Necessity of vitamin B_{12} for growth of rats fed on an odd- or even-carbon-number fat

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1. The effect of vitamin B_{12} on growth was studied in young male and female rats fed on diets sufficient ($+B_{12}$) or deficient ($-B_{12}$) in vitamin B_{12} containing 30 % of the dietary energy as fat, either maize oil (CO) or triundecanoin (TUD).

2. Vitamin B_{12} deficiency severely depressed growth. After 6 weeks the weight gain of CO($-B_{12}$) rats was only 72 % of that of CO($+B_{12}$) rats and the gain of TUD($-B_{12}$) rats was only 47% of TUD($+B_{12}$) rats.

3. After fasting 24 or 96 h TUD-fed rats, both $+B_{12}$ and $-B_{12}$, had greater glycogen reserves and higher plasma glucose levels than CO-fed rats.

4. It is concluded that vitamin B_{12} is required for the metabolism and utilization of both an odd-carbon-number medium-chain fat, TUD, and an even-C-number long-chain fat, CO, during growth in rats.

The β-oxidation of odd-carbon-number fatty acids yields propionate and acetate residues as the end-products of metabolism, whereas metabolism of even-C-number fatty acids would yield primarily acetate. Several studies have demonstrated that vitamin B_{12} is required in the metabolism of propionate under in vitro conditions (Smith & Monty, 1959; Stadtman, Overath, Eggerer & Lynen, 1960) and in the ruminant (Marston, Allen & Smith, 1961). In an earlier study from this laboratory (Dryden & Hartman, 1971), it was found that feeding C_3, C_5, C_7, and C_9 fatty acids to vitamin B_{12}-deficient rats depressed growth. The present study was designed to compare the growth of rats and their response to fasting when given diets sufficient or deficient in vitamin B_{12}. Experimental diets containing either an even-C-number long-chain fat, maize oil (CO), or an odd-C-number medium-chain fat, triundecanoin (TUD), either containing no additional vitamin B_{12} or supplemented with vitamin B_{12}, were fed to male and female rats for 6 weeks.

EXPERIMENTAL

Sprague–Dawley rats (83–94 g) were placed on one of the four diets at 4 weeks of age. Each group of twenty-one males and twenty-one females was individually housed during the 6-week feeding period. Rats were killed by decapitation immediately or after fasting for 24 or 96 h. Water was provided during the fast.

Two diets contained TUD and two diets contained CO to supply 30 % of the dietary energy. One TUD and one CO diet contained a vitamin B_{12} supplement ($+B_{12}$) and the remaining two diets were fed without additional vitamin B_{12} ($-B_{12}$), providing four diets: CO($-B_{12}$), CO($+B_{12}$), TUD($-B_{12}$), TUD($+B_{12}$). The basal semi-purified diet had the following composition (g/kg): dextrin 521·6, soya-bean protein 277·8, salt mixture 35·0, methionine 4·0, added vitamins 1·6. The vitamin mixture contained (/kg diet): retinol 7·5 mg, ergocalciferol 0·25 mg, D-tocopheryl acetate 100 mg, 2-methyl-1,4-naphthoquinone 2·5 mg, biotin 0·5 mg, folic acid 2·0 mg, thiamine hydrochloride 8·0 mg, riboflavin 16·0 mg, Ca pantothenate 50 mg, *myo*-inositol 50 mg, nicotinic acid 50 mg, choline choloride 1·2 g, butylated hydroxyanisole 30 mg. The CO diets contained 160 g CO/kg, whereas the TUD

diets contained 144 g TUD and 16 g CO. Vitamin B^{12}, when given, was included at 0·05 g cyanocobalamin/kg, at the expense of the vitamin mix.

For the measurement of body fat, the whole carcass (minus liver, perirenal fat, biceps femoris and blood) was frozen untill analysed. After the gastrointestinal tract removal, the carcasses were homogenized in distilled water–ice (1:1, v/v). Dried samples of the carcass homogenates, minced-muscle samples and faeces were extracted for fat using diethyl ether.

Blood, perirenal fat, carcass fat and biceps femoris fat were extracted with chloroform–methanol (2:1, v/v) by the method of Storry & Millard (1965). Methyl esters were formed by the procedure of Christopherson & Glass (1969) in sealed vials. The esters were analysed using programmed (65–180°) gas–liquid chromatography (model 7620; Hewlett-Packard, Palo Alto, Calif.) on 100 g EGSS-X/kg Gas-Chrom P (100–200 mesh; Applied Science Laboratories, State College, Pa.) in a 4 mm i.d. × 1830 mm glass column.

Blood plasma was analysed for glucose by the glucose oxidase method (Worthington Biochemical Corp., 1961). Plasma and liver cholesterol were determined by the procedure of Sobel & Mayer (1945), and plasma triglycerides by the method of Kaplan & Lee (1965). Non-esterified fatty acids (NEFA) were estimated by the method of Annison (1960). Liver and muscle glycogen were analysed by the anthrone procedure of Seifter, Dayton, Novic & Muntwyler (1950).

Statistical comparisons were made by analysis of variance with mean separation by the Student–Newman–Keuls (SNK) test ($P < 0·05$) (Zar, 1974). Statistical evaluation of simple linear regressions was by analysis of covariance (heterogeneity of slopes) with mean separation by the related SNK test.

