Biochemical Reviews

The Physiological Role and Control of Mammalian Fatty Acyl-Coenzyme A Desaturases

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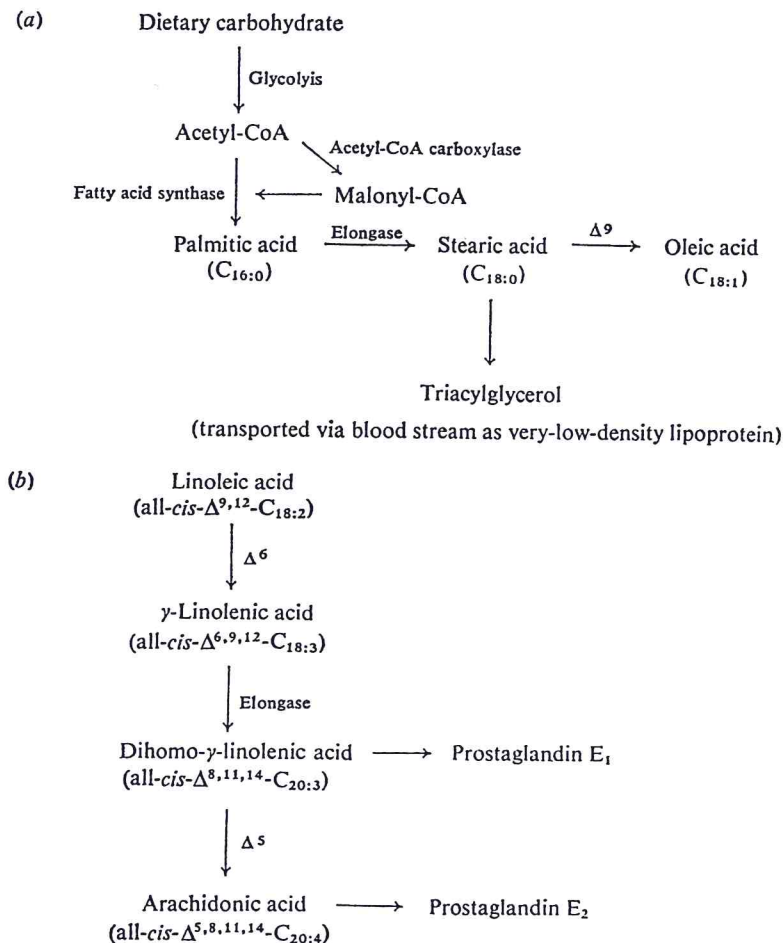*Basic Studies Unit, Biosciences Division, Unilever Research, Colworth House, Sharnbrook, Bedford MK44 1LQ, U.K.*

This article reviews our present understanding of mammalian desaturases. In particular it aims at identifying the physiological roles of liver desaturases and the way in which these enzymes are influenced by dietary fat and carbohydrate. Furthermore it underlines the needs for the regulation of lipid-metabolizing enzymes in order to maintain the optimum range of fatty acids for cell-membrane formation and to maintain an adequate flow of substrates for prostaglandin biosynthesis. Finally, it emphasizes the gaps in our understanding of unsaturated-fatty acid biosynthesis, namely the detailed chemistry of desaturation reactions and the biochemical control mechanisms that influence the activities of the enzymes involved.

