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Changes in Lipid Synthesis in Rat Liver during Development

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1. Lipogenesis, as measured by the incorporation of ^{14}C-labelled glucose or acetate into fatty acids in liver slices, is high in foetal and adult rat liver but is low in the liver of the suckling rat, especially with glucose as substrate. 2. The rate of synthesis of non-saponifiable lipids from glucose is about 15 times as great in the liver of the 18-day foetus as in adult liver. Activity in the newborn is negligible. 3. Glucose incorporation into fat is strongly concentration-dependent in liver slices from the adult and 2-week-old rat, but less markedly so in liver slices from the foetus. 4. Changes in the activity of hepatic citrate-cleavage enzyme (ATP–citrate lyase) occur in parallel with the changes in the extent of fatty acid formation, supporting the participation of this enzyme in lipogenesis. However, NADP–malate dehydrogenase, a potential source of reduced nucleotide coenzyme for lipogenesis in the adult, could not be detected in foetal rat liver.

In contrast with the extensive studies of age-dependent changes in hepatic carbohydrate metabolism (Dawkins, 1966), there are few reports on the effects of development on lipogenesis in liver. Popják (1954) and Villee & Hagerman (1958) measured the rate of lipid synthesis in livers from foetal and adult rats, using acetate as the substrate. Although acetate is incorporated into lipid by rat liver, the utilization of glucose for lipogenesis, particularly in the foetal liver, would be a more physiological measure of lipid synthesis. In the present study we have examined the effects of age and of substrate concentration on the incorporation of ^{14}C-labelled glucose and acetate into different classes of lipids in rat liver slices. In addition, the activities of certain key enzymes of lipogenesis have been measured.

MATERIALS AND METHODS

Chemicals. NAD^+, NADH, $NADP^+$, NADPH and CoA were obtained from P-L Biochemicals Inc., Milwaukee, Wis., U.S.A. [U-^{14}C]Glucose, sodium [1-^{14}C]acetate and $NaH^{14}CO_3$ were purchased from New England Nuclear Corp., Boston, Mass., U.S.A. Glucagon-free porcine insulin was a gift from Dr O. K. Behrens of Eli Lilly Co., Indianapolis, Ind., U.S.A. Cholesteryl palmitate, cholesterol, tripalmitin, palmitic acid and lecithin, standards for thin-layer chromatography, and NAD–malate dehydrogenase (EC 1.1.1.37) were obtained from Calbiochem, Los Angeles, Calif., U.S.A.

Animals. Wistar albino rats were obtained from Carworth Farms, New City, N.Y., U.S.A., and were fed *ad libitum* on Purina rat chow. The adult rats used were males. Although no attempt was made to study diurnal effects, all experiments were started at the same time of day.

Liver extracts. Liver homogenates (10%, w/v) in 0·15M-KCl were centrifuged at 100000*g* in a Spinco model L ultracentrifuge for 1hr. The supernatants were used for the assays of NAD–malate dehydrogenase, isocitrate dehydrogenase (EC 1.1.1.42), NADP–malate dehydrogenase (EC 1.1.1.40) and citrate-cleavage enzyme (ATP–citrate lyase, EC 4.1.3.8).

Assay of NAD–malate dehydrogenase. This enzyme was assayed at 37° as described by Ochoa (1955).

Assay of isocitrate dehydrogenase. This enzyme was assayed at 37° as described by Plaut (1962).

Assay of ATP–citrate lyase. Oxaloacetate formation from citrate, CoA and ATP was measured as the oxidation of NADH by coupling with NAD–malate dehydrogenase according to Srere (1962). The final concentrations of reactants in the assay cuvette at 37° were: tris (adjusted to pH7·4 with 2N-KOH), 15mM; $MgCl_2$, 10mM; potassium citrate, 20mM; β-mercaptoethanol, 10mM; coenzyme A, 0·5mM; NADH, 0·3mM; ATP (adjusted to pH7·4), 5mM; excess of NAD–malate dehydrogenase; liver extract. The extinction change at 340mμ with either ATP or CoA omitted was subtracted from that of the complete system. The extinction changes in these blanks were approx. 10% of those in the complete assay.