RESULTS

Weight gains and efficiency of food utilization

Rats fed on $-B_{12}$ diets did not grow as well as rats fed on $+B_{12}$ diets (Fig. 1). Weight gains at 6 weeks of males and females on TUD($+B_{12}$) were 90 % of gains on CO($+B_{12}$). Growth was depressed in rats fed on CO($-B_{12}$), and weight gain was only 72 % of gains on CO($+B_{12}$). Growth was severely depressed in rats fed on TUD($-B_{12}$), and weight gain was only 47 % of gains on TUD($+B_{12}$).

The relationship between weight gain and food intake (Table 1) indicated that with $+B_{12}$ diets, TUD was as efficiently utilized for growth as CO in both males (0·41 *v.* 0·41) and in females (0·27 *v.* 0·28). With $-B_{12}$ diets, the efficiency of food utilization was significantly depressed for males and females fed on TUD. On CO diets, the male rats utilized the even-C-number fat almost as well without as with vitamin B_{12} (0·38 *v.* 0·41), but female rats showed a lower efficiency without vitamin B_{12}.

Fatty acid composition of adipose tissue

The fatty acid composition of carcass fat is shown in Table 2. Fatty acid patterns did not show marked compositional differences between male and female rats or between rats on a $-B_{12}$ diet and those receiving the $+B_{12}$ diet.

Comparison of adipose tissue between TUD-fed and CO-fed rats indicated that TUD increased the content of undecanoate from less than 10 to approximately 300 mmol/mol total fatty acids. The $-B_{12}$-fed rats had a slightly lower $C_{11:0}$ fatty acid content than those fed the vitamin B_{12} supplement. The odd-C-number fatty acids longer than $C_{11:0}$, $C_{13:0}$, $C_{15:0}$ and $C_{17:0}$, also increased so that the proportion of odd-C-number fatty acids was approximately 350 mmol/mol total fatty acids. This enrichment with odd-C-number fatty acids was accompanied by major compensatory decreases in $C_{18:1}$ and $C_{18:2}$. Oleate $C_{18:1}$, decreased

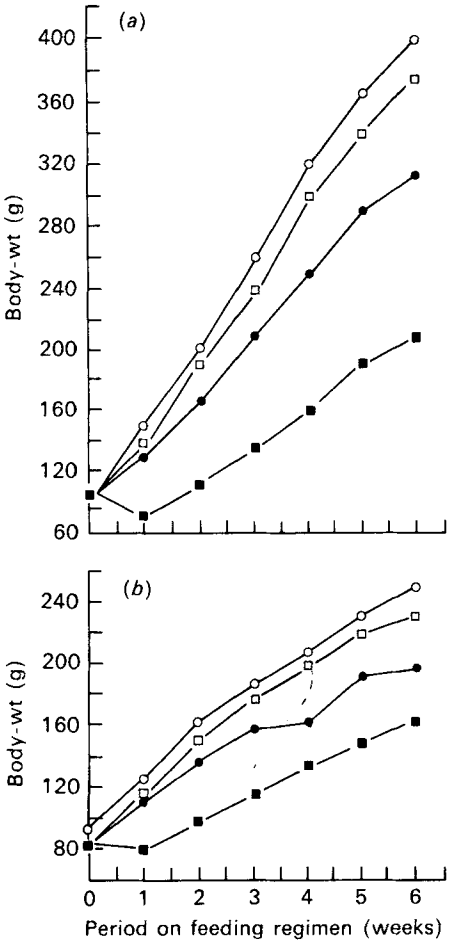

Fig. 1. Body-weights of (*a*) male and (*b*) female rats fed on the following diets: maize oil (CO) without vitamin B_{12} ($-B_{12}$) (●); CO with vitamin B_{12} ($+B_{12}$) (○); triundecanoin (TUD)($-B_{12}$) (■); TUD($+B_{12}$) (□). For details of diets, see p. 615. Each point represents mean values from twenty-one rats. Slope values bearing different superscripts were significantly different and were for CO($-B_{12}$), CO($+B_{12}$), TUD($-B_{12}$) and TUD($+B_{12}$) respectively: males, $38{\cdot}0^{d}$, $51{\cdot}8^{c}$, $24{\cdot}5^{e}$, $48{\cdot}5^{c}$; females, $17{\cdot}2^{d}$, $24{\cdot}0^{c}$,$16{\cdot}4^{d}$, $22{\cdot}8^{d}$.

from 320 to 200 mmol/mol total fatty acids, and linoleate, $C_{18:2}$, decreased from 350 to 130 mmol/mol total fatty acids. There were no marked changes in the saturated even-C-number fatty acids, stearate,$C_{18:0}$, or in palmitate, $C_{16:0}$.

There were no major differences in the dry matter (DM) or fat content between CO-fed and TUD-fed rats (Table 2). On both diets, the amounts of fat were relatively similar for males and females but were affected by the presence or absence of vitamin B_{12}. Rats fed on the $-B_{12}$ diets had significantly higher water contents and lower lipid contents than rats fed on the $+B_{12}$ diets.