To achieve these objectives it will first be necessary to define the reactions catalysed by these enzymes. In conjunction with fatty acid synthetase and elongases, the desaturases are responsible for the biosynthesis of unsaturated fatty acids. Although large numbers of these fatty acids have been isolated and characterized as products of normal mammalian lipid metabolism, it is generally becoming accepted that they result from the combined effects of four desaturases (Inkpen *et al.*, 1969; Brenner, 1974; Ninno *et al.*, 1974; Gurr & James, 1975) and the microsomal malonyl-CoA-dependent elongases (Sprecher, 1974). The function of the mitochondrial acetyl-CoA-dependent elongases in relation to desaturation reactions, which occur exclusively in the microsomal fraction, is as yet obscure. The four desaturase enzymes introduce double bonds at fixed positions from the carboxyl group of the fatty acid and are hence designated Δ^9-, Δ^6-, Δ^5- and Δ^4-fatty acyl-CoA desaturases. The specificity of these enzymes is now being established (Brett *et al.*, 1971; Ninno *et al.*, 1974; Enoch *et al.*, 1976). The action of these enzymes usually alternates with that of the elongases and in this way the characteristic methylene-interrupted double-bond pattern of polyunsaturated fatty acids is established. Scheme 1 shows an example of this with reference to the formation of arachidonic acid (all-*cis*-$\Delta^{5,8,11,14}$-$C_{20:4}$) from the essential fatty acid, linoleic acid (all-*cis*-$\Delta^{9,12}$-$C_{18:2}$).

Introduction to the physiological roles of desaturases

To appreciate the biochemical significance of the desaturases, it will be necessary to consider them in the context of known metabolic pathways as shown in Scheme 1. Pathway (*a*) summarizes the fate of excess dietary carbohydrate and the reactions leading to the synthesis of triacylglycerol. Pathway (*b*) shows the conversion of linoleic acid into dihomo-γ-linolenic acid and arachidonic acid, which give rise to the physiologically active molecules prostaglandin E_1 and E_2 respectively (van Dorp, 1975). In this sense the Δ^9-fatty acyl-CoA desaturase is intimately involved in carbohydrate–lipid conversion and energy metabolism, whereas the Δ^6- and Δ^5-fatty acyl-CoA desaturases are responsible for the biosynthesis of molecules with specific physiological functions (Horton, 1974).

The main function of the Δ^9-fatty acyl-CoA desaturase is therefore to convert the saturated fatty acids into the mono-unsaturated fatty acids. Oleic acid, a product of this

(a)   Dietary carbohydrate

|
| Glycolyis
↓

Acetyl-CoA   Acetyl-CoA carboxylase

Fatty acid synthase | ←——— Malonyl-CoA
↓

Palmitic acid $\xrightarrow{\text{Elongase}}$ Stearic acid $\xrightarrow{\Delta^9}$ Oleic acid
($C_{16:0}$)   ($C_{18:0}$)   ($C_{18:1}$)

|
↓

Triacylglycerol

(transported via blood stream as very-low-density lipoprotein)

(b)   Linoleic acid
(all-*cis*-$\Delta^{9,12}$-$C_{18:2}$)

|
| Δ^6
↓

γ-Linolenic acid
(all-*cis*-$\Delta^{6,9,12}$-$C_{18:3}$)

|
| Elongase
↓

Dihomo-γ-linolenic acid ——→ Prostaglandin E₁
(all-*cis*-$\Delta^{8,11,14}$-$C_{20:3}$)

|
| Δ^5
↓

Arachidonic acid ——→ Prostaglandin E₂
(all-*cis*-$\Delta^{5,8,11,14}$-$C_{20:4}$)

Scheme 1. *Metabolic pathways for the conversion of (a) carbohydrate into triacylglycerol and (b) the essential fatty acid linoleic acid into prostaglandins*

Δ^9, Δ^6 and Δ^5 refer to specific fatty acyl-CoA desaturases.

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reaction has itself three main physiological roles. (1) It can be incorporated into phospholipid and in this way influence the physical properties and hence the biological activity of membranes and may be able to substitute for other unsaturated fatty acids in this role. (2) It is a substrate for triacylglycerol biosynthesis and this may be of importance in influencing the properties of these lipids to facilitate their transport and storage. (3) Triacylglycerols are transported from the liver to the adipose tissue as very-low-density lipoproteins. Goh & Heimberg (1973, 1976) suggest that the observed stimulation of cholesterol biosynthesis by oleic acid reflects the requirement of cholesterol as a component of very-low-density lipoproteins for the secretion of hepatic triacylglycerols.