Assay of NADP–malate dehydrogenase. The spectrophotometric assay described by Ochoa (1955) was used unless otherwise noted. The final concentrations of reactants at 37° were: tris (adjusted to pH7·4), 30mM; $MnCl_2$, 1·5mM; NADP, 0·3mM; sodium malate, 0·75mM; liver extract. Extinction changes without malate were subtracted from the rates in the complete system. As these blanks were high and possibly could represent malate present in the liver extract, and therefore not a true blank, in several experiments cuvettes containing all reactants

except malate were maintained at 37° until the extinction change was negligible. The increased rate of NADP reduction upon addition of malate agreed closely with the rates calculated by subtracting the blank.

In the radioactivity assay, [^{14}C]bicarbonate fixation in the presence of pyruvate, $MgCl_2$ and NADPH was measured. The concentrations of reactants in a final volume 1·0ml. were: tris (adjusted to pH7·4), 50mM; $NaH^{14}CO_3$, 50mM (2μc); sodium pyruvate, 10mM; NADPH, 1·5mM. Liver extract was added and the tubes were incubated for 5, 10 and 15min. at 37°. The reaction was stopped and CO_2 liberated by the addition of 0·5ml. of 10% trichloroacetic acid. The tubes were centrifuged and the CO_2 remaining in the supernatant was removed by gassing with unlabelled CO_2 for 5min. The radioactivity in a 1·0ml. sample of this solution was measured in a liquid-scintillation detector by using Diotol (Herberg, 1960). The average amount of radioactivity fixed in blanks without NADPH or without pyruvate was subtracted from determinations with the complete assay system. Under the conditions used, the incorporation was linear with both time and amount of enzyme up to 5000 counts/min. The blank incorporation in both this and the pyruvate-carboxylase assay did not vary with time or amount of extract and was approx. 250 counts/min.

Isotope experiments. Liver slices were prepared in the cold as described by Ballard & Oliver (1964*a*), rinsed in Krebs–Ringer bicarbonate buffer, pH7·4, weighed, and 300mg. was transferred to 25ml. Erlenmeyer flasks containing 5ml. of Krebs–Ringer bicarbonate buffer, pH7·4 (Umbreit, Burris & Stauffer, 1959). The substrate concentrations, except where indicated, were: [^{14}C]acetate, 50mM (2·5μc); [^{14}C]glucose, 50mM (2·5μc). Insulin (0·1 unit) was added with the glucose. The tissue slices were incubated at 37° for 3hr., except as noted in Table 1, in an atmosphere of O_2+CO_2 (95:5, v/v) in a shaking water bath (90 strokes/min.).

After incubation, the liver slices were removed from the buffer, rinsed in 0·9% NaCl and transferred to 10ml. of chloroform–methanol (2:1, v/v). The lipid was extracted from the tissue by shaking overnight in the chloroform–methanol at room temperature. This method of extraction was found to compare favourably with saponification of the tissue in methanolic KOH or homogenization of the slices in chloroform–methanol, and, because of simplicity, was used throughout this study. The extracted lipid was washed three times by the method of Folch, Lees & Sloane-Stanley (1957) and saponified in methanolic KOH (95ml. of methanol and 5g. of KOH in 5ml. of water) at 50° for 30min. Completeness of saponification under these conditions was verified by thin-layer chromatography with the solvent system described below. The saponifiable and non-saponifiable lipid fractions were isolated as described previously (Hanson, 1965) and dissolved in 10ml. of toluene containing, per l., 230ml. of ethanol, 4g. of 2,5-diphenyloxazole and 15mg. of 1,4-bis-(5-phenyloxazol-2-yl)benzene. The radioactivity was measured in a Packard Tri-Carb 314 EX liquid-scintillation spectrometer at 10°. The counting efficiency, as determined by internal standardization, was 20–30% and all counts were corrected to 100% efficiency on this basis.