Fatty acid composition of faeces

The fatty acid pattern of the faeces reflected the type of fat fed. Thus C_{18} acids comprised approximately 650 mmol/mol total faecal fatty acids in CO-fed rats and approximately 360 mmol/mol total faecal fatty acids were undecanoic in TUD-fed rats. Odd-C-number

Table 1. *Effect of vitamin B_{12} (B_{12}) on growth and efficiency of food utilization of rats fed on maize oil (CO) or triundecanoin (TUD)**

(Mean values for twenty-one rats/group)

Dietary treatment ...	CO(−B_{12})	CO(+B_{12})	TUD(−B_{12})	TUD(+B_{12})
♂				
Wt gain in 6 weeks (g)	222	305	117	279
Daily wt gain (g)	5·3	7·3	2·8	6·6
Food intake (g/d)	13·8	17·9	8·9	16·4
Efficiency†	0·38[a]	0·41[b]	0·31[c]	0·41[b]
♀				
Wt gain in 6 weeks (g)	112	160	75	144
Daily wt gain (g)	2·7	3·8	1·8	3·4
Food intake (g/d)	11·4	13·7	8·0	12·7
Efficiency†	0·23[a]	0·28[b]	0·20[a]	0·27[b]

[a, b, c] Values with different superscripts were significantly different ($P < 0·05$, Student–Newman–Keuls multiple comparison test (Zar, 1974)) in animals of the same sex.

* For details of diets, see p. 615.

† Efficiency of food utilization = g wt gain/g food consumed.

saturated fatty acids longer than $C_{11:0}$, primarily $C_{15:0}$ and $C_{17:0}$, were almost two times greater in faeces from TUD-fed as compared to CO-fed rats. The presence or absence of vitamin B_{12} and sex of the rats did not affect the fatty acid composition of the faeces. However, $-B_{12}$ rats excreted considerably less fat in their faeces (260 g/kg DM) than $+B_{12}$ rats (380 g/kg DM).

Effect of TUD and vitamin B_{12} on organ weights and vaginal patency

In males fed on TUD, all organs were larger on a per unit body-weight basis without than with vitamin B_{12} (Table 3). In females, the uteri were heavier with than without vitamin B_{12} but differences were not significant; liver and kidneys, however, were larger without vitamin B_{12}. There did not appear to be significant differences in organ weights between the rats fed on CO and those given TUD.

Without vitamin B_{12} vaginal opening was delayed in female rats (Table 3). With vitamin B_{12} vaginas opened approximately 8 d after being placed on the diet, i.e. when the rats were 36 d old. In rats fed on a CO($-B_{12}$) diet, vaginas opened slightly later, when the rats were 40 d old. In contrast, TUD($-B_{12}$)-fed females had a mean vaginal opening time of 57+ d. This mean was confounded by the fact that four of the twenty-one vaginas were not open at the end of the experiment and were included in the results as 6-week values.

Effect of starvation on rats fed on CO or TUD

Body-weight losses during the 4 d fast did not differ consistently although almost all groups lost 10–25 % of their body-weight (Table 4). All organs examined except liver (testes, uterus, adrenals and kidneys) maintained their weight during the fast (values not shown). The decreases in liver weight were marked and, relatively, much greater than those in body-weight. Liver weight decreased 25–30 % during the first 24 h of the fast and an additional 10–20 % during the next 72 h of the fast. On a body-weight basis almost all the high relative loss in liver weight occurred during the first 24 h; thereafter, liver weight decreased in direct proportion to body-weight.

Liver glycogen levels decreased greatly during the first 24 h of fasting, to only 5–31 % of pre-fast levels (Fig. 2). While it appeared that liver glycogen increased between 24 and

Table 2. *Carcass composition of rats fed on maize oil (CO) or triundecanoin (TUD), with (+B_{12}) or without (−B_{12}) vitamin B_{12}**

(Mean values for twenty-one rats/group)

Dietary treatment ...	Sex	CO(−B_{12})	CO(+B_{12})	TUD(−B_{12})	TUD(+B_{12})
Water (g/kg body-wt)	♂	638^a	596^b	657^d	604^b
	♀	637^a	544^b	660^d	587^b
Fat (g/kg dry matter)	♂	264^a	368^d	244^a	367^d
	♀	272^a	416^d	242^a	373^d
Fatty acid (mmol/mol)					
11:0	♂	3^b	5^b	274^a	326^d
	♀	3^b	4^b	240^a	272^d
12:0	♂	3^a	3^a	8^d	10^d
	♀	4^a	4^a	8^d	10^d
13:0	♂	—	—	12^d	14^d
	♀	—	—	12^d	13^d
14:0	♂	12^a	14^a	15^a	16^a
	♀	13^a	14^a	13^a	17^a
15:0	♂	5^b	3^b	18^d	13^a
	♀	3^b	2^b	18^d	14^a
16:0	♂	190^a	209^d	198^d	209^d
	♀	207^d	217^d	194^d	210^d
16:1	♂	26^a	30^a	28^a	38^d
	♀	37^{ab}	44^d	32^b	41^{ad}
17:0	♂	5^b	4^b	16^d	9^a
	♀	5^b	2^b	18^d	10^a
18:0	♂	61^{ad}	49^{ab}	70^d	44^b
	♀	68^d	53^a	72^d	50^a
18:1	♂	316^d	328^d	188^a	183^a
	♀	317^d	323^d	220^a	215^a
18:2	♂	361^b	342^b	146^a	113^a
	♀	324^b	317^b	143^a	124^a
18:3	♂	4^b	4^b	7^a	2^a
	♀	5^b	5^b	6^a	3^a
Others	♂	6^b	6^b	7^b	2^b
	♀	4^b	6^b	3^b	3^b

$^{a, b, c, d}$ Values with different superscripts in horizontal columns were significantly different among different dietary groups ($P < 0{\cdot}05$).

