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The Effects of Streptozotocin Diabetes and of Dietary Protein Content on the Composition and Metabolism of Testicular Lipids (41963)

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Abstract. The effect of streptozotocin-induced diabetes on the fatty acid composition and metabolism in testes of rats on diets varying in protein content has been investigated. The protein content of the diet (40, 20, 5%) had little or no effect on essential fatty acid metabolism during the 2 weeks following injection of streptozotocin, but the 5% diet resulted in a high rate of mortality for diabetic rats. Increased amounts of octadeca-9,12-dienoic (linoleic or 18:2) acid and of eicosa-8,11,14-trienoic (dihomo-γ-linolenic or 20:3) acid and decreased amounts of eicosa-5,8,11,14-tetraenoic (arachidonic or 20:4) acid were observed in testes of some but not all diabetic compared to pair-fed control rats 2 weeks after injection of streptozotocin. Incorporation of ^{14}C from [^{14}C]18:2 into testicular lipids of these rats was determined 26 hr after intratesticular injection. In some rats there was a greater amount of ^{14}C in eicosa-11,14-dienoic acid (dihomolinoleic acid or 20:2) and 20:3 and less ^{14}C in 20:4 of testes of diabetic than in those of control rats. The suggested impairment in conversion of 18:2 to 20:4 was studied further by using [^{14}C]20:3 as the substrate for intratesticular injection. Four hours after administration of the [^{14}C]polyene there was more ^{14}C in 20:3 and less ^{14}C in 20:4 and in docosa-7,10,13,16-tetraenoic (adrenic or 22:4) acid in testes of diabetic than in those of control rats. The results indicate that in diabetic rats at least one enzyme responsible for the decreased conversion of 18:2 to 20:4 is the Δ^5-desaturase. © 1984 Society for Experimental Biology and Medicine.

Polyunsaturated fatty acids of the linoleic acid family appear to have an important role in reproduction in the male rat (1, 2). Conditions which result in decreased amounts of testicular polyenes, such as essential fatty acid deficiency, also result in testicular degeneration (3). Experimentally induced diabetes in the rat is also associated with testicular degeneration (4). In the liver the desaturation of octadeca-9,12-dienoic (linoleic or 18:2) acid was reported to be impaired by experimental diabetes (5), and there appeared to be an inhibition in the conversion of octadeca-6,9,12-trienoic (γ-linolenic or 18:3) acid to eicosa-5,8,11,14-tetraenoic (arachidonic or 20:4) acid (6). In alloxan-diabetic rats with pronounced testicular atrophy there was a marked decrease in the amount of intratesticularly injected [^{14}C]18:2 that was converted into 20:4 and docosa-4,7,10,13,16-pentaenoic (22:5) acids (7).

The decreased desaturase activity in livers of diabetic rats (8, 9) is reflected in the lower relative amount of 20:4 found in liver and serum phospholipids (10). Diets rich in protein may be effective in opposing the effects of diabetes on fatty acid metabolism in liver (11).

Because of the importance of polyenoic acid metabolism in reproduction in the male rat, we have investigated the effect of diabetes on the lipid and fatty acid composition and on the testicular metabolism of [^{14}C]polyene precursors of 20:4 and 22:5 acids at a relatively short time period after injection of streptozotocin. A short time period was used in order to study lipid changes before the testes had undergone significant degeneration. Because of the possible effect of high protein diets on diabetes (11) the effect of varying the protein content of the diet was also studied.

