Lipid Synthesis *in vivo* by Tissues of the Maternal and Foetal Guinea Pig

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The rate of lipid biosynthesis *in vivo* was determined in pregnant guinea pigs after maternal and foetal injections of $^3\text{H}_2\text{O}$. Synthesis in the maternal tissues was low and in the foetal liver and adipose tissues relatively high. In the foetal liver it reached a peak at about two-thirds of gestation, whereas that in the foetal adipose tissue occurred later. These results were used to support the view that lipid synthesis in the foetal guinea-pig liver at two-thirds of gestation is largely from short-chain fatty acids, whereas in foetal adipose tissue glucose is probably the major substrate.

The tissues of the pregnant guinea pig have very low rates of lipid synthesis as measured by glucose, acetate and amino acid incorporation, whereas the rate of synthesis in the liver and adipose tissue of the foetus is relatively high (Jones, 1973; Jones & Ashton, 1976). The developmental changes in the rate of lipid synthesis from acetate by the foetal guinea-pig liver are different from those for synthesis from glucose, glucose incorporation being much slower than acetate incorporation (Jones, 1973). Thus it is not clear which substrate incorporation is representative of the situation *in vivo* or what the rate of fatty acid synthesis is. The use of $^3\text{H}_2\text{O}$ incorporation into lipid gives a better estimate of the rate of fatty acid synthesis (Fain & Scow, 1966; Windmueller & Spaeth, 1966, 1967; Jungas, 1968; Clark *et al.*, 1974) as it is independent of the source of acetyl groups for synthesis. The incorporation of $^3\text{H}_2\text{O}$ into lipids by the pregnant guinea pig has been followed after maternal and foetal injection. The origin of and substrates for incorporation into foetal lipids are discussed.

Experimental

Guinea pigs of the Dunkin–Hartley strain were used and the time of mating was determined as described by Elvidge (1972). Guinea pigs were given an intraperitoneal injection of either 1 or 2 mCi of $^3\text{H}_2\text{O}$ /kg and the maternal and foetal tissues obtained 15, 30 or 60 min later. For the foetal injection, weight was estimated from growth curves and an intraperitoneal injection of approx. 40 mCi/kg administered through the maternal body wall and uterus. Tissues were homogenized in 20 vol. (w/v) of chloroform/methanol (3:1, v/v). The chloroform/methanol extract was evaporated to dryness and then saponified with 3 ml of 10% (w/v) KOH at 90°C for 2 h. The extract was acidified with 10M- H_2SO_4 and lipids were extracted with 3 × 20 ml of light petroleum (b.p. 40–60°C). This was washed with 4 × 50 ml of water and then dried over anhydrous Na_2SO_4 .

Samples were counted for radioactivity in a toluene-based scintillation fluid [6 g of 2,5-diphenyloxazole and 1,4-bis-(5-phenyloxaz-2-yl)benzene in 1 litre of toluene] with a Phillips liquid-scintillation counter and corrected to 100% counting efficiency with an external standard. Radioactivity in the non-saponifiable fraction of any of the tissues represented no more than 5–12% of the total lipid incorporation. The specific radioactivity of $^3\text{H}_2\text{O}$ in body water was determined from $^3\text{H}_2\text{O}$ radioactivity in plasma and used to determine the tissue rates of incorporation into fatty acids. No significant differences were observed in $^3\text{H}_2\text{O}$ specific radioactivity in plasma from the maternal inferior vena cava and foetal umbilical vein after maternal intraperitoneal injections of $^3\text{H}_2\text{O}$.

$^3\text{H}_2\text{O}$ was obtained from The Radiochemical Centre, Amersham, Bucks., U.K.

Results

In all tissues studied the incorporation into the non-saponifiable fraction was small (<5–12% of total) and it is likely that the incorporation measured largely represents incorporation into fatty acids. This has been demonstrated for incorporation into liver lipids (C. T. Jones & G. F. Fellows, unpublished work).

