of labelled fatty acids. Quantitatively, however, the difference was less with the incorporation technique. Gluconeogenic substrates, which by themselves had no effect on triacylglycerol concentrations, stimulated neutral lipid formation from fatty acids. Glucose and lysine did not have such a stimulatory effect. Inhibition of gluconeogenesis from lactate by mercaptopicolinic acid likewise inhibited triacylglycerol formation. This inhibitory effect was seen with oleate as well as with oleate plus lactate. When [2-^{14}C]lactate was used the incorporation of label into triacylglycerol was found in the glycerol moiety exclusively. Addition of DL-β-hydroxybutyrate (5 mM) to the incubation medium in the presence of oleate or oleate plus lactate led to a significant increase in triacylglycerol formation. In contrast with the gluconeogenic substrates, DL-β-hydroxybutyrate had no stimulatory effect on fatty acid uptake. The results suggest that renal triacylglycerol formation is a quantitatively important metabolic process. The finding that gluconeogenic substrates, but not glucose, increase lipid formation, indicates that the glycerol moiety is formed by glyceroneogenesis in the proximal tubules. The effect of ketone bodies seems to be caused by the sparing action of these substrates on fatty acid oxidation. The decrease of triacylglycerol in the absence of exogenous substrates confirms previous conclusions that endogenous lipids provide fatty acids for renal energy metabolism.

Examinations of kidney lipid composition (Morgan *et al.*, 1963; Druilhet *et al.*, 1975) revealed that the predominant neutral lipids are triacylglycerols and cholesterol. Dog kidneys perfused for 24 h without added oleate lost 65% of their neutral lipids and 58% of their triacylglycerols (Huang *et al.*, 1971). In kidney slices Hohenegger (1976) observed a 40% decrease in triacylglycerols after 4 h incubation of kidney cortex slices. Addition of oleate prevented this loss.

Endogenous fatty acids have been proposed as the major fuel of respiration in the renal cortex (Weidemann & Krebs, 1969). This assumption is supported by the low respiratory ratio of 0.75 in the kidney cortex (Dickens & Simer, 1930; Hohenegger, 1976). Therefore, it was concluded (Huang *et al.*, 1971; Hohenegger, 1976) that oleate can replace endogenous fatty acids as metabolic fuel to meet the energy requirements of kidney cortex. Only a smaller proportion of fatty acids taken up *in vivo* (Gold & Spitzer, 1964; Park *et al.*, 1974) or by kidney cortex preparations *in vitro* (Weidemann & Krebs, 1969) were recovered in respiratory CO_2. This was in agreement with the observation that O_2 consumption was less than could be accounted for by assuming complete oxidation of the fatty acids taken up (Lee *et al.*, 1962; Barac-Nieto & Cohen, 1968). This phenomenon has been explained by assuming 'incomplete oxidation' of fatty acids (Barac-Nieto & Cohen, 1971). Since ketogenesis from long-chain fatty acids is negligible in kidney (Weidemann & Krebs, 1969), other products of

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fatty acid metabolism have to be postulated. In accordance with previous conclusions derived from isotope experiments (Tinker & Hanahan, 1966; Weidemann & Krebs, 1969; Barac-Nieto, 1976) triacylglycerols were found to be the main product of exogenous fatty acid metabolism.

In the present study we examined net synthesis and degradation of tubular triacylglycerols by a fully enzymic method. The results indicate a regulation of renal triacylglycerol metabolism by metabolic substrates. Some results have been presented previously in a preliminary form (Guder & Wirthensohn, 1978; Wirthensohn & Guder, 1979).

Materials and Methods

Tubule preparation and incubation

Isolated tubule fragments from rat kidney cortex (male Sprague–Dawley; Ivanovas, Kisslegg, Germany) were prepared by collagenase treatment as described previously (Guder & Wieland, 1971) with some recent modifications (Guder, 1979). Tubules equivalent to 1–2mg of tissue protein were incubated in a final volume of 1ml of Krebs–Henseleit bicarbonate buffer (Krebs & Henseleit, 1932), containing 1% (w/v) albumin (fraction V; Serva, Heidelberg, Germany) defatted by the method of Chen (1967) or containing 1mM-oleate (C. Roth, Karlsruhe, Germany) bound to albumin (Guder & Wieland, 1972). All substrates were added as neutral solutions freshly prepared as sodium salts. The tubules were incubated in 25ml plastic vials for 30min at 37°C with O_2/CO_2 (19:1) as gas phase in a shaking water bath.