Materials and Methods. Sprague–Dawley rats (procured either from Harlan, Inc., Indianapolis, Ind., or Sasco, Inc., Omaha, Nebr.) of 9 to 10 weeks of age were used. In Experiment 1 the rats were fed one of three diets: A, the AIN-76 diet (12) but containing 40% protein; B, the AIN-76 standard diet containing 20% protein; or C, the AIN-76 diet but containing only 5% protein. The variable protein content was balanced by

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adjusting the sucrose content. Rats fed diets A and B were injected intraperitoneally with one 75 mg/kg body wt dose of streptozotocin dissolved in 0.9% saline immediately before use. Control rats (nondiabetic) were injected intraperitoneally with an equal volume of 0.9% saline and were pair-fed to the rats injected with streptozotocin. The rats were pair-fed because the food intake of the streptozotocin-injected rats decreased significantly during the first few days after injection. Two weeks after the induction of diabetes the rats were injected intratesticularly with 1 to 2 μCi [1-^{14}C]18:2 (New England Nuclear Corp., Boston, Mass.; sp act 54.9 mCi/mmole) complexed to serum albumin. The rats were decapitated 26 hr later. Because of high mortality rates of the rats on the 5% protein diet the dose of streptozotocin for this group was reduced to 60 mg/kg body wt and they were injected intratesticularly with [^{14}C]18:2 1 week after induction of diabetes.

In Experiment 2 all the rats were fed only the AIN-76 standard diet (20% protein). They were injected intraperitoneally with one 75 mg/kg body wt dose of streptozotocin. Control rats were injected with an equal volume of 0.9% saline and were pair-fed to the diabetic. Two weeks later all rats were injected intratesticularly with 1 μCi of [1-^{14}C]20:3 (New England Nuclear Corp., sp act 54.9 mCi/mmole) complexed to serum albumin. The rats were killed 4 hr after injection of the radioisotopic compound.

Some of the animals of both experiments were kept in Roth metabolic cages after ^{14}C administration for collection of expired $^{14}CO_2$.

Glucose determinations. The serum glucose concentration was determined by a glucose oxidase assay (Sigma Chemical Co. No. 510, St. Louis, Mo.).

Analyses of lipids. Total lipids were extracted with chloroform/methanol (2/1 v/v) and aliquots used for the determination of phospholipids by the method of Doizaki and Zieve (13) and triacylglycerols by the method of Soloni (14).

Methods for thin-layer chromatography of total lipids have been reported previously (13, 15). Lipid classes were identified by using known standards spotted on a lane next to the samples. For ^{14}C determination the silica gel containing each lipid was scraped directly into a counting vial. For gas chromatographic analysis of fatty acids from phospholipids or triacylglycerols the silica gel was transferred to a Teflon-lined screw-capped tube and the lipids transesterified according to the methods of Morrison and Smith (16).

Analytical gas chromatography of methyl esters was done using a Varian Aerograph Model 1520 gas chromatograph equipped with flame ionization detector and a column (8 ft × $\frac{1}{8}$ in. i.d.) packed with 10% SP 2340 on 110/120-mesh Supelcoport. The temperature was programmed from 175 to 225°C (at 5°C/min. Known standards were used for qualitative and quantitative calibration. Measurement of peak areas was done using a Hewlett–Packard Model 3390 A Reporting Integrator.

^{14}C incorporation. Radioisotopic determinations were done using a Packard Tri-Carb Model 3000 scintillation spectrometer. Separation of fatty acid methyl esters for ^{14}C determination was accomplished as reported previously (15) using a Varian Model 1800 gas chromatograph equipped with a thermal conductivity cell, the exit port of which was connected to a heated Packard Instrument Company fraction collector. The methyl esters were trapped in glass wool packed into glass cartridges. The glass wool and cartridge were transferred to counting vials for ^{14}C determination. Eighty to ninety percent of the radioactivity injected into the column was usually recovered in the fatty acids collected.

Expired $^{14}CO_2$ was trapped in ethanolamine and the ^{14}C determined by scintillation spectrometry using the method of Jeffay and Alvarez (17).

When necessary appropriate corrections were made for quenching.

Statistical analysis. Data were analyzed for statistical significance by the two-tailed Student t test except where it is noted that the one-tailed t test was used (18).

