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SOME INFLUENCES OF DIETARY CARBOHYDRATE ON LIVER AND DEPOT LIPIDS

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It has long been known that dietary carbohydrate can be laid down in the body as fat and that the fattening animal on a diet high in carbohydrate stores a more saturated type of body lipid than when on a normal diet. Previous work has shown that changes in the liver lipid in rabbits fed on a constant protein intake with varying amounts and types of carbohydrate are not only related to the amount of the carbohydrate consumed, but also to its type (Macdonald, 1962). For example, sucrose appears to give rise to more fat in the liver than does an equivalent amount of starch. These changes in liver lipid, together with those in depot fat, have been further investigated to learn in more detail the influence that the amount and type of dietary carbohydrate has on the pattern of fatty acids in these two sites, and the results are reported here. These experiments can be compared to the state of malnutrition in children brought about by deficient protein intake accompanied by varying degrees of carbohydrate excess; the greater the relative carbohydrate excess the greater the amount of hepatic and depot lipid (Macdonald, 1961).

METHODS

Adult rabbits were grouped in pairs of similar weights. The feeding was planned to give each pair the same amount of protein, but different amounts of carbohydrate. Two categories of diet were constructed, *A* and *C*, and three types of carbohydrate were employed. The composition of the diets was as follows:

	Diet (parts by weight)	
	A	*C*
Green food	25	25
Dried yeast	2	2
Salt mixture	5	5
Carbohydrate	68	18
Calorie value	390 cal/100 g	184 cal/50 g

One animal of each pair was given an unlimited supply of diet *A* and the number of grams consumed was measured. On the following day half this number of grams of diet *C* was fed to the other animal, thus providing it with the same amount of protein as its partner, but a smaller number of calories owing to the lower carbohydrate content of diet *C*. This pattern of feeding was continued throughout the experimental period. With one exception all the

animals on diet *C* ate the quantity of food provided for them, so that uniformity of protein intake was maintained in each pair of animals, the non-eater being rejected from the series. There was also one on diet *A* which would not eat the diet and this was also rejected. The carbohydrate of the diet was supplied in the form of maize starch or liquid glucose B.P.C. (a starch hydrolysate), or sucrose, and there were eight animals (four pairs) on each type of carbohydrate. These three carbohydrates were chosen as providing a gradation in molecular size. The protein was provided from powdered green food (Bruce & Parkes, 1946) which was mixed with the carbohydrate. Vitamins A and D (approximately 3000 and 600 i.u. respectively) were sprayed on the food each day.

The animals were weighed weekly and the daily amount of food consumed was measured. All the animals lost weight, even those on *ad libitum* feeding, and this was probably due to the fact that the protein intake was approximately half the normal amount. When one third of the initial body weight was lost the rabbit was killed by a blow on the back of the head. If the animal on diet *A* lost a third of its body weight before its pair-mate on diet *C*—as happened in the sucrose series—then both were killed at the same time. This arbitrary weight for killing was decided on to avoid any pre-mortal infiltration of fat into the liver.

At post-mortem the liver was removed, weighed and dried in a desiccator over sulphuric acid. The lipid was extracted from the dry liver with petroleum ether (b.p. 40–60° C) in a Soxhlet for 24 hr. A similar procedure was carried out on a sample of depot fat (where present) taken from the peri-renal region. The extracted lipids were methylated (Stoffel, Chu & Ahrens, 1959) and the proportions of the various methyl esters determined by gas chromatography (Farquhar, Insull, Rosen, Stoffel & Ahrens, 1959). Cholesterol was measured by the method of Zlatkis, Zak & Boyle (1953). Every 3 weeks the amount of carbohydrate in a 24 hr sample of faeces was determined (Viles & Silverman, 1949).

RESULTS

Daily food intake. The mean value for each animal on diet A showed that the dietary starch-fed animals ate most (26·7 g/kg/24 hr), next were the liquid glucose-fed animals (19·0 g/kg/24 hr) and the sucrose-fed animals ate the least (16·0 g/kg/24 hr). The differences between the starch group and the other two groups are significant.

Experimental survival time. The mean survival time was calculated for the rabbits on *ad libitum* feeding only (diet *A*), as the survival time of animals on diet *C* was to a large extent determined by their pair-mates on diet *A*, which determined the food consumption of animals on diet *C*. Survival time was greatest for the starch-fed group (130 days) and least for the sucrose (67 days) with the liquid glucose-fed group in between (93 days). The differences between these means are not significant.

