# Lipid composition and metabolism in liver and brain of vitamin $B_{12}$-deficient rat sucklings

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1. Rat sucklings (18-d-old) bred from vitamin $B_{12}$-deprived dams were compared with vitamin $B_{12}$-supplemented dams' offspring, which were considered normal rat sucklings.

2. The vitamin $B_{12}$-deficient rat sucklings had lower body-weight, liver weight and brain weight.

3. Vitamin $B_{12}$ deficiency was also evident from the tenfold lower concentrations of vitamin $B_{12}$ in liver and cerebellum.

4. The concentration of liver lipid was markedly increased in vitamin $B_{12}$-deficient rats; triacylglycerol accounted for most of the increase. In brain the lipid concentration was slightly decreased ($P < 0.05$).

5. The methylation of ethanolamine phosphoglyceride to choline phosphoglyceride was reduced in both liver and brain in vitamin $B_{12}$-deficient rats, as measured after the administration of [$^{14}$C]ethanolamine. A slight decrease in choline phosphoglyceride concentration could be a consequence of this finding. The composition of phospholipids was otherwise normal.

6. Odd-chain fatty acids (pentadecanoate and heptadecanoate) accumulated in both liver and brain of the vitamin $B_{12}$-deficient rat sucklings and constituted approximately 1 % of total fatty acid.

7. The biosynthesis of fatty acid and cholesterol from intraperitoneally-injected $^3H_2O$ and [$^{14}$C]propionate was unchanged in vitamin $B_{12}$ deficiency.

A progressive demyelination is one of the most conspicuous findings in vitamin $B_{12}$ deficiency neuropathy (Russell *et al.* 1900; Greenfield & O'Flynn, 1933; Pant *et al.* 1968). The metabolic mechanism behind this derangement is obscure. Vitamin $B_{12}$ dependency has been established for the isomerization of methylmalonate to succinate and the methylation of homocysteine to methionine, but metabolic changes in vitamin $B_{12}$ deficiency also involve folate turnover, as expressed in the 'folate trap' hypothesis (see Herbert, 1976). Propionate and methylmalonate and their derivatives which accumulate in vitamin $B_{12}$ deficiency (Frenkel *et al.* 1974) inhibit fatty acid synthesis in vitro (Cardinale *et al.* 1970; Frenkel & Kitchens, 1977). Becuase the activity of some enzymes involved in fatty acid synthesis concomitantly increase, especially in the liver (Frenkel *et al.* 1973; Frenkel *et al.* 1976), it is difficult to evaluate the consequences of vitamin $B_{12}$ deficiency for fatty acid synthesis in vivo. Fatty acid synthesis in adult rats with moderate or severe vitamin $B_{12}$ deficiency was not markedly changed (Fehling, Jägerstad & Arvidson, 1978; Fehling, Jägerstad, Åkesson *et al.* 1978). As fatty acid synthesis in brain is most active during the first postnatal weeks (Volpe & Kishimoto, 1972), the effects of vitamin $B_{12}$ deficiency on fatty acid synthesis from $^3H_2O$ have now been studied in 18-d-old rats. No changes in the rate of fatty acid and cholesterol synthesis could be observed in vitamin $B_{12}$ deficiency although significant effects on tissue lipid concentrations were evident.

The inhibition of methionine synthesis in vitamin $B_{12}$-deficient adult rats is probably the cause of a decreased methylation of ethanolamine phosphoglyceride and a slight decrease in the proportion of choline phosphoglyceride in liver (Åkesson *et al.* 1978). These observations were confirmed in rat sucklings where corresponding changes were demonstrated also in brain.

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## EXPERIMENTAL

### *Animals and their management*

Rats were bred from females of the Wistar strain given a vitamin $B_{12}$-deficient diet from 4 weeks of age. The control animals were supplemented with vitamin $B_{12}$ in the drinking-water (20 $\mu$g/l). The rats were housed in stainless-steel cages with elevated wire-mesh floors as previously described (Fehling, Jägerstad, Åkesson *et al.* 1978). For mating, two male rats were introduced into a cage with seven vitamin $B_{12}$-deprived females who had been on the diet for 2 months. Two other males were placed with six control rats. After 3 d, the males were switched from one cage to the other, and after another 3 d, they were removed. After 12 d, the female rats were transferred to individual plastic cages with floors covered with wood-shavings. They remained in individual cages with their pups until these were killed after 18–19 d.

### *Diet*

Except for the content of methionine, the diet was identical with the one used in a previous experiment (Fehling, Jägerstad, Åkesson *et al.* 1978). Its gross composition was (g/kg diet): sugars 330, soya-bean protein 571, soya-bean oil 48, vitamin and salt mixtures 55. It contained 6·7 g L-methionine/kg and less than 2 $\mu$g vitamin $B_{12}$/kg.

