Effect of feeding a carbohydrate-free diet on the growth and metabolism of preruminant kids

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1. Experiments were done using 8-d-old kids to determine the metabolic effect of feeding a carbohydrate-free diet, and the effects of supplementation of this diet with a small amount of glycerol, sodium propionate or glucose.

2. The experimental (carbohydrate-deficient) diets permitted growth nearly equal to that with the control diet (cow's milk). The kids given the experimental diets generally had lower levels of blood glucose than those given the control diet.

3. With all experimental diets there were increases in the concentrations of plasma lipid and total liver lipid and a decrease in the concentration of liver glycogen; supplementation of the carbohydrate-free diet with glycerol, sodium propionate or glucose had no additional effect on these values.

4. The ingestion of cow's milk produced hyperglycaemia 2 h after feeding, while in kids given the carbohydrate-free diet there was no increase in blood glucose level. The concentration of plasma free fatty acids in the kids given the carbohydrate-free diet was higher than that in control animals 24 h after feeding, suggesting that the kids given the experimental diet preferentially utilize free fatty acids as an energy source.

It is well known that ruminants utilize volatile fatty acids as their main energy sources. In simple-stomached animals the main product of carbohydrate digestion is glucose. In ruminants, the greater proportion of dietary carbohydrate is degraded to volatile fatty acids in the rumen. Although the amount of dietary carbohydrate escaping rumen digestion depends on various factors, such as the extent of lignification, and starch content of the food, it is apparent that glucose requirement is not met fully by glucose absorbed from the intestine.

In ruminants, the liver is important in gluconeogenesis and glucose is synthesized from propionate, some amino acids, lactate and glycerol.

In preruminant animals, a sufficient amount of lactose is supplied by milk. Lactose is digested into glucose and galactose, which are absorbed from the small intestine. Therefore, the importance of gluconeogenesis is thought to be much lower in preruminant animals than in ruminant animals.

Several workers have studied the metabolic effect of feeding carbohydrate-free diets to chicks (Renner, 1964; Renner & Elcombe, 1967). They found that neutral fat could isoenergetically replace dietary carbohydrate without affecting growth and blood glucose level of chicks. Feeding a carbohydrate-free diet reduced weight gain of young rats, but it had no marked effect on blood glucose level, in either the fed or the fasted state (Goldberg, 1971). Replacing dietary carbohydrate by soya-bean-oil fatty acids markedly reduced both the growth and blood glucose level of chicks (Brambila & Hill, 1966). However, this could be partially overcome by a low level of glucose supplementation.

Table 1. *Composition (g/kg) of the experimental, carbohydrate-deficient diets fed to kids*

	Expt 1			Expt 3			
Diet ...	Bu	Gly	Prop	Bu	BuGl	Gly	GlyGl
Ingredients							
Sodium caseinate	550	500	530	550	500	520	470
Unsalted butterfat	368	298	318	368	338	298	268
Glycerol	–	120	–	–	–	100	100
Sodium propionate	–	–	70	–	–	–	–
Glucose	–	–	–	–	80	–	80
Lecithin	10	10	10	10	10	10	10
Mineral mixture*	64	64	64	64	64	64	64
Micro-mineral mixture†	3	3	3	3	3	3	3
Vitamin mixture‡	4	4	4	4	4	4	4
Antibiotic§	1	1	1	1	1	1	1
Gross energy (MJ/kg)	24·0	22·8	23·1	24·0	23·2	22·9	22·1

* Contained (g/kg diet): $CaHPO_4.2H_2O$ 34·7, KCl 19·4, $KHCO_3$ 3·3, K_2SO_4 3·3, $MgCl_2.6H_2O$ 3·3.
† Contained (mg/kg diet): ZnO 61·8, $FeSO_4.7H_2O$ 126·0, $MnSO_4.5H_2O$ 109·8, $CuSO_4.5H_2O$ 39·0, KI 0·7, $CoSO_4.7H_2O$ 1·2, $Na_2MoO_4.2H_2O$ 0·6.
‡ Contained (mg/kg diet): retinoic acid 3·44, ergocalciferol 0·025, thiamin 4, riboflavin 6, pteroylmonoglutamic acid 0·6, nicotinamide 40, pyridoxine 6, pantothenic acid 20, cyanocobalamin 0·004, ascorbic acid 150, DL-α-tocopherol 4, biotin 0·08.
§ Providing 55 mg chlortetracycline (Lederle Japan Ltd, Nihonbashi, Tokyo, Japan)/kg diet.