Liver lipids

The amount and proportion of total fat, amount of total cholesterol and proportion of each of the principal fatty acids in the liver lipid (Table 1) was plotted against the mean daily carbohydrate intake expressed as g/kg initial body weight/24 hr. Lines were fitted to the data by the method of least squares and correlation was tested using Student's *t* test. A correlation was considered as not arising by chance if $P > 0{\cdot}05$. As the protein

TABLE 1. Results obtained in adult rabbits on diets containing varying amounts and type of carbohydrate. *A*, High-carbohydrate diet; *C*, low-carbohydrate diet

	Maize starch						Liquid glucose								Sucrose							
	A	*C*	*A*	*C*	*A*	*C*	*A*	*C*	*A*	*C*	*A*	*C*	*A*	*C*	*A*	*C*	*A*	*C*	*A*	*C*	*A*	*C*
Diet																						
Carbohydrate intake (g/kg initial body wt./24 hr)	25·8	12·7	20·9	8·8	22·0	8·8	17·2	8·3	17·6	8·6	13·3	5·1	16·8	6·2	16·0	7·5	12·9	7·3	16·1	6·4	10·1	5·4
Protein intake (g/kg initial body wt./24 hr)	2·0	2·3	1·6	1·6	1·7	1·6	1·3	1·5	1·4	0·6	1·0	0·9	1·3	1·1	1·3	1·4	1·0	1·3	1·3	1·2	0·8	1·0
Time on diet (days)	92	38	110	71	188	71	50	50	148	116	72	72	100	72	51	51	63	63	86	86	66	66
Lipid (g/100 g dry liver)	8·5	8·5	12·5	8·2	7·7	6·6	10·8	10·4	24·7	6·7	17·7	8·1	4·9	11·3	46·4	6·6	17·3	6·5	22·3	3·6	4·5	8·5
Liver lipid (g)	0·7	0·4	1·4	0·7	1·7	0·6	3·6	1·0	4·1	0·5	1·9	0·6	0·9	1·3	6·8	0·3	1·4	0·9	2·5	0·5	0·9	0·7
Liver cholesterol (g)	0·13	—	0·19	0·17	0·41	0·27	0·48	0·13	0·31	0·10	0·23	0·10	0·38	0·25	0·44	0·12	0·21	0·10	0·22	0·11	0·07	0·13
Cholesterol (g/100 g depot fat)	—	—	2·2	—	2·2	—	2·5	2·6	2·1	—	3·2	3·2	—	2·6	2·6	—	2·3	2·6	—	—	—	—
Fatty acids (%)																						
Liver: Myristic	3·1	0·6	1·3	0·6	2·9	0·4	2·3	0·9	3·9	1·3	0·5	1·6	1·4	0·4	0·5	—	0·6	0·4	0·9	0·4	1·6	—
Liver: Palmitic	32·7	35·0	31·2	27·2	31·8	33·8	31·9	37·8	34·4	25·8	33·1	32·9	34·5	30·3	33·4	—	25·5	27·3	32·5	20·8	34·5	—
Liver: Palmitoleic	5·5	2·0	6·4	3·1	5·3	2·8	4·9	2·9	6·1	2·0	4·4	3·8	6·6	4·1	6·6	—	9·0	3·5	4·5	3·1	5·8	—
Liver: Stearic	0	0	0	0	7·3	16·9	4·1	0	8·6	12·2	8·8	0	0	9·8	5·5	—	0	0	0	0	5·0	—
Liver: Oleic	37·7	43·7	46·3	38·6	37·7	24·8	37·1	39·5	35·2	31·5	32·0	38·0	50·0	33·8	38·1	—	35·3	34·6	39·2	42·2	30·8	—
Liver: Linoleic	21·0	18·7	14·8	30·5	15·0	21·3	19·7	18·9	11·8	27·2	21·1	23·7	7·5	21·6	15·9	—	29·6	34·2	22·9	33·5	22·3	—
Adipose tissue: Myristic	—	—	0·5	—	6·7	—	1·5	0·9	4·7	—	2·2	1·3	—	3·1	1·5	—	3·0	3·8	0·3	—	—	—
Adipose tissue: Palmitic	—	—	35·5	—	38·5	—	25·1	32·8	33·6	—	28·0	26·2	—	33·7	24·5	—	43·7	22·8	20·5	—	—	—
Adipose tissue: Palmitoleic	—	—	11·4	—	11·9	—	5·9	2·1	7·5	—	7·6	5·2	—	3·8	4·4	—	8·1	7·1	3·4	—	—	—
Adipose tissue: Stearic	—	—	0	—	5·6	—	0	0	3·6	—	0	0	—	0	4·8	—	0	0	0	—	—	—
Adipose tissue: Oleic	—	—	41·8	—	26·3	—	40·2	44·2	36·0	—	40·8	37·5	—	31·4	47·7	—	20·3	35·4	50·4	—	—	—
Adipose tissue: Linoleic	—	—	10·8	—	11·0	—	27·3	20·0	15·2	—	21·4	29·8	—	28·0	17·1	—	24·9	30·9	25·4	—	—	—

