Further Studies on the Tumor-Promoting Action of Fat*†

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There is abundant evidence that diets high in fat tend to increase the rate of formation of certain types of induced tumors (1, 2, 4, 18). In the presence of borderline amounts of carcinogen, dietary fat may also increase the total number of tumors produced (6). Not all types of tumors, however, are sensitive to dietary fat (2, 9, 18), and at least a part of the effect appears to be due to local fat in direct contact with the area in which the tumor develops (4, 11, 13, 20). The present report deals with further studies on the extent and limitation of the "fat effect," and includes attempts to explain the mechanism of action involved.

METHOD

The experimental procedure was essentially that reported previously (6). Tumors were produced on the backs of albino mice by the application twice weekly of a 0.2 per cent solution of methylcholanthrene in dioxane. The experiments were performed in a number of series in each of which the animals were divided into groups of 25, comparable in age and weight. All groups within a series were treated with the same amount of hydrocarbon, but each in addition received some special dietary treatment. Except in certain specific experiments food and water were given *ad libitum*, and all mice were examined twice monthly for tumors.

A basal ration used in compounding many of the diets had the following composition:

Corn meal	445
Skim milk powder	222
Linseed oil meal	167
Soybean oil meal	111
Alfalfa leaf meal	33
NaCl (fine, iodized)	11
$Ca_3(PO_4)_2$	11

A diet relatively low in fat was used as the control ration in most of the experimental series; it consisted of 93 parts of the basal ration, 5 parts of brewers' yeast, and 2 parts of cod liver oil. Diets high in fat were prepared from the same ingredients with fat replacing an equivalent amount of the basal ration. When still other ingredients were included in the diet, they were likewise substituted at the expense of the basal ration. Semisynthetic diets were prepared as indicated in the separate sections dealing with the experiments themselves.

EXPERIMENTAL

Synthetic triglycerides.—It was observed previously that ethyl laurate stimulated the production of tumors to nearly the same extent as the natural fats themselves, while glycerol and the unsaponifiable matter from vegetable oil increased tumor formation only slightly (6). In the present studies, fatty acids from hydrogenated vegetable oil (primex) were prepared free from unsaponifiable matter, resynthesized into triglycerides (7), and fed at a level of 10 per cent to mice receiving the usual carcinogenic treatment. The percentage of tumors that developed in these animals was found to be the same as on diets containing 10 per cent of natural fat—37 to 43 per cent at 6 months, as compared to 18 per cent on the low fat control diet (Table I). It appeared, therefore, that neither the sterols nor the other substances in unsaponifiable matter were necessary for demonstrating the tumor-promoting effect of fat.

TABLE I: THE EFFECT OF SYNTHETIC TRIGLYCERIDES ON THE FORMATION OF INDUCED SKIN TUMORS IN MICE

(0.2 per cent methylcholanthrene, twice weekly, 2 months)

Diet	Effective total	Tumor formation 2 mos., per cent	4 mos., per cent	6 mos., per cent
Low fat control	22	0	4	18
10% lard (m.p. > 37° C.)	23	0	30	43
10% synthetic triglycerides *	22	0	27	41
10% lard (m.p. < 37° C.)	24	0	25	37

* Synthetic triglycerides of fatty acids from hydrogenated vegetable oil (primex).

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Effect of heating the fat.—The heated vegetable oil, primex, has been reported to be more effective in increasing tumor formation than the unheated fat. Subsequent experiments, however, indicated that the original results were not representative for heated fats in general. Lard, coconut oil, and corn oil were heated at 300° C. for 1 hour as previously described (6), incorporated into the basal ration as 10 per cent of the diet, and fed to mice receiving the usual carcinogenic treatment. The incidence of tumors in these groups was then compared with that in 3 others fed 10 per cent of the respective unheated fats. In addition, another group of mice was fed a diet containing 10 per cent of coconut oil plus 1 per cent of cholesterol that had been heated to 300° C. for 1 hour.

