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The Lipid Composition of Rat-Liver-Cell Sap

By G. S. GETZ,* W. BARTLEY, F. STIRPE,† B. M. NOTTON, A. RENSHAW

Department of Biochemistry and Medical Research Council, University of Oxford

AND D. S. ROBINSON‡

Sir William Dunn School of Pathology, University of Oxford

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The fatty acid composition of the total lipids of each of the different compartments (nuclei, mitochondria, fluffy layer, microsomes, supernatant and fatty supernatant) of the rat-liver cell was determined by Getz & Bartley (1961). The fatty acid composition of the different lipid classes (neutral lipids, aminophosphatides and choline-phosphatides) within a particular compartment of the liver cell, the cell sap, is described in this study.

METHODS

The cell sap was prepared from 92 g. of rat-liver pulp (obtained from eight male rats of the Wistar strain, average age 8 months and average weight 400 g.). It was obtained as the supernatant fluid that separated after sedimenting the particles from a homogenate of the livers in 0.25M-sucrose solution. The nuclear fraction and the mitochondria were isolated by the procedure of Werkheiser & Bartley (1957) and the fluffy layer was retained and washed in the same way as the mitochondrial sediment. The microsomes were isolated from the combined supernatants of the mitochondrial and fluffy layer fraction by centrifuging for 60 min. at 30 000g in no. 20 rotor of the Spinco centrifuge. A clear supernatant was obtained with a layer of fatty material floating on the surface. The floating fatty material was removed with a Pasteur pipette and the fat on the sides of the centrifuge tubes removed by careful wiping with fat-free filter paper. The combined material ('floating fatty supernatant') was then extracted with 20 vol. of chloroform-methanol (2:1, v/v). The cell sap was removed

from above the microsomes with a Pasteur pipette. To avoid the extraction of large volumes of cell sap with organic solvents, the lipids were first co-precipitated with protein, with trichloroacetic acid as described by Getz & Bartley (1961), and then extracted from the trichloroacetic acid precipitates with 20 vol. of chloroform-methanol (2:1, v/v).

Analysis of cell sap. One portion of the extract was used for the total lipid determination and another was saponified and the fatty acids liberated were esterified with diazomethane, in each case by procedures outlined by Getz & Bartley (1961). The remainder of the lipid extract was chromatographed on silicic acid (Mallinkrodt Chemical Works; mesh 100, prepared according to the method of Hirsch & Ahrens, 1958). The loading procedure and elution sequence were carried out as described by Getz, Bartley, Stirpe, Notton & Renshaw (1961) except that the triglycerides were eluted with 500 ml. of 10% ether in light petroleum (b.p. 60–80°) and the remaining neutral glycerides with 300 ml. of diethyl ether before the first phospholipid fraction was eluted with 100 ml. of chloroform-methanol (7:1, v/v). The elution sequence was as shown in Table 1. Each lipid fraction was subjected to appropriate analysis by methods described by Getz *et al.* (1961). In addition, the methyl esters of the fatty acids of each fraction were prepared by interesterification and microsublimation (Stoffel, Chu & Ahrens, 1959) and the mixtures of methyl esters so obtained were analysed by gas chromatography.

Preparation of lipid extracts for silicic acid chromatography. Insoluble material was deposited from the chloroform-methanol extract of the cell sap during storage and during concentration of the extract for putting on to the silicic acid column. The extract was therefore evaporated to dryness under N₂ and extracted with warm chloroform. The chloroform extract was filtered and the residue on the filter paper washed three times with chloroform. The combined chloroform extracts were evaporated to dryness, dissolved in a small amount of light petroleum (b.p. 60–80°) and applied to the silicic acid column. The residue filtered off from the chloroform solution was saponified and the fatty

* Present address: Department of Chemical Pathology, Witwatersrand University Medical School, Johannesburg, South Africa.

† Present address: National Institutes of Health, Bethesda, U.S.A.