Incubation was stopped by transferring the vessels into an ice bath. After 10–15min the samples were decanted into Eppendorf cups and centrifuged for 5s at 10000*g* at 4°C. Of the supernatant 500μl was added to 50μl of 30% (v/v) $HClO_4$ and neutralized with $KHCO_3$ for the determination of glucose. Another 200μl of the supernatant was transferred into 3ml glass-stoppered tubes for the extraction of lipids. The sediment was heated for 3min at 100°C and used for triacylglycerol determination.

Analyses

Glucose was determined by the hexokinase method (Bergmeyer *et al.*, 1974). Fatty acids were extracted with di-isopropyl ether/ethanol (19:1, v/v), (Laurell, 1966) and determined as copper-soaps by using diethyl dithiocarbamate (Duncombe, 1963) with the modification of Laurell & Tibbling (1967).

Triacylglycerol determination

Tubular triacylglycerols were determined by a fully enzymic method described recently (Guder & Wirthensohn, 1979). Briefly, the tubule sediment was digested with sodium dodecyl sulphate and Pronase E in Tris buffer, pH 7.4. To one of two identical samples 10μl of a lipase/esterase mixture (Boehringer, Mannheim, Germany) was added to hydrolyse triacylglycerols (Wahlefeld, 1974); 10μl of water was added to the control. After an additional 30min incubation at 37°C glycerol was determined in the neutral $HClO_4$ extracts (Eggstein & Kreutz, 1966). The difference in glycerol content between lipase-treated and untreated (free glycerol) samples was taken as the glycerol content of triacylglycerols.

For comparison triacylglycerols were determined after organic-solvent lipid extraction (Folch *et al.*, 1957; Laurell, 1966). Saponification of the extracted triacylglycerol was performed with ethanolic KOH and glycerol was determined enzymically (Eggstein & Kuhlmann, 1974). In addition neutral lipids were separated by t.l.c. on silica gel plastic sheets of 0.2mm thickness (Merck, Darmstadt, Germany) by using light petroleum (b.p. 60–70°C)/diethyl ether/acetic acid (35:15:1, by vol.) as solvent system. Phospholipids were separated with chloroform/methanol/water (14:6:1, by vol.). The lipid spots were visualized with I_2 vapour. In the experiments with preincubation 2ml of the tubule suspension were incubated in 250ml plastic flasks containing 10ml of Krebs–Henseleit buffer, glutamine (5mM) and albumin-bound oleate (0.8mM) or albumin (1%) for 1h at 37°C. Then tubules were washed twice with buffer and further incubated as described above.

Radioactive-isotope experiments

In experiments with labelled substrates either [2-^{14}C]lactate, [1-^{14}C]oleate or [1-^{14}C]palmitate (The Radiochemical Centre, Amersham, Bucks., U.K.) were added to the incubation medium in the concentrations indicated in the legends to Figs. 4 and 5. In these experiments lipids were extracted and separated by t.l.c. as described above. The visualized spots of the neutral lipids (triacylglycerols, diacylglycerols, monoacylglycerols, fatty acids and cholesteryl esters) were cut out and transferred into counting vials containing 10ml of Scintigel (Roth, Karlsruhe, Germany). Radioactivity was determined with a Packard Tri-Carb liquid-scintillation counter by using external standard ratios to exclude quenching effects.

Materials

Collagenase CLS type II was obtained from Worthington, Freehold, NJ, USA; 3-mercaptopicolinic acid came from Smith, Kline and French Labs., Philadelphia, PA, U.S.A.; glycerol-free KOH was from Riedel-de Haen, Hannover, Germany. The other substrates came from Serva (L-lactate, succin-

ate and lysine), Boehringer (α-oxoglutarate, pyruvate and DL-β-hydroxybutyrate), Roth Karlsruhe (glutamine and dihydroxyacetone) and Merck (fructose, glucose, proline and palmitate). Basic chemicals were of analytical grade from Merck. Phospholipid standards were purchased from Sigma, Heidelberg, Germany.