### *Injection of isotopes*

$^3H_2O$, [$^{14}$C]propionate and [$^{14}$C]ethanolamine were obtained from the Radiochemical Centre, Amersham, Bucks., UK. In one experiment 100 $\mu$l [$^{14}$C]ethanolamine (1 $\mu$Ci) in saline (9 g sodium chloride/l)/10 g body-weight was injected intraperitoneally into fifteen 18-d-old rats. In another experiment, 0·5 mCi $^3H_2O$, 0·9 $\mu$Ci [$^{14}$C]propionate, and 0·1 mmol propionate/10 g body-weight in a volume of 100 $\mu$l were injected into seventeen rats. The rats were killed 2 or 24 h later. In a preliminary experiment, 0·5 mCi $^3H_2O$/10 g body-weight was injected into 10-d-old rats, which were killed at different time intervals after injection.

### *Sampling of tissues*

Liver and brain were excised under diethyl ether anaesthesia, washed in ice-cold saline, blotted on filter paper, weighed, and immersed in liquid nitrogen. After the brain was weighed, the cerebellum was taken for vitamin $B_{12}$ analysis and the cerebrum for lipid analysis. The tissues were stored in sealed plastic bags at $-80°$ until analysed.

### *Analytical procedures*

Methods for lipid extraction, alkaline hydrolysis for isolation of esterified plus free fatty acid and cholesterol, fatty acid analysis by gas–liquid chromatography, radioactivity measurement, and vitamin $B_{12}$ determination are described elsewhere (Åkesson *et al.* 1978; Fehling, Jägerstad, Åkesson *et al.* 1978). Individual phospholipids were separated by thin-layer chromatography on silica gel H using the developing solvents, diethyl ether–acetic acid (99:1, v/v) then chloroform-methanol-conc. ammonia (60:30:5, by vol.). The phospholipids were eluted (Åkesson *et al.* 1970) and quantitated by phosphorus determination (Belfrage *et al.* 1970). Sphingolipid was determined according to Kisic & Rapport (1974). Statistical evaluation was performed by Student's *t* test or the Mann–Whitney *U* test (one-tailed). Level of significance was chosen as $P < 0·05$. The one-tailed test was used since the individual values clearly indicated the region of rejection of the null hypothesis.

Table 1. *Vitamin $B_{12}$ concentrations (ng/g wet tissue), body and organ weights (g) in 18-d-old rats deprived of or supplemented with vitamin $B_{12}$*

(Mean values with their standard errors; no. of animals in parentheses)

| Vitamin $B_{12}$ concentration: | Vitamin $B_{12}$-deficient (16) Mean | SE | Vitamin $B_{12}$-supplemented (16) Mean | SE |
|---|---|---|---|---|
| Cerebellum | 5·0 | 0·4 | 45·0 | 1·1 |
| Liver | 9·0 | 0·5 | 67·0 | 3·0 |
| Wt: | | | | |
| Body | 15·0 | 0·6 | 25·0 | 0·6 |
| Brain | 0·89 | 0·05 | 1·22 | 0·02 |
| Liver | 0·52 | 0·03 | 0·90 | 0·03 |

Differences between vitamin $B_{12}$-deficient and supplemented animals were all highly significant ($P < 0·001$, Student's $t$ test).

Table 2. *Concentrations of total lipid (mg/g wet weight), fatty acid (mg/g wet tissue), and phospholipid (μmol/g wet tissue) in liver and brain of 18-d-old rats deprived of or supplemented with vitamin $B_{12}$*

(Mean values with their standard errors; no. of animals in parentheses)

| | Vitamin $B_{12}$-deficient Mean | SE | Statistical significance of difference between groups $P$* | Vitamin $B_{12}$-supplemented Mean | SE |
|---|---|---|---|---|---|
| Liver: | | | | | |
| Total lipid | 127·2 | 7·3 (16) | < 0·001 | 54·0 | 2·5 (15) |
| Total fatty acid | 52·9 | 3·3 (4) | < 0·001 | 31·7 | 1·6 (4) |
| Total phospholipid | 28·4 | 2·0 (8) | < 0·01 | 37·4 | 1·2 (7) |
| Brain: | | | | | |
| Total lipid | 47·8 | 1·7 (15) | < 0·05 | 52·4 | 1·2 (15) |
| Total fatty acid | 25·4 | 0·7 (4) | | 27·4 | 1·0 (4) |
| Total phospholipid | 34·6 | 1·3 (7) | | 36·6 | 2·7 (8) |

* Student's $t$ test.

## RESULTS

### *Animals and their vitamin $B_{12}$ status*

Four of the seven vitamin $B_{12}$-deprived rats delivered litters of nine, eight, eight, and six animals. Four of the six control rats delivered litters of ten, nine, eight, and six animals. One litter of nine and one of eight pups from vitamin $B_{12}$-depleted and control mothers were chosen for the main experiments. The pups of the vitamin $B_{12}$-depleted dams were smaller than the control pups; otherwise, they appeared healthy. Table 1 shows the weights and the organ concentrations of vitamin $B_{12}$. Brain weight was less influenced by vitamin $B_{12}$ deprivation than were liver and total body-weight. The content of vitamin $B_{12}$ was extremely low in both liver and brain; actually, the values were as low as those recorded in a long-term experiment on adult rats where neurological signs were induced (Fehling, Jägerstad, Åkesson *et al.* 1978).

