

# The Influence of Dietary Casein Level on Tumor Induction with 2-Acetylaminofluorene\*

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## INTRODUCTION

The present experiments were conducted to determine the carcinogenicity of 2-acetylaminofluorene when it was fed in partially purified diets that varied in casein level between 9 and 60 per cent. Studies involving this broad range of dietary protein level have not been reported previously with this carcinogen.

Harris (7) concluded that variations in dietary protein level had no appreciable effect on the production of liver tumors with 2-acetylaminofluorene in rats. Purified diets containing 13 and 20 per cent of casein were employed. Morris and associates (10) found that rats developed more tumors when purified diets containing 18-24 per cent of casein were used than when the diet contained 12 per cent of casein.

In these studies, it was noted that dietary protein levels in the range used by the above investigators had no influence on tumor induction by 2-acetylaminofluorene. However, it was observed that diets containing 40 or 60 per cent of casein had a definite protective effect against the induction of mammary tumors and possibly other types of tumors.

## PROCEDURE AND RESULTS

Weanling female rats of the Alabama Experiment Station (AES) strain, 40-60 gm. in body weight and 20-22 days old, were used. They were kept in individual screen-bottomed cages. Fresh feed and water were supplied daily, ad libitum. A record of feed consumption was kept during the first 16 weeks of the experiment. The animals were

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weighed and examined for visible tumors at weekly intervals.

The basal diet had the following percentage composition: alcohol-extracted casein, 9.0; degerminated corn grits, 20.0; sucrose, 50.7; salts,<sup>1</sup> 4.0; lard, 15; L-cystine, 0.3; and cod liver oil, 1.0. Fifty ml. of an aqueous solution containing the following vitamins (mg.) was mixed into each kilogram of the dry ingredients: thiamin, 2; riboflavin, 4; pyridoxine, 6; calcium pantothenate, 10; niacin, 20; *i*-inositol, 200; and choline chloride, 2,000. Alpha-tocopherol and  $\alpha$ -tocopheryl acetate were mixed into the cod liver oil to furnish 25 mg of each/kg of diet. All diets contained 300 mg/kg of 2-acetylaminofluorene, which was added to the fat-free dry ingredients in acetone solution and the solvent removed with an air current.

Increases in the casein component of the diet were at the expense of equal weights of the sucrose component. The other dietary changes that were tried are given in Table 1.

The basal diet is similar to diet C-5 used in earlier studies (2-5), except that the supplement of pyridoxine was increased from 2 mg to 6 mg/kg of diet to eliminate the possibility of the occurrence of a deficiency of this vitamin on the high casein diets. Varying the pyridoxine supplement between 2 and 10 mg/kg of diet was demonstrated in preliminary trials to be without influence on the carcinogenicity of 2-acetylaminofluorene.

The animals were continued on experiment until they died, unless indicated otherwise. At termination, all tumors and grossly abnormal tissues were carried through routine procedures for histologic study; a complete description of the autopsy material will appear in another publication.

The present results confirm the earlier observations (2-5), in that a high incidence of mammary tumors was obtained when the 9 per cent casein diet was fed (Table 1, groups 1 and 11). When this diet was fed, liver and ear duct tumors appeared

<sup>1</sup> W. D. Salmon, *J. Nutrition*, 33:155, 1947.

with about the same frequency as reported earlier.

Increasing the dietary protein to 12, 20, or 27 per cent had no influence on the final incidence of mammary, ear duct, or liver tumors (groups 2, 3, and 6). However, when the dietary casein was increased to 40 or 60 per cent, there was a marked reduction in the incidence of mammary tumors (groups 4 and 5) when the food consumption was about equal to that on the lower protein diets

tein diet again resulted in a marked protection against mammary tumor induction by 2-acetylaminofluorene (group 12). With ad libitum feed consumption of the 60 per cent casein diet, a moderately high incidence of mammary tumors again occurred (group 13).

Irrespective of the level of feed consumption and the growth rate, the 60 per cent casein diets had a real and beneficial effect on survival. Ani-

TABLE 1  
INFLUENCE OF DIETARY CASEIN LEVEL ON TUMOR INDUCTION  
BY 2-ACETYLAMINOFLUORENE (AAF)