Results

Evaluation of enzymic triacylglycerol determination

Triacylglycerols in serum can easily be measured with enzymic methods without prior extraction and saponification (Wahlefeld, 1974). Recently we found that this method can also be applied to tissues if the cells are properly pretreated (Guder & Wirthensohn, 1979). This pretreatment included Pronase and sodium dodecyl sulphate digestion after boiling of the tubules. As can be seen from Table 1, omission of boiling resulted in relatively high non-esterified-glycerol concentrations compared with the standard procedure. The heating step simultaneously led to an increase in glycerol content of triacylglycerols, indicating that non-esterified glycerol was probably derived from endogenous lipids by the action of tissue lipase. The absence of Pronase, sodium dodecyl sulphate or both slightly decreased the recovery of the glycerol content of triacylglycerol. Therefore, both substances were added to the standard procedure described in the Materials and Methods section.

To exclude that glycerol measured was derived from sources other than triacylglycerol the results were compared with those obtained with standard procedures. Enzymic determination of the glycerol moiety of acylglycerols after organic-solvent extraction and saponification with ethanolic KOH resulted in a 15% decrease in glycerol content, which is the range of recovery with this method. With the fully enzymic method, added trioleoylglycerol was recovered by 94% (mean for three determinations). Moreover, enzymic digestion of tubular lipids with lipase/esterase mixture led to a complete loss of the triacylglycerol spot in t.l.c. and an obvious increase in the fatty acid fraction.

When phospholipids were separated by t.l.c., lipase treatment was found to increase lysophospholipids, indicating a hydrolysis of fatty acids from phospholipids (W. Stoffel, personal communication). For this reason fatty acids could not be taken as a measure of triacylglycerol.

Triacylglycerol synthesis

Freshly prepared tubules from starved rats contained 22.7 ± 1.1 μmol of triacylglycerols/g of protein (mean ± S.E.M. for 27 preparations). During incubation triacylglycerol content decreased by 30% in 30 min when tubules were incubated in the absence of fatty acids (Guder & Wirthensohn, 1979). This decrease stopped after 1 h when 50% of triacylglycerols had disappeared (Fig. 1). The presence of 0.8 mM-oleate in the medium led to a steady increase in tubular triacylglycerol content. The rate of triacylglycerol formation was linearly dependent on medium oleate concentration up to 0.8 mM (Fig. 2), reaching a plateau at 1 mM. At this saturating

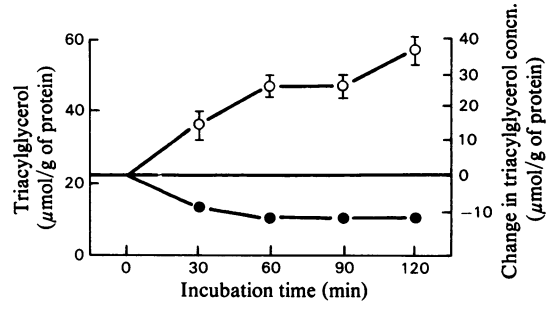

Fig. 1. *Effect of oleate* (0.8 mM) *on triacylglycerol concentrations during incubation of kidney cortex tubules*
Tubules were incubated with 1% albumin (●) or 0.8 mM-oleate (○). Results are means for two (albumin) or three experiments (oleate) respectively. The bars represent S.E.M.

Table 1. *Influence of boiling, Pronase and sodium dodecyl sulphate on the determination of the glycerol moiety of acylglycerols in kidney tubules*
Isolated tubule fragments from starved rats (3.3 mg of protein/ml) were distributed in 0.1 ml portions and treated as indicated. The standard procedure included boiling for 3 min, Pronase/sodium dodecyl sulphate digestion and glycerol determination as described in the Materials and Methods section.