There is a lack of information about the metabolic effect of feeding a carbohydrate-free diet to preruminant animals. This study was done, therefore, to determine the effect on the growth and metabolism of preruminant kids of feeding a carbohydrate-free diet, and the effect of the addition of a small amount of glycerol, sodium propionate or glucose to this diet.

EXPERIMENTAL

Diets

The composition of the experimental diets is given in Table 1.

Expt 1. Four diets were used, three experimental purified diets (carbohydrate-deficient diets) and a control diet (cow's milk). The carbohydrate-free diet (diet Bu) contained unsalted butterfat and sodium caseinate as the only energy source. In diet Gly, 120 g glycerol/kg was added to diet Bu as the glucose precursor, replacing 50 g sodium caseinate and 70 g butterfat/kg. In diet Prop, 70 g sodium propionate/kg was added to diet Bu as the glucose precursor, replacing 20 g sodium caseinate and 50 g butterfat/kg. In a previous study, it was found that a purified diet containing (g/kg) 391 lactose, 220 unsalted butterfat and 300 sodium caseinate had the same nutritive value as cow's milk (Kameoka & Tanabe, 1975). The results of our previous study indicated that weight gain of preruminant kids given a high-fat diet (420 g butterfat/kg) was significantly lower than that of kids given a medium-fat diet (220 g butterfat/kg) during the first 3 weeks after birth, although these two groups received the same amount of dietary energy (Tanabe & Kameoka, unpublished results). This suggests that preruminant kids are not able to adapt well to a high-fat diet during the

Table 2. *Expts* 1 *and* 3. *Amount of cow's milk* (*control diet*) (*ml*) *or milk-substitute* (*experimental diets*) (*g*)* *offered daily to each kid*

	Diet†	Experimental period			
		Week 1	Week 2	Week 3	Week 4
Expt 1	Control	500	600	700	—
	Experimental: Bu	60·0	72·0	84·0	—
	Gly	62·5	75·0	87·5	—
	Prop	61·5	73·8	86·1	—
Expt 3	Control	500	600	700	800
	Experimental: Bu	60·0	72·0	84·0	96·0
	BuGl	62·5	75·0	87·5	100·0
	Gly	63·0	75·6	88·2	100·8
	GlyGl	65·5	78·6	91·7	104·8
	Water (ml)‡	500	600	700	800

* Air-dry matter basis.
† For details of diets, see Table 1 and pp. 48–9.
‡ Milk-substitutes were mixed with water in the ratios indicated, and homogenized thoroughly.

first few weeks after birth. In the present study, in order to avoid any detrimental effect of the very high-fat diet, the fat level of the experimental diets was limited to about 370 g/kg, and instead of replacing lactose by only butterfat, lactose was replaced by both butterfat and sodium caseinate. Therefore, the protein levels of the experimental diets were much higher than that of cow's milk.

Expt 2. Two diets were used, a carbohydrate-free diet with the same composition as diet Bu in Expt 1, and a control diet (cow's milk).

Expt 3. Five diets were used, four carbohydrate-deficient diets and a control diet (cow's milk). Diet Bu was a carbohydrate-free diet with the same composition as diet Bu in Expt 1. In diet BuGl, 80 g glucose/kg was added to diet Bu, replacing 50 g sodium caseinate and 30 g butterfat/kg. In diet Gly, 100 g glycerol/kg was added to diet Bu, replacing 30 g sodium caseinate and 70 g butterfat/kg. In diet GlyGl, 80 g glucose/kg was added to diet Gly, replacing 50 g sodium caseinate and 30 g butterfat/kg.

The average values for gross energy (MJ/kg) in cow's milk in Expts 1, 2 and 3 were 2·82, 2·76 and 2·78 respectively. The values for gross energy in the experimental diets are given in Table 1. The energy intake of kids given the experimental diets was similar to that of kids given cow's milk in all three experiments (see Table 2). This amount of food energy was expected to be sufficient for almost maximum growth of kids.

