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The Carcinogenicity of *p*-Dimethylaminoazobenzene in Diets Containing the Fatty Acids of Hydrogenated Coconut Oil or of Corn Oil*

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The striking retardation in the development of hepatomas in rats fed *p*-dimethylaminoazobenzene when hydrogenated coconut oil is included in the diet has been the subject of two communications from these laboratories (6, 7). When this fat was fed at a level of 5 per cent in a synthetic diet the incidence of liver tumors was found to be only 8 per cent or less at 6 months, in contrast to a usual incidence of 60 to 80 per cent at this time when the hydrogenated coconut oil was replaced by corn oil. Although great differences exist between the chemical and physical properties of these fats (3, 7), no conclusive basis has been found for the anticarcinogenic action of hydrogenated coconut oil. Thus the presence or absence of antioxidants in these fats did not affect the tumor incidence. The effect of hydrogenated coconut oil was maintained despite the presence of amounts of ethyl linolate sufficient for the growth and health of rats on a fat-free diet. Furthermore, the formation of hepatic tumors did not depend upon the development or prevention of the syndrome characteristic of a deficiency of essential fatty acids. Only slight protection against hepatoma formation was obtained with a diet containing trilaurin at a level equivalent to the lauric acid contributed by 5 per cent of hydrogenated coconut oil. Similar results were produced with raw coconut oil. Although many small tumors in nearly normal livers were obtained with a diet free of added fat, subsequent trials have revealed that the low-fat synthetic diet generally produces a low incidence of tumors. High incidences were observed when either hydrogenated coconut oil or corn oil were fed in diets containing crude casein and rice bran extract. This is consistent with the accelerating effect that this crude diet has on hepatoma formation as compared with the synthetic diet (8).

The present study deals with the effect of the fatty acid constituents of hydrogenated coconut oil and of corn oil on the induction of liver tumors in rats fed *p*-dimethylaminoazobenzene in synthetic diets. In each case the fat was hydrolyzed and the fatty acids were fed with glycerol at levels equivalent to 5 per cent of the corresponding fat. Lauric acid, the major fatty acid constituent of coconut oil, was fed also as a sole source of dietary lipid. These groups were controlled by two others, one fed corn oil and one that received a diet free of added fat. In two smaller series diets containing either olive oil or its chief constituent, oleic acid, as the source of lipid were compared with the corn oil diet. No diets containing hydrogenated coconut oil were included in any of the three series; the tumor incidences obtained with this fat in ten previous groups have always ranged between zero and 8 per cent at 6 months (6, 7).

METHODS

As in our previous studies (6, 7, 8), young, adult, male albino Sprague-Dawley rats from 150 to 210 gm.

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in weight were fed 0.06 per cent of *p*-dimethylaminoazobenzene for 120 days. The animals were kept in groups of 7 and 8 in screen-bottomed cages; food and water were given *ad libitum*. The dye was added to the diets by dissolving it with heat in the fat employed; for the low-fat diet an acetone solution of the dye was evaporated on the dry ration. The rations were mixed in amounts sufficient for 1 to 2 weeks and stored at 0° C. After 120 days the livers were examined by laparotomy and the animals continued on the same diet as before, but without the azo dye. At 6 months a final examination of the livers was made.

The composition of the various diets is indicated in Table I. All were supplemented with the following levels of crystalline B vitamins per kilogram: 3.0 mgm. thiamin chloride, 2.0 mgm. riboflavin, 2.5 mgm. pyridoxine hydrochloride, 7.0 mgm. calcium pantothenate, and 30.0 mgm. choline chloride. Each rat received also 1 drop of halibut liver oil monthly.

TABLE I: COMPOSITION OF DIETS *

Constituents	Diets, gm./kgm.						
	1, 6, 8	2	3	4	5	7, 9	10
Cerelose †	790	787	786	816	840	790	795
Casein (purified ‡)	120	120	120	120	120	120	120
Salts mixture	40	40	40	40	40	40	40
Corn oil (Mazola)	50						
Fatty acids of corn oil		48					
Fatty acids of hydrogenated coconut oil			47				
Glycerol		5	7				
Lauric acid				24			
Olive oil (commercial)						50	
Oleic acid (U.S.P. XI)							45
p-Dimethylaminoazobenzene	0.6	0.6	0.6	0.6	0.6	0.6	0.6

* See text under Methods for the levels of crystalline vitamins added to these diets.
† Pure glucose monohydrate, obtained from Corn Products Refining Co.
‡ For preparation see Reference (6).