Enzymology of the Δ^9-fatty acyl-CoA desaturase

All attempts to purify mammalian desaturases have been focused on the stearoyl-CoA desaturase of rat livers (Shimakata *et al.*, 1972; Strittmatter *et al.*, 1974). Studies with mitochondrial supernatant fractions (Brett *et al.*, 1971) or with partially purified enzyme
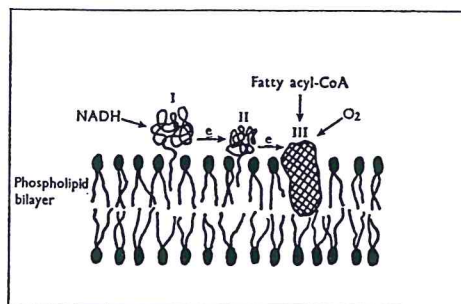
Fig. 1. *Diagrammatic representation of the Δ^9-fatty acyl-CoA desaturase complex*

The location of the three proteins, NADH–cytochrome b_5 reductase (I), cytochrome b_5 (II) and the desaturase (III) are shown in the phospholipid bilayer of the endoplasmic reticulum. The first two proteins serve to transfer electrons from NADH to the non-haem iron of the desaturase protein (III).

(Jeffcoat *et al.*, 1977) show that the desaturase, although capable of desaturating a range of fatty acids of chain length C_{10} to C_9, show a maximum activity with stearic acid and markedly less activity with haptadecanoic acid and nonadecanoic acid. However, any hypothesis that is proposed to explain the specificity and mechanism of the Δ^9-fatty acyl-CoA desaturase must take into account the requirement for molecular oxygen, NADH, a substrate in the form of a CoA ester and the fact that not only is the double bond inserted between the C-9 and C-10 atoms of the fatty acid, but that it is specifically the D-hydrogen atoms which are removed during desaturation. The latter point in conjunction with the kinetic evidence indicating that the hydrogen atoms were removed in a concerted reaction (Morris, 1970) preclude the possibility that desaturases are mixed-function oxidases acting via hydroxy acid intermediates, at least in the systems studied, and therefore by inference also in the rat liver situation.

Attempts to purify the stearoyl-CoA desaturase were hampered by the fact that desaturases are membrane-bound, and only with the knowledge of detergent solubilization of membranes did a better understanding of the enzymic complex emerge. Both Holloway (1971) and Shimakata *et al.* (1972), using these techniques have demonstrated the involvement of a haem-containing protein, cytochrome b_5 and a flavin-containing protein, NADH–cytochrome b_5 reductase (EC 1.6.2.2). Detailed protein-chemistry studies by Spatz & Strittmatter (1971, 1973) revealed that both these proteins are folded to form a globular hydrophilic domain containing the active centre and a hydrophobic tail, which serves to anchor the single polypeptide chain of the protein in the phospholipid bilayer of the endoplasmic reticulum (Fig. 1). Detailed studies using both detergent- and trypsin- or lysosomal-solubilized proteins, which enable the whole molecule and the hydrophilic domain respectively to be isolated, have shown that (1) the hydrophobic tail is important for binding the molecules to the phospholipid (Sullivan & Holloway, 1973); (2) the removal of the phospholipid does not affect the activity of the NADH–cytochrome b_5 reductase towards potassium ferricyanide, which can be used as an artificial electron acceptor, but does significantly decrease its activity towards cytochrome b_5 (Rogers & Strittmatter, 1973); (3) both the hydrophilic and hydrophobic domains are required for maximum interaction of the cytochrome b_5 and its reductase (Okuda *et al.*, 1972) and (4) as a consequence of the above requirements it has further been demonstrated that only the 'intact' cytochrome b_5 molecule having both domains can reconstitute the stearoyl-CoA desaturase activity of cytochrome b_5-depleted

microsomal preparations. It has been concluded from these observations that both the cytochrome b_5 and its reductase are anchored in a phospholipid bilayer via the hydrophobic tail of the respective proteins. The phospholipid serves only to orientate the two proteins with respect to each, but does not influence the activities of the individual proteins (Fig. 1). However, from model studies it has been demonstrated that changes in the phospholipid bilayer brought about by alterations of temperature can influence the protein–protein interactions which are dependent upon the translational diffusion of the non-polar domains (Strittmatter & Rogers, 1975).