Table 3. *Phospholipid composition (% total phospholipid) in liver and brain of 18-d-old rats deprived of or supplemented with vittamin $B_{12}$*

(Each value represents pooled extracts from eight rats)

| Lipid fraction | Liver | | Brain | |
|---|---|---|---|---|
| | Vitamin $B_{12}$-deficient | Vitamin $B_{12}$-supplemented | Vitamin $B_{12}$-deficient | Vitam'n $B_{12}$-supplemented |
| 1* | 1·4 | 1·3 | 0·4 | 0·3 |
| 2* | 4·6 | 5·7 | 3·6 | 2·5 |
| Ethanolamine phosphoglyceride | 26·5 | 26·5 | 32·1 | 31·3 |
| Choline phospho-glyceride | 44·2 | 47·4 | 43·1 | 45·6 |
| Serine phosphoglyceride + inositol phospho-glyceride + sphingomyelin | 20·4 | 17·4 | 19·0 | 19·3 |
| 3* | 1·5 | 1·1 | 0·8 | 0·5 |
| 4* | 1·4 | 0·7 | 1·0 | 0·5 |

* Fractions obtained by thin-layer chromatography which were not further characterized.

### *Tissue lipid content and composition*

Liver of vitamin $B_{12}$-deficient rats contained twice as much lipid per g as normal livers, whereas lipid concentration was slightly reduced in brains of deficient rats (Table 2). These results are in contrast to findings in 1-year-old vitamin $B_{12}$-deficient rats, which had normal total lipid concentrations in liver, brain, and spinal cord (Fehling, Jägerstad, Åkesson *et al.* 1978).

The phospholipid concentration in liver was lower in the vitamin $B_{12}$-deficient rats, but the phospholipid composition of brain and liver was very similar in deficient and supplemented rats (Table 3). The slightly lower proportion of choline phosphoglyceride in deficient rats might reflect a reduced phospholipid methylation, as discussed later. Gas–liquid chromatography of fatty acid methyl esters showed that 59·5% of the total fatty acids in vitamin $B_{12}$-deficient liver was in triacylglycerol. The corresponding value for supplemented animals was 30·3%, indicating that accumulation of triacylglycerol was the major reason for the high liver lipid concentration in vitamin $B_{12}$-deficient rats. Also cholesteryl ester accumulated in these livers.

The concentration of total sphingolipid in brain was 0·115 and 0·112 $\mu$mol/mg total lipid in control and vitamin $B_{12}$-deficient animals respectively.

### *Fatty acid composition*

The fatty acid composition of the major lipid classes in brain was similar in vitamin $B_{12}$-deficient and supplemented rats (Table 4). In total lipids the relative concentration of heptadecanoate was much higher in the vitamin $B_{12}$-deficient animals and minor increases were observed for myristate, palmitoleate, and linoleate at the expense of stearate and oleate. (Pentadecanoate was used as internal standard for quantitation of methyl esters and was therefore not determined in tissue lipids). These analyses were performed on rats injected with propionate. *A priori*, it could not be excluded that the accumulation of odd-chain fatty acids was due to propionate administration. In a previous experiment less odd-chain fatty acids were found in vitamin $B_{12}$-deficient than in supplemented rats after propionate loading (Fehling, Jägerstad & Arvidson, 1978). However, in 10-d-old rats not given propionate, the same accumulation of odd-chain fatty acids was evident in vitamin $B_{12}$ deficiency (Table 5).

Table 4. *Fatty acid composition (% total fatty acid weight) of major lipids in brain of 18-d-old rats deprived of or supplemented with vitamin* $B_{12}$

(Individual lipids were isolated from pooled extracts from eight rats; values for total lipid are means with their standard errors)