During this study several incubation media were used to determine the buffer which most effectively supported lipogenesis in liver slices. Krebs–Ringer bicarbonate buffer was finally decided upon since consistently higher rates of lipogenesis were found than with the high-K^+ buffer of Hastings (Hastings, Teng, Nesbett & Sinex, 1952) or a Ca^{2+}-free Krebs–Ringer bicarbonate buffer.

To test whether glucose and acetate conversion into fatty acids and non-saponifiable lipid was proceeding continuously throughout the normal 3hr. incubation period, liver slices from foetal, newborn and adult rats were incubated for various times from 0 to 4hr. Table 1 shows that incorporation of the isotopic precursor into lipid continued in all cases throughout the 4hr. period.

Chromatographic separation. Approx. 1g. of liver slices from term foetuses and adult rats was incubated in 10ml. of Krebs–Ringer bicarbonate buffer, pH7·4 (Umbreit *et*

Table 1. *Effect of incubation time on lipogenesis from glucose and acetate by liver slices from rats of various ages*

The methods used are described under 'Isotope experiments' in the Materials and Methods section. In those cases where sufficient liver could not be obtained from a single animal, livers from litter mates were pooled. Each value is the mean of duplicates.

Labelled product	Labelled substrate	Source of liver	Incubation time (hr.).....	Substrate incorporated (mμmoles/g. of liver) 1	2	3	4
Fatty acid	Glucose	Foetus (21 days)		32	98	169	—
		Young (2 weeks)		8	10	17	18
		Adult		110	341	666	726
	Acetate	Foetus (21 days)		282	1538	1388	2401
		Young (2 weeks)		43	86	117	281
		Adult		458	1220	2060	2190
Non-saponifiable lipids	Glucose	Foetus (21 days)		72	137	212	—
		Young (2 weeks)		3	4	9	12
		Adult		11	49	95	191
	Acetate	Foetus (21 days)		434	847	1011	1566
		Young (2 weeks)		33	123	159	363
		Adult		19	76	234	370

al. 1959), containing [^{14}C]acetate, 50mM (25μc). The tissue was incubated for 3hr. and the lipid extracted in 10ml. of chloroform–methanol (2:1, v/v) and washed as described above. The volume of the extract was reduced to 2·0ml. by heating in a water bath at 50–60°. Samples (100μl.) were spotted on Eastman Kodak Co. Chromatogram sheet (type K301R2) of silica gel and developed in the solvent system light petroleum (b.p. 38–54°)–diethyl ether–acetic acid (81:18:1, by vol.) by ascending chromatography for 60min. This system resolved the five major lipid components found in mammalian liver: phospholipid, cholesterol, fatty acids, triglycerides and cholesterol esters. The lipids were detected by spraying with a 0·5% solution of Rhodamine B in ethanol (Stahl, 1965) and viewing the sheets under ultraviolet light (360mμ). Each of the lipid components, corresponding to standard spots, was cut from the sheet and the radioactivity determined by counting the spot directly in the toluene liquid-scintillation medium described above.

Nitrogen determinations. The nitrogen content of the liver slices, after extraction of lipids by chloroform–methanol, and of the liver extract taken for enzyme assay was determined by nesslerization (Johnson, 1941). Because the protein content of foetal liver is considerably lower than that of adult liver (Burch *et al.* 1963), all results in the developmental study have been expressed both on a wet-wt. basis and as μmoles of substrate utilized/mg. of nitrogen.

RESULTS

Effects of substrate concentration on glucose and acetate incorporation into lipid. Liver slices from foetal, 2-week-old and adult rats were incubated at various glucose concentrations. As shown in Table 2, increasing the glucose concentration from 1mM to 100mM produced a sixfold rise in isotope incorporation into fatty acid in foetal liver; the corresponding increases in the 2-week-old animal and the adult were about 35-fold and 180-fold. Differences of similar magnitude were found when incorporation into the non-saponifiable lipid was measured. As we wished to measure the maximum rate of lipogenesis in the age study, a glucose concentration of 50mM was chosen.