‡ Member of the External Staff of the Medical Research Council.

Table 1. *Elution sequence used to fractionate the lipids of the cell sap on silicic acid*

Fraction no.	Solvent	Volume (ml.)	Main lipid expected
1	1% Ether in light petroleum (60-80°)	350	Sterol esters
2	4% Ether in light petroleum (60-80°)	60	
3	10% Ether in light petroleum (60-80°)	500	
4	100% Ether	300	Triglycerides
5	Chloroform-methanol (7:1, v/v)	100	Lower neutral glycerides
6	Chloroform-methanol (4:1, v/v)	200	Non-nitrogen phosphatide, serine phosphatide
7	Chloroform-methanol (3:2, v/v)	100	Ethanolamine phosphatide
8	Chloroform-methanol (3:2, v/v)	400	Serine and inositol phosphatide
	Chloroform-methanol (1:4, v/v)	200	Lecithin
			Sphingomyelin and other lipids

Table 2. *Analysis of lipid fractions from cell sap of rat liver*Results are expressed as μ moles/g. dry wt. of cell sap.

No. of fraction	Lipid esters in fraction	Total P in fraction	Ester/phosphate	Total choline in fraction	Choline/phosphate	Total amino N in fraction	Amino N/phosphate	Methyl esters/phosphate
1	20.2	0	—	—	—	—	—	—
2	19.6	0.03	—	—	—	—	—	—
3	4.8	0.04	—	—	—	—	—	—
4	8.2	1.6	5.1	0	—	1.3	0.8	2.3
5	1.7	1.4	1.3	0	—	1.6	1.1	2.0
6	3.5	2.8	1.2	0	—	1.3	0.46	1.8
7	8.7	5.6	1.6	5.6	1.0	0.4	0.07	1.9
8	1.8	2.8	0.6	0	—	0.1	0.035	0.7

Table 3. *Distribution of lipids within the cell sap compared with the mean distribution throughout the cell*

A is the

$$\frac{\text{Wt. of lipid fraction in cell sap}}{\text{Wt. of same lipid fraction in total homogenate}} \times 100.$$

B compares the amount of a lipid class/unit dry wt. of cell sap with the amount of the same lipid class in unit dry wt. of the whole cell. The dry weights were determined by the method of Werkheiser & Bartley (1957) as the trichloroacetic acid-insoluble material.

Fraction no.	Percentage distribution of esters within lipid class	A	B
1	29.6	32.1	1.34
2+3	35.7	8.7	0.35
4	12.0	13.4	0.56
5	2.54	0.75	0.03
6	5.07	3.1	0.13
7	12.8	1.3	0.055
8	2.56	19.6	0.73
Total		4.8	0.33

acids liberated were esterified. Yields of 3.28 μ moles of fatty acid ester/g. dry wt. of cell soluble sap were obtained. The fatty acid composition of this material is reported in Table 4. The triglyceride fraction from the 'floating fatty supernatant' contained a large proportion of free fatty acids. These were removed by extracting the fraction with 0.5% potassium carbonate in 50% methanol. After esteri-

fication, their fatty acid composition was examined (Table 7).

Some difficulty was experienced in obtaining the theoretical ester:phosphorus ratio when the fatty acid esters were measured directly on the various phospholipid fractions. After saponification and methylation of the fatty acids, the analyses of the methyl esters recovered were much nearer those expected from the phosphorus content of the phospholipids.

RESULTS

The results of the silicic acid fractionation and of the analysis of the various fractions of the cell sap are shown in Table 2. More than half the total lipid was in the form of neutral lipid.

In Table 3 (column A) the distribution of the various lipid fractions in the cell sap, is related to their distribution in the whole liver, the data obtained for the lipid composition of the rat liver given by Getz *et al.* (1961) being used.