Attempted purification of the desaturase protein and its resistance to solubilization by detergents would suggest that this protein is more intimately associated with the membrane than either of the electron-transport proteins (Jeffcoat et al., 1977) (Fig. 1). Studies by Strittmatter et al. (1974) have successfully resulted in the purification of the desaturase protein which is a single polypeptide chain of mol.wt. 53000 containing one non-haem iron atom per enzyme molecule. Progressive removal of the iron, which can be reduced in the absence of stearoyl-CoA by NADH and the electron-transport proteins, results in a corresponding loss of enzyme activity. It has thus been concluded that it is the function of the electron-transport protein to transfer electrons from NADH to the non-haem iron of the desaturase protein. The iron in this reduced form can then bind molecular oxygen which after activation by a mechanism, which is not yet understood, bring about the desaturation of the enzyme-bound stearoyl-CoA. However, preliminary electron-paramagnetic-resonance studies (Strittmatter et al., 1974) have failed to identify any iron signals which would be consistent with a high-spin iron intermediate.

Biochemical control of desaturases

Detailed biochemical analyses of desaturases are particularly difficult, because not only are the enzymes bound in an insoluble form to membranes, but the substrates are micellar at the concentrations that are convenient for studies *in vitro*. Although neither the Δ^6- nor the Δ^5-fatty acyl-CoA desaturase have been studied in the same detail as the stearoyl-CoA desaturase, studies *in vitro* have been carried out (Ninno et al., 1974; Do & Sprecher, 1976) and useful comparisons made. As yet, however, no direct evidence is available concerning the involvement of cytochrome b_5 and NADH–cytochrome b_5 reductase in the Δ^6- or Δ^5-fatty acyl-CoA desaturation reactions. However, both desaturases do require molecular oxygen and a reduced nicotinamide nucleotide, and the CN^- sensitivity of the Δ^6- (Brenner & Peluffo, 1969) and the Δ^5- (R. Jeffcoat, unpublished work) fatty acyl-CoA desaturases have been demonstrated.

Although it is now generally accepted that the desaturases and the accompanying electron-transport proteins are bound to the endoplasmic reticulum, little is known about the way in which the various cytoplasmic and membrane-bound complexes interact with each other. Catala et al. (1975) first reported that microsomal fractions, which were free of cytoplasmic proteins, were unable to desaturate linoleoyl-CoA. Addition of the cytoplasmic fraction to the same concentration as found in the crude preparations results in the full restoration of enzyme activity. Since the acyl-CoA has been used as substrate, it was concluded that the cytoplasmic proteins provide some component other than the thiokinase, which converts the unesterified fatty acid to its acyl-CoA. Although a single active cytoplasmic component has not yet been isolated and characterized, Jeffcoat et al. (1976) have shown that several cytoplasmic fractions are capable of restoring the Δ^6-fatty acyl-CoA desaturase activity of washed rat liver microsomal preparations. The observations of Catala et al. (1975) provide the first evidence that full desaturase activity can only be expressed by an interaction of membrane bound desaturases and a hitherto unidentified cytoplasmic component(s).

Studies by Baker et al. (1976) have resulted in the isolation of a cytoplasmic protein from rat liver and have identified this as catalase. Addition of this protein to microsomal preparations not only stimulates the desaturation of 1-alkyl-2-acyl-*sn*-glycero-3-phosphoethanolamine and stearoyl-CoA, but it is also suggested that it may be the hitherto unidentified cytoplasmic protein required for the Δ^6-desaturation of linoleoyl-CoA (Catala et al., 1975).