Little difference was found between the stimulatory activity of the heated and the unheated fats, and at the end of 5 months the tumor incidence in each of the groups varied from 61 to 78 per cent (Table II). Furthermore, when 1 per cent of heated cholesterol was added to the diet containing coconut oil, the tumor incidence was no greater than when coconut oil alone was fed. At a level of 7 per cent, primex and lard heated to 300° C. for 4 hours were less effective in increasing tumor formation than the corresponding unheated fats (Table II, groups 9 to 13). However, this apparent reduction in the ability of heated fats to stimulate tumor formation was not interpreted as a genuine effect, but rather as the result of a reduced consumption of the diet by the mice (see below), presumably because of disagreeable substances present in the heated fats.

TABLE II: THE EFFECT OF HEATED FAT ON TUMOR FORMATION

(0.2 per cent methylcholanthrene, twice weekly, 2 months)

Group	Diet	Effective total	Tumor formation: 2 mos., per cent	4 mos. per cent	5 mos., per cent
1	Low fat control	21	0	28	33
2	10% corn oil	21	19	71	76
3	10% heated corn oil	24	16	71	71
4	10% coconut oil	24	4	66	66
5	10% heated coconut oil	23	4	70	78
6	10% coconut oil + 1% heated cholesterol	22	9	63	68
7	10% lard	18	11	61	61
8	10% heated lard	16	26	69	69
9	Low fat control	22	0	23	27 *
10	7% primex †	17	0	59	59 *
11	7% heated primex	22	0	27	32 *
12	7% lard	26	8	38	42 *
13	7% heated lard	25	0	20	24 *

* Tumor incidence at 6 months.
† Hydrogenated vegetable oil.

Tumors relatively insensitive to dietary fat.—Tannenbaum has recently reported that diets high in fat stimulate the formation of induced skin tumors and of spontaneous breast tumors in mice, but not of primary lung tumors nor of induced subcutaneous sarcomas (18). Earlier studies from this laboratory likewise revealed the greater sensitivity to dietary fat of skin tumors as contrasted with subcutaneous sarcomas (2, 4, 6). The possibility remained, however, that an effect of fat might become evident on the formation of subcutaneous sarcomas if merely borderline amounts of carcinogen were injected (14, 18). Accordingly, mice fed high or low fat diets were injected subcutaneously with only 20 μgm. of methylcholanthrene in corn oil. The effect of dietary fat was also tested on the development of spontaneous breast tumors in rats, of tumors of the submaxillary gland, and of induced subcutaneous sarcomas (Table III).

The mice receiving the 20 μgm. of methylcholanthrene subcutaneously showed essentially the same incidence of tumors on the diet high in fat as on the low fat control diet (16 and 17 per cent), even though the experimental conditions were presumably ideal

TABLE III: THE INSENSITIVITY OF CERTAIN TYPES OF TUMORS TO DIETARY FAT

Group	Type of tumor	Methylcholanthrene injected, mgm.	Fat * added to diet, per cent	Effective total	Tumor formation: 5 mos., per cent	7 mos., per cent	10 mos., per cent	30 mos., per cent
1	Induced subcutaneous sarcoma (mice)	0.02	0	23	9	17	—	—
2	" " " "	0.02	15	30	13	16	—	—
3	Spontaneous breast carcinoma (rats)	0	0	33	—	0	3	9
4	" " " "	0	30	32	—	0	0	6
5	Submaxillary gland tumor	1	0	5	0	40	40	—
6	" " "	1	30	5	0	40	40	—
7	Induced subcutaneous sarcoma (rats)	1	0	8	25	62	—	—
8	" " " "	1	30	8	38	62	—	—
9	Induced subcutaneous sarcoma (rats)	2	0	6	16	50	83	—
10	" " " "	2	30	7	43	57	85	—

* Hydrogenated vegetable oil, primex.

for demonstrating any accelerating influence that might exist. The presence of 30 per cent of fat in the diet also failed to affect the incidence of spontaneous mammary carcinomas, of induced submaxillary gland tumors, or of induced subcutaneous sarcomas in the rat.