Whereas only about 4.8% of the total lipid esters of the liver are found in the cell sap 32.1% of the presumed sterol esters (fraction 1) and 20% of the presumed sphingomyelin (fraction 8) are found in this fraction. By contrast, only 1.3% of the lecithin (fraction 7) is present in the cell sap.

The concentration of each lipid ester in the cell sap can also be related to the concentration of the same ester in the whole liver. This has been done in Table 3 (column B), concentrations being

Table 4. *Fatty acid composition of the cell sap of rat liver*

Values are given as percentages of the total methyl esters calculated from the gas chromatogram. The area of each peak was measured and expressed as a percentage of the total area under all the peaks.
 br, branched chain; h.br, highly branched; uns, unsaturated.
 Number of double bonds is given, e.g. as 18:1. This designates a fatty acid 18 carbon atoms long with one double bond; the position of the double bond is not given.

Fatty acid	Nominal lipid class							Chloroform-insoluble fraction
	Sterol esters	Triglycerides	Lower neutral glycerides	Fraction eluted with chloroform-methanol (7:1)	Ethanolamine phosphatide	Serine phosphatides + inositides	Lecithin	Sphingolipid
*11-br	—	—	—	—	—	—	—	—
12-br	0-05	—	—	—	—	—	—	—
12-0	0-12	0-22	0-17	0-19	0-05	—	0-05	—
13-0	—	—	—	0-04	—	—	—	—
14-1	—	0-25	—	—	—	—	—	—
*14-h.br	—	0-21	—	—	—	—	—	—
14-0	0-04	0-97	1-06	0-30	0-07	0-04	—	—
*15-h.br	0-87	—	1-52	0-54	0-25	0-13	0-55	0-34
*15-h.br	—	—	0-38	—	0-09	0-03	—	—
*15-br	—	—	—	0-56	—	—	—	—
15-0	—	—	0-25	0-08	—	—	—	—
*16-h.br	0-20	0-15	0-40	0-36	0-09	0-19	0-67	0-40
16-1	0-54	—	—	—	0-31	0-06	—	—
*16-h.br	0-41	—	—	0-54	0-07	—	—	—
16-0	2-4	2-5	2-1	0-64	0-92	0-61	1-5	1-23
*17-h.br	27-0	26-9	21-4	18-1	22-7	15-8	25-2	19-6
17-0	—	—	—	—	0-06	—	—	—
*17-uns	0-43	0-42	0-53	0-34	0-41	0-36	0-38	0-31
*18-h.br	0-63	0-69	0-31	0-82	0-90	0-76	0-99	0-64
18-0	—	—	—	0-30	0-36	0-39	0-28	—
18-1	0-07	0-39	—	0-34	—	0-06	—	—
18-2	15-0	10-0	13-9	26-8	22-0	26-4	18-7	16-4
*18-2 or 3	20-6	23-1	18-2	10-4	11-5	8-3	13-4	12-6
19-0	21-2	21-1	20-2	8-9	14-2	8-9	21-0	19-6
*19-br	—	—	1-25	—	—	—	—	—
20-0	—	—	0-66	0-27	0-33	0-18	—	—
20-1	—	0-85	—	—	0-22	0-42	—	—
20-2	—	0-19	—	—	0-23	0-39	—	0-65
*20-2 or 3	—	—	3-68	—	—	—	—	0-39
20-3	0-56	0-57	1-07	1-6	1-1	1-6	0-89	2-13
20-4	9-0	7-0	10-4	20-2	18-2	26-1	14-3	21-0
22-5	—	1-25	Trace	1-6	0-51	2-2	—	0-56
22-6	1-02	2-8	2-6	6-9	5-4	7-2	2-2	4-2

* Tentative identification only.

expressed in terms of the dry weight of either the cell sap or the liver pulp. This relationship expresses the richness of the cell sap, as compared with the liver as a whole, in particular lipid fractions, and if the lipids were evenly distributed on a weight basis these would be unity. It is evident that, in these terms, the cell sap is enriched only in the cholesterol esters, and is particularly poor in phospholipid (fractions 5, 6 and 7).