Animals and experimental procedures

Japanese native kids were used in all three experiments. As it had been found (Tanabe & Kameoka, unpublished results) that there was no significant difference in growth rate between male and female kids, both males and females were used. The kids were removed from their dams on the second day after birth. They were given cow's milk (400 ml/d) until the fourth day. Three kids were randomly assigned to

each treatment and they were housed in individual cages. Cow's milk was replaced gradually by the experimental diet until the seventh day.

After the preliminary period, the kids were given experimental diets for 3 weeks in Expt 1 and for 4 weeks in Expts 2 and 3. The amounts of control and experimental diets offered to kids are given in Table 2. The amounts of control diet and milk-substitute (diet Bu) offered to kids in Expt 2 were similar to those used in Expt 3. The experimental diets were mixed with water in the ratios indicated in Table 2, and homogenized well using a Waring blender. The kids were nipple-fed the ration in two equal feeds daily at 09.00 and 16.00 hours, after warming the milk to about 37°.

In Expts 1 and 3, the effect of feeding the carbohydrate-free diet, and the effect of supplementation of the carbohydrate-free diet with a small amount of glycerol, sodium propionate or glucose on growth, and on the concentrations of several blood and liver constituents were studied. Expt 2 was done to determine the effect of fasting for 48 h on the concentrations of some blood metabolites of kids previously given cow's milk or the carbohydrate-free diet.

Blood samples were taken at 09·00 hours (just before the morning feed) from the jugular vein during the second and third week of the experimental period in Expt 1 and during the third and fourth week in Expt 3. In Expt 2, during the fourth week of the experimental period, blood samples were taken just before the morning feed (09.00 hours), and 2, 24, 30 and 48 h after feeding. In Expts 1 and 3, the kids were killed at 09.00 hours (just before the morning feed) on the last day of the experimental period, and liver samples were taken.

Analytical methods

For blood glucose, whole blood and plasma were diluted (0·5 ml sample with 2·0 ml water) and deproteinized by the addition of 1·5 ml each of 0·3 M-barium hydroxide and zinc sulphate solution (50 g/l). The glucose content of blood samples was measured by the glucose oxidase (*EC* 1.1.3.4) method of Bergmeyer & Bernt (1965).

Blood lipid estimations were done on samples taken during the third week of the experimental period in Expt 1 and during the fourth week in Expt 3. Triglycerides in plasma were estimated by the method of Carlson (1963). Plasma cholesterol was estimated by the method of Abell, Levy, Brodie & Kendall (1952). Phospholipids in plasma (0·2 ml sample) were precipitated with a solution of 5 ml trichloroacetic acid (50 g/l) (Kushiro & Fukui, 1967) and the precipitate was digested with 10 M-sulphuric acid. Phosphorus was then estimated by the method of Bartlett (1958). Free fatty acids in plasma were estimated by the method of Duncombe (1963), as modified by Itaya & Ui (1965).

Plasma urea was estimated by the method of Conway (1957).

Liver lipid was extracted with chloroform–methanol (2:1, v/v) and estimated by the method of Folch, Lees & Sloane Stanley (1957). Fractionation of liver lipid was done only with the samples obtained in Expt 3. For liver lipid fractionation, liver lipid extracts were taken to dryness under reduced pressure in a rotary evaporator. The dry residues were dissolved in an appropriate volume of chloroform, and liver tri-

Table 3. *Expts* 1 *and* 3. *Effect of feeding cow's milk* (*control*) *and carbohydrate-deficient* (*milk-substitute*) *diets for* 3 *weeks in Expt* 1 *and for* 4 *weeks in Expt* 3 *on weight gain of kids*

(Mean values with their standard errors for three kids/treatment)

	Diet*	Initial body-wt (kg)		Wt gain (kg)	
		Mean	SE	Mean	SE
Expt 1	Control	1·65	0·37	1·25	0·05
	Experimental: Bu	1·84	0·16	1·17	0·01
	Gly	1·87	0·10	1·13	0·03
	Prop	1·94	0·14	1·20	0·02
Expt 3	Control	1·73	0·28	1·99	0·05
	Experimental: Bu	1·85	0·10	1·86	0·05
	BuGl	1·96	0·26	2·01	0·15
	Gly	1·88	0·19	1·96	0·08
	GlyGl	2·01	0·12	1·85	0·07

* For details of diets, see Table 1 and pp. 48–9.

glycerides and liver free fatty acids were then determined as described for plasma samples. For liver phospholipids, the dry residues were digested with 10 M-sulphuric acid, and phosphorus was estimated by the method described previously for plasma samples. For liver cholesterol, the dry residues were dissolved in ethanol, and cholesterol was estimated as described for plasma samples.

