Tissue Fatty Acid Changes and Tumor Incidence in C3H Mice Ingesting Cottonseed Oil

IAN J. TINSLEY*, GLENN WILSON and ROBERT R. LOWRY,
Department of Agricultural Chemistry, Oregon State University, Corvallis, OR 97331

ABSTRACT

The incidence of spontaneous mammary tumors in C3H mice at 35 wk was higher in mice fed rations containing cottonseed oil than in mice fed fats of comparable fatty acid composition. The time to 50% (T_{50}) incidence was also shorter in the first group. The fatty acid composition of tissue lipids from mice fed the cottonseed oil showed changes indicating the presence of cyclopropene fatty acids–higher levels of saturates and lower oleate/stearate and palmitoleate/palmitate ratios. A possible association between the development of a spontaneous mammary tumor in the C3H mouse and the presence of cyclopropene fatty acids in the cottonseed oil is indicated.
Lipids 17:115-117, 1982.

INTRODUCTION

Sterculic and malvalic acids, cyclopropenoid fatty acids present in cottonseed, have been shown to have a synergistic effect on the induction of liver tumors in rainbow trout by aflatoxin B_1 (1). Subsequently, it has been demonstrated that both are primary hepatocarcinogens in this organism (2). Further experiments have shown that hepatocellular carcinomas can be induced in rainbow trout by feeding diets containing either glandless cottonseed kernels or a lightly processed cottonseed oil (3).

At this stage, these cyclopropenoid fatty acids have not been shown to have either a synergistic or direct carcinogenic effect in mammals. A recent study in our laboratory provides evidence suggesting that these components could be affecting the development of a spontaneous mammary tumor in mice. Mice fed rations containing cottonseed oil developed tumors at a higher rate than animals fed rations containing other oils of comparable fatty acid composition. In addition, the fatty acid composition of tissues from mice fed the cottonseed oil suggested the presence of cyclopropenoid fatty acids.

MATERIALS AND METHODS

The development of spontaneous mammary tumors was observed in female C3H mice fed nutritionally adequate (4) semisynthetic rations containing 10% fat. A total of 20 different fats were used including 11 natural fats and oils and mixtures of those fats and oils. These fats and mixtures were selected such that the levels of different fatty acids were not significantly correlated and statistical procedures were used to define the effect of individual fatty acids on the development of the tumor system. The composition of the 20 fats and other experimental details have been described (5). A food-grade cottonseed oil was used in one experimental diet and was combined with butterfat (60:40) in another.

Four animals from each dietary group were sacrificed after 4 months on experiment and samples of subcutaneous fat from the mammary region and the liver were excised. The fatty acid compositions of the triglyceride fraction of the subcutaneous fat and of the liver lipid were determined (6,7). These analyses were intended to monitor feeding efficiency, confirming fatty acid patterns expected from the different fats. Similar samples were taken from animals (6-7 from each dietary group) with tumors upon termination of the experiment.

RESULTS AND DISCUSSION

The major conclusions of the overall study was that the substitution of linoleate for the other fatty acids enhanced tumorigenesis whereas a comparable substitution of stearate tended to reduce tumor development (5). For the purposes of this analysis, short-term tumor incidence data and calculated time to 50% tumor incidence ($\hat{T}_{50}$) are repeated for those fats showing the 6 lowest values for the second parameter (Table 1). By contrast, mice ingesting diets containing 10% tallow and 10% lard showed the longest $\hat{T}_{50}$, i.e., 68.5 and 70.1 wk, respectively. Data are also included for animals fed olive oil or butter–the butter for comparison with those fed the cottonseed/butter mixture. The fatty acid composition of the dietary fat is also given (Table 2).