Fatty acid composition of the cell sap

The data in Table 4 show that the total fatty acids of the cell sap are only slightly less saturated than those of the liver pulp (Getz *et al.* 1961). However, there are large differences within the individual lipid classes. Thus the sterol ester fraction of the cell sap contains much more stearate and arachidonate, and less oleate and docosahexanoate, than does the corresponding fraction of the pulp. Both the tri- and lower-glyceride fractions have more stearate and arachidonate and less unsaturated C₁₈ esters than are in the liver pulp.

The phospholipids of the cell sap have compositions similar to those of the liver pulp. Within the cell sap itself the phospholipids contain less palmitate and C₁₈ unsaturated acids than do the neutral lipids. The highest arachidonate content is found in the inositide-containing fraction. Docosahexaenoic acid is approximately evenly distributed between the silicic acid fractions 4, 5 and 6. By contrast, in the whole-liver pulp this acid was found at highest concentration in the amino phosphatide (chloroform-methanol, 4:1) fraction.

The lipid fraction of the cell sap eluted from the silicic acid column by the chloroform-methanol (7:1) mixture might be expected to contain cardiolipin by analogy with the corresponding fraction obtained from the whole-liver pulp. However, the low linoleate and the high arachidonate and stearate of the cell-sap fraction suggest that cardiolipin is only a minor component.

The material which was insoluble in chloroform and which was removed from the lipid extract before the silicic acid chromatography had a fatty acid composition similar to that of the 'sphingolipid' fraction.

Composition of the 'floating fatty supernatant'

In a previous study of the fatty acid composition of the 'floating fatty supernatant' separated from a rat-liver homogenate, it was shown to resemble closely that of the adipose tissue (Getz & Bartley, 1961). The present data (Table 6) show that this fraction, also like the adipose tissue, contains over 90% of its fatty acid esters in the form of neutral glycerides. Relative to the cell lipids as a whole, the neutral lipids are markedly concentrated and

the phospholipids are much diluted in this fraction. The sterol esters are slightly more concentrated than in the whole cell but not as greatly as in the cell sap (cf. Table 3). The data in Table 5 show that the triglyceride fraction eluted from the silicic acid column had a high content of free fatty acids. These fatty acids resembled quite closely those fatty acids present in the triglycerides but differed in particular in the greater amount of palmitate present (Table 7). They do not therefore arise entirely as a result of triglyceride hydrolysis during the fractionation procedure.

Phospholipids were present in very low concentrations in the 'floating fatty supernatant'. The analysis of the various phospholipid fractions eluted from the silicic acid columns (Table 5) suggests that the elution pattern was anomalous. For this reason, the fatty acid composition of the various lipid fractions containing phosphorus which were obtained (Table 7) cannot easily be related to that of the phospholipid fractions of the cell sap or of the whole liver.

Metabolic lability of the 'floating fatty supernatant'

Experiments were carried out to determine whether the fatty acids of the 'floating fatty supernatant' were rapidly exchanged with the fatty acids of the diet. A 'floating fatty supernatant' fraction was prepared from the livers of

Table 5. *Analysis of lipid fractions from 'fatty supernatant' of rat liver*

Results are expressed as μ moles/g. dry wt. of 'fatty supernatant'.

Fraction no.	Esters (μ moles)	Free acid (μ moles)
1	3.9	18.2
2	321.0	109
3	52.2	3.8
4	6.6	—
5	9.9	—
6	1.0	1.9
7	6.7	8.2
8	1.9	—

Table 6. *Distribution of lipids within the 'fatty supernatant' compared with the mean distribution throughout the cell*

For explanation of A and B see Table 3.