Liver glycogen was estimated by the method of Hassid & Abraham (1957).

In Expt 1, total liver nitrogen was estimated by the Kjeldahl method. In Expt 3, the liver was homogenized with 20 vol. water, and liver protein was precipitated (5 ml liver homogenate) by the addition of 5 ml trichloroacetic acid solution (100 g/l). Protein-N content of the precipitate was determined by the Kjeldahl method.

RESULTS

Growth. Values for weight gain of the kids in Expts 1 and 3 are given in Table 3. Kids given the control diet (cow's milk) gained weight at an almost maximum rate in Expts 1 and 3. Animals given the experimental diets showed good growth in both Expts 1 and 3. There was no significant difference in weight gain between the experimental groups and the control group in either experiment. All kids remained in good health throughout the experiment and there were no instances of food refusal. The incidence of diarrhoea in the animals given the experimental diets was very low.

Blood composition. The concentration of glucose in samples of whole blood and plasma taken just before the morning feed (17 h after the previous evening feed) are given in Table 4. In Expt 1, there was a tendency for the glucose concentrations in both whole blood and plasma of the kids given the experimental diets to be lower than those of kids given the control diet, but the difference was not significant. In Expt 3, the concentrations of glucose in whole blood were generally lower in the experimental kids than in the control animals. During the fourth week of the experimental period,

Table 4. *Expts* 1 *and* 3. *Effect of cow's milk* (*control*) *and carbohydrate-deficient* (*milk-substitute*) *diets on the concentrations of blood glucose* (*mmol/l*) *in kids*

(Mean values with their standard errors for three kids/treatment)

Experimental period ...		Week 2				Week 3				Week 4	
		Whole blood		Plasma		Whole blood		Plasma		Whole blood	
Diet†		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Expt 1	Control	4·51	0·25	5·15	0·34	3·92	0·24	4·94	0·51	—	—
	Experimental: Bu	3·91	0·48	4·68	0·54	3·57	0·40	4·62	0·40	—	—
	Gly	3·25	0·46	4·10	0·43	2·88	0·37	3·93	0·46	—	—
	Prop	3·09	0·66	3·87	0·70	2·83	0·39	3·89	0·56	—	—
Expt 3	Control	—	—	—	—	3·98	0·37	—	—	4·27	0·29
	Experimental: Bu	—	—	—	—	3·50	0·33	—	—	3·36	0·21*
	BuGl	—	—	—	—	2·94	0·51	—	—	3·62	0·14
	Gly	—	—	—	—	3·29	0·64	—	—	3·13	0·22*
	GlyGl	—	—	—	—	3·84	0·14	—	—	2·90	0·37*

Values significantly different from control group: * $P < 0·05$. † For details of diets, see Table 1 and pp. 48–9.

Table 5. *Expts* 1 *and* 3. *Effect of cow's milk* (*control*) *and carbohydrate-deficient* (*milk-substitute*) *diets on the concentrations of plasma lipids in kids*

(Mean values with their standard errors for three kids/treatment)

	Diet†	Triglycerides (mg/l)		Cholesterol (mmol/l)		Phospholipids (mg/l)	
		Mean	SE	Mean	SE	Mean	SE
Expt 1	Control	297	103	5·20	0·18	2390	130
	Experimental: Bu	688	199	9·03	0·65**	3470	260**
	Gly	535	165	7·22	0·23**	2960	90
	Prop	567	206	7·60	0·34**	3250	90**
Expt 3	Control	163	43	4·32	0·21	2070	100
	Experimental: Bu	520	99	7·53	0·34**	3550	80**
	BuGl	383	118	6·83	0·83**	3270	150**
	Gly	340	62	5·33	0·39	2840	170**
	GlyGl	173	15	5·15	0·10	2430	70*

Values significantly different from control group: * $P < 0·05$, ** $P < 0·01$.
† For details of diets, see Table 1 and pp. 48–9.

the kids given diets Bu, Gly and GlyGl had significantly lower concentrations of blood glucose than those given the control diet, but during the third week there were no significant differences in the concentrations of blood glucose between the experimental and control groups.