* For details of diets, see p. 615.

96 h of fasting (Fig. 2), expressing liver glycogen levels in absolute terms (Table 4), taking into account the fact that liver weight was decreasing, revealed that there was no repletion of glycogen during the fast. In both males and females, rats fed on TUD(+B_{12}), had a significantly higher liver glycogen content than the other groups after the 96 h fast. Muscle glycogen concentrations decreased approximately 35 % in all groups during the first 24 h of fasting (Table 4) but changed little during the next 72 h. Concentrations of cholesterol and total lipids in the liver increased, but in relation to the decreasing liver weight the total amounts of these constituents remained constant during the fast.

The plasma constituents, cholesterol, NEFA, glucose, and triglycerides, generally were similar among treatments and differed little between rats fed on CO and TUD (Table 5). Levels appeared to be slightly higher in males than in females. Glucose decreased 30–40 % in all dietary groups during starvation. The decrease was slightly less in TUD-fed rats than

Table 3. *Organ weights of rats fed on maize oil (CO) or triundecanoin (TUD) with ($+B_{12}$) or without ($-B_{12}$) vitamin B_{12} for 6 weeks**

(Mean values for seven rats/group for organ wt and for twenty-one rats/group for vaginal patency)

	♂				♀			
Dietary treatment ...	CO($-B_{12}$)	CO($+B_{12}$)	TUD($-B_{12}$)	TUD($+B_{12}$)	CO($-B_{12}$)	CO($+B_{12}$)	TUD($-B_{12}$)†	TUD($+B_{12}$)
Mean body-wt (g)	303	404	184	370	191	241	163	216
Organ wt (g/kg body-wt)								
Testes	$10{\cdot}0^{a}$	$8{\cdot}5^{a}$	$13{\cdot}4^{b}$	$9{\cdot}5^{a}$	—	—	—	—
Uterus	—	—	—	—	$1{\cdot}31^{a}$	$1{\cdot}58^{a}$	$1{\cdot}10^{a}$	$1{\cdot}48^{a}$
Liver	$39{\cdot}3^{a}$	$37{\cdot}8^{a}$	$44{\cdot}1^{b}$	$39{\cdot}3^{a}$	$39{\cdot}4^{a}$	$34{\cdot}4^{b}$	$43{\cdot}9^{c}$	$38{\cdot}9^{a}$
Kidneys	$9{\cdot}3^{a}$	$7{\cdot}7^{a}$	$10{\cdot}8^{b}$	$8{\cdot}0^{a}$	$9{\cdot}2^{ab}$	$7{\cdot}7^{b}$	$10{\cdot}7^{a}$	$7{\cdot}7^{b}$
Adrenals	$0{\cdot}15^{a}$	$0{\cdot}13^{a}$	$0{\cdot}19^{b}$	$0{\cdot}14^{a}$	$0{\cdot}27^{a}$	$0{\cdot}26^{a}$	$0{\cdot}28^{a}$	$0{\cdot}29^{a}$
Vaginal patency (weeks)‡	—	—	—	—	$1{\cdot}76^{a}$	$1{\cdot}14^{a}$	$4{\cdot}15^{b}$	$1{\cdot}14^{a}$

$^{a, b, c, d}$ Values with different superscripts were significantly different ($P < 0{\cdot}05$) in animals of the same sex.

* For details of diets, see p. 615.

† Four vaginas not open at end of experiment and averaged with 6-week values in calculation of vaginal patency time.

‡ Week by end of which vaginas opened.

Table 4. *Effect of starvation on body-weight, liver constituents, and muscle glycogen in rats fed on maize oil (CO) or triundecanoin (TUD) with (+B_{12}) or without (−B_{12}) vitamin B_{12} for 6 weeks**