EXP. NO.	GROUP NO.	DIETARY CASEIN LEVEL (per cent)	AV. BODY WEIGHT		AV. DAILY INTAKE PER RAT		No. RATS	No. RATS WITH TUMORS				AV. SURVIVAL (wks.)
			Initial (gm.)	16 wks. (gm.)	Feed (gm.)	AAF (gm.)		Mammary	Ear duct	Liver	None	
1	1	9	52	173	6.6	1.98	17	14(22)*	12(25)*	10	0	27
	2	20	51	197	7.0	2.10	8	7(21)	5(25)	4	0	28
	3	27	49	210	7.0	2.10	4	3(21)	2(24)	3	0	30
	4	40	49	196	7.0	2.10	8	2(25)	3(28)	4	0	32
	5	60	53	168	6.5	1.95	9	0	2(37)	4	3	40†
2	6	12	48	198	7.2	2.16	18	16(19)	12(24)	8	0	27
	7	12‡	56	216	7.6	2.28	6	6(20)	3(25)	2	0	28
	8	12§	57	220	7.6	2.28	6	6(21)	4(24)	3	0	27
3	9	60	47	222	7.7	2.31	6	5(23)	4(31)	4	0	42
	10	60#	46	216	8.0	2.40	12	10(18)	7(23)	4	0	32
4	11	9	47	162	6.2	1.86	7	5(21)	4(24)	5	0	28
	12	60	48	184	6.4	1.92	8	1(31)	3(31)	2	3	32**
	13	60	46	214	7.2	2.16	8	5(25)	6(29)	4	0	32**

\* The numbers in parentheses are the average tumor induction periods in weeks.

† All these animals were still alive after the 40-week experimental period. They were sacrificed at that time to determine the liver tumor incidence.

‡ The diet was supplemented with 1 per cent of desoxyribonucleic acid.

§ The diet was supplemented with 5 per cent of yeast nucleic acid.

# The diet was supplemented with 30 µg. of vitamin B<sub>12</sub> and 2 mg. of folacin per kilogram.

|| Rats of this group were pair-fed with those of group 11.

\*\* All these animals were still alive after the 32-week experimental period. They were sacrificed at that time to determine the incidence of liver tumors.

(groups 1 and 11). When the 60 per cent casein diet (group 5) was used, three out of nine animals remained tumor-free for 40 weeks, whereas on the low protein diets all animals had tumors of one or more types, usually by the 25th week, and the average survival was only 27 weeks.

In contrast with the marked reduction in mammary tumors in group 5 is the high incidence of mammary tumors in group 9, which was also fed the high casein diet but which consumed larger amounts of feed and gained weight more rapidly.

To test the relation of mammary tumor induction to body weight gain and feed consumption level, the study represented by groups 11, 12, and 13 was conducted. Animals in groups 11 and 13 were allowed to consume their diets ad libitum. The animals in group 12 were paired with those in group 11, and the feed offered to those on the 60 per cent casein diet (group 12) was limited to the amount consumed by those on the 9 per cent casein diet (group 11). With equalized caloric intakes (viz., equalized feed intakes), the 60 per cent pro-

teins receiving this diet survived 40 weeks or longer, whereas those on the 9 per cent casein diet were usually dead by the 30th week. Supplementation of the 60 per cent casein diet with vitamin B<sub>12</sub> and folacin<sup>2</sup> hastened the induction of mammary tumors (group 10). This supplement also appeared to intensify the toxicity of the carcinogen, since it reduced the average survival period from 42 weeks to 32 weeks.

Supplementing the 12 per cent casein diet with desoxyribonucleic acid or with yeast nucleic acid (ribonucleic acid) did not influence the tumor incidence or induction time (groups 7 and 8).

The influence of high protein intake on mammary tumor induction is emphasized when the results with the 40 and 60 per cent casein diets are compared to the results obtained when the casein ranged between 9 and 27 per cent of the diet. The over-all incidence of mammary tumors in animals

<sup>2</sup> The folacin used in this study was donated by the Lederle Laboratories, Pearl River, New York, and all other vitamins were donated by Merck & Co., Inc., Rahway, N.J.

receiving the diets containing 9–27 per cent of protein was 86 per cent (57 out of 66), contrasted with an incidence of 45 per cent (23 out of 51) for the animals on the high casein diets. The high casein diet, combined with restricted feed intake (groups 4, 5, and 12), resulted in only three mammary tumors in 25 rats (12 per cent incidence), contrasted with an incidence of 80 per cent (nineteen of 24 rats, groups 1 and 11) with a comparable feed consumption of the 9 per cent casein diet.

There is suggestive evidence that the incidence of ear duct tumors may also be influenced. For example, the rats on the high casein diets had a 49 per cent incidence of this tumor, contrasted with an incidence of 63 per cent in the remainder. Moreover, the induction period for ear duct tumors was considerably longer in rats fed the high protein diets than in rats consuming similar amounts of the low protein diets.