The concentrations of plasma lipids in Expts 1 and 3 are given in Table 5. In Expt 1, the concentrations of plasma triglycerides in the experimental kids were higher than those in the control animals but the differences were not significant. In Expt 3, the experimental diets except diet GlyGl produced an increase in the concentrations of plasma triglycerides but the differences between the experimental and control groups were not significant. In Expt 1, significant increases in the concentrations of plasma phospholipids and cholesterol were found in the kids receiving the experimental diets, except in values for phospholipids with diet Gly. In Expt 3, the concentrations of plasma phospholipids in the experimental kids were significantly higher than those in the control animals. In Expt 3, the experimental diets generally produced higher concentrations of plasma cholesterol compared with the control diet and the values obtained for the kids given diet Bu and diet BuGl were significantly higher than those for control animals.

The results of Expt 2 are shown in Figs. 1 and 2. For samples taken just before the morning feed, the concentrations of glucose in both whole blood and plasma of the kids given the carbohydrate-free diet were similar to those of the kids given the control diet (Fig. 1). The concentrations of blood glucose in the kids given the control diet increased rapidly in the 2 h after feeding, while those in the kids given the carbohydrate-free diet remained constant. Thereafter, the concentration of blood glucose decreased gradually with time in both groups, and the blood glucose *v.* time curve for the kids given the carbohydrate-free diet did not differ from that of the kids given the control diet.

In samples taken just before the morning feed, the concentration of plasma free

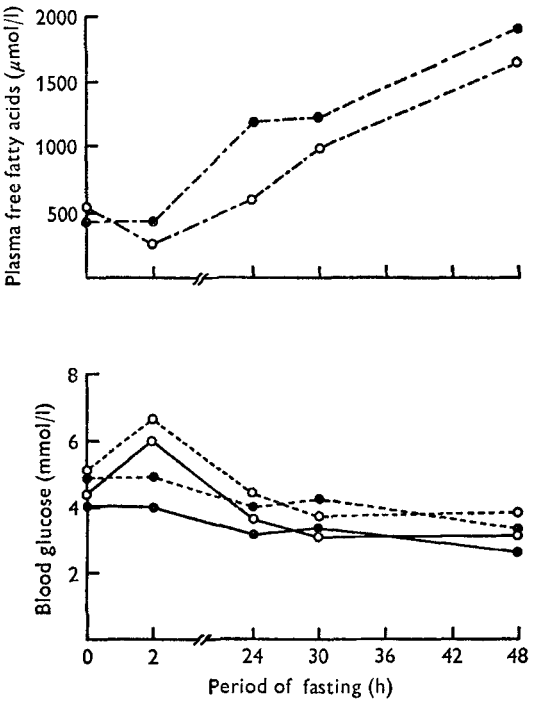

Fig. 1

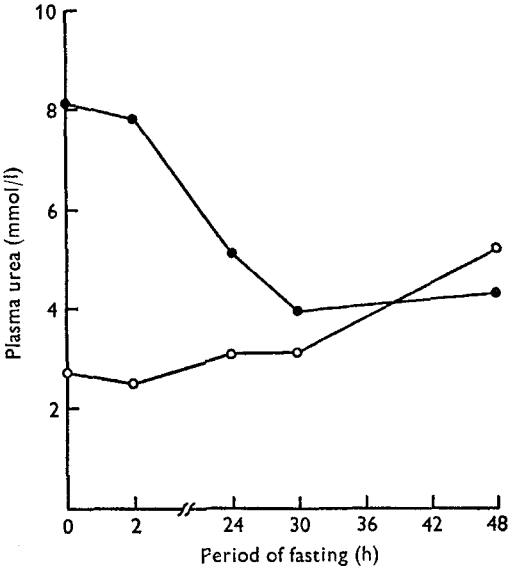

Fig. 2

Fig. 1. Expt 2. Effect of fasting on blood glucose and plasma free fatty acid concentrations in the kids given cow's milk (control) (○) or a carbohydrate-free (milk-substitute) diet (●). (——), Glucose in whole blood; (----), glucose in plasma; (–·–·–), free fatty acids in plasma. For details of diets, see Table 1 and pp. 48–9.