intake between pairs and the experimental survival times within and between pairs of animals were not the same, the protein and the time on the diet were variables in addition to the carbohydrate. However, no correlation at all could be found between any aspect of the liver lipid and either protein intake or experimental survival time.

Total liver lipid. When previous data on the amount of liver lipid and mean daily carbohydrate intake of rabbits under identical conditions to those described here (Macdonald, 1962) are combined with the results obtained here, a significant positive correlation between the amount of

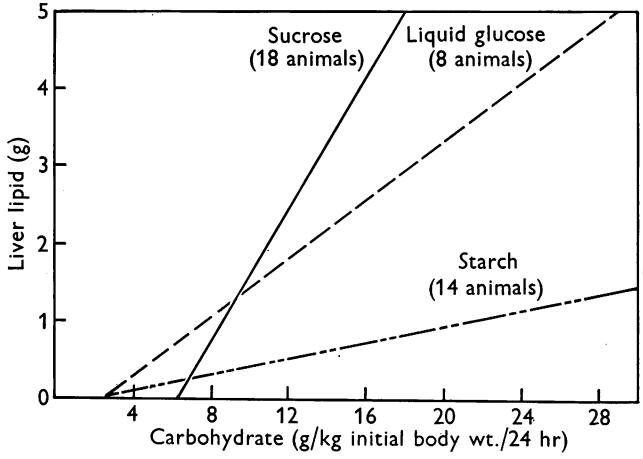

Fig. 1. Mean daily carbohydrate intake plotted against amount of liver lipid. Comparison of slopes: starch and liquid glucose, $P = 0{\cdot}005$; starch and sucrose, $P = 0{\cdot}005$; sucrose and liquid glucose, $P = 0{\cdot}005$–$0{\cdot}001$.

liver lipid and the carbohydrate intake is found. The difference between the slopes of these correlations for starch and sucrose is highly significant, the steeper slope of the sucrose data confirming previous findings that weight-for-weight dietary sucrose is associated with more liver fat than is starch in the diet. A similar significant correlation is found for liquid glucose and the slope of this line is intermediate between those for starch and for sucrose (Fig. 1). If the results in this series are considered separately, similar significant correlations are found except in the starch-fed animals, where the slope of the line between amount of liver lipid and carbohydrate intake has a probability value of 0·2–0·1.

Liver cholesterol. As the mean daily carbohydrate intake increases, there is an increase in the total amount of cholesterol in the liver. However, no increase in liver cholesterol is seen with starch alone (Fig. 2); comparison of the slopes between starch and liquid glucose and starch and sucrose show significant differences. This increase in liver cholesterol with increase

in the amount of sucrose intake is comparable with the increase in sterol esters previously described in sucrose-fed rabbits (Macdonald, 1962).

Fatty acids. The greater the daily carbohydrate intake, the greater the proportion of myristic and palmitoleic acids and the smaller the proportion of linoleic acid when findings with all the carbohydrates are grouped together. This effect on linoleic acid varies with different carbohydrates. Sucrose is associated with a more rapid drop in this fatty acid than is starch (Fig. 3). There is no such correlation with palmitic acid, though significant differences are shown when types of carbohydrate are considered separately. For instance, with dietary sucrose the palmitic acid proportion

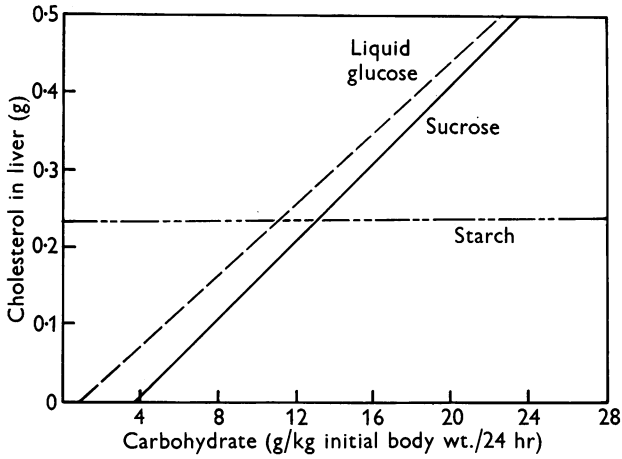

Fig. 2. Mean daily carbohydrate intake plotted against amount of cholesterol in the liver.

