Influence of Dietary Carbohydrate, Fat, and Protein on Lipogenesis in Rats

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The incorporation of ^{14}C of acetate-1- ^{14}C into the lipids of the liver and carcass, and the changes in the concentrations of nucleotides and citric acid in the liver were studied in the rats fed individual nutrients; starch, casein or corn oil. And the metabolism of citric acid-1,5- ^{14}C was also investigated after the feeding of nutrients. Lipogenesis in the liver and carcass was more markedly stimulated with starch than with casein or corn oil. In the liver of rats fed starch, the concentration of ATP doubled and that of citric acid was one-half of those with casein or corn oil, respectively. And the conversion of citric acid to carbon dioxide and lipids was stimulated with starch.

In the previous paper,¹⁾ it was reported that lipogenesis was stimulated with an increase in the content of starch both in the starch-casein or starch-corn oil diets, as shown by determination of the incorporation of ^{14}C of acetate-1- ^{14}C into the lipids of the liver and carcass of rats fed diets containing starch and casein, or starch and corn oil at different ratio. Furthermore, the level of ATP increased and that of citric acid decreased with an increase in lipogenesis in the liver.

The present work was carried out to study the effect of individual nutrients, such as carbohydrate, fat, and protein, on lipogenesis in rats, and the relationship between these effects and the concentrations of nucleotides and citric acid in the liver, and between these effects and citric acid metabolism in the rats.

EXPERIMENTAL

Experimental animals. Male rats of the Wistar-Donryu strain weighing approximately 150 g were

trained for 2 weeks to consume a 25% casein diet¹⁾ for only 2 hr a day.

Experiment 1. After the training period, the rats were divided into 4 groups. One group was killed before experimental feeding. The other 3 groups were given 5 g of α -starch, 5 g of casein, and 2 g of corn oil with 3 g of cellulose powder, respectively. Each of the nutrients, containing nearly the same amount of calories, was consumed within 2 hr. After the end of 2 hr of feeding, the rats of each group were injected intraperitoneally with 10 μCi of acetate-1- ^{14}C , having a specific activity of 10.0 mCi/mmole, and placed immediately in a metabolic chamber. The respiratory carbon dioxide was collected by trapping into 2 N sodium hydroxide solution. One hour later the rats were decapitated. The livers were quickly removed from them, placed in acetone containing solid carbon dioxide, and used for analysis. The carcasses, from which the dietary residue in the stomach had been removed, were kept in a deep freezer until they were used for analysis. The frozen carcasses were ground by passing through a meat grinder several times. The radioactivities of the carbon dioxide and lipids in the liver and carcass, and the amounts of glycogen and nucleotides in the liver were measured by the same

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1) T. Kimura and K. Ashida, *Agr. Biol. Chem.* **33**, 915 (1969).

methods as those described in the previous paper.¹⁾ The results on the incorporation of ^{14}C were shown with the relative recovery ratio, which was obtained by comparing the radioactivity of each of the three fractions, *i.e.*, the respiratory carbon dioxide, the lipids of the liver, and those of the carcass, with the sum total of radioactivity found these three fractions.

Experiment 2. Rats trained in such manner as mentioned above were used. After the training period, they fed the nutrients for 2 hr a day as in Experiment 1. The rats were decapitated 2, 6 and 18 hr later. The livers were quickly removed from them, placed in acetone containing solid carbon dioxide, and used to assay the concentration of nucleotides in the same manner as in Experiment 1.

Experiment 3. As in Experiment 1 and 2, rats were trained and fed three nutrients for 2 hr a day. They were decapitated 1 hr later. The livers were quickly removed, placed in acetone containing solid carbon dioxide, and used to assay the concentration of citric acid by the method of Saffran and Denstedt.²⁾ In addition, some liver samples were treated with acetic anhydride and pyridine, cooled then in ice-water, and centrifuged in order to remove the turbidity of glycogen. The concentration of citric acid was determined by measuring the absorption at 420 $\text{m}\mu$ of the resulting supernatant.

Experiment 4. Rats trained as mentioned above were fed the three nutrients in the same manner as in Experiment 1, 2 and 3. Then they were injected intraperitoneally with 5 μCi of citric acid-1,5- ^{14}C , having a specific activity 3.0 mCi/mmole , and placed immediately in a metabolic chamber. The respiratory carbon dioxide was collected by trapping in 2 N sodium hydroxide solution. Thirty minutes later the rats were decapitated and the livers were removed from them. In order to assay the total activity of ^{14}C in the liver or the carcass, the pooled pieces of each tissue from 4 animals were immersed in 3 volumes of 1% sodium hydroxide solution, heated for digestion, and then cooled. After neutralization, water was added to the solution to make up a definite volume. The aliquot was plated for counting. In order to assay the activity of ^{14}C in the 5% trichloroacetic acid-soluble fraction in the liver and the carcass, the pooled pieces of each

tissue from 4 animals were homogenized with 5 volume of 5% trichloroacetic acid solution two times and centrifuged. The resulting supernatant was neutralized and made up to a definite volume. The aliquot was mixed an equal volume of 5% gelatin solution, and the mixture was plated for counting.