		♂				♀			
Dietary treatment ...		CO(−B_{12})	CO(+B_{12})	TUD(−B_{12})	TUD(+B_{12})	CO(−B_{12})	CO(+B_{12})	TUD(−B_{12})	TUD(+B_{12})
Constituent	Duration of fast (h)								
Body-wt (g)	0	303^{bd}	404^{ad}	184^{cd}	370^{ad}	191^{bcd}	241^{ad}	163^{cd}	216^{abd}
	24	312^{bd}	372^{ad}	203^{cd}	371^{ad}	194^{bd}	230^{ad}	153^{cd}	236^{ad}
	96	267^{bd}	347^{ad}	186^{cd}	305^{abe}	158^{be}	215^{ad}	119^{cd}	194^{ad}
Liver wt (g)	0	$11{\cdot}8^{bd}$	$15{\cdot}3^{ad}$	$8{\cdot}1^{cd}$	$14{\cdot}2^{ad}$	$7{\cdot}6^{ad}$	$8{\cdot}3^{ad}$	$7{\cdot}1^{ad}$	$8{\cdot}4^{ad}$
	24	$8{\cdot}4^{be}$	$10{\cdot}6^{ae}$	$7{\cdot}4^{cd}$	$11{\cdot}5^{ae}$	$5{\cdot}6^{be}$	$6{\cdot}1^{abe}$	$5{\cdot}1^{bd}$	$6{\cdot}8^{ae}$
	96	$6{\cdot}7^{bf}$	$8{\cdot}3^{af}$	$5{\cdot}3^{cd}$	$8{\cdot}0^{af}$	$4{\cdot}6^{bce}$	$5{\cdot}6^{ae}$	$3{\cdot}9^{cd}$	$5{\cdot}4^{af}$
Liver wt (g/kg body-wt)	0	39^{ad}	38^{ad}	44^{bd}	38^{ad}	40^{ad}	34^{bd}	44^{cd}	39^{ad}
	24	27^{ce}	28^{ce}	36^{ae}	31^{bce}	29^{be}	26^{be}	33^{ae}	29^{be}
	96	25^{cf}	24^{af}	28^{af}	26^{af}	29^{be}	26^{be}	33^{ae}	28^{be}
Liver glycogen (mg/liver)	0	719^{ad}	892^{ad}	403^{ad}	703^{ad}	384^{ad}	383^{ad}	318^{ad}	379^{ad}
	24	50^{ce}	76^{bce}	121^{ae}	132^{abe}	44^{abe}	15^{be}	65^{ae}	88^{ae}
	96	73^{be}	72^{be}	105^{be}	203^{af}	36^{bce}	25^{ce}	46^{be}	97^{ae}
Liver cholesterol (g/kg)	0	$4{\cdot}37^{ad}$	$4{\cdot}84^{ad}$	$3{\cdot}26^{bd}$	$3{\cdot}40^{bd}$	$4{\cdot}31^{bd}$	$4{\cdot}96^{ad}$	$3{\cdot}68^{bd}$	$4{\cdot}22^{bd}$
	24	$6{\cdot}31^{bd}$	$8{\cdot}31^{ae}$	$4{\cdot}12^{ce}$	$4{\cdot}60^{ce}$	$5{\cdot}87^{abe}$	$6{\cdot}55^{ae}$	$4{\cdot}39^{ce}$	$5{\cdot}20^{be}$
	96	$10{\cdot}66^{ae}$	$8{\cdot}48^{be}$	$4{\cdot}89^{cf}$	$6{\cdot}02^{cf}$	$6{\cdot}96^{abf}$	$7{\cdot}42^{ae}$	$5{\cdot}31^{cf}$	$5{\cdot}93^{bce}$
Liver lipids (g/kg)	0	61^{ad}	62^{ad}	60^{ad}	62^{ad}	54^{bd}	64^{bd}	66^{bd}	79^{ad}
	24	69^{bd}	87^{ae}	66^{bd}	69^{bd}	67^{ae}	74^{ad}	67^{ad}	82^{ad}
	96	89^{ad}	89^{ae}	61^{ad}	75^{ad}	68^{be}	116^{ae}	61^{bd}	82^{bd}
Muscle glycogen (g/kg)	0	$4{\cdot}6^{ad}$	$4{\cdot}5^{ad}$	$4{\cdot}3^{ad}$	$4{\cdot}1^{ad}$	$3{\cdot}9^{ad}$	$3{\cdot}9^{ad}$	$3{\cdot}7^{ad}$	$4{\cdot}0^{ad}$
	24	$3{\cdot}2^{ae}$	$3{\cdot}2^{ae}$	$3{\cdot}7^{ad}$	$2{\cdot}9^{ae}$	$3{\cdot}1^{ae}$	$2{\cdot}7^{ae}$	$2{\cdot}4^{ae}$	$1{\cdot}9^{ae}$
	96	$3{\cdot}4^{ae}$	$2{\cdot}8^{abe}$	$1{\cdot}9^{be}$	$3{\cdot}0^{abe}$	$2{\cdot}5^{ae}$	$2{\cdot}6^{ae}$	$2{\cdot}1^{ae}$	$2{\cdot}5^{ae}$

[a, b, c] Values with different superscripts in horizontal columns were significantly different ($P < 0{\cdot}05$) among different dietary groups; [d, e, f] values with different superscripts in vertical columns were significantly different ($P < 0{\cdot}05$) at different time periods within a group.

* For details of diets, see p. 615.