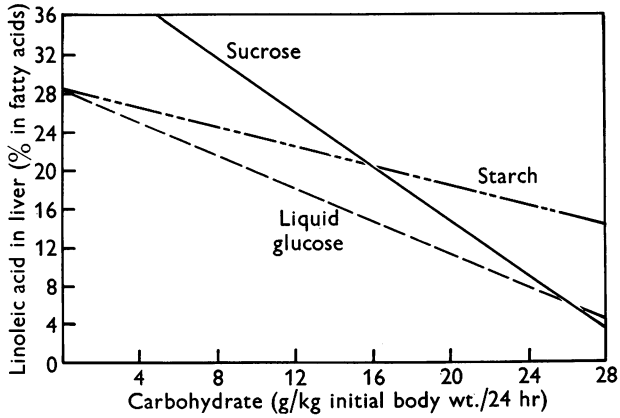

Fig. 3. Mean daily carbohydrate intake plotted against the proportion of linoleic acid in the liver fatty acids. Comparison of slopes: starch and sucrose, $P = 0{\cdot}05$–$0{\cdot}025$; others not significant.

rises with increasing intake, but with liquid glucose it tends to fall and with starch there seems to be no alteration. Comparison of the slopes of these lines shows that the differences are significant when sucrose is compared with starch and with liquid glucose (Fig. 4).

It is unfortunately not possible to assess the *amount* of each fatty acid fraction in the liver because liver lipid is not entirely made up of fatty acids. The increase in proportions of palmitoleic and decrease in linoleic acids in the liver lipid is of interest because of similar findings in the liver lipid of children who had died as a result of malnutrition from a diet high in carbohydrate and low in protein (Macdonald, unpublished).

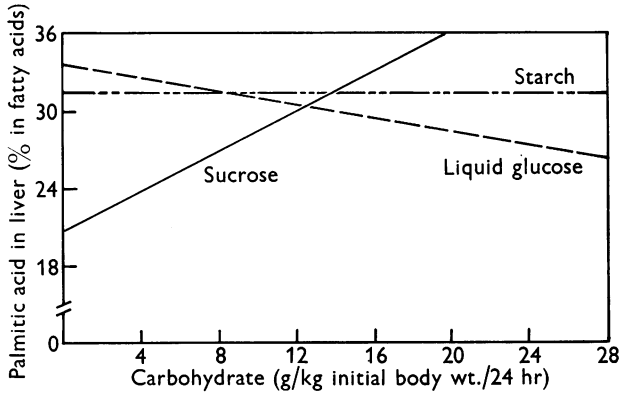

Fig. 4. Mean daily carbohydrate intake plotted against the proportion of palmitic acid in the liver fatty acids. Comparison of slopes: starch and liquid glucose, not significant; starch and sucrose, $P = 0{\cdot}025$; sucrose and liquid glucose, $P = 0{\cdot}025$–$0{\cdot}01$.

Depot fat

In the depot fat it is not possible to estimate total amounts, so the results are perforce expressed as percentage compositions (Table 1).

Cholesterol. The greater the mean daily carbohydrate intake, the smaller the percentage of cholesterol in depot fat. This may mean that with these diets more fat is being laid down as the carbohydrate intake increases and that this fat contains less cholesterol, or that cholesterol is being selectively removed. This latter view is favoured in view of the probable reduction of depot fat while on the diet (the body weight decreases) and the increased amount of cholesterol in the liver may be an accumulation of some of the depot cholesterol.