Fraction no.	Percentage distribution	A	B
1	0.97	1.12	0.26
2	92.50	23.8	5.5
3	1.63	1.9	0.45
4	2.44	0.77	0.18
5	0.26	0.17	0.038
6	1.67	0.18	0.042
7	0.46	3.76	0.79
Total	—	1.18	5.11

Table 7. *Fatty acid composition of the 'floating fatty supernatant' of rat liver*

Values are given as percentages of the total methyl esters (see Table 4). br, branched chain; h.br, highly branched; uns, unsaturated (see Table 4).

Fatty acid	Total acids	Sterol esters	Tri-glycerides	Free acids	Lower neutral glycerides	Fraction eluted with chloroform-methanol (7:1)	Amino phosphatides	Inositides	Chloroform-insoluble fraction	Lecithin	Sphingo-lipid
9-0	—	—	—	—	0.06	—	—	—	—	—	—
10-0	—	—	—	—	0.02	—	0.17	—	—	—	—
11-0	—	—	0.09	—	0.05	—	0.62	—	—	—	1.04
12-0	0.06	0.15	0.18	0.09	0.09	1.41	1.74	—	—	0.21	1.59
*13-h.br	—	—	—	—	0.03	—	—	—	—	—	—
13-br	—	—	—	—	—	—	—	—	0.58	—	—
13-0	—	—	—	—	—	—	—	—	—	—	1.18
*14-h.br	—	0.35	0.12	0.08	0.11	0.94	0.96	—	7.0	0.30	—
14-0	1.1	0.77	0.88	1.48	0.72	1.33	1.51	1.49	4.7	0.80	1.51
14.1	—	0.21	0.07	0.07	0.28	—	0.23	—	1.75	0.16	—
*15-br	—	0.24	—	—	0.08	—	1.17	—	—	—	—
15-br	0.56	0.24	—	0.09	0.13	1.00	0.92	—	0.64	0.13	1.8
15-0	—	0.44	—	0.72	0.28	1.17	1.22	0.72	1.9	0.53	1.32
*16-h.br	—	0.51	—	—	—	—	1.08	—	1.36	—	—
*16-h.br	—	—	0.66	0.09	0.37	10.7	6.6	—	—	0.35	6.9
16-0	26.0	16.3	22.3	31.3	21.7	30.0	27.6	20.2	23.8	27.7	22.0
16.1	2.9	5.3	2.8	3.1	2.4	2.3	2.15	2.6	5.6	1.59	2.5
*17-h.br	—	—	—	—	0.18	—	0.63	—	—	—	—
17-0	0.39	0.24	0.18	0.25	0.26	—	0.83	1.14	—	0.81	—
17-br	0.31	1.16	0.41	0.36	0.43	1.31	1.12	1.6	1.41	0.54	—
*17-uns	—	—	—	0.32	—	—	—	—	—	0.28	—
*18-h.br	—	0.27	0.21	0.24	0.27	—	0.74	—	—	—	0.75
18-0	2.0	1.8	1.5	1.45	1.39	6.1	13.3	20.2	20.8	19.6	21.3
18.1	28.0	35.3	31.9	28.4	31.3	20.9	14.6	18.4	16.1	12.9	10.4
18.2	27.0	23.2	29.6	27.3	32.6	15.6	12.2	16.6	7.7	16.7	7.1
18.3	0.56	0.55	0.22	1.6	—	—	—	—	—	—	—
*18.1	—	—	—	—	—	—	—	0.87	—	—	—
*19-?	—	—	—	—	—	—	—	1.19	—	—	—
*19-h.br	—	0.21	—	—	—	—	—	0.45	—	—	—
*19-br	0.66	—	0.06	—	0.20	—	—	—	—	0.47	—
19-0	—	—	0.18	—	—	—	—	—	—	—	—
20-0	0.55	—	—	0.23	—	—	—	—	—	—	—
20.1	0.76	—	0.74	0.43	0.59	—	—	—	—	—	—
20.2	0.45	—	0.27	0.36	0.58	—	—	—	—	—	1.6
20.3	0.55	0.76	0.46	0.46	0.81	—	—	1.68	—	1.25	7.3
20.4	2.0	10.7	1.6	1.5	2.4	2.1	7.8	12.8	6.7	13.6	—
20.5	0.51	0.62	0.59	—	—	—	—	—	—	—	—
21-uns	0.83	—	—	—	—	—	—	—	—	Trace	11.8
22.5	1.4	—	1.11	0.83	1.21	2.2	—	—	—	2.06	—
22.6	3.1	0.76	2.9	1.01	1.72	2.9	3.0	—	—	—	—