Similar studies carried out by Jeffcoat *et al.* (1976) have shown that rat liver micro-somal preparations, when washed free of cytoplasmic components, although not com-pletely losing their stearoyl-CoA desaturase activity, showed considerably decreased activity. The non-linear relationship between enzyme activity and microsomal protein concentration shown by such preparations could be overcome by the addition of optimum concentrations of either bovine serum albumin or a purified cytoplasmic fraction from rat liver. This latter component has been purified to near homogeneity and has been shown to have a dimer mol.wt. of 46 000 and a monomer mol.wt. of 23 000. This, in conjunction with a characteristic high isoelectric point, suggested that this protein was ligandin, which had been isolated earlier in connexion with its corticosteroid- and carcinogen-binding properties (Litwack *et al.*, 1971). The involvement of this protein, which represents 4.5 % of the protein present in the liver cell, was established by its stearoyl-CoA-binding properties. A second protein was also isolated (Jeffcoat *et al.*, 1976) and shown to be identical with the Z-protein previously isolated by Mishkin & Turcotte (1974). This protein has been reported by those authors to stimulate the activity of *sn*-glycerol 3-phosphate acyltransferase (EC 2.3.1.15). Jeffcoat *et al.* (1976) were un-able to demonstrate any significant effect of this protein on the Δ^9-fatty acyl-CoA desaturase, but showed that it was capable of restoring 74 % of the Δ^6-fatty acyl-CoA desaturase activity of washed rat liver microsomal preparations. Although evidence is accumulating to support the stimulation of lipid-metabolizing enzymes by cytoplasmic proteins, the detailed physiological role of these molecules is not yet understood. Do they in fact have a physiological role, and if so do they act as regulators of enzyme activity or are they carrier molecules, transporting fatty acids from one complex to another?

Studies by Jeffcoat *et al.* (1977) have shown that with partially purified Δ^9-fatty acyl-CoA desaturase, there is no requirement for either bovine serum albumin or cytoplasmic protein. This is in contrast with the crude microsomal enzyme, which is stimulated by both these proteins. From their studies with bovine serum albumin and washed microsomal preparations, the authors have shown that in the presence of albumin the rate of hydrolysis of stearoyl-CoA and oleoyl-CoA is about one-half the rate in its absence. They conclude that the substrate binds to the albumin under conditions that favour the desaturation rather than the hydrolysis of the substrate by endogenous acylthioester hydrolase. In this sense the bovine serum albumin under optimum con-ditions could be said to protect the substrate from competing enzyme reactions. Since bovine serum albumin and ligandin have a similar effect on the activity of the Δ^9-fatty acyl-CoA desaturase, it could be concluded that naturally occurring fatty acid binding proteins could, by dietary or hormonal control of their concentration influence the activities of lipid-metabolizing enzymes (Nervi *et al.*, 1975; Mishkin *et al.*, 1976; Wu-Rideout *et al.*, 1976).

Physiological control of fatty acyl-CoA desaturases

Scheme 1 shows the involvement of fatty acyl-CoA desaturases in the context of lipogenesis and prostaglandin biosynthesis. An understanding of these reactions is therefore relevant if the control mechanisms of fatty acyl-CoA desaturases is to be fully appreciated. For example, considering the enzymes of lipogenesis it is known that under conditions of high dietary carbohydrate such that the calorie intake exceeds energy output, fatty acids are synthesized and transported as triacylglycerol in the form of very-low-density lipoproteins. Further, it is now well established that under such conditions there is an increase in the activity of the enzymes involved in the biosynthesis of saturated fatty acids (Volpe & Vagelas, 1976), mono-unsaturated fatty acids (Oshino & Sato, 1972), elongases (Sprecher, 1974), triacylglycerol formation (Waddell & Fallon, 1973) as well as other lipogenic enzymes (Muto & Gibson, 1970). The most marked stimulation of enzyme activity occurs when animals are deprived of food for 24–48 h before being fed with the high carbohydrate diet. Oshino & Sato (1972) have demonstrated that there is a significant stimulation of stearoyl-CoA desaturase activity when rats are deprived of food before re-feeding. However, this was not observed if the rats were injected with cycloheximide immediately after deprivation of food and before