Treatment	Glycerol measured (nmol/tube)	
	+ Lipase	− Lipase
No boiling	93	30
Standard procedure	121	8
− Pronase	109	8
− Sodium dodecyl sulphate	115	8
− Pronase, − sodium dodecyl sulphate	113	8

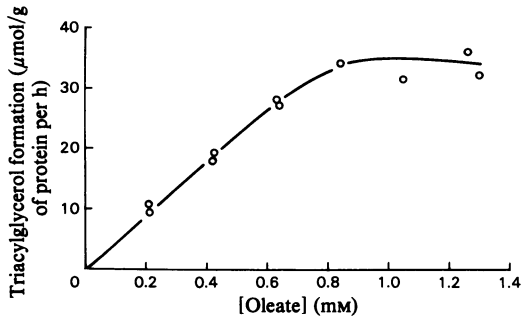

Fig. 2. *Dependence of triacylglycerol formation on oleate concentration*

Kidney tubules were incubated in Krebs–Henseleit bicarbonate buffer in a final volume of 1 ml with increasing concentrations of oleate. Results are from two different tubule preparations.

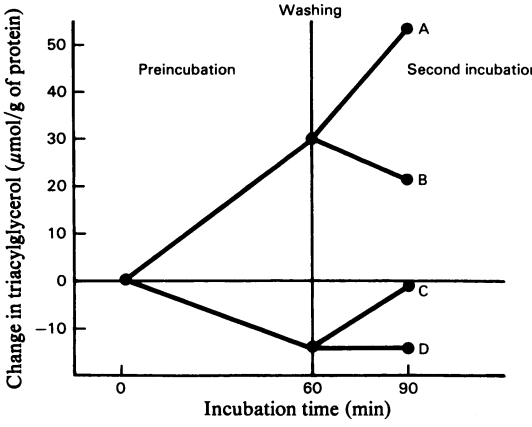

Fig. 3. *Effect of preloading tubule cells with triacylglycerol on the further triacylglycerol metabolism*

Kidney tubules from starved rats were preincubated for 60 min with 0.8 mM-oleate and 5 mM-glutamine or without substrates as described in the Materials and Methods section. After washing of the tubules a second 30 min incubation was performed (with both tubule populations). Results are means for two experiments. In the preincubation, oleate and glutamine were present in A and B and absent in C and D. For the second incubation, oleate and glutamine were present in A and C and absent in B and D.

oleate concentration, oleate was removed at a linear rate of 144 ± 16 μmol/g of protein per h (Guder & Wirthensohn, 1979) over 60 min.

To show that these newly synthesized triacylglycerols could be used as endogenous substrates, we removed medium fatty acids by changing the buffer after 60 min, followed by a second 30 min incubation (Fig. 3). As can be seen, preloading in the first incubation did not prevent additional triacylglycerol synthesis in the second incubation, when

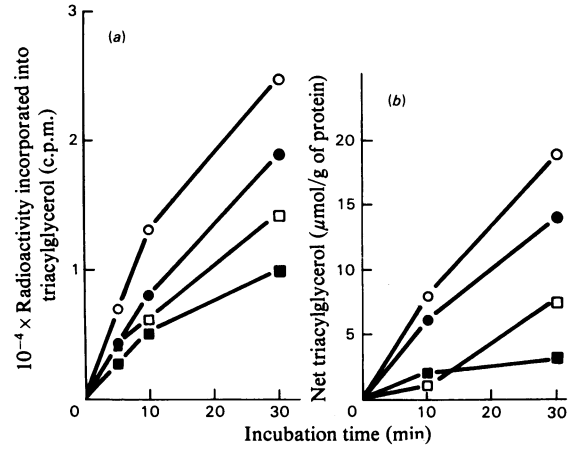

Fig. 4. *Comparison of oleate and palmitate as precursors of triacylglycerol formation*

Kidney tubules from starved rats were incubated with [1-^{14}C]oleate or [1-^{14}C]palmitate (235 000 c.p.m./tube or 255 000 c.p.m./tube respectively) with and without 5 mM-glutamine (*a*). In (*b*), the same experiment was done with unlabelled fatty acids. Incorporation of label into triacylglycerols was measured after extraction of the tubule sediment and separation on t.l.c. (see the Materials and Methods section). Net triacylglycerol synthesis was determined enzymically as described in the Materials and Methods section. Symbols: ●, 0.8 mM-oleate; ○, oleate + glutamine; ■, 0.8 mM-palmitate; □, palmitate + glutamine.

oleate support was continued. In the absence of oleate in the medium a decrease of the newly formed triacylglycerol was observed. Tubules depleted of endogenous triacylglycerol by substrate-free preincubation resynthesized them after oleate addition.