Attempts to Explain the Action of Fat

Local and systemic effects.—The greasy fur of mice fed a high fat diet suggested that the increased tumor incidence observed could have been due in part to a local action of the fat upon the skin. Such an effect was demonstrated when applications of oil increased the rate of formation of tumors due to ultraviolet irradiation (13), or to the carcinogenic hydrocarbons (4, 6, 11, 20). In the present experiments, local applications also increased the total number of tumors produced. The carcinogen was applied twice weekly for the first 2 months of the experiment, and either oleic acid, potassium oleate solution, cottonseed oil, or a 5 per cent solution of cholesterol in cottonseed oil was applied at the site of carcinogenesis on days when the hydrocarbon was not painted. A definite increase in tumor incidence resulted from the local applications: 41 to 59 per cent of the treated animals had tumors at the end of 5 months, as compared to 26 per cent in the control group, which received no applications of oil (Table IV). These results suggested that much of the tumor-promoting activity of dietary fat was exerted locally. However, some fat, no doubt, was absorbed through the skin and the animals were also able to lick off an undetermined amount of the applied oil. Thus enough oil may have entered the general circulation for a systemic effect to be operative as well. The importance of a systemic effect was suggested by the observation that as little as 7 per cent of fat added to a grain mixture containing 5 per cent of fat was sufficient to increase the number of tumors produced (Table II, groups 9 to 13), although no appreciable greasiness of the skins was evident under these conditions. Tannenbaum has also reported increased tumor production in the absence of visible greasiness (18).

Table IV: Tumor Development in the Presence of Oils Applied Locally

(0.2 per cent methylcholanthrene in dioxane, twice weekly, 2 months)

Oil painted	Effective total	Tumor formation 2 mos., per cent	3 mos., per cent	4 mos., per cent	5 mos., per cent
None	23	0	21	21	26
Oleic acid	17	0	23	35	41
Potassium oleate	17	0	17	41	59
Cottonseed oil	17	0	35	46	59
Cholesterol in cottonseed oil	19	5	15	37	52

Purina fox chow was fed to all groups.

Accordingly, mice treated with methylcholanthrene were given emulsified fat incorporated in the drinking water, rather than in the ration. Thus the carcinogenic area of the skin was spared the effects of local contact with dietary fat. The emulsion consisted of 7 per cent of lard suspended in water containing 3 per cent of gum arabic as the emulsifying agent, and was sufficiently stable so that the loss of fat during feeding did not exceed 3 per cent of the total. The animals receiving the emulsion as drink were fed the control diet low in fat, and the incidence of tumors was compared with that in comparable groups on the low fat control diet or on a diet containing 10 per cent of lard. The ingestion of the emulsion varied from week to week, corresponding to intakes of lard that ranged from 7.8 per cent to 21.4 per cent by weight of the total food consumed. Mice fed the emulsion developed more tumors than those on the low fat diet alone, thus confirming the existence of a systemic effect of dietary fat, but the lard fed as an emulsion appeared to be less than half as effective in increasing tumor formation as lard incorporated in the ration. Tumor incidence in the emulsion-fed group was 29 per cent at the end of 6 months, as compared to 18 and 43 per cent respectively in the groups on the low fat control diet and on the diet containing 10 per cent of lard (Table V, groups 1 to 3).

Metabolic derivatives of fat.—One suggested explanation of the fat effect was that products of the incomplete metabolism of fat were the agents responsible for the increased tendency toward tumor formation. Accordingly, acetone bodies and a short-chain fatty acid were fed to mice developing skin tumors, and also to rats developing subcutaneous sarcomas or tumors of the submaxillary gland. One group of mice was fed 1 per cent of ethyl acetoacetate incorporated in the low fat control diet, while 2 per cent of acetone was given in the drinking water. A second group was fed a diet containing 10 per cent of coconut oil plus 0.3 per cent of butyric acid. Tumors were induced by the local application of 0.2 per cent of methylcholanthrene in dioxane twice weekly for 2 months, and the incidence of tumors was compared with that in groups on the control diet and on a diet containing 10 per cent of coconut oil. As usual the addition of fat increased the incidence of tumors appreciably (Table VI), but the presence of butyric acid failed to augment this increase: 66 *versus* 63 per cent respectively. Furthermore, the group receiving the low fat control diet plus added acetone bodies de-