Fig. 2. Expt 2. Effect of fasting on the concentration of plasma urea in the kids given cow's milk (control) (○) or a carbohydrate-free (milk-substitute) diet (●). For details of diets, see Table 1 and pp. 48–9.

fatty acids in the kids given the carbohydrate-free diet was similar to that in the kids given the control diet. The concentration of plasma free fatty acids in control kids decreased by about 50% 2 h after feeding, but the value in the experimental kids remained almost constant. Thereafter, the concentration of plasma free fatty acids in both groups increased gradually with time until 48 h after feeding. At 24 and 48 h after feeding, the kids given the carbohydrate-free diet had higher concentrations of plasma free fatty acids than the kids given the control diet.

Just before the morning feed and 2 h after feeding, the concentration of plasma urea in the kids given the carbohydrate-free diet was higher than in the control group (Fig. 2). The concentration of plasma urea in kids given the carbohydrate-free diet was markedly reduced 24 h after feeding and thereafter the value remained constant until 48 h after feeding. The concentration of plasma urea in the control group remained low and fairly constant until 30 h after feeding, but the value increased 48 h after feeding.

Liver composition. Values for liver weight and composition are given in Table 6. In Expt 1, there were no significant differences in relative liver weights (g/kg body-

Table 6. *Expts* 1 *and* 3. *Effect of cow's milk* (*control*) *and carbohydrate-deficient* (*milk-substitute*) *diets on the weight and composition of liver of kids*

(Mean values with their standard errors for three kids/treatment)

	Diet§		Liver wt (g/kg body-wt)		Nitrogen† (g/kg liver wt)‡		Glycogen (g/kg liver wt)‡		Total lipid: g/kg liver wt‡		Total lipid: mg/kg body-wt	
			Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Expt 1	Control		26·4	0·6	27·2	1·0	56·7	12·2	40·7	0·8	1080	30
	Experimental:	Bu	30·2	1·4	35·8	0·5**	7·0	1·4**	55·7	2·2*	1690	110*
		Gly	27·1	1·9	35·4	0·5**	3·1	0·9**	57·7	6·0*	1530	70*
		Prop	24·4	1·5	35·1	0·6**	6·7	2·4**	48·3	0·8	1300	30
Expt 3	Control		22·9	2·7	25·5	0·6	41·6	13·5	46·1	4·9	1030	30
	Experimental:	Bu	31·3	2·1	30·0	0·3**	13·4	2·7	53·7	1·5	1620	30**
		BuGl	26·6	1·1	30·5	0·5**	14·0	3·1	53·2	0·7	1420	80**
		Gly	29·3	2·0	29·6	0·2**	13·9	4·4	51·7	2·9	1510	50**
		GlyGl	28·1	0·6	29·4	0·4**	9·6	4·3	52·5	1·0	1480	60**

Values significantly different from control group: * $P < 0{\cdot}05$, ** $P < 0{\cdot}01$. † Total N values for Expt 1, protein-N values for Expt 3.
‡ Fresh wt basis. § For details of diets, see Table 1 and pp. 48–9.

Table 7. *Expt 3. Effect of cow's milk (control) and carbohydrate-deficient (milk-substitute) diets on the concentrations (mg/kg body-wt) of liver lipids in kids*

(Mean values with their standard errors for three kids/treatment)

Diet†		Triglycerides Mean	Triglycerides SE	Cholesterol Mean	Cholesterol SE	Phospholipids Mean	Phospholipids SE	Free fatty acids Mean	Free fatty acids SE
Control		86	12	177	18	619	47	112	7
Experimental:	Bu	205	15**	209	5	952	47**	153	36
	BuGl	226	38**	180	11	797	37**	107	11
	Gly	273	46**	165	8	873	59**	119	6
	GlyGl	235	13**	189	23	828	22**	133	4

Values significantly different from control group: ** $P < 0.01$.
† For details of diets, see Table 1 and pp. 48–9.

weight) between the experimental and control groups. In Expt 3, the relative liver weights of the kids given the experimental diets were greater than those of the kids given the control diet, but the difference was not significant. In Expt 1, the concentration of total N in the livers from the experimental groups was significantly higher than that for the control group.

In Expt 1, the concentration of liver glycogen in the kids given the experimental diets was significantly lower than that in the kids given the control diet. In Expt 3, feeding the experimental diets produced a decrease in the concentration of liver glycogen, but the difference between the experimental and control groups was not significant.