The amounts and radioactivities of the lipids were estimated in the same manner as in the previous experiments.

Organic acids in the liver were isolated by the method of Bryant *et al.*³⁾ A liver specimen weighing 4 g, which had been pooled from 4 rats, was homogenized with 25 ml of 95% ethanol solution and centrifuged at $10,000\times g$ for 10 min. The resultant supernatant was evaporated under reduced pressure and dissolved in approximately 10 ml distilled water. This solution was passed through Amberlite IRA 40 and then 60 ml of distilled water was passed to remove sugars and amino acids. After elution with 25 ml of 1 N ammonium carbonate, the solution containing the acids and excess eluant were evaporated to dryness and the residue was brought to the required volume with distilled water. This concentrated extract was chromatographed on Toyo Roshi No. 50 filter paper by using ascending chromatography with a solvent system of butanol/formic acid/water (10:2:15). The chromatogram was scanned for radioactivity by the Aloka JPL-105 radioactive chromatogram counter, and to identify the acids 0.04% chlorophenol red solution in ethanol⁴⁾ was used as the indicator for the standard acids.

RESULTS

Experiment 1. The incorporation of ^{14}C into respiratory carbon dioxide and the lipids of the liver and carcass, and the concentration of nucleotides in the liver 1 hr after injection of acetate-1- ^{14}C following 2 hr feeding with nutrient

The results presented in Table I show the relative recovery ratio of ^{14}C in the respiratory carbon dioxide, the lipids of the liver and those of carcass. The feeding of starch resulted in

3) F. Bryant and B. T. Overell, *Biochim. Biophys. Acta*, **10**, 471 (1953).

4) R. J. Block, E. L. Durrum and G. Zweig, "A Manual of Paper Chromatography and Paper Electrophoresis", Academic Press Inc., New York, N.Y. 1955, p. 169.

2) M. Saffran and O. F. Denstedt, *J. Biol. Chem.*, **175**, 849 (1948).

increase in the incorporation into the lipids of the liver and carcass, but the feeding of casein or corn oil did not cause any significant alteration. The level of glycogen in the liver was markedly elevated with the feeding of starch.

The concentration of nucleotides in the liver is given in Table II. In comparing the level of nucleotides, it seemed preferable to calculate the relative value of each nucleotide. This

value was expressed as percentage to the level of ADP, which was relatively constant against changes in the dietary components. Table III shows these relative values of nucleotides. The feeding of starch induced marked changes in the level of nucleotides. The level of ATP and the sum total of GTP and UTP increased with the feeding of starch, but decreased a little or was unaffected with the feeding of casein or corn oil. On the other hand, the

TABLE I. EFFECTS OF DIETARY STARCH, CASEIN OR CORN OIL ON INCORPORATION OF ^{14}C OF ACETATE- $1\text{-}^{14}\text{C}$ INTO RESPIRATORY CARBON DIOXIDE, AND LIVER AND CARCASS LIPIDS¹⁾

	No. of rats	Liver glycogen % of wet wt.	Relative recovery ratio ²⁾		
			Respiratory CO_2 %	Liver lipids %	Carcass lipids %
Before feeding	5	$0.45 \pm 0.30^3)$	95.6 ± 0.9	0.4 ± 0.12	4.0 ± 0.60
Starch	5	4.76 ± 0.69	70.0 ± 1.8	8.6 ± 2.28	21.4 ± 2.81
Casein	5	0.25 ± 0.09	93.7 ± 1.0	1.9 ± 0.96	4.4 ± 0.75
Corn oil	5	0.21 ± 0.08	95.4 ± 1.2	0.5 ± 0.10	4.1 ± 1.18

1) Rats were allowed to gain access to feed for 2 hr, prior to injection with acetate- $1\text{-}^{14}\text{C}$ being housed in a metabolic chamber for 1 hr.

2) The ratio of radioactivity in each of the three fractions, *i.e.*, the respiratory carbon dioxide, and lipids of the liver, and those of the carcass, to the sum total of radioactivity found in these three fractions.

3) Mean $\pm$ S. E.