It is significant that the lowest $\hat{T}_{50}$ values were observed in mice fed cottonseed oil even when it comprised only 5% of the diet (Table 1). The differential associated with feeding cottonseed oil is even more pronounced if one considers tumor incidence at 35 wk. Tumor incidence in animals ingesting the other 13

TABLE 1

Dietary Fat and Tumor Incidence

Dietary fat	$\hat{T}_{50}$ (wk)	Mice with palpable tumor (%)	
		35 wk	45 wk
Cottonseed	56.3	19	30
Butter (0.6), cottonseed (0.4)	56.3	18	18
Safflower	56.6	10	21
Safflower (0.5), olive (0.5)	59.0	5	15
Span	59.5	8	18
Corn	59.9	9	21
Olive	61.3	0	12
Butter	62.0	8	21
Approx. standard error	± 3-5	± 4	± 6

TABLE 2

Fatty Acid Composition[a] (% by wt)

	14:0	16:0	18:0	18:1	18:2	18:3
Cottonseed	0.59	14.9	3.08	22.5	56.5	2.78
Butter (0.6), cottonseed (0.4)	6.52	27.3	7.98	24.6	30.3	1.61
Safflower	–	6.2	2.48	10.4	80.8	
Safflower (0.5), olive (0.5)	–	9.0	2.51	24.3	46.0	
Span (rapeseed–low erucic)	–	3.9	1.92	60.6	22.7	9.48
Corn	–	10.6	2.11	25.8	60.6	1.0
Olive	–	12.9	2.70	75.7	8.12	
Butter	12.7	39.7	13.8	28.3	2.5	

[a]Each value represents the average of results of analyses of 8 diet samples. Standard deviations are less than 10% and are omitted for clarity.

rations was 8% or less at 35 wk with one exception (a ration containing coconut/safflower, 50:50) where a 15% incidence was observed. The data suggest that lauric acid may be a factor in this case. The tumorigenic effect of the cottonseed oil thus appears to be more pronounced than that of corn oil which has a comparable fatty acid composition, and equivalent to safflower oil which contains considerably higher levels of linoleate. The latter contrast may be tenuous, in that there is some evidence that the tumorigenic response to dietary linoleate is not necessarily linear over an extended range (8).

The feeding of cottonseed oil to C3H mice increases the level of both palmitate and stearate in the triglycerides of subcutaneous fat (Table 3). A significant reduction in the oleate/stearate and palmitoleate/palmitic ratios is also observed. Comparison of the means of the 2 subsets, cottonseed-no cottonseed, using the Students' t-test, indicated a highly significant ($p<.001$) effect of the cottonseed oil in all cases. In animals fed the cottonseed/butter mixture, the effect of the cottonseed is over and above that produced by feeding butter alone. These responses were observed in animals fed for 4 months or until tumor development and sacrifice. Similar changes were observed in liver and tumor lipids. Data from animals fed the other 13 rations were consistent with these observations, i.e., lower saturate content and higher monoene/saturate ratios.

These changes are clearly indicative of the presence of cyclopropene fatty acids in the cottonseed oil and reflect the inhibition of the Δ9-desaturase system (9). Such changes in fatty acid composition may be observed before functional changes are produced (10). Analysis of the cottonseed oil by nuclear magnetic resonance (NMR) spectroscopy did not show the characteristic resonance peaks of the cyclopropene protons (11), suggesting that, if the cyclopropene acids were present, the level was considerably less than 1%.

Allen et al. (10) have produced comparable changes in fatty acid composition in chickens, feeding 2.8 mg/kg/day of methyl sterculate for 12 months. If a comparison between species is valid, a 35-g mouse consuming 4 g of diet a day would require ca. 25 ppm of cyclopropene acid in the diet or 250 ppm (.025%) in the oil.

TABLE 3

Saturate and Monoene Fatty Acids from Triglycerides of Mammary Tissue—4-Month Feeding

	Fatty acid level (% by wt)				Monoene/saturate ratios	
	16:0	16:1	18:0	18:1	16:1/16:0	18:1/18:0
Cottonseed	15.6 ± 4.5[a]	3.33 ± .23	3.60 ± .87	34.8 ± 2.5	.23 ± .07	9.9 ± 1.9
Butter (0.6), cottonseed (0.4)	20.3 ± 1.6	6.15 ± 1.10	3.25 ± .77	45.0 ± 1.2	.31 ± .06	14.5 ± 3.8
Safflower	10.3 ± 0.4	3.83 ± .46	1.33 ± .17	21.9 ± 0.5	.37 ± .05	16.7 ± 1.8
Safflower (0.5), olive (0.5)	10.2 ± 0.7	3.63 ± .36	1.32 ± .17	49.7 ± 2.2	.36 ± .03	37.8 ± 3.5
Span	8.73 ± 1.0	3.17 ± .69	1.18 ± .15	65.1 ± 1.5	.37 ± .07	56.1 ± 7.1
Corn	12.4 ± 0.9	4.13 ± .38	1.40 ± .20	36.7 ± 1.0	.34 ± .03	26.7 ± 5.1
Olive	10.2 ± 0.2	4.43 ± .38	1.07 ± .03	76.9 ± 1.3	.43 ± .04	76.1 ± 21
Butter	17.5 ± 1.4	12.3 ± 0.3	1.70 ± .10	60.1 ± 1.7	.67 ± .02	35.4 ± 2.6

[a]Mean and standard deviation.

