# Inability of Fetal Liver to Synthesize Plasma Phospholipids* (33762)

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Synthesis of plasma phospholipids in adult animals is carried out almost exclusively in the liver. This was shown by means of $^{32}P$ phosphate or $^{14}C$ fatty acid incorporation in hepatectomized dogs (1–3) or in the rat (4). The cycle of liver involvement in plasma phospholipid metabolism became complete when Entenman *et al.* (5) and Zilversmit and Bollman (4) showed that plasma phospholipid removal and turnover were also largely a hepatic function.

The site of production of plasma phospholipids in the fetus had not been investigated. Injection of $^{32}P$ phosphate into pregnant animals resulted in labeling of fetal plasma phospholipids (6). This could have been the result of maternal hepatic synthesis followed by transplacental transfer; or of placental synthesis followed by secretion of phospholipids into fetal circulation; or of synthesis and secretion by fetal liver or other organs. Nielsen (7) was the first to show that maternal phospholipids did not cross the placenta. Popjàk and Beeckmans (8) confirmed this finding and suggested from comparisons of specific activity that fetal liver synthesized plasma phospholipids. Our previous work (9, 10) also confirmed absence of placental transfer but the data pointed strongly to placental, rather than fetal hepatic, plasma phospholipid synthesis. Both mechanisms may be operative. However, results presented in this communication indicate that fetal liver does not contribute to plasma phospholipid production.

*Methods and Materials.* The animals used were white New Zealand rabbits kept on standard pellets. The control series consisted of nonpregnant adult females about 2500 g in weight. They were starved overnight and throughout the experiment. Water was offered *ad libitum*. Each animal was injected with 1.5 mCi of $^{32}P$ phosphate (Abbott) in 2 ml 0.9% NaCl intraperitoneally. Blood samples (no more than 2 ml each) were collected at 5 min, 1, 2, 3, 4.5, 6, 12, and 24 hr after isotope injection into tubes containing EDTA disodium salt as anticoagulant. The experimental series consisted of 84 fetuses from 17 pregnant rabbits at term. The does were sacrificed by intravenous injection of air. Fetuses were removed quickly by hysterotomy and were injected intraperitoneally with 30–40 $\mu$Ci of $^{32}P$ phosphate in 0.2 ml 0.9% NaCl. They were then placed in a humid atmosphere in a 37° oven without feeding. At time intervals described above the fetuses were bled from the heart and hepatectomized. Every fetus provided one blood sample. Each litter was invariably split into at least three time collections. Thus each time collection represented samples from several litters. Lipid extracts of plasma, liver, and carcass were obtained, purified, and analyzed for lipid P and radioactivity as described before (9).

*Results.* Incorporation of $^{32}P$ phosphate into adult rabbit plasma phospholipids was evident at the first blood collection, 5 min after isotope injection (Fig. 1). The values increased exponentially thereafter but actual counts represented a very small fraction of the injected radioactivity.

The $^{32}P$ incorporation into fetal plasma phospholipids was quite different (Fig. 2). Almost no counts were obtained till the 3-hr blood collection after which a sharp rise in activity was evident. The general pattern then became similar to that in the adult animal but fetal counts were approximately 100 times higher than adult. This is emphasized by the different order of magnitude of counts

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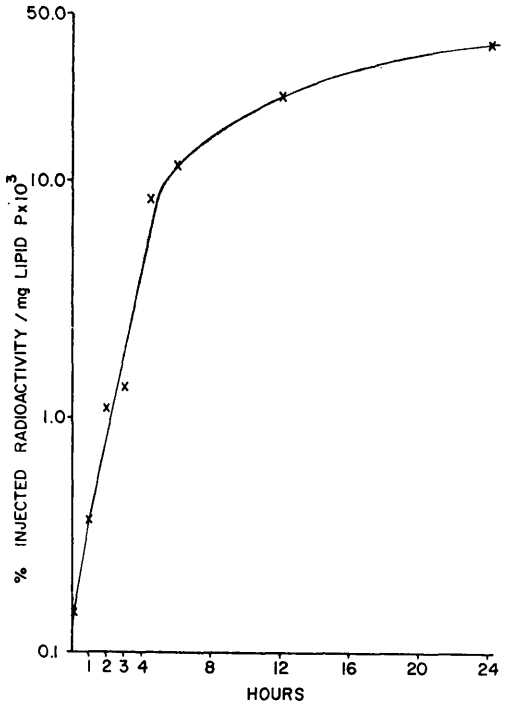


FIG. 1. Incorporation of $^{32}P$ into plasma phospholipids of adult rabbits following a single intraperitoneal injection; values are averages of 4 animals.

employed (Figs. 1 and 2).

Incorporation of $^{32}P$ into phospholipids of fetal liver and carcass was immediate (Fig. 2) with the general pattern like that of adult rather than fetal plasma phospholipids. However, carcass phospholipids reached almost a plateau after about 6 hr, at a much lower level of incorporation than either fetal plasma or liver.