Contrary to the findings with glucose, an increase in acetate concentration from 1mM to 50mM

Table 2. *Effect of concentration of glucose on its incorporation into lipids by liver slices from rats of various ages*

Incorporation was measured in Krebs–Ringer bicarbonate buffer (Umbreit *et al.* 1959) containing [^{14}C]glucose, by determining the amount of radioactivity in either fatty acids or non-saponifiable lipids after 3hr. incubation.

	[U-^{14}C]Glucose incorporated (mμmoles/g. of liver/3hr. incubation)					
	Into fatty acid			Into non-saponifiable lipids		
Concn. of glucose (mM)	Term foetus	2-week-old	Adult	Term foetus	2-week-old	Adult
1	73	0·33	3·6	66	0·63	1·9
2	78	0·75	6·9	74	1·1	3·7
5	108	1·8	25·0	99	3·2	11·9
10	236	2·1	65	180	5·0	41
20	253	4·0	173	234	6·6	96
50	314	7·8	586	308	9·7	104
100	464	11·4	668	359	16·3	114

Table 3. *Effect of concentration of acetate on its incorporation into lipids by liver slices from rats of various ages*

Incorporation was measured in Krebs–Ringer bicarbonate (Umbreit *et al.* 1959) containing [^{14}C]acetate, by determining the amount of radioactivity in either fatty acids or non-saponifiable lipids after 3hr. incubation.

	[1-^{14}C]Acetate incorporated (mμmoles/g. of liver/3hr. incubation)					
	Into fatty acid			Into non-saponifiable lipids		
Concn. of acetate (mM)	Term foetus	2-week-old	Adult	Term foetus	2-week-old	Adult
1	629	25	291	517	30	90
2	700	55	512	634	53	103
5	574	121	832	631	133	197
10	856	54	1314	800	86	153
20	1403	44	2373	912	78	204
50	1659	84	2243	835	121	254

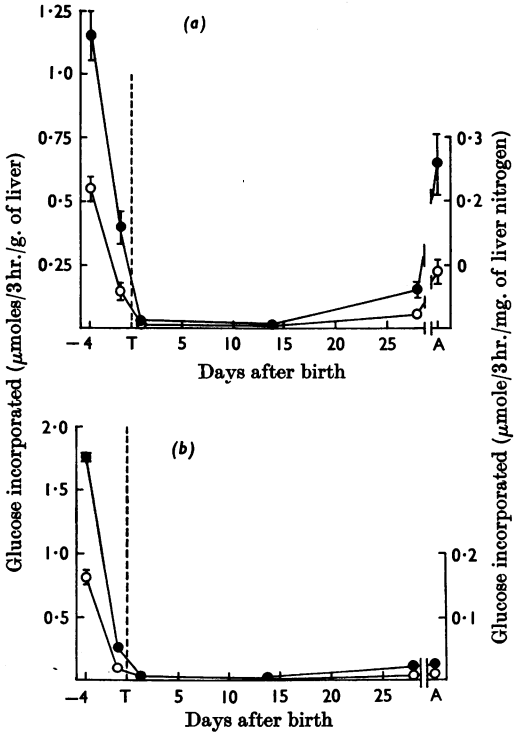

Fig. 1. (*a*) Incorporation of [^{14}C]glucose into fatty acids in liver slices. (*b*) Incorporation of [^{14}C]glucose into non-saponifiable lipids in liver slices. The methods of incubation and lipid extraction are described in the Materials and Methods section. T, term; A, adult. Each point represents the mean ± S.E.M. of from six to eight determinations. Incorporation is expressed as μmoles/3hr./g. of liver (●) or as μmole/3hr./mg. of nitrogen (○).