| | Vitamin $B_{12}$-deficient | | | | | Vitamin $B_{12}$-supplemented | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Ethanolamine phosphoglyceride | Choline phosphoglyceride | Serine phosphoglyceride + inositol phosphoglyceride + sphingomyelin | Total lipid Mean | Total lipid SE | Ethanolamine phosphoglyceride | Choline phosphoglyceride | Serine phosphoglyceride + inositol phosphoglyceride + sphingomyelin | Total lipid Mean | Total lipid SE |
| 14:0 | | | | 1·3 | 0·2* | | | | 0·5 | 0·1 |
| 16:0 | 10·3 | 53·7 | 5·2 | 27·1 | 0·2 | 7·2 | 49·8 | 14·7 | 26·2 | 0·5 |
| 16:1 | | 1·3 | | 1·6 | 0·1* | 0·7 | 1·9 | | 1·2 | 0·1 |
| 17:0 | 0·1 | 0·3 | 0·2 | 0·4 | 0·0* | 0·1 | 0·1 | | 0·1 | 0·0 |
| 18:0 | 25·1 | 10·2 | 44·9 | 21·4 | 0·2* | 25·9 | 12·0 | 42·0 | 22·4 | 0·2 |
| 18:1 | 7·4 | 20·9 | 7·8 | 14·9 | 0·2* | 10·8 | 23·1 | 10·9 | 16·4 | 0·3 |
| 18:2 | 0·3 | 0·9 | 0·1 | 1·1 | 0·0* | 0·8 | 0·6 | | 0·9 | 0·1 |
| 20:3 | 0·1 | | | 0·3 | 0·0 | 0·3 | | | 0·3 | 0·0 |
| 20:4 ($n-6$) | 24·5 | 9·0 | 14·5 | 16·0 | 0·1 | 22·9 | 8·4 | 12·1 | 15·6 | 0·1 |
| 22:4 ($n-6$) | 7·2 | 0·7 | 4·6 | 2·9 | 0·1 | 7·7 | 0·5 | 2·8 | 3·7 | 0·6 |
| 22:5 ($n-6$) | 2·2 | 0·2 | 2·4 | 0·7 | 0·1 | 2·1 | | 1·6 | 0·9 | 0·1 |
| 22:5 ($n-3$) | | | | 0·0 | | | | | 0·5 | 0·3 |
| 22:6 ($n-3$) | 22·8 | 2·8 | 20·3 | 12·5 | 0·2 | 21·5 | 3·6 | 15·9 | 11·4 | 1·1 |

* Statistical significance of difference between vitamin $B_{12}$-deprived and supplemented animals (Mann–Whitney $U$ test): $0{\cdot}01 < P < 0{\cdot}05$.

Table 5. *Proportions of odd-chain saturated fatty acids (% total fatty acid weight) in liver and brain total lipid of individual 10-d-old rats deprived of or supplemented with vitamin* $B_{12}$

| | Fatty acid | Vitamin $B_{12}$-deficient | Vitamin $B_{12}$-supplemented |
|---|---|---|---|
| Liver | 15:0 | 0·75; 0·61 | 0·15; 0·09 |
| | 17:0 | 1·13; 1·08 | 0·20; 0·21 |
| Brain | 15:0 | 0·70; 0·39 | 0·06; ND |
| | 17:0 | 0·59; 0·42 | 0·05; 0·06 |

ND, not determined.

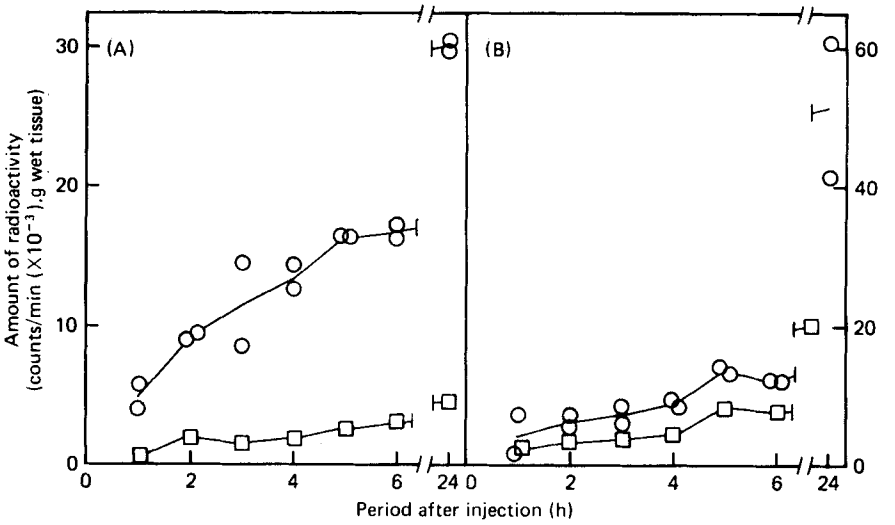


Fig. 1. Time-course for the incorporation of $^3H$ (counts/min ($10^{-3}$) per g wet tissue) into fatty acid (□) and total lipid (○) after intraperitoneal injection of $^3H_2O$ into vitamin $B_{12}$-supplemented rats. A, liver; B, brain.

The proportion of heptadecanoate was higher in liver than in brain in the vitamin $B_{12}$-deficient animals (Tables 4–6), especially in the phospholipids. Total fatty acid composition of liver changed dramatically in vitamin $B_{12}$ deficiency, mainly due to the accumulation of triacylglycerol.

*Tissue uptake of injected isotopes*

In a preliminary experiment, the time-course for incorporation of $^3H_2O$ into tissue lipids of 10-d-old rats was studied (Fig. 1). The amount of $^3H$ both in total lipid and in fatty acid increased continuously in brain and liver 1–6 h after injection, and the increase continued up to 24 h. For a comparison between vitamin $B_{12}$-deficient and supplemented rats, the time-intervals 2 and 24 h were chosen. The radioactivity amount at 2 h would preferentially reflect initial uptake, and the change in lipid radioactivity between 2 and 24 h could give information about lipid breakdown.