TABLE V: RELATION BETWEEN CALORIC INTAKE AND THE INCIDENCE OF TUMORS IN MICE ON VARIOUS DIETS

(0.2 per cent methylcholanthrene, twice weekly, 2 months)

Diet	Effective total	Cal./25 gm. mouse/day	Tumor formation: 4 mos., per cent	Tumor formation: 6 mos., per cent
SERIES A				
1. Low fat control	22	13.5	4	18
2. Control + lard emulsion	25	14.7	16	29
3. 10% lard	23	15.7	30	43
SERIES B				
4. Low fat control	23	10.8	4	4
5. 10% primex	22	11.1	4	22
6. 10% primex + riboflavin	25	12.1	40	50
SERIES C				
7. Low fat control	22	13.5	4	18
8. 10% lard + 7% casein	21	15.3	28	43
9. 10% lard (m.p. > 37° C.)	23	15.7	30	43
10. 10% triprimex *	22	16.6	27	41
11. 10% lard (m.p. < 37° C.)	24	17.6	25	37
SERIES D				
12. Low fat control (♀)	17	13.0	0	6
13. Low fat control (♂)	14	13.4	14	14
14. 15% primex † (restricted)	20	13.1	10	15
15. 10% primex + riboflavin	20	14.6	10	30
16. 15% primex	22	17.2	27	32
17. 10% primex	18	17.9	11	28
18. Semisynthetic with cooked starch	21	10.8	9	19
19. Semisynthetic with glucose	23	12.1	22	30

* Synthetic triglycerides of fatty acids from hydrogenated vegetable oil.
† Hydrogenated vegetable oil.

TABLE VI: THE EFFECT OF METABOLIC DERIVATIVES OF FAT ON THE FORMATION OF SKIN TUMORS IN MICE

(0.2 per cent methylcholanthrene, twice weekly, 2 months)

Diet	Effective total	Tumor formation: 2 mos., per cent	Tumor formation: 3 mos., per cent	Tumor formation: 5 mos., per cent
Low fat control	21	0	28	33
Low fat control + acetone bodies *	23	0	17	26
10% coconut oil	24	4	58	66
10% coconut oil + 0.3% butyric acid	24	8	54	63

* 1 per cent ethyl acetoacetate in diet; 2 per cent acetone in drinking water.

veloped essentially the same percentage of tumors as the control group itself: 26 *versus* 33 per cent.

The presence of 15 per cent of sodium butyrate in the diet of rats injected with 2 mgm. of methylcholanthrene failed to increase the incidence of subcutaneous sarcomas. Similarly, the incidence of tumors arising in the submaxillary gland of rats after the insertion of 1 mgm. of methylcholanthrene into this organ was likewise unaffected by feeding a combination of acetone bodies: 1 per cent of ethyl acetoacetate and 5 per cent of sodium butyrate in the diet, and 3 per cent of acetone in the drinking water. Hence it appeared unlikely that dietary fat increased tumor incidence through the medium of the acetone bodies.

Riboflavin and protein.—The riboflavin requirement of growing rats is reported to be increased on diets high in fat (8), while an inadequate intake of riboflavin tends to promote the incidence of tumors in rats fed *p*-dimethylaminoazobenzene (5, 9). The possibility therefore existed that the increased incidence of tumors on the high fat diets was due in part to an effective riboflavin deficiency brought about by the extra fat in the diet. Accordingly, 10 mgm. of riboflavin were added per kg. of a typical high fat diet, containing 10 per cent of hydrogenated vegetable oil in addition to the 5 per cent of fat contained in the basal ingredients. When this diet was fed to groups of mice receiving the usual carcinogenic treatment, the incidence of tumors was not only as high, but in some series it was actually higher than in comparable groups of animals receiving the same diet without the added riboflavin (Table V, groups 4, 5, 6, 13, 15, and 17). In a similar manner it was shown that additional protein failed to counteract the tumor-promoting action of the fat (Table V, groups 7 to 11).