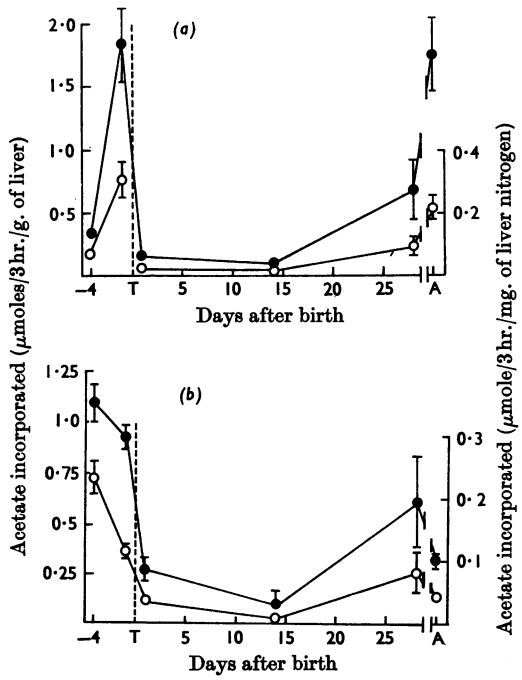

Fig. 2. (*a*) Incorporation of [^{14}C]acetate into fatty acids in liver slices. (*b*) Incorporation of [^{14}C]acetate into non-saponifiable lipids in liver slices. The methods of incubation and lipid extraction are described in the Materials and Methods section. T, term; A, adult. Each point represents the mean ± S.E.M. of from six to eight determinations. Incorporation is expressed as μmoles/3hr./g. of liver (●) or as μmole/3hr./mg. of nitrogen (○).

produced only a relatively small increase in isotope incorporation into either fatty acid or non-saponifiable lipids (Table 3).

Effect of developmental state on glucose and acetate incorporation into lipid. The rate of incorporation of glucose into fatty acid (Fig. 1*a*) decreased from 1·18 μmoles/g./3hr. in the liver of the 18-day foetus to a minimum of 0·013 μmole/g./3hr. in that of the 2-week-old animal and then increased to 0·64 μmole incorporated/g. of liver/3hr. in the adult. Similar age-dependent changes were found when the rate of glucose incorporation into cholesterol was measured, although the rate in adult liver was very much lower than was found in the 18-day foetus (Fig. 1*b*).

The incorporation of acetate into fatty acid (Fig. 2*a*) occurred at a rate of 0·33 μmole/g./3hr. in the liver of the 18-day foetus and increased to 1·83 μmoles/g./3hr. in that of the term foetus. There was a fall in incorporation rate in the 2-week animal, with a subsequent increase to 1·77 μmoles/g./3hr. in the adult. With acetate as substrate, as with glucose, the incorporation rate into cholesterol was considerably lower in adult liver than in the livers of 18-day foetuses.

When these results are expressed as μmoles incorporated/mg. of tissue nitrogen/3hr., the ratios of foetal rates to adult rates are higher than when expressed as μmoles incorporated/g. of liver/3hr.

Although preliminary experiments had indicated that radioactivity incorporated into the saponifiable fraction was in fatty acid, and radioactivity in the non-saponifiable fraction was in cholesterol, the relative distribution of radioactivity among the various lipid fractions of the chloroform–methanol extract was determined before saponification. These experiments (Table 4) showed that 35–40% of the radioactivity incorporated with liver slices from both term foetuses and adults was in phospholipid. Little radioactivity appeared in cholesterol esters or in free fatty acids at either age. A greater proportion of the radioactivity incorporated in

Table 4. *Percentage distribution of radioactivity in lipid fractions*

The separation of lipid fractions is described in the Materials and Methods section. Each value is the mean of three determinations with [1-^{14}C]acetate as substrate.