Lipid-$^3H$ increased between 2 and 24 h after the injection of $^3H_2O$ also in vitamin $B_{12}$-deficient rats (Table 7). On both occasions, liver lipid $^3H$ was higher in vitamin $B_{12}$-deficient animals; this might be related to their higher liver lipid concentration (Table 2). In brain the incorporation of $^3H$ into lipids was not changed in vitamin $B_{12}$ deficiency (Table 7).

The liver uptake of [$^{14}C$]propionate at 2 h was similar in vitamin $B_{12}$-deficient and-supplemented rats. The concentration of $^{14}C$ was unchanged at 24 h in the deficient rats but

Table 6. *Fatty acid composition of major lipids in liver of 18-d-old rats deprived of or supplemented with vitamin $B_{12}$*

(Individual lipids were isolated from pooled extracts from eight rats; values for total lipid are means with their standard errors for four rats)

| | Vitamin $B_{12}$-deficient | | | | | | Vitamin $B_{12}$-supplemented | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Fatty acid | Triacyl-glycerol | Ethanol-amine phospho-glyceride | Choline phospho-glyceride | Serine phospho-glyceride + inositol phospho-glyceride + sphingomyelin | Total lipid Mean | SE | Triacyl-glycerol | Ethanol-amine phospho-glyceride | Choline phospho-glyceride | Serine phospho-glyceride + inositol phospho-glyceride + sphingomyelin | Total lipid Mean | SE |
| 14:0 | 6·0 | | 1·1 | | 5·1 | 0·3* | 3·6 | | | | 2·1 | 0·0 |
| 16:0 | 21·4 | 14·4 | 27·9 | 16·0 | 20·2 | 0·5* | 28·2 | 20·9 | 31·9 | 10·1 | 24·0 | 0·1 |
| 16:1 | 0·4 | | | | 0·6 | 0·1 | 0·3 | 0·1 | | | 0·6 | 0·0 |
| 17:0 | 0·5 | 1·4 | 1·2 | 1·0 | 0·8 | 0·0* | | | 0·1 | 0·1 | 0·1 | 0·0 |
| 18:0 | 3·6 | 26·5 | 16·7 | 37·1 | 7·1 | 0·4* | 3·2 | 26·3 | 15·7 | 40·6 | 13·6 | 0·5 |
| 18:1 | 11·4 | 2·9 | 3·4 | 3·5 | 10·3 | 0·2 | 16·6 | 1·2 | 4·1 | 2·2 | 10·1 | 0·3 |
| 18:2 | 31·8 | 6·9 | 10·8 | 7·3 | 28·3 | 0·5* | 31·6 | 4·3 | 13·4 | 4·1 | 20·3 | 0·5 |
| 18:3 | 1·3 | | | | 1·2 | 0·1* | 1·0 | | | | 0·5 | 0·1 |
| 20:3 | 1·3 | | | | 1·1 | 0·2 | 1·0 | | | | 0·8 | 0·0 |
| 20:4 ($n-6$) | 11·5 | 21·5 | 28·0 | 24·2 | 13·7 | 0·3* | 5·9 | 24·2 | 24·7 | 33·9 | 17·4 | 0·3 |
| 22:4 ($n-6$) | 2·2 | 0·5 | | 1·9 | 1·5 | 0·1* | 2·1 | | | | 0·8 | 0·1 |
| 22:5 ($n-6$) | 0·8 | 0·7 | | | 0·7 | 0·1 | 1·0 | | | | 0·5 | 0·1 |
| 22:5 ($n-3$) | 3·1 | 1·7 | 1·2 | 1·4 | 2·7 | 0·2* | 2·7 | 2·0 | 1·7 | 1·3 | 1·8 | 0·2 |
| 22:6 ($n-3$) | 4·7 | 23·4 | 9·7 | 7·6 | 6·9 | 0·1* | 2·9 | 21·0 | 8·4 | 7·6 | 7·6 | 0·1 |

* Statistical significance of difference between rats deprived of or supplemented with vitamin $B_{12}$ (Mann–Whitney $U$ test): $0{\cdot}01 < P < 0{\cdot}05$.