The fatty acids were prepared by hydrolysis of the original oils with alcoholic base, followed by acid decomposition of the soaps. 150 gm. of U.S.P. KOH pellets were placed in a 3 liter, round-bottom flask, 650 cc. of 95 per cent ethanol added, and the contents heated nearly to boiling on a steam bath. After 500 gm. of oil had been cautiously added to the hot solution, a condenser was attached and the mixture was refluxed for 30 minutes. The hydrolysate was cooled to about 40° C. in a stream of tap water, and 650 cc. of cold distilled water were added. With continued cooling and agitation, 275 cc. of concentrated HCl were added in small portions. The mixture was transferred to a large separatory funnel and shaken vigorously to insure complete decomposition of the soaps; the fatty acids were allowed to layer and the aqueous phase was drawn off and discarded. The acids were then washed twice with about 500 cc. of distilled water, care being taken to avoid emulsification. After drying over anhydrous Na_2SO_4 the acids were filtered through paper and 50 gm. portions placed in 125 cc. Erlenmeyer flasks. The flasks were warmed slightly to keep the acids in the liquid state, and then evacuated with an oil pump for approximately 5 to 10 minutes; after this nitrogen gas was allowed to enter, and after several exhaustions the acids were sealed under an atmosphere of nitrogen. The fatty acids were stored at 0° C., and each served for the preparation of about 1 kgm. of diet. Rancidity in the diet containing the fatty acids of corn oil was easily controlled by preparing it frequently and by storage in the cold. No attempt was made to free the fatty acids of unsaponifiable matter, and tests on the tocopherol content of the fatty acids of the corn oil indicated that about 75 per cent of this constituent remained in the acid portion.

RESULTS

Most of the rats on the various diets remained in good physical condition; they gained moderately in weight, and the survival at 4 months was 100 per cent in 9 of the 10 groups (Table II). Normal incidences of tumors were observed in the control groups of the first 2 series, which were fed the diets containing corn oil (Table II, Groups 1 and 6); at 4 months the incidences were 33 and 8 per cent, and by 6 months they were 80 and 77 per cent respectively. In the third series, the control group fed corn oil had the unusually high incidence of 73 per cent at 4 months, which increased to 93 per cent at 6 months (Group 8).

When the corn oil in the diet was replaced by its constituent fatty acids (Group 2) 53 per cent of the animals developed tumors by 6 months. Since the incidence at this time on the control corn oil diet generally varies from 60 to 80 per cent, probably no great significance can be attached to the reduction apparently caused by the fatty acids of corn oil. The feeding

of the fatty acids of hydrogenated coconut oil (Group 3) lowered the incidence of hepatomas to zero, even by 6 months; this effect is comparable to our oft-repeated experience with the original glyceride (6, 7). The chief constituent of coconut oil, lauric acid, also did not allow any tumors to develop by 6 months (Group 4). A definite but less striking inhibition was obtained when a diet containing no added fat was fed; only 20 per cent of the rats had liver tumors by 6 months (Group 5). A scaly dermatitis of the paws and tail was noted in the rats of the groups whose diets lacked any source of essential fatty acid (Groups 3, 4, and 5); no dermatitis was observed in the groups receiving corn oil or its fatty acids.

The 2 series represented by Groups 6 to 10 tested the effects of olive oil and its main constituent, oleic acid, on the carcinogenicity of *p*-dimethylaminoazobenzene. When the corn oil in the control diet was replaced by olive oil a lowering in the incidence of liver tumors resulted; 33 and 53 per cent were attained by 6 months (Groups 7 and 9). In the group fed oleic acid (Group 10) the final hepatoma incidence of 87 per cent was not significantly different at 6 months from that obtained with the control diet. No dermatitis was evident in any of these groups; olive oil is known to contain a small amount of linoleic acid in ester form, and apparently the oleic acid used in these experiments also contained this factor as a contaminant.