Fraction	Percentage distribution in lipids: From term foetus	From adult
Phospholipid	35	39·8
Cholesterol	36·6	8·8
Free fatty acid	1·8	0·5
Triglyceride	21·6	49·9
Cholesterol ester	3·9	0·5

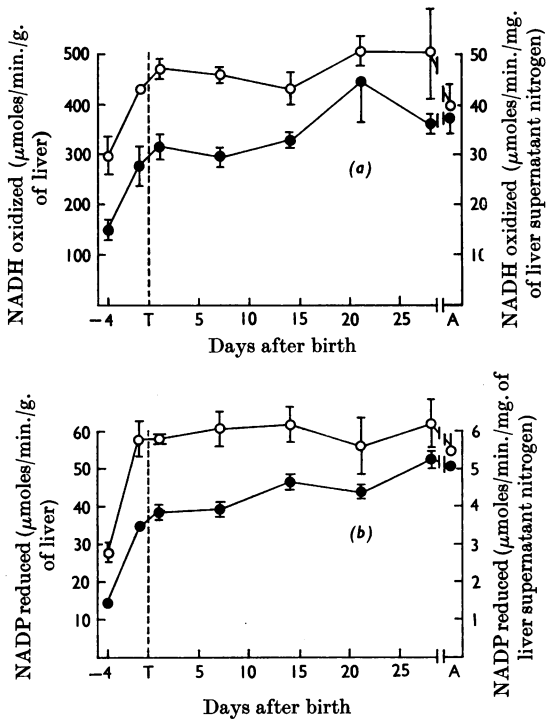

Fig. 3. (*a*) NAD–malate-dehydrogenase activity determined as NAD formation from oxaloacetate and NADH in 100000*g* liver supernatants at 37°. (*b*) Isocitrate-dehydrogenase activity measured as NADPH formation from NADP and isocitrate in 100000*g* liver supernatants at 37°. T, term; A, adult. Each point represents the mean ±S.E.M. of six determinations. Activities are expressed as μmoles/min./g. of liver (●) or as μmoles/min./mg. of nitrogen (○).

foetal livers was in cholesterol than in triglyceride, with the converse true in adult livers. This is also clear from the experiments shown in Fig. 2(*a*).

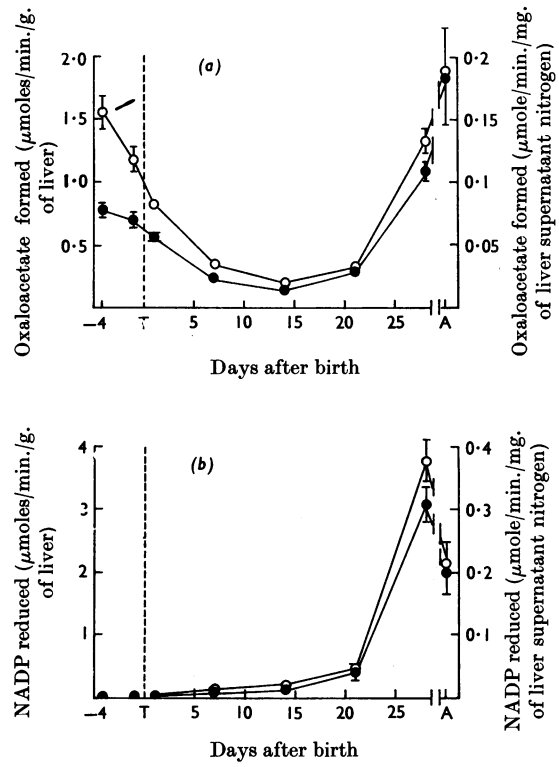

Fig. 4. (*a*) ATP–citrate-lyase (citrate-cleavage enzyme) activity determined as oxaloacetate formation from ATP, citrate and CoA in 100000*g* liver supernatants at 37°. (*b*) NADP–malate-dehydrogenase activity measured as NADPH formation from NADP and malate in 100000*g* liver supernatants at 37°. T, term; A, adult. Each point represents the mean ±S.E.M. of six determinations. Activities are expressed as μmoles/min./g. of liver (●) or as μmoles/min./mg. of nitrogen (○).