Comparison of oleate and palmitate as precursors

Triacylglycerol formation from oleate and palmitate was compared by measuring the incorporation of 1-^{14}C-labelled fatty acids into triacylglycerol (Fig. 4*a*) and net triacylglycerol determination (Fig. 4*b*). With both methods a significantly lower triacylglycerol synthesis was found from palmitate compared with oleate. Quantitatively the difference was more pronounced when net triacylglycerol was measured. The Figure also shows that addition of glutamine resulted in an increase of triacylglycerol synthesis from both fatty acids. This effect of glutamine and lactate (Barac-Nieto, 1978; Guder & Wirthensohn, 1979) was not seen in the absence of non-esterified fatty acids. Therefore, a stimulatory effect of these gluconeogenic substrates on fatty acid esterification was postulated. This was supported by the results summarized in Table 2 showing that all gluconeogenic substrates tested exhibited this stimulatory effect on triacylglycerol formation. Glucose and

Table 2. *Triacylglycerol and glucose formation in the presence of oleate and and other metabolic substrates*
Isolated tubule suspensions were prepared and incubated as described in the Materials and Methods section. Triacylglycerol and glucose were determined enzymically (see the Materials and Methods section). Metabolic rates were calculated from the differences between 0 and 30 min of incubation. Results are given as means ± S.E.M.. Significances of changes due to added substrates were tested by paired-data *t* test. The number of tubule preparations tested is given in parentheses. $*P < 0.025$, $**P < 0.01$, $***P < 0.005$. Abbreviation used: n.s., not significant.

Substrate present	Triacylglycerol formation (μmol/g of protein per h)	Substrate effect (μmol/g of protein per h)	Gluconeogenesis (μmol of glucose/g of protein per h)
Oleate (0.8 mM)	27.1 ± 1.5 (26)		66.0 ± 4.8 (24)
+ α-Oxoglutarate (10 mM)	42.3 ± 3.0 (7)	12.7 ± 3.3***	225.2 ± 32.2 (6)
+ Succinate (10 mM)	35.3 ± 3.3 (6)	4.3 ± 1.8*	212.0 ± 50.0 (5)
+ L-Proline (5 mM)	36.4 ± 4.2 (5)	12.4 ± 3.1***	165.0 ± 21.8 (5)
+ Fructose (5 mM)	41.3 ± 6.1 (4)	11.8 ± 4.0*	506.5 ± 66.8 (4)
+ Dihydroxyacetone (10 mM)	41.8 ± 7.6 (6)	11.4 ± 6.6 n.s.	286.2 ± 11.6 (6)
+ Pyruvate (10 mM)	38.3 ± 6.2 (6)	11.3 ± 3.6**	434.5 ± 29.9 (6)
+ D-Glucose (5 mM)	30.6 ± 3.9 (8)	0.1 ± 2.6 n.s.	
+ L-Lysine (5 mM)	25.6 ± 4.4 (4)	1.0 ± 6.1 n.s.	42.3 ± 19.8 (3)
+ L-Lactate (5 mM)	35.6 ± 3.9 (7)	8.9 ± 2.4**	389.4 ± 28.5 (5)
+ L-Lactate + L-lysine	35.8 ± 4.5 (7)	8.8 ± 4.1*	419.6 ± 31.7 (5)

Table 3. *Effect of mercaptopicolinic acid on triacylglycerol synthesis and gluconeogenesis*
Kidney tubules were prepared and incubated with 0.8 mM-oleate as described in the Materials and Methods section. Mercaptopicolinic acid (0.1–0.5 mM), L-lactate (5 mM) or both were added to the incubation medium. Results are given as means ± S.E.M. Significance of changes due to added mercaptopicolinic acid was tested by the paired data *t* test. *n* is the number of tubule preparations tested. $*P < 0.01$.