Fatty acids. As in the liver lipid, the proportion of palmitoleic acid rises and that of linoleic acid falls as the level of daily carbohydrate consumption rises. There is no correlation between any finding in depot fat and length of time on the diet. There is, however, correlation between

the percentage of cholesterol, of palmitoleic acid and of linoleic acid in the depot fat with the mean daily protein intake. These are of the same order of significance as are found with the carbohydrate: this finding is probably due to the fact that of those animals in whom sufficient depot fat was found for analysis a very high proportion (8 out of 12) were on Diet *A*. If all the animals had been on the same diet and if a significant correlation with carbohydrate intake had been found, the same significant correlation would have been found with protein, because the ratio of carbohydrate to protein was constant. For this reason the significant correlation between some fatty acids in depot fat and protein intake may be misleading, as three-quarters of the samples analysed came from animals on the same diet.

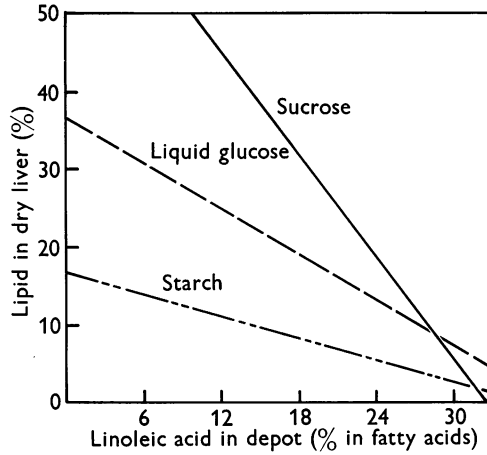

Fig. 5. Proportion of linoleic acid in depot fatty acids plotted against proportion of total lipid in dry liver. Comparison of slopes: sucrose and liquid glucose, $P = 0{\cdot}005$–$0{\cdot}001$.

Liver lipid and adipose tissue. When the proportion of linoleic acid in the liver lipid is plotted against that in adipose tissue there is a high degree of positive correlation, and when the fitted line is forced through zero correlation is still high. This means that in these animals the proportion of linoleic acid in the liver fat is similar to that in the adipose tissue.

As there is a correlation between the proportion of linoleic acid in the adipose tissue and carbohydrate intake and between the proportion of liver lipid and carbohydrate intake, can the proportion of linoleic acid in the adipose tissues of these animals be taken as a guide to the proportion of lipid in dry liver? Plotting shows that this is so for the liquid-glucose and sucrose diets, there being insufficient data for the starch series. However, the negative correlation found is not the same for sucrose as for liquid glucose and starch seems as if it might be still different (Fig. 5).

It would seem feasible, therefore, to estimate the percentage of lipid in the liver by depot-fat biopsy if the animal is on a diet containing a constant type of carbohydrate for which the correlation factor is known. For any given reduction in percentage of linoleic acid in the depot fat a greater proportion of lipid in dry liver is seen with the sucrose diet than with the liquid-glucose diet. This means that the mechanism concerned with the increase in liver lipid is not exactly the same as that which results in a reduction of linoleic acid in the adipose tissue.

Carbohydrate in faeces. Estimations of the percentage of carbohydrate in the dry faeces did not show any significant difference related to the quantity or character of the carbohydrate employed, so presumably most of the ingested carbohydrate was either absorbed or used by the gut flora. There may be different rates and amounts of absorption of the carbohydrate eaten, which differences are not revealed in the faeces because the activity of the bacterial community kept the faecal percentage of carbohydrate constant. This is unlikely; also any marked increase in carbohydrate-splitting bacteria might result in diarrhoea, and this was not seen.

DISCUSSION

Perhaps the most interesting finding in the response of the liver and depot lipids is that the pattern of fatty acid alters with the value of the carbohydrate intake. In both these sites the fatty-acid pattern is altered in a similar manner, showing an increase in the proportion of palmitoleic acid and decrease in linoleic acid as the carbohydrate intake increases. This means that the alteration in the composition of liver and depot fat cannot be explained by a simple transference of depot fat to the liver or vice versa: had this been so the change in pattern of fatty acids with depot fat would have been mirrored in the liver lipid or vice versa.

Similar alterations to those reported here were found in the fatty-acid pattern in the liver and depot fat of children who had died as a result of malnutrition involving excess dietary carbohydrate (Macdonald, unpublished). The significance of the fall in proportion of linoleic acid is of interest in view of the fact that many of the current hypotheses on ischaemic heart disease in man involve dietary deficiency of unsaturated fatty acids (Davidson, Meiklejohn & Passmore, 1959). The deficiency, if present, has been thought to be due to reduced intake, but it may be that increased carbohydrate intake aggravates any shortage of linoleic acid. A correlation exists between sucrose intake and the mortality from coronary artery disease (Yudkin, 1957) and this becomes more significant when it is noted that in these experimental animals the proportion of linoleic acid in the liver falls more rapidly with dietary sucrose than with dietary starch.