Caloric intake.—In line with earlier reports (3, 10, 12, 15, 16), Tannenbaum (17) recently observed that more tumors developed in full-fed control mice than

in animals fed only two-thirds of the caloric level adequate for growth. This effect of underfeeding was evident in all strains of mice and with all types of tumors studied: spontaneous tumors of the breast and lung, and carcinomas and sarcomas induced respectively by the local application or by the subcutaneous injection of benzpyrene. A similar effect of underfeeding has been reported by Visscher and his associates (19). In one series a reduction in caloric intake by one-third completely prevented the appearance of mammary carcinomas for 16 months although the full-fed control animals exhibited a tumor incidence of 67 per cent at this time.

These pronounced effects of underfeeding suggested that the converse might also be true to some extent; *viz.*, an increased ingestion of calories above an *ad libitum* level of intake might result in an increased incidence of tumors. Accordingly, measurements were made of the amounts of food consumed by the mice on our various experimental diets, and the caloric equivalent calculated on the basis of 4.1, 4.1, and 9.3 calories per gm. of carbohydrate, protein, and fat respectively. The results were expressed as calories consumed per 25 gm. of body weight of mouse per day.

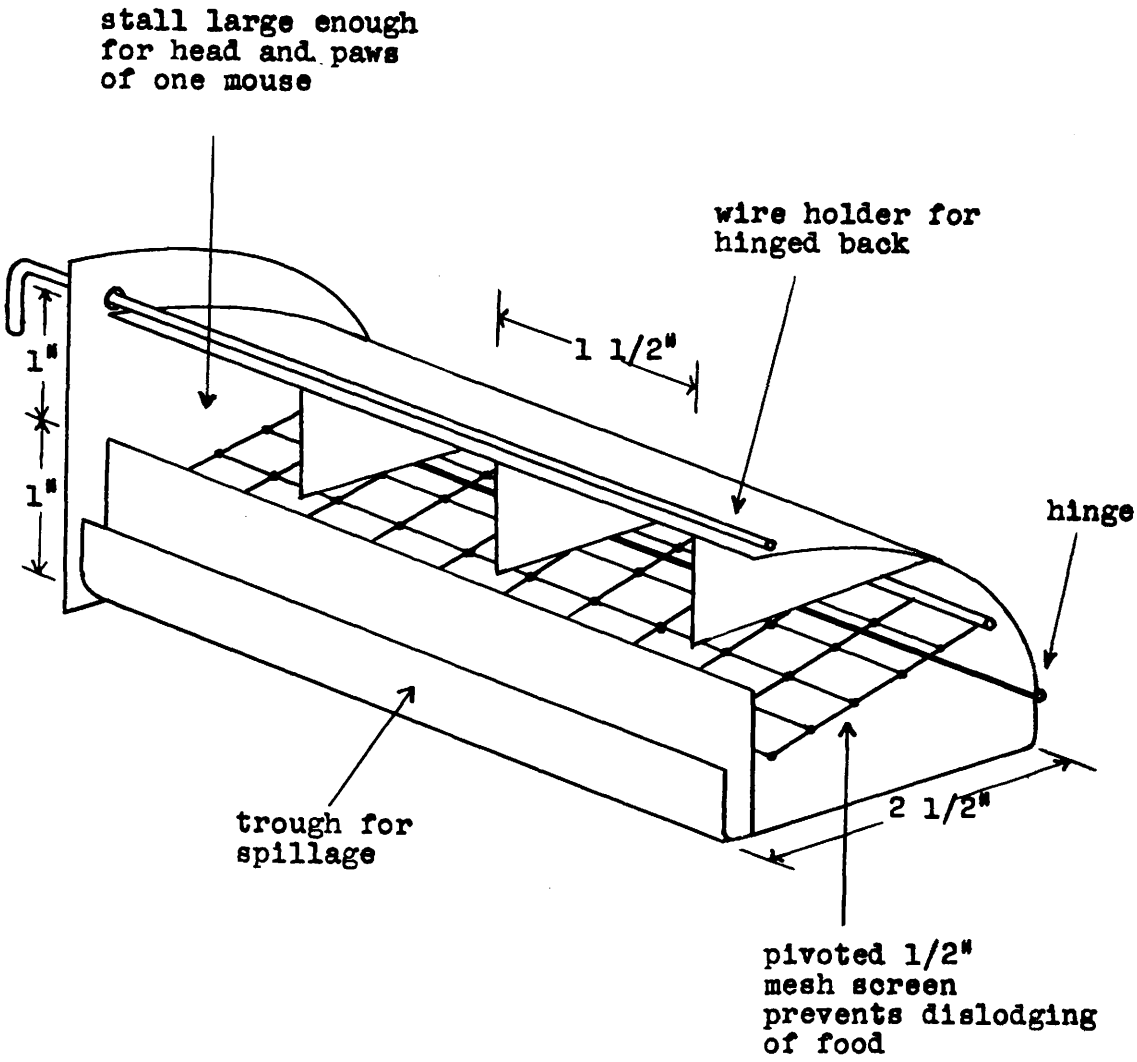

FIG. 1.—Cut-away diagram of mouse feeder.

In each series the number of calories consumed *ad libitum* was least in the group on the low fat control diet (Table V), while the mice on the high fat diets consumed from 12 to 30 per cent more calories than the control animals. Within each series there was a rough correspondence between the number of calories ingested and the number of tumors that ultimately appeared. When mice receiving 15 per cent of hydrogenated vegetable oil were restricted in caloric intake to the number consumed by the low fat control group,

the tumor-promoting effect of the fat was definitely reduced (Table V, groups 12, 13, 14, and 16). An effect of calories was also noted in two low fat semi-synthetic diets containing either cooked starch or glucose monohydrate (cerelose) as the source of carbohydrate. The mice receiving starch consumed 10.8 calories per day, and 19 per cent of these animals developed tumors; those on glucose consumed 12.1 calories per day, and the tumor incidence in this group was 30 per cent.