Substrates and inhibitor present	*n*	Triacyglycerol formation (μmol/g of protein per h)	Mercaptopicolinic acid effect (μmol/g of protein per h)	Gluconeogenesis (μmol of glucose/g of protein per h)
Oleate (0.8 mM)	8	28.0 ± 3.2		66.1 ± 8.7
Oleate + mercaptopicolinic acid (0.1–0.5 mM)	7	19.8 ± 3.8	−8.4 ± 2.6*	40.4 ± 6.4
Oleate + L-lactate (5 mM)	6	39.8 ± 6.6		402.5 ± 33.4
Oleate + mercaptopicolinic acid + L-lactate	6	32.7 ± 7.3	−7.2 ± 2.6*	104.8 ± 13.2

lysine did not increase triacylglycerol synthesis from oleate. Due to large variation, probably caused by the washing procedure needed to remove medium dihydroxyacetone, effects of this substrate were also not statistically significant. Interestingly, lysine when added together with lactate stimulated glucose formation without any additional effect on triacylglycerol synthesis.

Inhibition of gluconeogenesis with mercaptopicolinic acid (0.1–0.5 mM) led to a significant decrease in triacylglycerol formation from oleate in the absence and presence of lactate (Table 3). In a similar experiment with oleate and labelled lactate (Fig. 5) mercaptopicolinate led to a dose-dependent decrease in incorporation of label into triacylglycerols, which reached 82% at 3 mM-mercaptopicolinate. At this concentration, net synthesis of triacylglycerol was inhibited by only 60%, whereas gluconeogenesis was completely suppressed (results not shown). When lipids labelled with [^{14}C]lactate were separated and triacylglycerol hydrolysed by lipase treatment, no label was recovered in the fatty acid fraction (Wirthensohn *et al.*, 1980).

Effect of ketone bodies

Incubation of tubules with 5 mM-DL-β-hydroxybutyrate, another non-gluconeogenic renal substrate, led to a significant stimulation of triacylglycerol formation from oleate (Table 4). This effect was additive to the stimulatory effect of lactate. In contrast with lactate, β-hydroxybutyrate did not significantly increase fatty acid uptake.

Discussion

The present paper confirms the previous finding that kidney cortex can synthesize triacylglycerols from exogenous fatty acids and endogenous glycerophosphate (Weidemann & Krebs, 1969). This was demonstrated by use of a fully enzymic method to measure intracellular triacylglycerols. This method needs no time-consuming organic-solvent extraction and can be applied to a large series of samples. Analytical precision is comparable with that of enzymic determination of water-soluble metabolites. Recovery of added triacylglycerol is close to 100%. Results can be obtained in 1 h. The

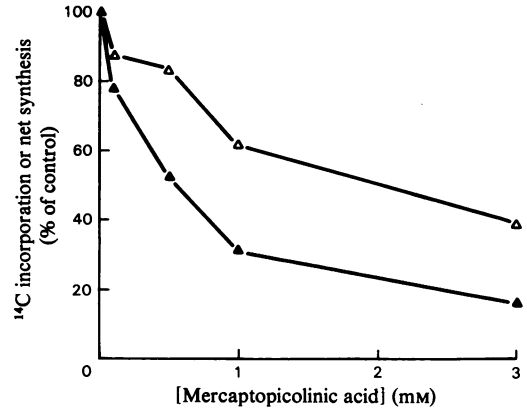

Fig. 5. *Effect of mercaptopicolinic acid on net triacylglycerol synthesis and incorporation of [2-^{14}C]lactate into triacylglycerol*

Kidney tubules were incubated with 0.8mM-oleate, various concentrations of mercaptopicolinic acid (0.1, 0.5, 1.0 and 3.0mM) and [2-^{14}C]lactate (5 mM) or unlabelled 5mM-lactate. Specific radioactivity was 0.2mCi/mmol. Incorporation of label into triacylglycerol (▲) was measured after extraction and separation on t.l.c. and net triacylglycerol synthesis (△) was determined enzymically as described in the Materials and Methods section. The incorporation of label in the absence of mercaptopicolinic acid (100%) was 7200c.p.m., and net triacylglycerol formation in the control (100%) was 37μmol/g of protein per h. Changes in incorporation or net synthesis are given as a percentage of the control experiment in the absence of mercaptopicolinic acid.

specificity is increased by denaturing endogenous tissue lipases before enzymic digestion. Although lipase hydrolyses fatty acids from phospholipids and triacylglycerol, glycerol seems to be released only from neutral lipids. With this method, net triacylglycerol synthesis in kidney *in vitro* is shown for the first time. The rate measured with oleate exceeds those calculated from previous isotope experiments (Weidemann & Krebs, 1969; Barac-Nieto, 1976). Since triacylglycerol synthesis requires 4mol of ATP/mol the present results can be taken as a measure of viability of the tubule preparation.