Incorporation into foetal and maternal tissues after a maternal injection of $^3\text{H}_2\text{O}$ was almost linear with time for at least 60 min (Table 1). It was low in all the maternal tissues with the highest values obtained in the liver and lung. No significant changes were observed between day 44 and 56 of pregnancy (Tables 1 and 2). Incorporation into the foetal liver, adipose tissue and brain was substantially higher than into the corresponding maternal tissues. There was an increase in lipid synthesis in foetal adipose tissue and a decrease in that in the foetal liver between 44 and 56 days (Tables 1 and 2). The possibility that a significant portion of the incorporation into foetal lipid was of maternal origin was investigated by

Table 1. *Lipid synthesis in guinea pigs 44–46 days pregnant*

An intraperitoneal injection of 1mCi of $^3\text{H}_2\text{O}$ /kg was given to pregnant guinea pigs. Lipids in the foetal and maternal tissues were isolated and counted for radioactivity as described in the Experimental section. The results are the means $\pm$ s.d. of five maternal injections.

	Time after injection (min) ...	Fatty acid synthesis (μmol of $^3\text{H}_2\text{O}$ incorporated/g of tissue)		
		15	30	60
Maternal				
Heart	—		0.16 $\pm$ 0.06	0.37 $\pm$ 0.13
Lung	1.23 $\pm$ 0.3		3.07 $\pm$ 0.53	4.44 $\pm$ 1.47
Kidney	0.33 $\pm$ 0.1		0.53 $\pm$ 0.19	1.63 $\pm$ 0.43
Adipose tissue	0.2 $\pm$ 0.07		0.39 $\pm$ 0.2	0.96 $\pm$ 0.36
Brain	—		0.1 $\pm$ 0.05	0.21 $\pm$ 0.12
Liver	0.56 $\pm$ 0.2		0.89 $\pm$ 0.27	2.0 $\pm$ 0.63
Foetal				
Heart	0.29 $\pm$ 0.16		0.93 $\pm$ 0.26	1.7 $\pm$ 0.42
Lung	0.26 $\pm$ 0.11		0.48 $\pm$ 0.18	1.11 $\pm$ 0.27
Kidney	0.31 $\pm$ 0.2		0.95 $\pm$ 0.37	1.63 $\pm$ 0.38
Adipose tissue	5.98 $\pm$ 2.1		14.27 $\pm$ 4.32	26.7 $\pm$ 9.6
Brain	0.34 $\pm$ 0.15		1.73 $\pm$ 0.51	3.14 $\pm$ 1.9
Liver	2.12 $\pm$ 0.91		4.97 $\pm$ 1.36	8.96 $\pm$ 2.8
Placenta	0.32 $\pm$ 0.14		0.63 $\pm$ 0.28	1.04 $\pm$ 0.37

Table 2. *Lipid synthesis in guinea pigs 54–56 days pregnant*

Details are as described for Table 1. The incorporation time was 60 min. The results are the means $\pm$ s.d. for four maternal injections.

	Fatty acid synthesis (μmol of $^3\text{H}_2\text{O}$ incorporated/g of tissue)	
	Maternal	Foetal
Heart	0.5 $\pm$ 0.23	1.0 $\pm$ 0.28
Lung	3.54 $\pm$ 0.98	0.87 $\pm$ 0.32
Kidney	1.43 $\pm$ 0.29	1.1 $\pm$ 0.41
Adipose tissue	1.42 $\pm$ 0.37	43.2 $\pm$ 12.6
Brain	0.26 $\pm$ 0.15	8.4 $\pm$ 3.1
Liver	1.79 $\pm$ 0.45	5.51 $\pm$ 1.3
Placenta	—	2.6 $\pm$ 0.39

direct injection of $^3\text{H}_2\text{O}$ into the foetus where the dilution effect of the maternal volume effectively prevented the production of maternal lipids with a significant specific radioactivity. After the foetal injection the rate of incorporation into all the maternal tissues was barely detectable, i.e. at least one-twentieth to one-hundredth of the rates observed in the foetal tissues. Because of the rapid rate of $^3\text{H}_2\text{O}$ equilibration it was not possible to determine the rate of lipid synthesis in these experiments. The relative rates of incorporation into the foetal lipids were the same as observed after a maternal injection. In five injections

into 45–47-day foetuses the mean foetal tissue incorporation values (d.p.m./60 min per g wet wt. $\pm$ s.d.) were: heart, 1326 $\pm$ 423; lung, 873 $\pm$ 269; kidney, 1123 $\pm$ 315; adipose tissue, 16701 $\pm$ 5826; brain, 1915 $\pm$ 367; liver, 5061 $\pm$ 1724.