re-feeding. It was thus concluded that the enhanced activity of the stearoyl-CoA desaturase was due to an increase in enzyme synthesis rather than activation of pre-existing enzyme. Similar studies with specific antisera for acetyl-CoA carboxylase (EC 6.4.1.2) and fatty acid synthetase have shown that with high dietary carbohydrate the increase in activity of these two enzymes is due to an increase in enzyme protein. From these studies it thus appears that acetyl-CoA carboxylase, fatty acid synthetase and stearoyl-CoA desaturase are all controlled together as a unit. However, the mechanism by which these enzymes are influenced by dietary components is not yet understood, but two types of control are emerging: metabolic and hormonal. The allosteric control of acetyl-CoA carboxylase and fatty acid synthesis, which may be of doubtful physiological significance, has been reviewed by Vagelos (1974) and Volpe & Vagelos (1976). However, if the control of these enzymes is linked as a unit with Δ^9-fatty acyl-CoA desaturase, then their control is also relevant to our understanding of unsaturated fatty acid biosynthesis. It has been suggested that an increased cellular concentration of citrate resulting from the oxidation of excess dietary carbohydrate might influence the activity of acetyl-CoA carboxylase (thought by some to be rate-limiting step of fatty acid synthesis) by stimulating the association of the inactive protomers of the enzyme (Volpe & Vagelos, 1976). Enhanced activity of this enzyme would thus result in a greater production of saturated fatty acids, which in turn might result in greater Δ^9-fatty acyl-CoA desaturase activity via adaptive enzyme formation. Some support for this hypothesis has been proposed by Mercuri *et al.* (1974), who demonstrated that, in the case of streptozotocin-induced diabetic rats, the decreased value of the Δ^9-fatty acyl-CoA desaturase activity could be restored to near normal by ingestion of saturated fatty acids.

The metabolic controls so far discussed may be considered as short-term controls influenced by transitory changes in metabolic concentration brought about by diet or the changing biochemical requirements of the animal.

Longer-term changes in enzyme activity due perhaps to changes in feeding patterns may well be brought about by a change in the balance between enzyme synthesis and degradation. Changes in the amount of Δ^9-fatty acyl-CoA desaturate protein brought about by dietary carbohydrate have already been described. Studies have been carried out by Jeffcoat & James (1977) on the influence of dietary fat on fatty acid synthetase and fatty acyl-CoA desaturases. Although many individual studies have been carried out on the dietary control of fatty acyl-CoA desaturases (Sprecher, 1972; Brenner, 1974) the studies by Jeffcoat and co-workers bring together data on the dietary control of fatty acid synthase and Δ^9-, Δ^6- and Δ^5-fatty acyl-CoA desaturases in a single series of experiments. The main findings not only re-emphasize the close control of fatty acid synthetase and Δ^9-fatty acyl-CoA desaturase, but also underline the very different control of Δ^5-fatty acyl-CoA desaturase. When 20% by wt. of the starch from the control diet was replaced by either sucrose or corn oil the activities of the fatty acid synthetase and Δ^9-, Δ^6- and Δ^5-fatty acyl-CoA desaturases relative to values with the control diet were 2.29, 1.54, 1.25 and 1.08 with sucrose, and 0.19, 0.25, 0.75 and 1.72 with corn oil. These results once again underline the different physiological roles of these enzymes particularly of the Δ^6- and Δ^5-fatty acyl-CoA desaturases (Castuma *et al.*, 1972), but the detailed control mechanism is not yet understood. In the case of fatty acid synthase and stearoyl-CoA desaturase more experimental data is available. The close correlation between the activities of these two enzymes would suggest that they are controlled as a unit. Both enzymes are induced by dietary carbohydrate and repressed by polyunsaturated fatty acid. Subsequent studies using rats fed on diets supplemented with sucrose or sucrose and ethyl linoleate showed that as the amount of dietary linoleic acid increased, both fatty acid synthase and Δ^9-fatty acyl-CoA desaturase activities decreased. This may be explained in terms of regulation of acetyl-CoA carboxylase and fatty acid synthetase since both these enzymes are repressed by dietary polyunsaturated fatty acids (Muto & Gibson, 1970). These authors have shown that dietary fats can influence a variety of enzyme activities. Rats fed a high carbohydrate diet show increased activities of acetyl-CoA carboxylase, fatty acid synthetase, citrate lyase (EC 4.1.3.6), 'malic' enzyme (EC 1.1.1.40) and glucose 6-phosphate dehydrogenase (EC 1.1.1.49). On oral