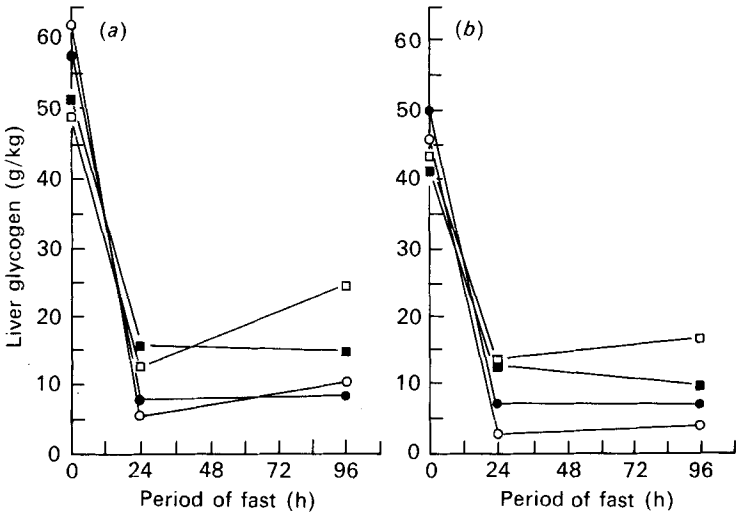

Fig. 2. Effects of starvation on liver glycogen of (*a*) male and (*b*) female rats fed on the following diets: Maize oil (CO) without vitamin B_{12} ($-B_{12}$) (●); CO with vitamin B_{12} ($+B_{12}$) (○); triundecanoin (TUD)($-B_{12}$) (■); TUD($+B_{12}$) (□). For details of diets, see p. 615. Each point represents mean values of seven rats.

in CO-fed rats and was least in TUD($-B_{12}$)-fed female rats. Plasma triglycerides decreased severely during the fast but NEFA remained almost constant.

In the plasma fatty acids (values not shown), the initial level of $C_{11:0}$ in rats fed on TUD was higher in males (100 mmol/mol total plasma fatty acids) than in females (40 mmol/mol total plasma fatty acids), although the proportion of undecanoic acid contributing to the total fatty acids of the carcass lipids (Table 2) was very considerable in both males and females. We have no explanation for this difference. Levels of plasma $C_{11:0}$ decreased sharply during the first 24 h of fasting and decreased to less than 10 mmol/mol total plasma fatty acids at 48 h. Linoleic acid ($C_{18:0}$), the only other plasma fatty acid which exhibited large changes, increased during the fasting and was higher in females than in males. The presence or absence of vitamin B_{12} generally did not affect changes in these two fatty acids during the fast. Plasma fatty acid composition of the CO-fed rats changed little during fasting.

DISCUSSION

Our results demonstrate that vitamin B_{12} is required for normal growth in rats given diets containing large amounts of fats. Growth of both male and female rats was severely depressed when rats were fed on $-B_{12}$ diets containing either an even-C-number long-chain fat, CO (20 % decrease) or an odd-C-number medium-chain fat, TUD (40 %). Rats fed on the TUD ($-B_{12}$) diet ate much less, gained much less, and utilized their food less efficiently than rats fed on the TUD($+B_{12}$) diet. These findings with a C_{11} triglyceride extend previous work from this laboratory in which growth depressions of similar magnitude were found when short-chain fatty acids (C_3, C_5, C_7 and C_9) were fed with a vitamin B_{12}-deficient diet (Dryden & Hartman, 1971). We also found that growth was depressed in rats fed on the even-C-number fat (CO) without vitamin B_{12}, in agreement with Dupont & Mathias (1969), who found that rats given 200 g CO/kg grew more slowly on vitamin B_{12}-deficient diets. They demonstrated directly that excretion of methylmalonate in the urine was greater for $-B_{12}$ than for $+B_{12}$ rats; apparently C_3 units were generated and further metabolized via

Table 5. *Effect of starvation on plasma constituents of rats fed on maize oil (CO) or triundecanoin (TUD) with ($+B_{12}$) or without ($-B_{12}$) vitamin B_{12} for 6 weeks**

(Mean values for seven rats/group)