However, the parallel between caloric intake and tumor incidence was not an absolute one. Small increases in caloric intake within a series sometimes accompanied a large increase in tumor incidence (Table V, groups 4, 5, and 6) while a relatively large difference in caloric intake between the control groups of two different series did not result in any appreciable change in the total number of tumors (Table V, groups 4, 12, and 13). It was therefore evident that factors other than caloric intake exerted some influence on the total number of tumors produced. The increases in caloric intake that appeared to affect tumor incidence in the present experiments were usually less than the differences of 33 per cent reported to be necessary to demonstrate the effects of caloric restriction. The association between the high caloric intake on high fat diets and an increased tendency toward tumor formation was therefore interpreted as suggestive rather than completely proved. It is of interest that a similar conclusion emerges from the results of experiments by Tannenbaum (18) if the caloric intakes of his animals on diets high or low in fat are calculated as in the present report.

The relative effectiveness on tumor development of fat *per se,* as distinct from the extra calories ingested in the form of fat, was estimated in two identical series of experiments involving restricted caloric intake. Each series consisted of 4 groups of 30 mice. The males and females of each group were kept in separate cages, and all mice were painted with a 0.2 per cent solution of methylcholanthrene in dioxane twice weekly for 10 weeks. Weighed amounts of food were fed daily to all groups in special containers (Fig. 1) that enabled the animals to get at the food with a minimum of contact, spillage, or contamination with excreta.

Two of the groups were fed a normal amount of food, 12 calories per 25 gm. of mouse per day, while the other 2 groups were restricted to 8 calories per 25 gm. mouse daily. All groups received the same amount of protein, salts, and vitamins, but the amounts of fat fed at each caloric level were varied so that a 25 gm. mouse ingested either 60 or 240 mgm. of corn oil daily. Thus the following variations were achieved: low fat, low calorie; high fat, low calorie; low fat, high calorie; and high fat, high calorie. The composition of the diets and the amounts fed daily are as follows:—

	Low fat, low cal.	High fat, low cal.	Low fat, high cal.	High fat, high cal.
Cooked starch	1.01 gm.	0.60 gm.	1.99 gm.	1.58 gm.
Casein	0.60 "	0.60 "	0.60 "	0.60 "
Brewers' yeast	0.24 "	0.24 "	0.24 "	0.24 "
Corn oil	0.06 "	0.24 "	0.06 "	0.24 "
Wesson salts (21)	0.12 "	0.12 "	0.12 "	0.12 "
Agar	0.06 "	0.06 "	0.06 "	0.06 "
Halibut liver oil	30 μgm.	30 μgm.	30 μgm.	30 μgm.
	2.09 gm.	1.86 gm.	3.07 gm.	2.84 gm.