TABLE II. CONCENTRATIONS OF NUCLEOTIDES IN LIVER AFTER FEEDING OF NUTRIENTS¹⁾

	CMP	AMP	GMP	IMP	UMP	ADP	UDPGlcNAc ²⁾ +UDPGlc	GDP	UDP	ATP	GTP +UTP
	$\mu\text{moles/g}$										
Before feeding	0.11 ³⁾	1.78	0.21	0.30	0.76	1.45	1.35	0.29	0.52	0.90	0.31
Starch	0.19	1.08	0.17	0.23	0.39	1.28	0.55	0.24	0.23	1.52	0.43
Casein	0.18	2.03	0.23	0.23	0.85	1.48	1.17	0.24	0.27	0.90	0.28
Corn oil	0.19	2.47	0.35	0.41	0.80	1.60	1.05	0.21	0.27	0.82	0.22

1) Rats were allowed to gain access to feed for 2 hr, and then kept away from food for 1 hr, prior to killing.

2) UDP-N-acetylglucosamine and UDP-glucose.

3) Each experiment was carried out with pooled liver of 5 rats.

TABLE III. RELATIVE CONCENTRATIONS¹⁾ OF NUCLEOTIDES IN LIVER AFTER FEEDING OF NUTRIENTS

	CMP	AMP	GMP	IMP	UMP	ADP	UDPGlcNAc ²⁾ +UDPGlc	GDP	UDP	ATP	GTP +UTP
Before feeding	8	126	14	21	52	100	93	20	36	62	22
Starch	15	85	13	18	30	100	43	18	18	119	33
Casein	12	137	15	15	56	100	79	16	18	61	19
Corn oil	12	155	22	26	50	100	66	38	17	52	14

1) The concentration of ADP is taken as the standard for nucleotide concentrations, which are shown in Table II.

2) UDP-N-acetylglucosamine and UDP-glucose.

level of AMP and UMP decreased with the feeding of starch, but increased a little or was not changed at all with the feeding of casein or corn oil.

Experiment 2. Periodic changes in the level of ATP and AMP in the liver after feeding starch or casein

After the feeding of starch, the concentration of ATP increased at 4 hr and then declined gradually till 20 hr, but the concentration of AMP decreased at 4 hr and increased gradually till 20 hr after feeding (Table IV). After the feeding of casein, however, the concentrations of ATP and AMP showed a trend nearly reverse to those of the feeding of starch till 8 hr.

TABLE IV. CHANGES IN RELATIVE CONCENTRATIONS¹⁾ OF ADENINE NUCLEOTIDES IN LIVER OF RATS WITH TIME AFTER FEEDING OF STARCH OR CASEIN

	AMP	ADP	ATP
Before feeding	134 ²⁾	100	86
4 hr after feeding of starch	79	100	136
8 hr after feeding of starch	96	100	107
20 hr after feeding of starch	115	100	57
4 hr after feeding of casein	174	100	53
8 hr after feeding of casein	147	100	80
20 hr after feeding of casein	162	100	57

1) The concentration of ADP is taken as the standard for nucleotide concentrations.

2) Each experiment was carried out with pooled liver of 5 rats.

Experiment 3. Changes in the concentration of citric acid in the liver after feeding with nutrients

Table V shows the concentration of citric acid in the liver 3 hr after the feeding of starch, casein, or corn oil. The concentration of citric acid following the feeding of casein or corn oil was nearly the same as that before the feeding. The feeding of starch, however, caused a significant reduction in the concentration of citric acid.

TABLE V. CONCENTRATION OF CITRIC ACID IN LIVER AFTER FEEDING OF NUTRIENTS¹⁾

	No. of rats	Citric acid $\times 10^{-4}M$
Before feeding	4	$2.4 \pm 0.32^2)$
Starch	4	1.3 ± 0.11
Casein	4	2.7 ± 0.11
Corn oil	4	2.6 ± 0.30

1) Rats were allowed to gain access to feed for 2 hr, and then kept away from food for 1 hr, prior to killing.

2) Mean $\pm$ S. E.

Experiment 4. Changes in the metabolism of citric acid-1,5-¹⁴C after feeding with nutrients

Since the concentration of citric acid in the liver had been found to vary with the dietary component, the metabolism of citric acid was investigated in this experiment. Table VI shows the relative recovery of ¹⁴C from the carbon dioxide, the liver, and carcass 30 min after injection with citric acid-1,5-¹⁴C which had been made 2 hr after feeding with nutrients. The incorporation of ¹⁴C into the respiratory carbon dioxide was greater and that into the liver and carcass was smaller in rats given starch than in those given corn oil or casein, and those before feeding. Almost all the radioactivity in the liver of rats fed starch, casein or corn oil was found in the 5%-TCA-soluble fraction and in the lipids. Although the incorporation of ¹⁴C into the liver was a little smaller in the rats given starch than in those of any other group, the incorporation of ¹⁴C into the lipids of the liver was extremely greater in the rats given starch than in those given casein or corn oil. The same, but not so remarkable, trend was observed in the lipids of the carcass.