		♂				♀			
Dietary treatment ...		CO($-B_{12}$)	CO($+B_{12}$)	TUD($-B12$)	TUD($+B_{12}$)	CO($-B_{12}$)	CO($+B_{12}$)	TUD($-B_{12}$)	TUD($+B_{12}$)
Constituent	Duration of fast (h)								
Glucose (mmol/l)	0	$7{\cdot}0^{ac}$	$6{\cdot}3^{ac}$	$6{\cdot}6^{ac}$	$6{\cdot}3^{ac}$	$4{\cdot}7^{bc}$	$5{\cdot}4^{abc}$	$4{\cdot}6^{bc}$	$6{\cdot}3^{ac}$
	24	$5{\cdot}1^{ad}$	$4{\cdot}6^{ad}$	$4{\cdot}4^{ad}$	$4{\cdot}9^{ad}$	$3{\cdot}2^{bd}$	$3{\cdot}8^{bd}$	$3{\cdot}7^{bc}$	$5{\cdot}5^{ac}$
	96	$4{\cdot}3^{ae}$	$3{\cdot}6^{be}$	$4{\cdot}6^{ad}$	$4{\cdot}7^{ad}$	$2{\cdot}7^{bd}$	$2{\cdot}7^{be}$	$4{\cdot}2^{ac}$	$4{\cdot}1^{ad}$
Cholesterol (mmol/l)	0	$2{\cdot}5^{ac}$	$2{\cdot}3^{ac}$	$2{\cdot}5^{ac}$	$2{\cdot}5^{ac}$	$2{\cdot}1^{ac}$	$2{\cdot}6^{ac}$	$3{\cdot}2^{ac}$	$2{\cdot}7^{ac}$
	24	$2{\cdot}0^{bcd}$	$1{\cdot}9^{bc}$	$2{\cdot}7^{ac}$	$2{\cdot}3^{abc}$	$2{\cdot}4^{ac}$	$2{\cdot}3^{ac}$	$3{\cdot}2^{ac}$	$2{\cdot}6^{ac}$
	96	$1{\cdot}4^{ad}$	$1{\cdot}3^{ad}$	$2{\cdot}0^{ac}$	$1{\cdot}5^{ad}$	$1{\cdot}4^{ac}$	$1{\cdot}6^{ac}$	$2{\cdot}0^{ad}$	$1{\cdot}7^{ad}$
NEFA (mg/l)	0	200^{bc}	430^{ac}	170^{bc}	290^{bc}	160^{bc}	170^{bc}	270^{ac}	140^{bc}
	24	280^{bc}	470^{ac}	220^{bc}	270^{bc}	240^{bd}	280^{bd}	370^{ac}	210^{bd}
	96	310^{abc}	380^{ac}	160^{bc}	230^{bc}	200^{acd}	320^{ad}	260^{ac}	200^{ad}
Triglycerides (mg/l)	0	720^{ac}	800^{ac}	610^{ac}	580^{ac}	470^{ac}	400^{ac}	450^{ac}	530^{ac}
	24	230^{ad}	280^{ad}	240^{ad}	310^{ad}	240^{bc}	290^{bc}	440^{ac}	320^{ad}
	96	210^{ad}	190^{ad}	170^{ad}	280^{ad}	150^{bc}	300^{ac}	190^{abd}	240^{abd}

[a, b] Values with different superscripts in horizontal columns were significantly different ($P < 0{\cdot}05$) in different dietary groups; [c, d, e] values with different superscripts in vertical columns were significantly different ($P < 0{\cdot}05$) at different time periods within a group.

* For details of diets, see p. 615.

methylmalonate. Their experiments suggested that unsaturated even-C-number fatty acids are also partially oxidized by the pathways for propionate catabolism.

TUD has been fed to growing animals in previous studies in vitamin B_{12}-adequate diets. Visscher (1946) reported that rats fed on TUD for 6 weeks gained only 75 % as much as rats fed on coconut oil. Mohrhauer & Holman (1967) found that rats fed on trivalerin and triundecanoin did not grow optimally and gained less weight than animals fed on triglycerides of even-C-number fatty acids. Van Itallie & Khachadurian (1969) also found that TUD-fed rats gained only 80 % as much as rats fed CO on diets presumably adequate in vitamin B_{12}. In contrast to these findings in rats, where feeding on TUD uniformly depressed growth, Pi-Sunyer (1976) recently reported that rats fed on TUD–CO (7:3, w/w) grew as well as rats fed on CO alone in a nutritionally-complete diet. Campbell & Hashim (1969) also found that TUD-fed dogs grew as well as animals fed on cottonseed oil, an even-C-number fat. Our results suggest that limitations in available vitamin B_{12} might be responsible for limited growth when odd-C-number fats are fed.

Vaginal patency often has been taken as an indication of the onset of sexual maturity in the rat. There was a severe delay in attaining sexual maturity, as measured by vaginal patency, in the rats fed on TUD($-B_{12}$). Results were similar when rats were fed $-B_{12}$ diets containing an even-C-number fat, 100 g cottonseed oil/kg (Dryden, Hartman & Cary, 1954).

In our experiments, rats fed on TUD rapidly incorporated C_{11} fatty acids into adipose tissue to approximately 300 mmol/mol total fatty acids; enrichment was slightly higher in those rats fed on $+B_{12}$ diets. Our levels of enrichment agree, in general, with levels published for rats (Visscher, 1946; Van Itallie & Khachadurian, 1969; Pi-Sunyer, 1976) and dogs (Campbell & Hashim, 1969) fed on TUD. The enrichment of adipose tissue in the rat with $C_{11:0}$ was associated with lower amounts of oleate and linoleate. In dogs, Campbell & Hashim (1969) found that oleate and palmitate decreased during the incorporation of undecanoate into tissue lipid. When triundecanoin was fed to lactating cows, C_{11} was rapidly transferred into milk fat but in only small amounts (Dryden, Bitman, Wrenn, Weyant, Miller & Edmondson, 1974). Concomitant decreases were observed in the even-C-number $C_{8:0-16:0}$ fatty acids of milk.