The combined results of the two series (Table VII) indicate definite effects both of fat and of calories. Regardless of the amount of fat fed, the incidence of tumors was much higher on the high calorie diets than on the low calorie diets. At each level of caloric intake the incidence of tumors was somewhat higher on the high fat diet than on the corresponding low fat diet. However, this extra effect of the fat, over and above the caloric effect, may have been merely an ex-

TABLE VII: THE RELATIVE EFFECTS OF FAT AND OF TOTAL CALORIES ON THE DEVELOPMENT OF SKIN TUMORS DUE TO METHYLCHOLANTHRENE

(0.2 per cent solution in dioxane, twice weekly, 10 weeks)

	Total mice with tumors/neg. survivors				Effective total	Tumor incidence, per cent
	3 mos.	4 mos.	5 mos.	6 mos.		
Low fat, low calorie	0/29	0 22	0/14	0/8	29	0
High fat, low calorie	3/25	5 19	8/9	8/9	28	28
Low fat, high calorie	15/39	26/24	29/10	29/8 *	54	54
High fat, high calorie	16/34	30/10	33/7	33/6	50	66

* During the last 2 months the negative survivors did not consume their full allotment of 12 calories per day.

pression of the degree of local contact of fat with the skin, which was minimized by the type of feeder used but was probably not prevented entirely. The greater importance of calories *per se* suggests that much of the systemic or cocarcinogenic activity of dietary fat, if not all, is exerted through the medium of a voluntarily increased intake of calories on diets high in fat.

This, however, does not settle the question of how dietary fat increases the incidence of skin tumors, since the mechanism by which extra calories enhance carcinogenesis is still obscure. It may be that tissues at the periphery of the body ordinarily receive borderline amounts of nutrients, and that the competition among normal cells for food is so high that incipient tumor cells are often suppressed by a kind of starvation. Wicks and Suntzeff (22) recently observed that applications of methylcholanthrene to the skin of mice result in a definite fall in the ratio of total lipid to protein in the epidermis, though not in the whole skin. Under these conditions a general increase in available calories throughout the body might be expected to result in relatively greater improvement in the nutrition of peripheral tumors than of tumor cells in other parts of the body.

SUMMARY

1. The fatty acids of hydrogenated vegetable oil, prepared free from unsaponifiable matter and resynthesized into triglycerides, had essentially the same tumor-promoting activity as natural fat. The prolonged heating of several natural fats did not greatly alter their tumor-promoting activity.

2. Much of the effect of the dietary fat was exerted locally. The application of oil to the skin increased the total number of tumors produced, while fat fed as an emulsion in the drinking water was only about half as effective in increasing tumors as fat incorporated into the diet.

3. Possible products of fat metabolism, such as acetone, ethyl acetoacetate, and butyric acid, did not appreciably increase incidence of the tumors in either rats or mice. An increased intake of riboflavin and protein failed to diminish the tumor-promoting action of the fat. Increased dietary fat did not increase the incidence of sarcomas in mice caused by the subcutaneous injection of methylcholanthrene, nor of spontaneous breast tumors of rats, nor of tumors induced by the injection of methylcholanthrene either subcutaneously or into the submaxillary gland of the rat.

4. Mice painted with methylcholanthrene consumed 12 to 30 per cent more calories on diets high in fat than on corresponding low fat diets. A rough correspondence was observed between the caloric intakes of mice on the various rations and the numbers of tumors that ultimately appeared. On an equivalent caloric intake, the incidence of tumors in mice on the high and low fat diets was more nearly equal. It is suggested that at least part of the tumor-promoting action of fat is due to an accompanying increased consumption of calories.

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