In Expt 1, there was a tendency for the concentration of total lipid in the livers from kids given the experimental diets to increase, and the values obtained with diets Bu and Gly were significantly higher than the control value. When total liver lipid was expressed as mg/kg body-weight, the values obtained with diets Bu and Gly were also significantly higher than the control value. In Expt 3, feeding the experimental diets resulted in the deposition of a greater amount of total lipid in the liver compared with the control diet, but the difference was not significant. When total liver lipid was expressed as mg/kg body-weight, the values for the experimental groups were significantly higher than that for the control group.

The concentrations (mg/kg body-weight) of the different liver lipid fractions obtained in Expt 3 are given in Table 7. Triglyceride and phospholipid concentrations tended to increase with the experimental diets compared with the control diet. There were no significant differences in the amounts of total cholesterol and free fatty acids in the livers of the experimental and control groups.

DISCUSSION

The results suggest that kids utilize carbohydrate-deficient diets as efficiently as cow's milk and that kids are able to tolerate a high-fat, high-protein diet. The evidence that kids given diets Gly or Prop showed good growth, similar to those given cow's

milk, indicates that kids have the ability to utilize glycerol and propionate efficiently. Our results are in agreement with those of Renner (1964), who reported that neither growth nor N retention of chicks was decreased when soya-bean oil was substituted isoenergetically for glucose in diets containing 55·2, 64·4, 73·6, 82·8 and 92·0 kJ/g protein. He found that the efficiency of energy utilization by chicks given a carbohydrate-free diet was similar to that with the control diet. Goldberg (1971) reported that rats given a diet in which carbohydrate was totally replaced by soya-bean oil gained less weight than those given a high-carbohydrate diet, but he did not consider food consumption. It was not clear, therefore, whether the reduced growth of rats given the carbohydrate-free diet was due to a lower food intake or to a reduced food conversion efficiency.

In the present study, some kids given the carbohydrate-free diet (diet Bu) had an extremely low blood glucose level, similar to that of adult goats. Some of the kids given the diets supplemented with a small amount of glycerol, sodium propionate or glucose (diets Gly, GlyGl, Prop, BuGl) had very low blood glucose levels, indicating that this supplementation of the diet was not completely effective in returning blood glucose concentrations to normal values. As there was a relatively large amount of glycogen in the liver of the kids given the control diet, killed just before the morning feed, it seemed that these kids were able to maintain blood glucose level by glycogenolysis. Liver glycogen values for the kids given the experimental diets indicated that gluconeogenesis was not of sufficient magnitude to maintain a liver glycogen level similar to that of the kids given the control diet. Our findings are in contrast to the results reported by Renner & Elcombe (1967). They found that chicks given a carbohydrate-free diet in which non-protein energy was supplied by soya-bean oil, maintained a normal level of blood glucose. Our results suggested that some of the preruminant kids given carbohydrate-deficient diets were not able to synthesize sufficient glucose by gluconeogenesis to maintain their blood glucose level. However, the evidence that kids given carbohydrate-deficient diets showed good growth, similar to that of kids given cow's milk, indicates that kids are able to tolerate a relatively low level of blood glucose.

In Expt 2, all kids given the carbohydrate-free diet had blood glucose levels, just before the morning feed, which were similar to those of kids given cow's milk. It seemed that ingestion of lactose produced an increase in the blood glucose level in the kids given cow's milk and a decrease in the level of plasma free fatty acids 2 h after feeding. As was expected, feeding a carbohydrate-free diet neither increased the blood glucose level nor affected the level of plasma free fatty acids 2 h after feeding. The evidence that fasting control kids for 24 h did not increase markedly the level of plasma free fatty acids suggests that glucose obtained by glycogenolysis was not used to increase the level of plasma free fatty acids, as 17 h after feeding, i.e. just before they would normally receive their morning feed, a relatively large amount of glycogen still remained in the liver of the kids given cow's milk. Fasting for 24 h increased the level of plasma free fatty acids in the kids given the carbohydrate-free diet, indicating that these animals can use free fatty acids as an energy source. The fact that an increase in the level of plasma free fatty acids was accompanied by a decrease in the

level of blood glucose 30 and 48 h after feeding suggests that animals in both groups utilize free fatty acids rather than glucose as an energy source.