Table 7. *Radioactivity (counts/min($\times 10^{-3}$) per g wet tissue) in liver and brain lipids 2 and 24 h after the injection of [$^{14}$C]ethanolamine*, $^{3}H_2O$ and [$^{14}$C]propionate into 18-d-old rats deprived of or supplemented with vitamin $B_{12}$*

(Mean values with their standard errors; no. of animals in parentheses)

| | Period after injection (h) | Vitamin $B_{12}$-deficient | | Statistical significance of differences between groups: $P$† | Vitamin $B_{12}$-supplemented | |
|---|---|---|---|---|---|---|
| | | Mean | SE | | Mean | SE |
| Liver: | | | | | | |
| [$^{14}$C]ethanolamine | 2 | 310 | 26 (4) | | 231 | 15 (3) |
| | 24 | 145 | 17 (3) | | 143 | 7 (4) |
| $^{3}H_2O$ | 2 | 14·5 | 0·8 (4) | 0·014 | 9·2 | 0·7 (4) |
| | 24 | 58·1 | 4·2 (4) | 0·014 | 35·3 | 3·3 (4) |
| [$^{14}$C]propionate | 2 | 3·3 | 0·3 (4) | | 3·3 | 0·5 (4) |
| | 24 | 3·7 | 0·3 (4) | 0·014 | 1·4 | 0·2 (4) |
| Brain: | | | | | | |
| [$^{14}$C]ethanolamine | 2 | 3·7 | 0·9 (4) | | 4·5 | 0·2 (3) |
| | 24 | 23·5 | 1·3 (3) | | 19·1 | 1·6 (4) |
| $^{3}H_2O$ | 2 | 4·1 | 0·2 (4) | | 4·0 | 0·4 (5) |
| | 24 | 35·7 | 2·2 (3) | | 35·8 | 1·8 (4) |
| [$^{14}$C]propionate | 2 | 1·6 | 0·1 (4) | | 2·0 | 0·2 (5) |
| | 24 | 2·5 | 0·1 (3) | | 2·4 | 0·2 (3) |

* [$^{14}$C]ethanolamine was administered to rats other than those receiving $^{3}H_2O$ and [$^{14}$C]propionate.
† Mann–Whitney $U$ test.

Table 8. *Methylation of ethanolamine phosphoglyceride (EPG) to choline phosphoglyceride (CPG) ($^{14}$C in CPG $\times$ 100:$^{14}$C in EPG plus CPG) in 18-d-old rats deprived or supplemented with vitamin $B_{12}$ and injected with [$^{14}$C]ethanolamine 24 h before death*

(Mean values with their standard errors; no. of animals in parentheses)

| | Vitamin $B_{12}$-deficient (4) | | Statistical significance of differences between groups: $P$* | Vitamin $B_{12}$-supplemented (4) | |
|---|---|---|---|---|---|
| | Mean | SE | | Mean | SE |
| Liver | 11·2 | 1·3 | 0·014 | 30·9 | 0·6 |
| Brain | 22·7 | 2·0 | 0·014 | 30·6 | 0·6 |

* Mann–Whitney $U$ test.

decreased to less than half in the supplemented rats (Table 7). This probably reflects a lower propionate oxidation in vitamin $B_{12}$ deficiency (Marston *et al.* 1961; Fish *et al.* 1968), which would lead to a slower turnover of [$^{14}$C]propionate. In brain, the total lipid $^{14}$C increased slightly between 2 and 24 h in both deficient and supplemented animals.

The incorporation of [$^{14}$C]ethanolamine in liver was larger at 2 h than at 24 h (Table 7). In brain, the incorporation was much lower on both occasions. It increased dramatically between 2 and 24 h. No differences between vitamin $B_{12}$-deficient and supplemented rats were observed.

*Choline phosphoglyceride synthesis by methylation*

The degree of ethanolamine phosphoglyceride methylation was expressed as 100 $\times$ $^{14}$C in choline phosphoglyceride:$^{14}$C in choline phosphoglyceride plus ethanolamine phosphoglyceride after injection of [$^{14}$C]ethanolamine. This value is markedly influenced by methio-

Table 9. *Radioactivity (counts/min ($\times 10^{-3}$) per g wet tissue) in liver and brain fatty acid and cholesterol 2 and 24 h after the injection of $^3H_2O$ and [$^{14}$C]propionate into 18-d-old rats deprived of or supplemented with vitamin $B_{12}$*

(Mean values with their standard errors; no. of animals in parentheses)

| | | Vitamin $B_{12}$-deficient | | Vitamin $B_{12}$-supplemented | |
|---|---|---|---|---|---|
| | | Mean | SE* | Mean | SE* |
| $^3H_2O$: | | | | | |
| Liver fatty acid | 2 | 1·8 | 0·2 (4) | 1·4 | 0·2 (4) |
| | 24 | 9·8 | 1·5 (4) | 7·1 | 0·6 (4) |
| Liver cholesterol | 2 | 0·36 | 0·10 (4) | 0·14 | 0·01 (4) |
| | 24 | 0·39 | 0·09 (4) | 0·51 | 0·07 (4) |
| Brain fatty acid | 2 | 1·7 | 0·1 (4) | 1·9 | 0·2 (5) |
| | 24 | 17·1 | 0·9 (3) | 20·2 | 1·5 (4) |
| Brain cholesterol | 2 | 0·40 | 0·03 (4) | 0·37 | 0·09 (5) |
| | 24 | 5·2 | 0·4 (3) | 5·5 | 0·4 (4) |
| [$^{14}$C]propionate: | | | | | |
| Brain fatty acid | 2 | 0·96 | 0·06 (4) | 1·18 | 0·10 (5) |
| | 24 | 1·24 | 0·13 (3) | 1·20 | 0·16 (4) |
| Brain cholesterol | 2 | 0·12 | 0·01 (4) | 0·17 | 0·01 (5) |
| | 24 | 0·29 | 0·03 (3) | 0·30 | 0·03 (3) |