Figure 1 shows the radiopaperchromatogram of the organic acid fraction of the liver 30 min after injection with citric acid-1,5-¹⁴C performed 2 hr after feeding the nutrients. In it, the highest peak of radioactivity was identified as citric acid. Accordingly, it was assumed that citric acid-1,5-¹⁴C had entered the liver almost as itself, and that it had hardly been metabolized into any other component of the organic acid 30 min after injection.

TABLE VI. METABOLISM OF CITRIC ACID-1,5-¹⁴C AFTER FEEDING OF NUTRIENTS¹⁾

	No. of rats	Respiratory CO ₂ %	Relative recovery ratio ²⁾					
			Liver			Carcass		
			Total %	5% TCA sol. fraction %	Lipids %	Total %	5% TCA sol. fraction %	Lipids %
Before feeding	4	46.6 ³⁾	2.3 ⁴⁾	1.50 ⁴⁾	0.07 ³⁾	51.1 ⁴⁾	39.3 ⁴⁾	0.36 ³⁾
Starch	4	63.1	1.8	1.32	0.40	35.1	23.9	0.81
Casein	4	50.5	2.3	2.14	0.10	47.2	32.6	0.35
Corn oil	4	50.4	2.4	2.20	0.08	47.2	36.0	0.52

1) Rats were allowed to gain access to feed for 2 hr, prior to injection with citric acid-1,5-¹⁴C, being housed in a metabolic chamber for 30 min.

2) The ratio of radioactivity in each of the seven fractions to the sum total of radioactivity found in the three fractions, *i.e.*, the respiratory carbon dioxide, the liver, and the carcass.

3) Average value of 4 rats.

4) From pooled liver or carcass of 4 rats.

FIG. 1. Radioactivity on Paper Chromatogram of Organic Acid Fraction of Liver after Feeding of Nutrients.

The method is described in the text.

DISCUSSION

In the present experiments, when the stimulation of lipogenesis in the liver was caused by the feeding of starch, the concentration of ATP nearly doubled and the concentration of citric acid reduced to half. On the other hand, the feeding of casein or corn oil, or fasting failed to stimulate lipogenesis in this organ (Table II and V). These observations suggest that there may be a correlation between lipogenesis in the liver and the changes of the concentration of ATP and citric acid in this organ in the rats fed individual nutrients, such as carbohydrate, fat or protein, as in the previous study,¹⁾ in which lipogenesis was stimulated with an increase in starch content both in the starch-casein and starch-corn oil diets. From these facts, the changes in lipogenesis with time after the feeding of starch or casein may be supposed by the periodic changes of the concentration of ATP in the liver shown in Table IV.

It was also observed in the previous study¹⁾ that there was a rapid drop in lipogenesis in the liver with an increase in corn oil content in the starch-corn oil diets, while lipogenesis decreased gradually with an increment in casein content in the starch-casein diets. These finding could be explained by the present data that the feeding of fat has little effect

on lipogenesis in the liver, whereas the feeding of casein led to low stimulation in lipogenesis in this organ (Table I).

The incorporation of ^{14}C of citric acid-1,5- ^{14}C into the lipids of the liver was higher with the feeding of starch than with that of casein or corn oil, as shown in Table VI. This finding seems to be consistent with the changes in the activity of citrate-cleavage enzyme in the liver of animals fed experimental diets as reported by Kornacker and Lowenstein.^{5,6)} The citrate-cleavage enzyme was suppressed on starvation and restored on refeeding after starvation. The increase in

enzyme activity that occurred on refeeding depended on the diet. It was largest on feeding a high carbohydrate and low fat diet, and smallest on feeding a high fat diet. The changes in activity of citrate-cleavage enzyme was correlated with fatty acid synthesis. On the other hand, Srere and Foster⁷⁾ proposed recently that no suppressed lipogenesis in starvation might be due to decreased levels of citrate-cleavage enzyme. The data of Table VI indicate that the feeding of starch stimulated citrate to be converted to lipids and carbon dioxide, although the primary cause of this conversion remains obscure.

5) M. S. Kornacker and J. M. Lowenstein, *Biochem. J.*, **94**, 209 (1965).

6) M. S. Kornacker and J. M. Lowenstein, *ibid.*, **95**, 832 (1965).

7) P. A. Srere and D. W. Foster, *Biochem. Biophys. Res. Comm.*, **26**, 556 (1967).