It was expected that protein catabolism in the kids given the carbohydrate-free diet would be increased because of the high protein content of the diet, and because of the necessity for these animals to synthesize glucose from protein. Higher concentrations of plasma urea just before the morning feed and 2 h after feeding in the kids given the carbohydrate-free diet support this concept. Walker (1967) reported that in lambs starvation for 4 d increased N excretion in urine. This indicates that starvation increases the rate of protein catabolism. Alexander (1962) found that the concentration of urea in blood of newborn lambs increased during starvation. In the present study, when kids given the carbohydrate-free diet were fasted for 24 h, the concentration of plasma urea was markedly reduced. The concentrations of plasma urea in the control group remained low and fairly constant until 30 h after feeding, but fasting these kids for 48 h increased the concentration of plasma urea, suggesting that fasting increases protein catabolism. The concentration of plasma urea 48 h after feeding in kids given the carbohydrate-free diet was similar to that in the control group. This indicated that the rate of protein catabolism in the kids given the carbohydrate-free diet did not differ greatly from that in the control group.

In general, feeding carbohydrate-deficient diets resulted in an increase in the concentration of plasma lipid compared with values obtained with the control diet. There were tendencies for the concentrations of plasma cholesterol, phospholipids and triglycerides to increase as the fat level in carbohydrate-deficient diets increased. It is apparent therefore, that an increase in the concentration of plasma lipid is mainly due to the high level of dietary fat.

It was found in Expts 1 and 3 that feeding carbohydrate-deficient diets generally produced an increase in the concentration of total liver lipid. It was postulated by Aoyama, Nakanishi & Ashida (1973) that one (or a combination of more than one) of the following factors would cause the accumulation of liver lipid: (*a*) increased lipid synthesis in the liver, (*b*) decreased oxidation of fatty acids in the liver, (*c*) increased mobilization of free fatty acids from adipose tissue, (*d*) impairment of the lipid transport from the liver. It is well known that a high-fat diet inhibits fatty acid synthesis in the liver and that it causes an increase in fatty acid oxidation (Brice & Okey, 1955; Hill, Linazasoro, Chevalier & Chaikoff, 1958). It is difficult therefore to ascribe the increased accumulation of lipid in the liver of the kids given the carbohydrate-deficient diets to increased lipid synthesis or decreased oxidation of fatty acids in the liver, as dietary fat levels in these diets were higher than in the control diet. Seakins & Robinson (1964) reported that fatty liver of rats induced by feeding white phosphorus was due to the reduction in the rate of formation in the liver of the protein moiety of the low-density lipoproteins of the plasma which carry triglycerides from the liver to the extrahepatic tissues. They found that the concentrations of cholesterol, phospholipids and esterified fatty acids in plasma were markedly reduced by feeding white P. In our present study, the concentrations of plasma cholesterol, phospholipids and triglycerides were generally higher in the kids given carbohydrate-deficient diets than in those given the control diet. It is unlikely therefore that impairment of

lipid transport from the liver may have caused an increased lipid accumulation in the liver. From the above discussion, it is apparent that the kids given the carbohydrate-deficient diets utilized free fatty acids to a greater extent than those given the control diet. This state may be considered to improve lipid accumulation in the liver.

Another possible explanation for lipid accumulation is that the kids given the carbohydrate-deficient diets may deposit dietary fat in the liver to a greater extent than kids given the control diet. The results of a previous study (Tanabe & Kameoka, unpublished results) suggested that the concentration of liver lipid (mg/kg body-weight) of kids given a high-fat diet (dietary fat level similar to that of diet Bu) with a sufficient amount of carbohydrate, was 1060. The concentrations of total liver lipid in the kids given the carbohydrate-deficient diets were much higher than in control kids (Table 6), which indicated that these diets may cause lipid accumulation in the liver. This discrepancy between liver lipid values probably reflects the effect of dietary carbohydrate level on liver lipid content.

Higher concentrations of total N (in Expt 1) and protein-N (in Expt 3) in the liver of the kids given the carbohydrate-deficient diets were due to higher protein levels in these diets.

We cannot attribute the results obtained with the kids given the carbohydrate-deficient diets to low levels of dietary carbohydrate only, as protein levels in these diets were also higher than that in control diet. Whether dietary protein level affects blood glucose, plasma lipid, and liver lipid awaits further study.

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