The present observation that the rate of net triacylglycerol synthesis in kidney tubules is linearly dependent on fatty acid concentrations is well in line with the observations of Gold & Spitzer (1964), Barac-Nieto & Cohen (1968) and Dies *et al.* (1970) that fatty acid uptake increases with arterial fatty acid concentration. As shown by Hirsch *et al.* (1963), mobilization of fatty acids *in vivo* led to an increase in triacylglycerol content of liver and kidney. In accordance with these observations, higher triacylglycerol concentrations were measured in tubules from starved compared with those from fed rats (Wirthensohn *et al.*, 1980). The

Table 4. *Effect of ketone bodies on fatty acid uptake and triacylglycerol formation*

Isolated tubule suspensions were prepared and incubated as described in the Materials and Methods section. Oleate added as a clear albumin complex was determined in the medium after sedimentation of the tubules. Triacylglycerol was measured enzymically in the tubule sediment and glucose was determined in the neutral deproteinized medium by the hexokinase method (see the Materials and Methods section). Metabolic rates were calculated from the differences between 0 and 30min of incubation. Results are given as means ± S.E.M. with the number of tubule preparations tested in parentheses. $*P < 0.0125$; $**P < 0.005$; $***P < 0.0005$ by the paired-data *t* test. Abbreviation used: n.s., not significant.

	Oleate uptake (μmol/g of protein per h)			Triacylglycerol formation (μmol/g of protein per h)			Glucose formation (μmol/g of protein per h)
Substrate present	Control	Effect of L-lactate	Effect of DL-β-hydroxybutyrate	Control	Effect of L-lactate	Effect of DL-β-hydroxybutyrate	
Oleate (0.8mM)	138.0 ± 23.7 (8)			27.9 ± 2.2 (8)			74.0 ± 7.0 (7)
Oleate + L-lactate (5mM)	190.5 ± 25.5 (8)	52.5 ± 20.3*		36.8 ± 2.8 (8)	9.0 ± 2.5**		365.7 ± 30.9 (7)
Oleate + DL-β-hydroxybutyrate (5mM)	147.9 ± 27.3 (8)		9.9 ± 12.1 (n.s.)	34.1 ± 2.9 (8)		6.3 ± 1.8**	71.5 ± 7.7 (6)
Oleate + L-lactate + DL-β-hydroxybutyrate	183.1 ± 24.2 (7)	50.5 ± 20.4*	14.9 ± 19.8 (n.s.)	43.4 ± 3.2 (8)	9.3 ± 1.6***	6.5 ± 1.7**	384.6 ± 30.6 (7)

rates of triacylglycerol synthesis were found to be similar in tubules isolated from both nutritional states. Therefore, arterial fatty acid concentration seems to be the major regulatory factor determining renal triacylglycerol concentrations.

The decrease in triacylglycerol content in the absence of fatty acids underlines the observation of Huang *et al.* (1971) in the perfused kidney and of Hohenegger (1976) in kidney cortex slices. The decrease supports the suggestion that fatty acids derived from endogenous triacylglycerols are consumed for renal energy demand (Weidemann & Krebs, 1969). Our preincubation experiments show that triacylglycerols formed in the presence of exogenous fatty acids are used up again in the absence of exogenous fuel. The decrease observed in the first 30 min of incubation (7 μmol/g of protein) would provide 42 μmol of fatty acid/g of protein per h. This would be sufficient to explain an O_2 uptake rate of 1000 μmol of O_2/g of protein per h. In fact, initial rates of 1600 μmol have been measured under similar conditions (Guder *et al.*, 1971), which decreased to about 800 μmol during incubation for 1 h.