Results. Diabetic rats had serum glucose concentrations of 400–1100 mg/100 ml with most values between 400 and 600 (mean, 493 ± 27). Serum glucose concentrations of control rats were always below 200 mg/100 ml (mean 128 ± 17). Diabetic rats generally

lost weight, especially during the initial portion of the 2-week period. Control rats gained weight consistently, except when pair-feeding severely restricted food intake because of the poor intake of diabetic rats during the first three or four days after injection of streptozotocin. By the end of the two week period the diabetic rats averaged about 100 g lower body weights than their pair-fed controls. Hematocrits and liver weights of the diabetic rats were not significantly different than those of their pair-fed controls.

The weights of the testes of diabetic rats were slightly lower than those of control rats on both the 40% (3.22 ± 0.10 g vs 3.55 ± 0.09 g; $P < 0.05$) and on the 20% (3.02 ± 0.21 g vs 3.64 ± 0.07 g; $P < 0.01$) protein diets. In the group given 5% protein the weights of testes of diabetic and control rats were similar (3.64 ± 0.12 g vs 3.66 ± 0.10 g), but these rats were killed 1 week after injection of streptozotocin.

There were no statistically significant differences in the concentrations of testicular triacylglycerols and of phospholipids between diabetic and control rats on any of the diets. However, testes of control rats on the 40% protein diet had slightly higher concentrations of phospholipids than did those of control rats on the 5% protein diet (13.1 ± 0.2 mg/g vs 11.6 ± 0.4 mg/g; $P < 0.05$).

There were significant differences in the fatty acid composition of the testicular phospholipids between diabetic and control rats (Table I). Of major interest is the higher percentage of 18:2 in diabetic rats (statistical significance achieved only for rats on the 5% protein diet) and in 20:3 (statistically significant in the 40% protein group) and the decreased 20:4 (statistically significant in the rats on the 20% protein diet). There was a trend toward slightly lower values for 22:4 in the diabetic rats, but there were no differences in values for 22:5 between diabetic and control rats. In the testicular triacylglycerols there were also significant differences in 20:3 values between diabetic and control rats on the 40 and 20% protein diets (0.9 ± 0.1 diabetic vs 0.6 ± 0.04 control, $P < 0.02$ for the 40% protein diet and 1.6 ± 0.2 diabetic vs 0.8 ± 0.1 control, $P < 0.02$ for the 20% protein diet).

In Experiment 2 (control and diabetic rats fed the standard diet and injected with [^{14}C]18:2) the significant changes were in 20:4 of phospholipids (17.0 ± 0.05 diabetic vs 17.8 ± 0.1 control, $P < 0.02$) and in 22:4 (1.1 ± 0.05 diabetic vs 1.4 ± 0.1 control, $P < 0.02$).

The incorporation of ^{14}C into testicular lipids of rats on the three diets was determined 26 hr after intratesticular injection of [^{14}C]18:2. In rats on the 20 and 5% protein

TABLE I. FATTY ACID COMPOSITION OF TESTICULAR PHOSPHOLIPIDS OF DIABETIC AND CONTROL RATS[a]

	Diet					
	40% Protein		20% Protein		5% Protein	
Fatty acid	Control (5)	Diabetic (5)	Control (8)	Diabetic (8)	Control (4)	Diabetic (4)
16:0	35.7 ± 0.6	34.4 ± 0.4	35.9 ± 0.5	37.9 ± 1.2	36.6 ± 1.1	36.3 ± 1.5
18:0	7.6 ± 0.2	7.1 ± 0.2	7.4 ± 0.1	7.0 ± 0.2	7.2 ± 0.5	6.6 ± 0.1
18:1	12.4 ± 0.2	12.5 ± 0.8	11.3 ± 0.3	12.2 ± 0.5	12.4 ± 1.1	12.3 ± 0.4
18:2	3.9 ± 0.2	4.7 ± 0.1	4.5 ± 0.2	5.1 ± 0.3	5.0 ± 0.1	6.2 ± 0.2[d]
20:3	0.7 ± 0.04	0.9 ± 0.04[b]	0.8 ± 0.04	1.0 ± 0.1	0.8 ± 0.1	0.8 ± 0.03
20:4	19.2 ± 0.4	18.1 ± 0.4	18.4 ± 0.4	15.9 ± 0.4[c]	17.6 ± 1.3	17.4 ± 0.6
22:4	1.1 ± 0.05	1.0 ± 0.4	1.2 ± 0.06	1.0 ± 0.1	1.4 ± 0.4	0.9 ± 0.05
22:5	16.7 ± 0.3	17.8 ± 0.5	16.4 ± 0.5	15.8 ± 0.7	15.9 ± 0.8	15.9 ± 1.2