* Values for *P* for the differences between groups were not below 0·057 (Mann–Whitney *U* test).

nine availability (Sundler & Åkesson, 1975*a*). The value in liver was markedly depressed in vitamin $B_{12}$ deficiency (Table 8), which agrees with previous findings in adult rats (Åkesson *et al.* 1978). It indicates that the low endogenous synthesis of methionine in vitamin $B_{12}$ deficiency leads to a lower methionine availability for phospholipid methylation, despite an adequate dietary supply of methionine. Also in brain, the value for the labelled phospholipid ratio was lower in vitamin $B_{12}$-deficient rats than in control rats. The values were similar in brain and liver from supplemented rats, but were twice as high in brain than in liver in vitamin $B_{12}$-deficient rats (Table 8).

### *Synthesis of fatty acid and cholesterol*

The incorporation of $^3H_2O$ into fatty acid and cholesterol was generally higher in brain than in liver (Table 9), in contrast to previous findings in adult rats (Fehling, Jägerstad, Åkesson *et al.* 1978). The synthesis of fatty acid and cholesterol from $^3H_2O$ was the same in vitamin $B_{12}$-deficient and supplemented rats (Table 9). Alkaline hydrolysis of liver lipids after [$^{14}$C]propionate injection showed that most of the $^{14}$C was in the polar part of lipid molecules. The incorporation of $^{14}$C into fatty acid was too low to permit accurate quantitation. In brain, a larger proportion of lipid $^{14}$C was in fatty acid (Table 9). The incorporation was the same in vitamin $B_{12}$-deficient and supplemented animals.

## DISCUSSION

*Organ and body-weights.* The reduction of brain weight in vitamin $B_{12}$ deficiency was less marked than the decrease in liver weight and body-weight. This has been observed also after general nutritional deprivation (Dobbing & Widdowson, 1965; Benton *et al.* 1966) and essential fatty acid malnutrition (Alling *et al.* 1974*a*, *b*). The value brain weight:body-weight decreases with age in rat (Alling & Karlsson, 1973) and the higher value in vitamin $B_{12}$-deficient animals could be due to the hypothetical fact that these animals were prematurely born. However, in other experiments, where the duration of the gestation was known to be normal, pups of vitamin $B_{12}$-deprived dams were still found to be small

compared with control pups. Thus the rat sucklings of the present experiment could well be the result of full-term gestation. This suggestion is supported by the random order in which the vitamin $B_{12}$-deprived and-supplemented dams delivered their litters.

*Tissue lipid composition.* The most remarkable finding was the accumulation of triacylglycerol in liver in the vitamin $B_{12}$-deficient rats. Vitamin $B_{12}$ might be lipotropic (Laird *et al.* 1965; Tuma *et al.* 1975); therefore the fatty liver could be caused by a reduced secretion of lipoproteins from the liver, as is observed in choline deficiency (Lombardi *et al.* 1969). In contrast, the total lipid concentrations in liver, brain, and spinal cord of 1-year-old vitamin $B_{12}$-deficient rats were normal whether the methionine content of the diet was 11·4 or 6·7 g/kg (Fehling, Jägerstad, Åkesson *et al.* 1978 and unpublished results). Because vitamin $B_{12}$ is lipotropic due to its function in methionine synthesis (Tuma *et al.* 1975), the difference indicates that the need for endogenously-synthesized methionine is higher in younger rats. The activity of the vitamin $B_{12}$-dependent tetrahydropteroylglutamate methyltransferase (*EC* 2·1·1·13) increases in methionine deprivation (Finkelstein *et al.* 1971). As this enzyme activity is highest during weaning, the rate of methionine synthesis probably also has a maximum during this period in normal rats. The role of betaine-homocysteine methyltransferase (*EC* 2.1.1.5) in methionine biosynthesis increases with age (Finkelstein *et al.* 1971). This could represent an alternative mechanism for the disappearance of fatty liver in adult vitamin $B_{12}$-deprived rats. It is also possible that excess dietary methionine will alleviate the fatty liver in vitamin $B_{12}$ deficiency.

A transient and less marked accumulation of liver triacylglycerol occurs in rats fed on an adequate diet, with a maximum at 4 d of age (Alling *et al.* 1976). The fatty liver we observed could to some extent represent a prolongation or an accentuation of the transient fatty metamorphosis in livers of animals fed on a complete diet or both.

The lipid content in brain was slightly depressed in vitamin $B_{12}$-deficient rats but the qualitative composition of phospholipid classes was similar to that of the control group. From a detailed study, Wells & Dittmer (1967) suggested that particularly cerebroside and sphingomyelin concentrations increased rapidly during the period of active myelination. We found no difference in total sphingolipid content in brain between vitamin $B_{12}$-deficient and control rats. The reported decrease in brain phospholipids in vitamin $B_{12}$ deficiency (Turner & Cevallos, 1968) was not confirmed (Table 2).