Comparison of fetal plasma and liver specific activity–time curves showed a higher liver phospholipid specific activity for nearly 10 hr after injection. At that point, the curves crossed. Plasma values continued to increase whereas liver values tended to level off. The actual counts obtained are shown in Table I. The sudden appearance of plasma phospholipid incorporation in the 3-hr specimen is very evident as is also a relatively fast incorporation into liver and carcass phospholipids. The total incorporation per gram of liver and carcass or per ml of plasma (Table II) reflects of course the difference in the phospholipid content of those tissues. For plasma this averaged 46.8 $\mu$g of lipid P/ml, for liver 798 $\mu$g/g, for carcass 272.0 $\mu$g/g. The average liver weight was 1.83 g, average carcass weight 36.3 g. Since 40 million cpm were injected into each fetus the total incorporation into fetal phospholipids after 24 hrs was over 11% of injected radioactivity. Liver phospholipids contained about 50% of that value. After 6 hr the total fetal incorporation was less than 5% of which liver contained close to 40%.

*Discussion.* It is clear from many reports that, both morphologically and metabolically, fetal liver is an organ different from its adult counterpart. Dawkins (11) observed marked mitochondrial multiplication in neonatal liver soon after birth. Fetal liver cells displayed a similarly poor development of endoplasmic reticulum (12), particularly of the smooth variety (13). This is significant because according to a number of reports discussed by Buckley *et al.* (14) plasma lipoproteins are probably formed in the

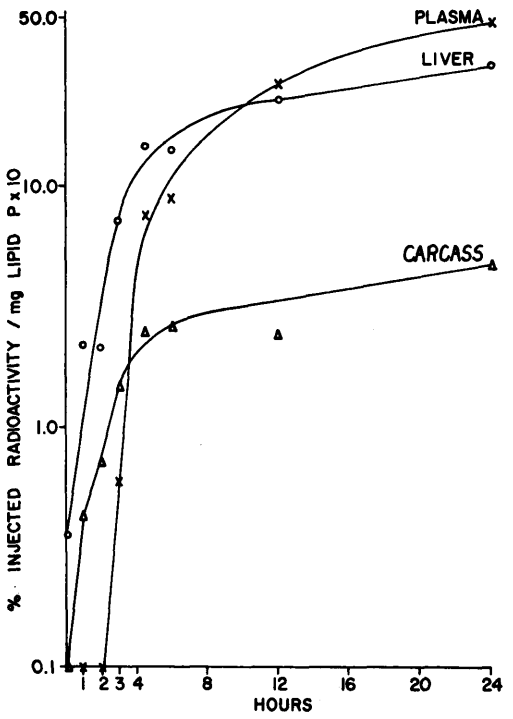


FIG. 2. Incorporation of $^{32}P$ into phospholipids of newborn rabbits following a single intraperitoneal injection.

TABLE I. Incorporation of $^{32}P$ into Fetal Phospholipids.

| Time | Plasma | Liver | Carcass |
|---|---|---|---|
| (min) | | | |
| 5 | 1.2 ± 0.3[a] | 14.5 ± 2.5 | 3.2 ± 1.2 |
| (hr) | | | |
| 1 | 1.8 ± 0.6 | 87.0 ± 9.2 | 18.0 ± 2.9 |
| 2 | 4.3 ± 1.4 | 81.9 ± 14.9 | 21.8 ± 4.4 |
| 3 | 24.5 ± 9.9 | 287.9 ± 53.1 | 61.5 ± 15.2 |
| 4.5 | 296.7 ± 55.4 | 565.0 ±109.1 | 76.0 ± 13.5 |
| 6 | 351.0 ± 68.0 | 550.0 ± 89.0 | 111.0 ± 12.3 |
| 12 | 1042.0 ±204.0 | 902.0 ±240.0 | 94.0 ± 20.9 |
| 24 | 1785.0 ±169.0 | 1198.0 ± 88.0 | 190.0 ± 35.0 |

[a] cpm/$\mu$g of P ± SEM; values equated to injection of 40,000,000 cpm of $^{32}P$ into each fetus; minimum in each group was 5.

smooth endoplasmic reticulum where lipids are esterified and combined with the proteins derived from the rough endoplasmic reticulum. Fetal liver was found deficient in many enzyme systems such as those concerned with carbohydrate (15) or tricarboxylic acid cycle metabolism (16). On the other hand enzymes concerned with anaerobic glycolysis displayed much activity.