TABLE II: THE EFFECT OF CERTAIN LIPIDS ON THE CARCINOGENICITY OF *p*-DIMETHYLAMINOAZOBENZENE

Group	Diet	Average starting weight, gm.	Average weight at 4 mos., gm.	Average food consumption gm./rat/day	Survival * at 4 mos.	Hepatomas † at 4 mos.	Hepatomas † at 6 mos.	Negative survivors at 6 mos.	Gross cirrhosis at 4 mos. none	Gross cirrhosis at 4 mos. moderate	Gross cirrhosis at 4 mos. severe	Dermatitis ‡
1	Corn oil	217	229	9.8	15/15	5/15	12/15	3	5	6	4	—
2	Fatty acids of corn oil	216	226	10.8	15/15	2/15	8/15	7	7	6	2	—
3	Fatty acids of hydrogenated coconut oil	212	229	11.0	15/15	0/15	0/15	15	15	0	0	+++
4	Lauric acid	208	218	11.0	15/15	0/15	0/15	13	15	0	0	+++
5	Low-Fat	216	232	11.5	15/15	1/15	3/15	12	14	1	0	+++
6	Corn oil	177	200	10.0	13/15	1/13	10/13	0	0	9	4	—
7	Olive oil	183	225	9.9	15/15	0/15	5/15	9	11	4	0	—
8	Corn oil	161	213	9.7	15/15	11/15	14/15	1	1	8	6	—
9	Olive oil	153	236	9.7	15/15	2/15	8/15	7	13	1	1	—
10	Oleic acid	152	207	9.6	15/15	2/15	13/15	2	13	1	1	—

* Survival = number living over number at start.
† Hepatomas = number with hepatomas over number surviving at 4 months.
‡ Dermatitis of paws and tail is indicative of a linoleic acid deficiency.

DISCUSSION

These experiments appear to support the conclusion that the fatty acids of hydrogenated coconut oil and of corn oil affect the carcinogenicity of *p*-dimethylaminoazobenzene in much the same way as do the original oils. The strong anticarcinogenic effect observed with a diet containing 2.4 per cent of lauric acid, the level of this acid equivalent to 5 per cent of hydrogenated coconut oil, suggests that it may be largely responsible for the inhibitory properties of the hydrogenated coconut oil. This is in contrast to previous experience, in which a diet containing the same amount of lauric acid in the form of trilaurin permitted a tumor incidence of 36 per cent by 6 months (7). However, coconut oil probably contains little trilaurin (3), and the lauric acid present as monolauryl- and dilaurylglycerides may be more easily liberated in the gastrointestinal tract than that contained in trilaurin, since trilaurin melts at 46° C. whereas hydrogenated coconut oil and several of its component glycerides (3) are liquid at body temperature. The finding that stearic acid is more digestible in the form of mixed glycerides than as tristearin (5) suggests that a similar situation may hold for hydrogenated coconut oil and trilaurin. Hence the diet containing trilaurin may have resembled more closely a low-fat diet than the hydrogenated coconut oil diet; this is supported to some extent by the fact that tumors developed on both of the former diets.

Although the development of tumors was slower with olive oil and oleic acid than with corn oil, the final incidence observed with oleic acid was not significantly different from that in the control group. However, olive oil appeared to exert a small but definite inhibition.

Our present experience with the diet containing no added fat is somewhat at variance with our previous data for this diet (7). This time only 3 animals out

of 15 developed tumors by 6 months, as compared with 9 of 13 reported for the previous low-fat group. Seven of the 9 tumors in the former series were small, and were discovered only at the examination made at 6 months. A third group fed the low-fat diet developed only 23 per cent of hepatomas by 6 months (4). Hence we must conclude that our low-fat synthetic diet generally induces a low percentage of liver tumors at 6 months. Opie (9) previously reached essentially the same conclusion from experiments with a partially purified diet low in fat.

In a previous paper (7) we suggested that the observations of Frazer (1, 2) may offer an explanation for the effect of hydrogenated coconut oil. Frazer presented evidence that the partition of absorbed fat between the portal and lymphatic systems may be affected by the nature of the fat fed. Thus rats fed scarlet red dissolved in olive oil deposited dye mainly in the fat depots, especially when the action of pancreatic lipase was inhibited by feeding a detergent. When excess pancreatic lipase was fed, or when oleic acid was substituted for the olive oil, the dye was deposited chiefly in the liver. In this way a portion of the *p*-dimethylaminoazobenzene absorbed might be diverted and subjected to extrahepatic destruction. However, lauric acid and the fatty acids of hydrogenated coconut oil would be expected to divert more of the dye to the liver. The strong anticarcinogenic action of these acids does not favor this hypothesis.

SUMMARY

Ten groups of 15 rats each were fed various lipids in synthetic diets containing 0.06 per cent *p*-dimethylaminoazobenzene for 4 months, followed by dye-free diets for 2 more months. When 2.4 per cent of lauric acid or 4.7 per cent of the fatty acids of hydrogenated coconut oil were fed, no liver tumors developed by 6 months. If, however, these lipids were replaced by 5 per cent of corn oil, or by 4.8 per cent of the fatty acids of corn oil, the tumor incidences at 6 months were 80 and 53 per cent respectively. The presence of 5 per cent of olive oil or 4.5 per cent of oleic acid in the diet permitted incidences of tumors of from 33 to 53 and 87 per cent respectively at 6 months. Twenty per cent of the rats fed a diet free of added fat developed hepatomas within this time.

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