Chain elongation of C_{11} occurred in the fatty acid of the rats fed on TUD and $C_{13:0}$, $C_{15:0}$ and $C_{17:0}$ fatty acids were found in the adipose tissue. The presence of these odd-C-number saturated fatty acids longer than C_{11} indicated synthesis from the terminal propionate unit of undecanoate. High odd-C-number fatty acids in adipose tissue were observed in C_{11} feeding experiments with rats (Van Itallie & Khachadurian, 1969; Pi-Sunyer, 1976) and dogs (Campbell & Hashim, 1969). Chain elongation was also demonstrated in the lactating cow; yields of odd-C-number long-chain fatty acids increased in milk lipids after intravenous infusion of odd-C-number triglycerides (Storry, Tuckley & Hall, 1969). The isolated bovine mammary gland also elongated odd-C-number fatty acids when perfused with propionic acid (James, Peeters & Lauryssens, 1956). When triundecanoin was infused directly into the bovine abomasum, thereby physically avoiding the rumen, or when encapsulated TUD was fed to lactating cows, thereby chemically by-passing the rumen, amounts of C_{11} were much larger than normal in milk fat (Dryden *et al.* 1974). Associated with this increase in $C_{11:0}$ were significant increases in $C_{13:0}$ and $C_{15:0}$, suggesting that chain elongation occurred in the digestive tract distal to the rumen (Dryden *et al.* 1974).

Large amounts of odd-C-number fatty acids longer than $C_{11:0}$ were present in the faecal fatty acids of our rats, suggesting microbial elongation in the digestive tract. Chain elongation has also been demonstrated in the protozoa, *Ochromonas danica*, when it was incubated with odd-C-number fatty acids (Gellerman & Schlenk, 1972). Another microbial transformation in the rumen and intestinal tract, biohydrogenation of unsaturated fatty

acids, has been well-documented (Dawson & Kemp, 1970). The faecal fatty acid composition of the rats fed on CO (550 mmol $C_{18:2}$/mol fatty acid) demonstrated that hydrogenation was occurring, and most of the dietary linoleate was saturated to $C_{18:1}$ and $C_{18:0}$. The observation that the fat content of faeces was lower for TUD- than CO-fed rats might be attributed to the chain length of the fat rather than to the odd or even-C-number type of the fatty acids. With humans, Cerda (1968) found that excretion of fatty acids in stools decreased when diets were changed from long- to medium-chain triglycerides. In faeces, fat comprised 230 g/kg DM in TUD-fed rats and 300 g/kg DM in CO-fed rats which probably indicated that absorption of the medium-chain C_{11} triglycerides was greater for TUD than for CO (Senior, 1968).

Starvation is a metabolic stress in which liver and muscle glycogen are utilized to provide available carbohydrate, then body fat is mobilized to provide additional energy. In our experiments, TUD- and CO-fed rats were compared to determine whether the propionate residues arising from odd-C-number fatty acid metabolism would limit the depletion of liver glycogen and blood glucose during starvation. Also the influence of vitamin B_{12} on these rats was assessed. Depletion of liver glycogen was less in TUD-fed than in CO-fed rats; '96 h fasting' levels as compared to 'initial' levels were 26, 29, 14 and 36 % in TUD-fed rats and only 10, 8, 9 and 7 % in CO-fed rats. This finding confirmed the results of Van Itallie & Khachadurian (1969). During fasting, our $+B_{12}$-fed rats maintained a higher liver glycogen level than $-B_{12}$-fed rats.

Several possibilities could explain the greater ability of TUD, in comparison to CO, to supply energy during starvation: (*a*) the generation of propionate residues by β-oxidation of the odd-C-number fat probably is one explanation for the enhanced resistance of TUD-fed rats to starvation (Van Itallie & Khachadurian, 1969); (*b*) the superiority of TUD over CO, however, might reside in its shorter chain length and much greater rate of metabolism. Scheig (1968) found that medium-chain fatty acids were much more readily oxidized than long-chain fatty acids. Thus, the mobilization and metabolism of these C_{11} stores might provide energy at a faster rate than would be possible from CO. The plasma C_{11} fatty acid content of TUD-fed rats decreased very rapidly during starvation, in contrast to a lack of change in plasma fatty acids in CO-fed rats. Campbell & Hashim (1969) also reported that, after cessation of TUD feeding in dogs, C_{11} disappeared from adipose tissue considerably faster than linoleate; (*c*) it also appears that medium-chain fatty acids such as undecanoate, in distinct contrast to the behaviour of long-chain free fatty acids, enter adipose cells directly (Hashim, 1968). After digestion and absorption, medium-chain fatty acids are rapidly transported into portal vein blood instead of entering the thoracic duct lymph (Senior, 1968). The liver also appears to be extraordinarily effective in removing and metabolizing the medium-chain fatty acids from the portal venous blood.

Although the higher glycogen levels in our TUD-fed rats indicated that metabolism of odd-C-number fatty acids spared liver glycogen during fasting, the present experiments did not permit us to distinguish between the glucose-sparing effect arising from the more rapidly catabolized medium-chain fatty acids (*b*) and the glycogenic effect arising from the generation of propionyl residues (*a*). Our experiments have shown, however, a clear role for vitamin B_{12} in the mobilization, utilization and incorporation of lipid into body tissue. Thus growth was severely depressed when rats were fed on an odd-C-number fat in the absence of adequate vitamin B_{12}, and moderately depressed when fed on an even-C-number fat in the absence of adequate vitamin B_{12}. Rats fed on either an even- or an odd-C-number fat had a greater body lipid content when given supplemental vitamin B_{12}. In addition, liver glycogen was maintained at higher levels in TUD-fed rats receiving adequate vitamin B_{12} when subjected to a 4 d fast.

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