* Tentative identification only.

Table 8. *Fatty acid distribution in 'fatty supernatant' of rats with and without corn oil in the diet*

Values are given as percentages of the total methyl esters (see Table 4).

Fatty acid	Corn oil	Control	After feeding with corn oil
11-0	—	0.04	—
12-0	0.6	0.17	0.08
14-0	0.7	1.16	1.14
14-1	—	—	0.10
15-0	0.2	0.38	0.48
16-br	—	0.23	—
16-0	15.4	25.06	24.6
16-1	0.23	4.69	4.2
*17-uns	—	0.38	0.38
17-0	—	—	0.36
*18-h.br	—	—	0.25
18-0	4.0	4.6	3.2
18-1	21.2	34.2	33.4
18-2	35.5	22.2	29.6
18-3	—	0.97	—
20-1	1.9	—	0.59
20-3	—	—	0.40
20-4	—	1.48	1.33
22-1	—	—	—
22-5	5.0	—	—
22-6	—	—	0.87
Others	15.3	—	—

* Tentative identification only.

eight rats that had been fed with 1 ml. of corn oil 4 hr. before they were killed. The fatty acid composition of this fraction was compared with that of a 'floating fatty supernatant' prepared from the livers of seven rats on their normal diet. The results are shown in Table 8. Little change occurred in the fatty acid pattern of the fraction after feeding with corn oil.

DISCUSSION

The present work is believed to provide the first detailed investigation of the lipid composition of the cell-sap fraction in a particular organ, although isolated studies on particular tissue-lipid components have been described previously. Ada (1949) reported that 9 % of the cell phospholipids were present in the cell sap. Our values are very much lower than this. The data of Kretchmer & Barnum (1951) on the mouse-liver soluble fraction show a low proportion of phospholipid (16.7 %), but the soluble fraction contained 12 % of the cell fat whereas our value was 4.8 %. It must be borne in mind that these authors ground their tissue in alkaline salt solution instead of sucrose before isolation of the cytoplasmic fractions. They also report a high percentage of choline-containing phospholipids. Marinetti, Erbland & Stotz (1958) found that neutral lipids made up 54.4 % of the fat of a soluble fraction corresponding to the cell sap prepared from pig heart whereas our value for rat-

liver-cell sap is 65.3 %. Lecithin formed 52.4 % of the total phosphatides of the pig-heart soluble fraction but only 40 % of the rat-liver-cell sap was lecithin.

Sphingomyelin is a large constituent of the pig-heart soluble fraction and, as judged in this study by the amount of the lipid fraction eluted with chloroform-methanol (1:4), it is also a main constituent of the rat-liver-cell sap.

Rice, Schotz, Alfin-Slater & Deuel (1953) and Schotz, Rice & Alfin-Slater (1953) have investigated the distribution of cholesterol and cholesterol esters in the rat-liver cell. They used young female rats of a different strain. They reported their 'F' layer, corresponding to the 'floating fatty supernatant' of this study, to contain 62 % of the recovered sterol esters of the whole liver. Our figures are very much lower than this.