Fetal liver is capable of vigorous lipogenesis *in vitro* (17). *In vivo* experiments demonstrated rapid $^{32}P$ incorporation into phospholipids of fetal liver and all other organs tested (6). Our experiments also demonstrated rapid phospholipid synthesis in both fetal liver and carcass. This process began immediately after injection of the precursor. That was not the case with plasma phospholipids. Plasma phospholipid production must be looked upon as the result of a secretory process which is probably unrelated to synthesis of tissue lipids. The only true measure of plasma phospholipid formation is plasma phospholipid turnover. The first evidence of incorporation was not obvious until the 3-hr specimen was collected. This meant that for at least 3 hr after injection (had the injection been intrauterine) and possibly much longer, the fetus would not have been able to produce plasma phospholipids. Throughout the experiment an assumption was made that for a number of hours after birth the newborn liver can be regarded metabolically as a fetal liver. Our interpretation therefore is that the fetus, including liver, is probably incapable of fulfilling that function before birth. The results also mean that by the third hour after birth, but not earlier, the various metabolic and morphologic changes in fetal liver (or other organs) necessary to generate plasma phospholipids have taken place. The relationship of specific activi-

TABLE II. Radioactivity Content of Fetal Phospholipids.

| Time | Plasma | Liver | Carcass |
|---|---|---|---|
| (min) | | | |
| 5 | 54 ± 21[a] | 11,870 ± 2790 | 1128 ± 422 |
| (hr) | | | |
| 1 | 64 ± 20 | 54,400 ± 11,200 | 4980 ± 940 |
| 2 | 139 ± 30 | 62,900 ± 13,800 | 8010 ± 1660 |
| 3 | 702 ± 343 | 330,800 ±109,700 | 16,000 ± 3030 |
| 4.5 | 14,010 ± 3010 | 335,500 ± 78,100 | 30,000 ± 4490 |
| 6 | 14,800 ± 3800 | 403,000 ± 63,200 | 31,500 ± 4170 |
| 12 | 62,300 ±11,100 | 456,700 ±130,600 | 23,750 ± 3869 |
| 24 | 106,800 ±14,600 | 1,244,100 ±235,000 | 60,000 ±16,700 |

[a] cpm/g (ml) ± SEM; values equated to injection of 40,000,000 cpm of $^{32}P$ into each fetus; minimum in each group was 5.

ty–time curves for liver and plasma phospholipids shown in Fig. 2 were similar to the precursor–product relationship described by Zilversmit *et al.* (18). This strongly suggested the liver as the site of neonatal synthesis of plasma phospholipids.

The apparent lack of $^{32}P$ incoporation into fetal plasma phospholipids could perhaps be due to a very slow turnover. Yet after injection of radioactive phospholipids into pregnant rabbits we found substantial radioactivity levels in fetal plasma phospholipids in a matter of minutes (10). In the proven absence of direct maternal transfer, a fast turnover of fetal plasma phospholipids must be assumed to be pointing to an origin other than fetal.

*Summary.* Following intraperitoneal injection of $^{32}P$ into newborn rabbits, appearance of radioactivity in plasma phospholipids was delayed for 3 hr. By contrast adult plasma phospholipids and newborn liver and carcass showed immediate incorporation. Results point to inability of fetal liver to produce plasma phospholipids.

1. Friedlander, H. D., Chaikoff, I. L., and Entenman, C., J. Biol. Chem. **158**, 231 (1945).

2. Fishler, M. C., Entenman, C., Montgomery, M. L., and Chaikoff, I. L., J. Biol. Chem. **150**, 47 (1943).

3. Goldman, D. S., Chaikoff, I. L., Reinhardt, W. O., Entenman, C., and Dauben, W. G., J. Biol. Chem. **184**, 727 (1950).

4. Zilversmit, D. B. and Bollman, J. L., Arch. Biochem. Biophys. **63**, 64 (1956).

5. Entenman, C., Chaikoff, I. L., and Zilversmit, D. B., J. Biol. Chem. **166**, 15 (1946).

6. Popjàk G., Nature **160**, 841 (1947).

7. Nielsen, P. E., Am. J. Physiol. **135**, 670 (1941-1942).

8. Popjàk, G. and Beeckmans, M. L., Biochem. J. **46**, 99 (1950).

9. Biezenski, J. J., Am. J. Obstet. Gynecol. in press.

10. Biezenski, J. J., "Proceedings of the International Symposium on Foeto-Placental Unit." Excerpta Medical Foundation, Amsterdam, in press.

11. Dawkins, M. J. R., Proc. Roy. Soc. (London), Ser. B, **150**, 284 (1959).

12. Peters, V. B., Kelly, G. W., and Dembitzer, H. M., Ann. N. Y. Acad. Sci. **111**, 87 (1963).

13. Dallner, G., Siekevitz, P., and Palade, G. E., Biochem. Biophys. Res. Commun. **20**, 135 (1965).

14. Buckley, J. T., Delahunty, T. J., and Rubinstein, D., Can. J. Biochem. **46**, 341 (1968).

15. Burch, H. B., Lowry, O. H., Kuhlman, A. M., Skerjance, J., Diamant, E. J., Lowry, S. R., and Von Dippe, P., J. Biol. Chem. **238**, 2267 (1963).

16. Dawkins, M. J. R., Brit. Med. Bull. **22**, 27 (1966).

17. Villee, C. A. and Hagerman, D. D., Am. J. Physiol. **194**, 457 (1958).

18. Zilversmit, D. B., Entenman, C., and Fishler, M. C., J. Gen. Physiol. **26**, 325 (1943).

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