[a] The rats were killed 2 weeks after intraperitoneal injection of streptozotocin. Results are expressed as weight percentages; means ± SE; figures in parentheses are the numbers of samples. Not all the fatty acids are listed; therefore, these percentages do not total 100%.
[b] Different than control rats, $P < 0.02$.
[c] Different than control rats, $P < 0.001$.
[d] Different than control rats, $P < 0.01$.

TABLE II. INCORPORATION OF ^{14}C FROM [1-^{14}C]LINOLEATE INTO FATTY ACIDS OF PHOSPHOLIPIDS OF TESTES OF DIABETIC AND CONTROL RATS[a]

	Diet					
	40% Protein		20% Protein		5% Protein	
Fatty acid	Control (5)	Diabetic (5)	Control (8)	Diabetic (8)	Control (4)	Diabetic (4)
16:0	3.0 ± 0.3	5.0 ± 0.5[b]	7.0 ± 1.4	11.6 ± 2.1	3.6 ± 0.2	5.1 ± 0.5[d]
18:2	60.0 ± 1.8	63.3 ± 1.7	57.8 ± 1.9	56.3 ± 1.6	62.8 ± 1.4	59.9 ± 1.5
20:2	1.8 ± 0.1	2.5 ± 0.1[b]	1.7 ± 0.1	2.5 ± 0.2[b]	2.2 ± 0.2	2.0 ± 0.2
20:3	5.5 ± 0.2	5.1 ± 0.1	5.5 ± 0.2	5.7 ± 0.4	5.1 ± 0.2	5.9 ± 1.2
20:4	19.6 ± 1.2	13.3 ± 0.8[b]	17.9 ± 1.0	13.3 ± 1.2[c]	14.4 ± 0.8	15.4 ± 0.4
22:4	1.6 ± 0.07	1.1 ± 0.1[b]	1.6 ± 0.1	1.3 ± 0.1	1.6 ± 0.1	1.7 ± 0.2
22:5	1.2 ± 0.1	1.4 ± 0.1	1.1 ± 0.06	1.7 ± 0.2[c]	1.2 ± 0.05	1.6 ± 0.02[e]

[a] The rats were killed 2 weeks after injection of streptozotocin and 26 hr after intratesticular injection of [^{14}C]linoleate. Results are expressed as percentages of recovered ^{14}C; means ± SE; figures in parentheses are the numbers of samples. Not all the fatty acids are listed; therefore, these percentages do not total 100%.

[b] Different than control, $P < 0.01$.

[c] Different than control, $P < 0.02$.

[d] Different than control, $P < 0.05$.

[e] Different than control, $P < 0.001$.

diets from 11 to 18% of the injected dose of ^{14}C was recovered in testicular lipids, and there were no statistically significant differences between diabetic and control rats. In the animals on the 40% protein diet there was less ^{14}C in lipids of testes of diabetic than in those of control rats (9.0 ± 1.1 vs 17.7 ± 0.8, $P < 0.001$). About 20 to 25% of the injected dose of ^{14}C was recovered in expired $^{14}CO_2$ in all groups, and no differences were detected between the diabetic and control rats.