Difference in metabolism of oleate and palmitate

Palmitate led to a much lower net triacylglycerol synthesis than oleate. Likewise less palmitate was incorporated into triacylglycerols. In accordance with this observation, kidney triacylglycerols contain fatty acids in the order: oleate > palmitate > linoleate (Druilhet *et al.*, 1975). The relative amounts of saturated and unsaturated fatty acids were found to be 38 and 62% respectively in the rabbit (Morgan *et al.*, 1963) and 39 and 71% in human kidney (Druilhet *et al.*, 1975). The fact that unsaturated fatty acids contribute two-thirds of the fatty acid pattern in triacylglycerols may be a possible explanation for the preferred incorporation of oleate into triacylglycerols compared with palmitate. In rabbit renal cortex Tinker & Hanahan (1966) found similar differences in fatty acid esterification. After 2 h of incubation 29.1% of the administered linoleic acid, but only 12.2% of palmitate was recovered in total lipids, mainly in triacylglycerols.

On the other hand there seem to exist species differences in the utilization of fatty acids. As reported by Gold & Spitzer (1964) dog kidney seems to remove palmitate exclusively. In contrast, Park *et al.* (1974) found that in dog kidney, besides palmitate, oleate and stearate were also extracted from plasma and metabolized to CO_2. Palmitate metabolism, however, exceeded that of stearate and oleate. Pig kidney was found to differ with respect to fatty acid composition of triacylglycerols. In this species, 50% saturated and 50% unsaturated fatty acids were found (Hagen, 1971).

Since renal fatty acid oxidation prefers the saturated palmitate, specific intracellular mechanisms seem to exist, which lead different fatty acids to their respective pathway. In fact a high specificity of esterifying enzyme for α- and β-esterification of α-glycerophosphate has been described in rat liver (Miki *et al.*, 1977). Such a positional specificity of re-esterifying enzymes can help to explain differences in rates calculated from isotope incorporation and net measurement. The observation that palmitate incorporation was 50% that of oleate, whereas net synthesis of triacylglycerol from palmitate was only 20% of that measured with oleate (Fig. 4) could be caused by a specific incorporation of palmitate into one of the three acyl positions of triacylglycerol. Positional analysis of the newly formed triacylglycerols is therefore needed to clarify this point.

The role of glyceroneogenesis for the provision of the glycerol moiety of acylglycerols in kidney

From the observation that all gluconeogenic substrates stimulated re-esterification rates from oleate we suggested that these gluconeogenic substrates may provide the glycerol moiety of the triacylglycerol formed. This was confirmed by the observation that ^{14}C-labelled lactate recovered in the renal lipids was not found in the fatty acid moiety. Glycerophosphate formation from gluconeogenic substrates would need phosphoenolpyruvate carboxykinase (EC 4.1.1.32) activity. Since this enzyme has been found to be exclusively located in the proximal tubule (Guder & Schmidt, 1974; Burch *et al.*, 1978) the observed effects can also be located in this nephron structure. The fact that mercaptopicolinate inhibited triacylglycerol formation also in the absence of gluconeogenic substrates could be interpreted on the basis of the assumption that endogenous glycerophosphate is provided by glyceroneogenesis from endogenous substrates. Therefore glyceroneogenesis, which has previously been described to occur in adipose tissue (Reshef *et al.*, 1970), seems to provide the glycerol moiety of lipid in kidney of fed and starved rats.

The finding that glucose did not stimulate triacylglycerol formation also supports this conclusion, since the proximal tubule is relatively lacking in glycolytic enzymes (Guder & Schmidt, 1976). Tubules prepared from the outer medulla, which contain more glycolytic enzymes (Guder & Schmidt, 1976), exhibit a stimulatory effect of glucose when studied under similar conditions (Wirthensohn *et al.*, 1980).

Effect of ketone bodies

Since ketone bodies do not provide carbon for gluconeogenesis, their stimulatory effect on triacylglycerol synthesis cannot be explained by glyceroneogenesis. The additive effect to that of lactate like-

wise indicates a different mechanism. Two different possibilities can be discussed. Ketone bodies could spare fatty acids from oxidation and thereby provide more intracellular substrate for re-esterification. This assumption is supported by the relative ineffectiveness of ketone bodies on fatty acid uptake. On the other hand, ketone bodies could increase fatty acid re-esterification in cells different from those where lactate provides the glycerophosphate moiety. Further studies are needed to clarify this point.

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