This level would not be detected by NMR but is substantially lower than that reported for some cottonseed oils (12,13).

It is not valid to conclude from these data that cyclopropene fatty acids enhance the development of mammary tumors in the C3H mouse. The response of the mice to the cottonseed oil is different from those fed comparable oils and tissue fatty acid data are suggestive of the presence of low levels of cyclopropene acids in the cottonseed oil. This possible association certainly warrants further investigation, given the tumorigenic effects observed in trout (3). Observations of Carroll and Khor would also tend to reinforce these conclusions (14). In rats exposed to 7,12-dimethylbenz(α)-anthracene and fed either corn, cottonseed or soybean oils (oils with comparable fatty acid compositions), invariably the trend, though not necessarily significant, is to observe the more tumorigenic effect in animals fed the cottonseed oil.

This analysis also illustrates the complex problem of defining the effects of different fats and oils on tumorigenesis. Not only is it necessary to establish the contribution of the major fatty acids, but it is important to consider some potentially active minor constituents. These could include the cyclopropene acids or food additives (15) and sterols (16).

ACKNOWLEDGMENTS

The research was supported in part by USPHS Grant no. CA20998 from the National Cancer Institute. Oregon State University Agricultural Experiment Station Technical Paper no. 6000.

REFERENCES

1. Sinnhuber, R.O., Lee, D.J., Wales, J.H., Landers, M.K., and Keyl, A.C. (1974) J. Natl. Cancer Inst. 53, 1285-1288.
2. Sinnhuber, R.O., Hendricks, J.D., Putnam, G.B., Wales, J.H., Pawlowski, N.E., Nixon, J.E., and Lee, D.J. (1976) Fed. Proc. 35, 505.
3. Hendricks, J.D., Sinnhuber, R.O., Loveland, P.M., Pawlowski, N.E., and Nixon, J.E. (1980) Science 208, 309-311.
4. National Research Council Nutritional Requirements of Laboratory Animals, p. 47, Washington, DC, National Academy of Sciences.
5. Tinsley, I.J., Schmitz, J.A., and Pierce, D.A. (1981) Cancer Res. 41, 1460-1465.
6. Saddler, J.B., Lowry, R.R., Krueger, H.M., and Tinsley, I.J. (1966) J. Am. Oil Chem. Soc. 43, 321-324.
7. Lowry, R.R., and Tinsley, I.J. (1975) J. Am. Oil Chem. Soc. 52, 298-299.
8. Carroll, K.K., and Hopkins, G.J. (1978) Lipids 14, 155-158.
9. Raju, P.K., and Reiser, R. (1967) J. Biol. Chem. 242, 379-384.
10. Allen, E., Johnson, A.R., Fogerty, A.C., Pearson, J.A., and Shenstone, F.S. (1967) Lipids 2, 419-423.
11. Pawlowski, N.E., Nixon, J.E., and Sinnhuber, R.O. (1972) J. Am. Oil Chem. Soc. 49, 387-392.
12. Nixon, J.E., Sinnhuber, R.O., Lee, D.J., Landers, M.K., and Harr, J.R. (1974) J. Natl. Cancer Inst. 53, 453-458.
13. Bailey, A.V., Harris, J.A., and Skau, E.L. (1966) J. Am. Oil Chem. Soc. 43, 107-110.
14. Carroll, K.K., and Khor, H.T. (1971) Lipids 6, 415-420.
15. McCay, P.B., and King, M.M. (1979) Fed. Proc. 38, 1073.
16. Littman, M.L., Taguchi, T., and Mosbach, E.H. (1966) Cancer Chemother. Rep. 50, 25-45.

[Received October 19, 1981]