administration of fatty acids, it was shown that the activity of all these enzymes was decreased to the normal low value. As the chain length and number of double bonds of the dietary fatty acids increased, the greater the response observed. However, since the inhibitory effect was a gradual one from myristic acid to arachidonic acid it is difficult to draw any definite conclusions about the specific effect of any one fatty acid. Studies from my own laboratory have demonstrated that, whereas dietary ethyl linoleate when administered as only 2.5% of the caloric intake causes 50% decrease in both fatty acid synthase and stearoyl-CoA desaturase, ethyl palmitate at the same caloric concentration had no effect on fatty acid synthase, but a 20% inhibitory effect of stearoyl-CoA desaturase.

The observed close correlation between dietary carbohydrate and the activities of acetyl-CoA carboxylase, fatty acid synthetase, elongases, Δ^9-fatty acyl-CoA desaturase and the triacylglycerol biosynthetic enzymes has suggested a linked control mechanism probably involving adaptive enzyme formation (Burton et al., 1969). However, in the absence of data for the elongases and the results of Wiegand et al. (1973), which suggest that dietary linoleic acid does not influence sn-glycerol 3-phosphate acyltransferase (EC 2.3.1.15), it would appear that fatty acid biosynthesis and subsequent triacylglycerol synthesis is regulated by acetyl-CoA carboxylase, fatty acid synthetase and the stearoyl-CoA desaturase, which appear to be controlled as a unit at least in part by dietary linoleic acid (Huntley, 1976; Inkpen et al., 1969).

The mechanism by which this might be effected is not yet clear, but in the case of the chick embryo it has been demonstrated that insulin causes a 5-fold increase in stearoyl-CoA desaturase activity. This effect was enhanced by thyroxine or tri-iodothyronine and totally inhibited by cycloheximide (Johsi & Wilson, 1976). In the diabetic rat Δ^9-fatty acyl-CoA desaturase activity is considerably lowered, but can be raised to near normal by the administration of dietary saturated fatty acids (Mercuri et al., 1974). This would suggest that insulin does not affect the amount of Δ^9-fatty acyl-CoA desaturase directly, but that enhanced activities of this enzyme are produced by an increase in endogenous or exogenous palmitic acid and stearic acid. Further support for this hypothesis is given by observations that dietary fructose, which is metabolized by insulin-independent reactions in the liver is also capable of inducing fatty acid synthase (Bruckdorfer et al., 1972) and stearoyl-CoA desaturase (Jeffcoat & James, 1977).

The observations made with the diabetic rat that the low activity of fatty acid synthase can be restored to normal values by insulin administration (Gibson et al., 1972) and the work of de Gomez Dumm et al. (1976) and de Alaniz et al. (1976) on the influence of insulin, glucagon, dibutyryl cyclic AMP and adrenaline on the Δ^6-fatty acyl-CoA desaturase provide good evidence for the hormonal influence on these enzymes. However, the outstanding question is still: 'is insulin the prime inducer, and if so how does dietary linoleic acid influence insulin production?' Although some studies have been made on the influence of prostaglandins (known metabolites of linoleic acid) on insulin production (Johnson et al., 1973), the answer to this question is considered beyond the scope of the present review.

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