Discussion

In both the maternal and foetal tissues the pattern of incorporation of $^3\text{H}_2\text{O}$ into lipid is similar to that for acetate and propionate (Jones, 1973; Jones & Ashton, 1976). It confirms that the rate of fatty acid synthesis by the tissues of the pregnant guinea pig appears to be very low, e.g. the rate of incorporation into lipid by the liver is at least one-tenth of that observed in the rat or mouse liver (Lowenstein, 1971; Salmon *et al.*, 1974).

It cannot be completely excluded that some of the incorporation of $^3\text{H}_2\text{O}$ into foetal tissue lipid was the result of maternal lipid synthesis. The arguments against this are that the $^3\text{H}_2\text{O}$ incorporation shows the same developmental changes as those observed for the incorporation of acetate and propionate by isolated foetal tissues. The low $^3\text{H}_2\text{O}$ incorporation into lipid in maternal tissues, suggesting a low rate of maternal synthesis, is confirmed by the low rates of incorporation into lipid of a range of substrates by maternal tissues *in vitro* (Jones, 1973; Jones & Ashton, 1975). In addition, if the transfer of maternal $^3\text{H}_2\text{O}$ -labelled lipid made a significant contribution to the foetal incorporation, then an initial lag in incorporation might be expected and the foetal incorporation pattern after foetal injection would probably differ from that after maternal injection. It thus seems likely that the maternal contribution to $^3\text{H}_2\text{O}$ incorporation into lipid in the foetal tissues is small. Similar conclusions have been reached for $^3\text{H}_2\text{O}$ incorporation into foetal lipid of the pregnant rat (Fain & Scow, 1966).

The apparent rate of lipid synthesis observed by the foetal liver *in vivo* is about four times higher than that observed in liver slices (Jones & Ashton, 1975). It changes in a manner similar to acetate, propionate and alanine incorporation into lipid with a peak at 45–50 days and declining towards term. Glucose incorporation on the other hand is relatively low at 45–50 days and reaches a peak at 56–57 days. This low glucose incorporation is unlikely to be the result of intracellular dilution of label, since the glycogen content of the liver at 45 days is very low (<0.5 mg/g) and it is more likely to be the result of low pyruvate carboxylase activity at this time (Kornfeld & Brown, 1963; Lea & Walker, 1967; Jones & Ashton, 1972; C. T. Jones, unpublished work). These results suggest that glucose is not the primary source of carbon for fatty acid synthesis by the foetal guinea-pig liver at about 45 days and that short-chain fatty acids may be much more important. This is supported by the

presence of acetate in the umbilical vein of the guinea pig (Jones & Ashton, 1976) and the presence of a large gradient of acetate across the liver and heart of the foetal guinea pig (C. T. Jones, unpublished work).

The changes in incorporation of $^3\text{H}_2\text{O}$ into foetal adipose-tissue lipid are similar to those observed for both glucose and acetate, but as acetate is probably effectively removed from umbilical-vein blood before reaching adipose tissue, it is likely those glucose represents the major substrate (Jones, 1973).

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References

- Clark, D. G., Rognstad, R. & Katz, J. (1974) *J. Biol. Chem.* **249**, 2028–2036
- Elvidge, H. (1972) *J. Inst. Anim. Technicians* **23**, 111–117
- Fain, J. N. & Scow, R. O. (1966) *Am. J. Physiol.* **210**, 19–25
- Jones, C. T. (1973) in *Foetal and Neonatal Physiology: Proc. Sir Joseph Barcroft Centenary Symp.* (Comline, K. S., Cross, K. W., Davies, G. S. & Nathanielsz, P. W., eds.), pp. 403–409, Cambridge University Press, Cambridge
- Jones, C. T. & Ashton, I. K. (1972) *Biochem. J.* **130**, 22 P–23 P
- Jones, C. T. & Ashton, I. K. (1976) *Biochem. J.* **154**, 149–158
- Jungas, R. L. (1968) *Biochemistry* **7**, 3708–3717
- Kornfeld, K. & Brown, D. H. (1963) *J. Biol. Chem.* **238**, 727–740
- Lea, M. A. & Walker, D. G. (1967) *Dev. Biol.* **15**, 51–61
- Lowenstein, J. M. (1971) *J. Biol. Chem.* **246**, 629–632
- Salmon, D. M., Bowen, N. L. & Hems, D. A. (1974) *Biochem. J.* **142**, 611–618
- Windmueller, H. G. & Spaeth, A. E. (1966) *J. Biol. Chem.* **241**, 2891–2899
- Windmueller, H. G. & Spaeth, A. E. (1967) *Arch. Biochem. Biophys.* **122**, 362–374