Likewise, there is suggestive evidence of a protective effect of high protein diets on liver tumor induction. For example, on equalized feed intakes, five of seven animals in group 11 (9 per cent casein diet) had liver tumors in 28 weeks, whereas only two of eight in group 12 (60 per cent casein diet) had liver tumors in 32 weeks. However, the influence of dietary casein level on liver tumor induction could not be determined accurately in these studies, since many of the animals were kept on experiment to determine the influence of dietary protein level on survival.

#### DISCUSSION

It is possible that the protective effect of protein observed in the present study is mediated through the liver, and at least suggestive evidence was obtained to indicate that there was less liver damage on the high protein diets than on the low protein diets. Gutmann and associates (6) have recently shown that the extent of disappearance of diazotizable material after the administration of 2-acetylaminofluorene in rats is dependent upon the amount of active liver tissue present. The role of dietary protein in carcinogenesis has received a great deal of attention, and excellent reviews are available (13, 14). It is generally agreed that the most striking protective effect of protein is on tumors originating in the liver, which are induced by the feeding of azo dyes (8, 9, 12).

The present results cannot be explained on the basis of the dietary protein functioning as a detoxifying agent for 2-acetylaminofluorene. At least, if this were the explanation, it would seem that high protein diets would have permitted better weight gains than the low protein diets, feed intake being equal. Diets containing 12–27 per cent

of casein were not protective against mammary tumor induction. They nevertheless promoted growth at least as good as that obtained with diets containing 60 per cent of casein.

Since the mammary tumor-inhibiting effects of high protein diets could be largely overcome when a liberal amount of feed was consumed (viz., a higher intake of carcinogen), certain predictions might be made relating level of carcinogen intake to carcinogenicity. In every case, when the food intake exceeded an average of 7.2 gm/rat daily (carcinogen intake of 2.16 mg. or more daily), the protective effect of high protein diets was largely overcome. When the food intake was restricted so that the daily intake of the carcinogen was limited to between 1.80 and 2.10 mg/rat daily, then a protective effect of high dietary casein could be demonstrated. From these and earlier studies (4, 5), it can be further calculated (based on individual food consumption records) that a daily carcinogen intake below 1.80 mg. will result in a low incidence of mammary tumors, even on the 9 per cent casein diet. At this low level of carcinogen intake, however, the reduced incidence of mammary tumors is probably caused at least in part by the low caloric intake, as pointed out earlier (4).

These evaluations serve again to emphasize the importance of controlling the levels of food and carcinogen intake when effects of dietary factors are to be tested. In this connection, it should be mentioned that the protective influence of the high casein diets may be due not to the protein itself but rather to the decreased concentration of sucrose in such diets.

Vitamin B<sub>12</sub> and folacin had a very definite effect in hastening the carcinogenic process, an effect that can be explained on the basis of a high feed and high carcinogen intake. This supplement promoted earlier tumor development and almost completely eliminated the beneficial effects of high protein intake on survival. Vitamin B<sub>12</sub> has been reported to enhance the carcinogenic effect of 4-dimethylaminoazobenzene (1), and it was noted in early studies that folacin (pteropterin) hastened the induction of mammary tumors in rats fed 2-acetylaminofluorene (3). The growth of a transplantable mammary adenocarcinoma was, however, inhibited more effectively when vitamin B<sub>12</sub> or folacin was administered with 8-azaguanine than when the latter was administered alone (11).

#### SUMMARY

When weanling rats consumed a partially purified diet containing 9 to 27 per cent of casein, 57 out of 66 developed mammary tumors (86 per cent incidence). The daily intake of 2-acetylaminofluo-

rene ranged between 1.8 and 2.1 mg/rat. On a corresponding intake of the carcinogen, diets containing 40 or 60 per cent of casein produced a marked reduction in mammary tumor incidence (three of 25 rats or 12 per cent incidence). Increased intakes of food and carcinogen exceeding 2.1 mg. daily largely overcame the protective effects of the high protein diets and resulted in a high incidence of mammary tumors (20 of 26 rats or 77 per cent incidence). Irrespective of level of carcinogen and feed intake, the high protein diets promoted generally improved well-being and prolonged the survival period from an average of 28 weeks to over 40 weeks.

Suggestive evidence was obtained indicating that high casein diets were also partially protective against the induction of ear duct and liver tumors.

Desoxyribonucleic acid or yeast nucleic acid, added to a 12 per cent casein diet, had no influence on tumor induction. The supplementation of a 60 per cent casein diet with vitamin B<sub>12</sub> and folacin decreased the average survival period from 42 weeks to 32 weeks.

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