In Table II are the results of ^{14}C recovered in various fatty acids of testicular phospholipids 26 hr after the intratesticular injection of [^{14}C]18:2. About 60% of the recovered radioactivity was still in 18:2. In all dietary groups more ^{14}C was recovered in hexadecanoic (palmitic acid or 16:0) of diabetic than of control rats. There was a lower incorporation of ^{14}C into 20:4 and a higher incorporation of ^{14}C into 20:2 of diabetic than of control rats in the 40 and 20% protein groups. In the rats fed the 40% protein diet there was also significantly less ^{14}C in 22:4 of diabetics compared to controls.

In the triacylglycerol fatty acids a greater incorporation of ^{14}C into 16:0 of diabetic compared to controls was seen in all groups but was statistically significant only in the rats on 20% protein (Table III). There was more ^{14}C in 20:3 of diabetic than in that of control rats for both the 40 and 20% protein groups, while the amount of ^{14}C in 20:2 was greater in diabetic rats of all groups (though statistically significant only for the rats on 40% protein). In this group there was also a statistically significant lower ^{14}C incorporation into 20:4 and 22:4 of diabetic compared to control rats.

Incorporation of ^{14}C from [^{14}C]20:3 into testicular lipids of diabetic and control rats was determined only in animals fed the 20% protein diet (Experiment 2). About 50% of the injected ^{14}C was recovered in lipids of the testes 4 hr after the intratesticular injection, and there were no differences between diabetic and control rats. Similar amounts of ^{14}C were expired as $^{14}CO_2$ (about 15% of the injected dose in 4 hr) by diabetic and control rats.

Results of the recovery of ^{14}C in fatty acids of testicular phospholipids and of triacylglycerols are given in Table IV. In the phospholipids there was more ^{14}C in 20:3 and less ^{14}C in 20:4 and 22:4 of the diabetic rats than in the controls. In the triacylglycerols similar results were obtained but only the difference in arachidonic acid was statistically significant.

TABLE III. INCORPORATION OF ^{14}C FROM [1-^{14}C]LINOLEATE INTO FATTY ACIDS OF TRIACYLGLYCEROLS OF TESTES OF DIABETIC AND CONTROL RATS[a]

	Diet					
	40% Protein		20% Protein		5% Protein	
Fatty acid	Control (5)	Diabetic (5)	Control (4)	Diabetic (4)	Control (4)	Diabetic (4)
16:0	5.7 ± 0.3	7.2 ± 0.7	4.8 ± 1.1	8.0 ± 0.2[e]	4.1 ± 0.6	4.7 ± 0.5
18:2	48.7 ± 1.6	42.7 ± 3.3	56.7 ± 5.3	40.4 ± 1.8[b]	60.4 ± 1.8	59.2 ± 2.6
20:2	3.8 ± 0.2	5.5 ± 0.3[b]	3.4 ± 0.9	6.2 ± 0.7	4.0 ± 0.4	4.6 ± 0.1
20:3	9.3 ± 0.4	12.5 ± 1.0[c]	7.7 ± 0.9	13.0 ± 1.2[c]	6.8 ± 0.4	7.4 ± 0.4
20:4	8.7 ± 0.7	6.1 ± 0.2[d]	6.4 ± 0.6	6.5 ± 0.2	5.2 ± 0.7	4.6 ± 0.5
22:4	6.3 ± 0.2	4.4 ± 0.2[b]	5.0 ± 0.4	4.7 ± 0.6	4.3 ± 0.2	4.0 ± 0.2
22:5	4.2 ± 0.2	4.1 ± 0.2	3.4 ± 0.8	4.0 ± 0.5	2.9 ± 0.3	3.4 ± 0.6

[a] The rats were killed 2 weeks after injection of streptozotocin and 26 hr after intratesticular injection of [^{14}C]linoleate. Results are expressed as percentages of recovered ^{14}C; means ± SE; figures in parentheses are numbers of samples. Not all the fatty acids are listed; therefore, these percentages do not total 100%.
[b] Different than control, $P < 0.001$.
[c] Different than control, $P < 0.02$.
[d] Different than control, $P < 0.01$.
[e] Different than control, $P < 0.05$.