The high glyceride content of the cell-sap fraction may be due at least in part to contamination with triglyceride from the 'floating fatty supernatant'. This is particularly rich in triglyceride. Of some interest is the apparent lack of rapid equilibration between dietary fatty acids and those of the 'floating fatty supernatant'. From other studies it is known that the quantity of dietary fatty acids which enters the liver in an acute experiment, such as that described, would be sufficient to replace the fatty acids of this fraction several times over (see Robinson & French, 1960). The minimal changes in the composition of the fatty acids of the 'floating fatty supernatant', which were observed after feeding with a fat of characteristic fatty acid composition, such as corn oil, suggest that such exchange does not occur to any marked extent. Recent studies of Stein & Shapiro (1959), who found that [1-¹⁴C]palmitate injected intravenously was incorporated into the triglycerides of the 'floating fatty supernatant' much less rapidly and extensively than into those of the mitochondria and microsomes, support this view.

SUMMARY

1. The cell sap of the rat liver has been separated, the constituent lipids fractionated on silicic acid and their fatty acid composition determined.
2. A similar study has been carried out on the 'floating fatty supernatant' of the rat liver.
3. Of the total liver fatty acid esters 4.6 % were in the cell sap and 5.1 % of the esters in the 'fatty supernatant'. Both these fractions were largely neutral lipid. About one-third of the cell sterol esters were in the cell sap and about the same proportion of the total lipid in this fraction was sterol ester. Over 90 % of the 'floating fatty supernatant' was triglyceride, representing about one-quarter of the total cell triglycerides.

4. Experiments in which corn oil was fed to rats showed that the 'fatty supernatant' did not rapidly equilibrate with the dietary fat.

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Effect of Carbohydrates on the Intestinal Synthesis of Thiamine in Rats

BY M. C. NATH AND S. K. MEGHAL

Department of Biochemistry, Nagpur University, Nagpur, India

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The intestinal synthesis of thiamine depends on dietary constituents, which affect both the type and number of intestinal micro-organisms (Kuno, 1953; Johansson, 1956; Balakrishnan, Baliga & Rajagopalan, 1957; Mameesh & Johnson, 1958; Nath & Meghal, 1960).

Banerji (1941) observed that the presence of much protein in the basal diet prevented the bradycardia and loss of weight usually associated with thiamine deficiency. Banerji & Yudkin (1942) reported that rats grow and thrive well in the absence of thiamine, provided that they do not have to metabolize dietary carbohydrate. According to Banerji & Yudkin (1942) and Yudkin (1951) the signs of thiamine deficiency are due to the toxic products of disturbed carbohydrate metabolism. As little as 10% of glucose in the diets led to polyneuritis and death (Yudkin, 1951), and sorbitol has been shown to be thiamine-sparing (Morgan & Yudkin, 1957; Jones & Baumann, 1958).

When potato starch is added to the thiamine-free basal diet, rats can grow well and this phenomenon was described by Fridericia, Freudenthal, Gudjohnson, Johanssen & Schaubye (1927) as

'refection'. According to them the end products of fermentation lowered the pH of the intestinal contents and this appeared to promote the growth of micro-organisms which synthesize vitamins of the B group. Nagase & Fugita (1956) reported that cellulose helps in the intestinal synthesis of thiamine in man.

Therefore, we have studied the effect of different carbohydrates on the faecal coliform count and daily excretion of thiamine in the urine and faeces of rats. Hydrolysed 'glucose cycloacetoacetate', which has been shown to contain glucose (1:2-dienol) and has a strong reducing action even in acid medium (Nath & Bhattathiry, 1956), not only helps in prevention of experimental diabetes (Nath & Behki, 1957*a, b*; Nath & Bhattathiry, 1959) and experimental hyperlipaemia induced by high concentrations of saturated fats or cholesterol in the diet (Nath & Saikia, 1959*a, b*), but also stimulates the intestinal biosynthesis of thiamine in rats kept on a thiamine-free diet (Nath & Meghal, 1960). Therefore we also studied the effect of hydrolysed 'glucose cycloacetoacetate' on the thiamine deficiency induced by adding either glucose or sucrose to a thiamine-free basal diet.