Effect of developmental state on enzyme activity. NAD–malate-dehydrogenase activity increased from 148 μmoles of NADH oxidized/min./g. of liver in 18-day foetal liver to 375 μmoles/min./g. of liver in adult liver (Fig. 3*a*). Isocitrate-dehydrogenase activity increased from 14·1 μmoles of NADP reduced/min./g. of liver to 50·9 μmoles/min./g. of liver over the same time period (Fig. 3*b*). When these results are expressed as units/mg. of nitrogen in the liver supernatant the percentage changes with age are considerably diminished.

ATP–citrate-lyase activity fell from 0·77 μmole of oxaloacete formed/min./g. in the liver of the 18-day foetus to 0·14 μmole/min./g. in that of the 2-week animal and then rose sharply to the adult activity of 1·84 μmoles/min./g. of liver (Fig. 4*a*). When expressed as units/mg. of nitrogen in the

liver supernatant, the activity in foetal liver was similar to that in adult liver.

NADP–malate-dehydrogenase activity, measured as NADP reduction, could not be detected in the liver until 1 week after birth. After this the activity increased to 3·09 μmoles/min./g. in 4-week animals. The adult activity was 1·97 μmoles/min./g. (Fig. 4*b*). To show more conclusively that NADP–malate-dehydrogenase activity was absent from the livers of very young rats, the enzyme was assayed in the reverse direction (see the Materials and Methods section). The average activity of the enzyme in livers from the 18-day foetus, term foetuses and 2-week-old rats was 0·01 μmole of $^{14}CO_2$ incorporated/min./g. of liver, compared with the value 0·17 μmole/min./g. of liver found in adult liver. Insufficient assays were carried out to permit statistical treatment, but the increase in activity agrees well with that found by using the NADPH-production assay.

DISCUSSION

As a constant concentration of blood glucose is maintained in mammalian foetuses by the maternal blood supply, the foetus can utilize carbohydrate as an energy source for growth, and does not depend on the synthesis of lipid to meet energy demands. Large amounts of lipid, mostly triglyceride, appear in foetal liver of some species at term and disappear in the first few days of life (Freedman & Nemeth, 1961). This triglyceride reserve is used by the animal immediately after birth before it becomes adjusted to a milk diet. After weaning, the diet changes to one composed predominantly of carbohydrate, and lipogenesis sharply increases.

Earlier work by Villee & Hagerman (1958) and by Popják (1954) has shown a high rate of lipogenesis from acetate and pyruvate in foetal liver. However, pyruvate and acetate probably represent a relatively minor substrate for lipogenesis in the rat foetus where there is an adequate supply of blood glucose. Our studies were therefore centred on determining the rate of lipogenesis from glucose during foetal life and during development and growth after birth.

Studies by DiPietro, Sharma & Weinhouse (1962) and Walker (1962) have shown that adult liver contains two enzymes for the phosphorylation of glucose. One, a glucokinase with a high K_m for glucose (10–40mM) is specific for glucose, whereas the other, a non-specific hexokinase, has a much lower K_m. The foetal liver of the rat and sheep lacks the high K_m glucokinase (Ballard & Oliver, 1964*b*) so that glucose phosphorylation is maximal at glucose concentrations well below the physiological range. Glucokinase activity first appears in rat liver about 2 weeks after birth (Ballard & Oliver, 1964*a*; Walker, 1965).

In the present paper we show (Table 2) that increasing the concentration of glucose over the range 1–100mM sharply increases lipogenesis in liver slices from 2-week-old and especially adult animals, but has a less marked effect on lipogenesis in slices from foetal liver. Cahill, Hastings, Ashmore & Zottu (1958) have also shown a striking effect of concentration on glucose incorporation into fatty acid in slices from adult liver. The effect on glycogen synthesis of increasing the medium glucose concentration over the range 1–200mM is, moreover, greater with slices from adult liver than with those from foetal liver (Ballard & Oliver, 1964*b*). We believe these findings to be consistent with the appearance in the liver of glucokinase at about 2 weeks of age.