*Lipid biosynthesis.* The stepwise methylation of ethanolamine phosphoglyceride to choline phosphoglyceride is dependent on methionine availability (Sundler & Åkesson, 1975*a*). It was decreased in vitamin $B_{12}$ deficiency, in accordance with previous results (Åkesson *et al.* 1978). Phospholipid methylation is most active in liver tissue (Björnstad & Bremer, 1966). It cannot be excluded that labelled choline phosphoglyceride found in the brain after the administration of labelled ethanolamine was actually formed in the liver and then transported to the brain by plasma lipoproteins. Ansell & Spanner (1971) proposed that choline in lipid-bound form is transported to the brain. More recent evidence suggested that methylation of ethanolamine phosphoglyceride (Morgenstern & Abdel-Latif, 1974) or even free ethanolamine in brain (Kewitz & Pleul, 1976) is important for its supply of choline as precursor of acetylcholine. Thus the higher value for $100 \times {}^{14}C$ choline phosphoglyceride:$^{14}C$ in choline phosphoglyceride plus ethanolamine phosphoglyceride in brain than in liver of vitamin $B_{12}$-deficient rats (Table 8) can be interpreted in two ways: either the brain is active in ethanolamine phosphoglyceride or ethanolamine methylation, or labelled choline phosphoglyceride formed in the liver is transported to the brain. The latter alternative is supported by the fact that choline phosphoglyceride is released to plasma from liver to a much greater extent than ethanolamine phosphoglyceride (Sundler & Åkesson, 1975*b*). This finding could also explain the distribution of $^{14}C$ between liver and brain 2 and 24 h after [$^{14}C$]ethanolamine injection. The synthesis of choline phosphoglyceride and ethanol-

amine phosphoglyceride *de novo* in brain increases during the first twenty postnatal days (McMurray, 1964). Ethanolaminephosphotransferase activity is higher than in other tissues (Ansell & Metcalfe, 1971) although phosphoethanolamine cytidyltransferase activity might be rather low (Porcelatti & Pirotta, 1970). Most of the labelled ethanolamine phosphoglyceride found in brain was probably formed in situ.

The proportion of choline phosphoglyceride was slightly lower in brain and in liver from vitamin $B_{12}$-deficient rats (Table 3). This agrees with previous findings in liver (Åkesson *et al.* 1978) and is most probably due to the reduced phospholipid methylation. If the phospholipid changes were due to a delayed development, an increase in brain choline phosphoglyceride would instead have been expected in vitamin $B_{12}$ deficiency (Berg Hansen & Clausen, 1969).

The high incorporation of $^3H_2O$ in brain fatty acid and cholesterol indicates a rapid lipid synthesis in brain in these young rats and supports information on fatty acid synthetase activity in brain and in liver during development (Volpe & Kishimoto, 1972). No difference between deficient and supplemented animals was observed. Previous studies on adult vitamin $B_{12}$-deficient rats indicated a slight retardation of fatty acid synthesis and a slight stimulation of cholesterol synthesis (Fehling, Jägerstad, Åkesson *et al.* 1978). Therefore at neither age could the increase in fatty acid synthesis suggested by enzyme activity determinations (Frenkel *et al.* 1973; Frenkel *et al.* 1976) be detected by measurements of fatty acid synthesis in vivo. The level of fatty acid synthetase is subject to regulation in both liver and brain, and the amount of this enzyme is of major importance for the rate of fatty acid synthesis (Volpe & Vagelos, 1973; Volpe & Marasa, 1975). As suggested previously (Fehling, Jägerstad, Åkesson *et al.* 1978), the incongruence between fatty acid synthesis in vivo and fatty acid synthetase activity in vitamin $B_{12}$ deficiency might be due to the inhibitory action of accumulated methylmalonyl-CoA and related metabolites.

Fatty acid synthesis is qualitatively changed as odd-chain fatty acids, like pentadecanoate and heptadecanoate, accumulated in all tissues tested (Fehling, Jägerstad, Åkesson *et al.* 1978). This abnormality was already present in 10–18-d-old rats (Tables 4–6), although it was less pronounced, especially in the brain. As discussed previously (Fehling, Jägerstad, Åkesson *et al.* 1978) the effect of odd-chain fatty acids on myelin structure and function is difficult to assess at present.

The present study has shown changes in the lipid concentration and composition of both brain and liver in vitamin $B_{12}$-deficient 18-d-old rats. Together with the depressed phosphoglyceride choline synthesis from methylation of ethanolamine phosphoglyceride this can be taken as evidence for changes in the composition of various cellular membranes. It is tempting to speculate about the role of these derangements for the composition and function of myelin; a substance rich in phospholipids and known to be particularly sensitive to vitamin $B_{12}$ deprivation.

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