The fall in the proportions of linoleic acid in the liver and depot lipids cannot be entirely due to a deficient intake. Any dietary linoleic acid would be in the green-food fraction of the diet and this fraction contained the protein and was kept relatively constant and showed no correlation with linoleic acid in the liver or depot lipids. Furthermore, the rate of fall of linoleic acid was more rapid with sucrose than with starch, even when the amount of dietary intake was similar.

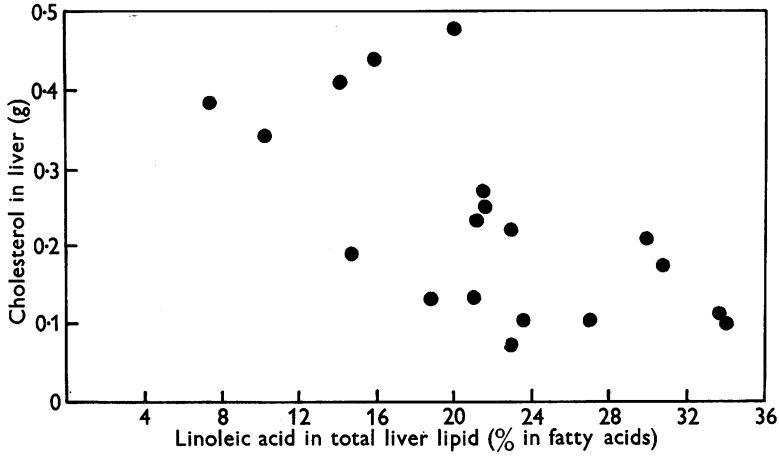

Fig. 6. Proportion of linoleic acid in liver fatty acids plotted against amount of cholesterol in liver. $P = 0{\cdot}005$.

It has been suggested that the rise in serum cholesterol found in ischaemic heart disease in man may be associated with an inadequacy of linoleic acid (Bronte-Stewart, Antonis, Eales & Brock, 1956). It may be that in these animals the fall in linoleic acid is the cause of the rise in their liver cholesterol. That there is an association can be seen from Fig. 6, where a negative correlation exists between the percentage of linoleic acid and amount of cholesterol in the liver.

The question as to whether all carbohydrates exert the same effects on fat metabolism can be answered in these animals. The significant differences found in rate of response or in type of response reveal that dietary carbohydrates are not all equal, as judged by their effect on lipids. The amount of lipid present in the livers of these animals shows, at any given level of carbohydrate intake, higher values with dietary sucrose than with starch. There does not seem to be any accumulation of cholesterol in the liver with dietary starch, but there is with increasing intake of liquid glucose and sucrose. Palmitic acid in liver lipid increases with sucrose intake but not with starch or liquid glucose, and the proportion of liver linoleic acid

falls faster with dietary sucrose than with liquid glucose or starch. Thus there is ample evidence to show that on the low protein intake used in these experiments the type of dietary carbohydrate does influence the size and pattern of the lipid response. An explanation of these findings is not possible at the moment, but as starch and liquid glucose are broken down to glucose, whereas sucrose is converted in the gut to glucose and fructose, the fructose may be a factor. This, however, cannot explain the differences found in the lipid response to starch and liquid glucose. The possibility cannot be excluded that the starch and liquid glucose used contained some essential food factor which was not present in the sucrose.

SUMMARY

In adult rabbits where the protein intake was relatively constant alterations in the carbohydrate intake showed that:

1. The amount of liver lipid was directly related to the mean daily carbohydrate intake. Weight for weight, sucrose is associated with more liver lipid than is liquid glucose B.P.C., and the latter with more liver lipid than is starch.

2. Variations in the intake of sucrose and liquid glucose are associated with corresponding variations in cholesterol in the liver, but this is not so for starch.

3. The proportion in the liver lipid of myristic and of palmitoleic acid rises, and that of linoleic acid falls, the greater the daily dietary carbohydrate intake. Sucrose is associated with a more rapid fall in linoleic acid than is starch.

4. In the adipose tissue an increase in dietary carbohydrate is associated with a fall in the proportions of linoleic acid and cholesterol and a rise in the palmitoleic acid.

5. The fall in linoleic acid is not due to a deficient intake.

6. These experiments show that the lipid response in rabbits to dietary carbohydrate is not only related to the level of intake, but also to the type of carbohydrate consumed.

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