Much smaller effects of increasing glucose concentration have been observed on: (*a*) glucose incorporation into fatty acids and non-saponifiable lipids in foetal liver slices: (*b*) acetate incorporation into both lipid classes in liver slices from foetal, 2-week and adult rats; (*c*) glucose incorporation (at concentrations less than 20mM) into glycogen in sheep liver slices (Ballard & Oliver, 1964*b*). We interpret all these as due to a limiting rate of diffusion of substrate into the slices. On this interpretation, incorporation rates in the inner cells of a slice would be expected to increase with a rise in substrate concentration so that the slice as a whole would show some dependence on concentration.

Effect of age on lipogenesis from acetate and glucose. During the suckling period, the diet of the rat contains proportionately more fat and less carbohydrate than is available to the foetus or to the weaned animal and rates of lipogenesis are extremely low. The function of the cholesterol formed in foetal liver is uncertain, although some is undoubtedly used for the synthesis of steroid hormones as has been demonstrated in human foetal liver (Slaunwhite, Karsay, Hollmer, Sandberg & Niswander, 1965). Much less cholesterol is synthesized in adult liver, where most of the labelled substrates are incorporated into fatty acid for triglyceride and phospholipid synthesis. Previous studies (Ballard & Oliver, 1963, 1965) showed negligible rates of gluconeogenesis in foetal rat liver and a sharp increase in this process at birth. Gluconeogenic activity is high throughout the suckling period and decreases when the animal reaches adulthood. These earlier studies on gluconeogenesis and the present work on lipogenesis indicate an inverse relationship between the two pathways which reflect the changes in nutrient available to the growing mammal.

Lipogenesis in liver and adipose tissue requires

a supply of extramitochondrial acetyl-CoA and NADPH. The pentose phosphate pathway generates some NADPH (Flatt & Ball, 1964) and the citrate-cleavage enzyme (ATP–citrate lyase) probably serves to supply cytoplasmic acetyl-CoA (Srere, 1959; Spencer & Lowenstein, 1962). The importance of citrate cleavage is underlined by the fact that the mitochondrial membrane is impermeable to acetyl-CoA (Spencer & Lowenstein, 1962). Young, Shrago & Lardy (1964) and Wise & Ball (1964) have presented evidence suggesting a conversion into pyruvate via malate of the oxaloacetate formed by citrate cleavage. This process couples the NAD- and NADP-malate dehydrogenases extramitochondrially with a resultant increase in cytoplasmic NADPH. Thus a mechanism may exist for overcoming the mitochondrial impermeability to acetyl-CoA while at the same time generating additional NADPH.

Both ATP–citrate lyase and NADP-malate dehydrogenase in rat liver and adipose tissue increase in activity during periods of rapid lipogenesis (Tepperman & Tepperman, 1964; Kornacker & Lowenstein, 1965; Leveille & Hanson, 1966). We have found that ATP–citrate-lyase activity also closely mirrors the age-dependent changes in hepatic lipogenesis. NADP-malate dehydrogenase, however, far from following this pattern, cannot even be detected in foetal liver, although this tissue has a very high rate of lipogenesis. These results are evidence for the cleavage of citrate as an active process in foetal liver and a possible control point in lipogenesis. The conversion of malate into pyruvate extramitochondrially, on the other hand, does not occur in foetal liver so that the generation of NADPH by the coupling of NAD- and NADP-malate dehydrogenases, as suggested in adult rat liver and adipose tissue, is not likely in foetal liver. It is possible that sufficient NADPH to support lipogenesis in the foetal liver is generated by the pentose phosphate pathway since the activity of glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase has been shown to be higher in foetal than in adult liver (Lea & Walker, 1964; Burch *et al.* 1963).

The changes in the activity of hepatic NAD-malate dehydrogenase and isocitrate dehydrogenase with age bear no relationship to the rates of lipogenesis found in liver, possibly because the activities of these enzymes are much greater than the rate of the overall lipogenic pathway.

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