The fatty acid composition of the liver phospholipids of diabetic and control rats on the three diets was also determined, and the results for some selected fatty acids are given in Table V. There was less 16:0 and more 18:2 in livers of diabetic rats (all three diets) than in those of control rats. In rats on the 40 and 20% protein diets there was less 20:4 in livers of diabetics than in those of controls.

Discussion. The effect of diabetes on the weight of the testes in rats used in this study is that expected in view of reports that after 2 weeks of the diabetic state the testes are in the early stages of a progressive degeneration (19, 20). These studies were done on rats only 1 or 2 weeks following injection of the diabetes-inducing drug in order to study lipid changes before the testis had undergone significant degeneration. Because of this short period of time it was expected that any compositional changes would be small and might not be observed in all diabetic rats.

TABLE IV. INCORPORATION OF ^{14}C FROM [1-^{14}C]EICOSA-8,11,14-TRIENOATE INTO FATTY ACIDS OF PHOSPHOLIPIDS AND OF TRIACYLGLYCEROLS OF TESTES OF DIABETIC AND CONTROL RATS[a]

	Phospholipids		Triacylglycerols	
Fatty acid	Control (4)	Diabetic (4)	Control (4)	Diabetic (4)
16:0	1.0 ± 0.1	1.1 ± 0.1	7.4 ± 0.4	7.9 ± 1.8
20:3	44.5 ± 1.6	55.9 ± 3.6[b]	55.1 ± 1.9	59.8 ± 3.6
20:4	47.2 ± 0.9	35.7 ± 3.5[c]	15.4 ± 0.5	12.2 ± 1.1[b]
22:4	1.8 ± 0.05	1.3 ± 0.1[d]	8.5 ± 1.2	6.0 ± 1.0
22:5	0.6 ± 0.03	0.6 ± 0.05	2.8 ± 0.4	2.1 ± 0.4

[a] The rats were killed 2 weeks after injection of streptozotocin and 4 hr after intratesticular injection of [^{14}C]eicosatrienoate. Results are expressed as percentages of ^{14}C recovered in fatty acids of each lipid fraction; means ± SE; figures in parentheses are numbers of samples. Statistical analysis by the one-tailed Student t test. Not all the fatty acids are listed; therefore, these percentages do not total 100%.
[b] Different than control, $P < 0.025$.
[c] Different than control, $P < 0.01$.
[d] Different than control, $P < 0.005$.

TABLE V. FATTY ACID COMPOSITION OF LIVER PHOSPHOLIPIDS OF DIABETIC AND CONTROL RATS[a]

Fatty acid	Diet					
	40% Protein		20% Protein		5% Protein	
	Control (5)	Diabetic (5)	Control (4)	Diabetic (4)	Control (4)	Diabetic (4)
16:0	15.7 ± 0.2	14.0 ± 0.5[b]	17.0 ± 0.5	13.4 ± 0.8[c]	19.9 ± 0.6	13.0 ± 0.4[b]
18:0	25.2 ± 1.0	26.8 ± 0.5	24.5 ± 0.5	28.6 ± 0.8[c]	21.4 ± 0.7	23.5 ± 2.8
18:2	12.1 ± 0.9	17.0 ± 0.9[c]	11.6 ± 1.1	17.7 ± 1.1[c]	14.5 ± 0.8	20.6 ± 0.6[c]
20:4	34.2 ± 0.5	28.6 ± 0.8[d]	34.0 ± 0.5	25.2 ± 1.4[c]	30.7 ± 1.0	29.4 ± 2.4

[a] The rats were killed 2 weeks after intraperitoneal injection of streptozotocin. Results are expressed as percentages weight; means ± SE; figures in parentheses are numbers of samples. Not all the fatty acids are listed; therefore, percentages do not total 100%.
[b] Different than control, $P < 0.02$.
[c] Different than control, $P < 0.01$.
[d] Different than control, $P < 0.001$.

This proved to be the case. Larger numbers of animals per group may have resulted in greater and more significant differences. The shorter time of 1 week (after injection of streptozotocin) for rats on the 5% protein diet was necessary because the rats did not survive any longer. Apparently, the diabetic plus poor nutritional states proved to be incompatible with survival.

The studies of testicular fatty acid composition led to the suggestion that there was a defect in the conversion of 18:2 to 20:4 in diabetic rats. The changes, at least in some of the diabetic rats, which supported this suggestion were increased 18:2 and 20:3 and decreased 20:4 in testes of the diabetic animals. The predominant pathway for conversion of linoleic to higher polyenes is as follows: 18:2 → 18:3 → 20:3 → 20:4 → 22:4 → 22:5.

Results of studies of incorporation of ^{14}C from intratesticularly injected [^{14}C]18:2 into testicular fatty acids indicated that the diabetic rats were accumulating ^{14}C in intermediates on the pathway from 18:2 to 20:4 and that they were incorporating less ^{14}C into 20:4 and 22:4 than were the control rats. Accumulation of ^{14}C in 20:3 and decreased incorporation into 20:4 indicated that at least one defect might be that of decreased Δ^5-desaturase activity, i.e., the enzyme which desaturates 20:3 to 20:4. Therefore, [^{14}C]20:3 was used as a substrate for intratesticular injection. The results confirmed the decreased incorporation of ^{14}C into 20:4 in testes of diabetic rats and thus pointed to the Δ^5-desaturation as at least one step responsible for the decreased conversion of 18:2 to 20:4.

Some of the changes in fatty acid composition observed in the testes of diabetic rats were also observed in the liver. The results obtained in liver agree with those reported by others (9, 10). A report by Holman *et al.* (10) noted results of changes in fatty acids in heart, aorta, and serum in rats made diabetic with streptozotocin and killed several weeks later. They suggested that diabetes resulted in impairment of Δ^5-desaturase activity. Marzouki and Coniglio have reported an impairment in the conversion of intratesticularly injected [^{14}C]20:3 to [^{14}C]20:4 in hypophysectomized rats (21). It appears, therefore, that this enzyme is under hormonal control in the testis as well as in the liver and may become rate limiting under certain conditions.

It has been reported that diabetes results in an inhibition in the conversion of 18:2 to 18:3 (Δ^6-desaturase) (8, 9). Our study did not allow this observation. It is also not possible from our studies to evaluate the effect of diabetes on elongation reactions in testes (18:3 to 20:3 and 20:4 to 22:4). The reduced incorporation of [^{14}C]20:3 into [^{14}C]22:4 acid observed in our studies could be due to an impairment in elongation of 20:4 to 22:4 or to the decreased supply of the substrate, 20:4. It is obvious that the conversion of 22:4 to

22:5 (Δ^4-desaturase) also needs additional study.

The larger amount of ^{14}C in 16:0 of testes of diabetic compared to control rats observed after intratesticular injection of [^{14}C]18:2 deserves notice since diabetes normally results in decreased *de novo* synthesis of fatty acids. (It is assumed that ^{14}C incorporation into 16:0 resulted from *de novo* synthesis using [^{14}C]acetyl CoA generated by the β oxidation of [^{14}C]18:2.) This was not reflected in the fatty acid composition of the testes nor was there a difference in ^{14}C in 16:0 between diabetic and control rats in testes of rats injected with [^{14}C]20:3. Furthermore, there was a statistically significant lower amount of 16:0 in liver phospholipids of diabetic rats than in those of controls on all three diets. The reason for the increased incorporation of ^{14}C from [^{14}C]18:2 into 16:0 of testes of diabetic rats is, therefore, not apparent.

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