

The Effect of Added Dietary Tryptophane on the Occurrence of Diethylstilbestrol-induced Mammary Cancer in Rats*

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A comparative study by White, White, and Mider (8) of the effect of a restriction of cystine, lysine, and tryptophane in the diet of dba mice showed that the development of methylcholanthrene-induced leukemia was inhibited by a restriction in cystine but was unaffected by a restriction in the essential amino acids, lysine, and tryptophane. A previous study by White and Anderson (7) showed that the restriction of dietary cystine completely inhibited the formation of spontaneous mammary cancer in C3H mice and that mice on a high-cystine diet developed mammary cancer in the same proportion as C3H mice on a Purina Dog Chow diet. The average latent period for the tumors in the mice on the high-cystine diet was significantly shorter than that for the tumors in the control groups. No mammary cancers were observed in the 45 C3H mice on the low-cystine diet, although all survived the average latent period for the occurrence of these tumors and 36 survived for 22 months. Of 42 mice on the high-cystine diet, 41 developed mammary tumors in an average of 8.4 months, while 341 of 350 C3H mice on Purina Dog Chow developed mammary tumors in an average of 10.4 months. The percentage of tumor mice in the two latter groups was similar, but the difference in average latent period of 2.07 ± 0.23 months is certainly significant and equivalent to one-fifth the average latent period observed for the tumors of the control group.

In a later report, White and White (6) showed that the implantation of diethylstilbestrol pellets partially compensated for the effect of the low-cystine diet, indicating that, in part, the inhibition of the mammary tumors resulted from lack of hormonal stimulation in the mice on the deficient diet. In this experiment, 17 of the 40 treated mice on the low-cystine diet developed mammary tu-

mors in an average of 11.18 months, while 23 of 27 mice on Purina Dog Chow developed mammary tumors in an average of 9.13 months and 23 of 28 mice on the high-cystine diet developed mammary tumors in an average of 8.43 months. The average latent period was significantly longer in the mice on the low-cystine diet than in the mice of the two other groups and was shortest in the mice on the high-cystine diet, although the difference between the latter group and the controls does not appear to be significant. These experiments indicate that a reduction below the optimum dietary intake of cystine inhibits mammary cancer formation and the occurrence of methylcholanthrene-induced leukemia, and that increased dietary cystine has an accelerating action on the development of mammary cancer.

The present experiments were undertaken for the purpose of exploring the effects of deficiencies and excesses of tryptophane on the occurrence of diethylstilbestrol-induced mammary cancer in rats. They included five experimental diets containing, respectively, 0.10, 0.14, 1.0, 1.4, and 4.3 per cent DL-tryptophane. No significant data were obtained from the first group because the rats failed to survive the average latent period for the induction of these tumors. Comparative data are not yet available for the groups which received 0.14 and 1.0 per cent tryptophane, but completed data for the two groups which received the greatest increment of tryptophane seemed of sufficient interest to report at this time.

MATERIAL AND METHODS

Pedigreed female rats of A×C line 9935 (2) between 4 and 5 months of age were used for the experiments. Cholesterol pellets containing 4–6 mg. of diethylstilbestrol¹ were implanted subcutaneously in the scapular region, to maintain a continuous state of hyperestrinism. A previous re-

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port (1) showed that 75 per cent of similarly treated female rats of this line, on an average daily consumption of 34 calories of a synthetic diet, developed multiple mammary cancers in 10 months.

In the present experiment, each rat was housed in an individual cage, with free access to water. The daily portion of 7 gm. of diet was weighed out and presented in a food cup, to which was added the daily supplement of crystalline vitamins. The

TABLE 1

THE NUMBER OF RATS IN EACH GROUP, THEIR AVERAGE INITIAL BODY WEIGHT, AND DAILY FOOD CONSUMPTION

Group	Number of rats	Body weight (gm.)	Daily ration (gm.)	Daily ration (calories)
Diet 3	12	162	6.9	32
Diet 7	12	153	7.0	33
Diet 5	15	160	6.8	32

diet of 6.5 per cent fat content (1). An attempt was made to recover and weigh all food that was spilled. The proportion of protein was chosen on the basis of McCoy's (3) finding that approximately 25 per cent was optimum for lactation.

Each rat was weighed and inspected for mammary cancers once a week. At death, post-mortem examination included a detailed description of every visible tumor, gross sectioning of lungs and mammary glands, and the inspection and weight of the liver, kidneys, adrenals, pituitary, and sex glands. Representative sections of each of these tissues and organs were preserved for microscopic examination. All cancer foci that could be identified were tabulated.

RESULTS

The number of rats in each group, their average initial body weight, and daily food consumption are

TABLE 2

THE NUMBER OF RATS WHICH SURVIVED FOR AT LEAST 160 DAYS, THEIR AVERAGE POST-MORTEM BODY WEIGHTS IN GRAMS, AND THE PERCENTAGE WEIGHTS OF SOME OF THE ORGANS

GROUP	NUMBER OF RATS	AVERAGE BODY WT.	ORGAN WEIGHTS IN PER CENT OF BODY WEIGHT					
			Liver	Kidney	Adrenal	Pituitary	Ovary	Uterus
Diet 3	12	121	5.5	1.4	.05	.06	.05	.66
Diet 7	12	117	4.4	1.2	.05	.06	.04	.65
Diet 5	15	99	5.4	1.5	.05	.03	.04	.75

three diets differed only in the composition of the protein as follows:

	Diet 7 (per cent)	Diet 3 (per cent)	Diet 5 (per cent)
Casein	26.0	0	0
Tryptophane-free casein hydrolysate ²	0	25.0	22.0
Salt mixture	4.0	4.0	4.0
Cellu flour	2.0	2.0	2.0
Dextrin	54.0	54.0	54.0
Crisco	15.0	15.0	15.0
Halibut liver oil	0.4	0.4	0.4
DL-tryptophane	0	1.4	4.3

plus the following crystalline vitamin³ supplement per kilogram:

Thiamin	4 mg.
Riboflavin	8 mg.
Pyridoxine HCl	4 mg.
Niacin	4 mg.
Calcium pantothenate	20 mg.
Choline HCl	2,000 mg.
Alpha-tocopherol	150 mg.

Each gram of diet was equivalent to 4.7 calories, and the daily portion fed was 33 calories, a little less than the *ad libitum* consumption of a synthetic

² Supplied through the courtesy of Dr. Charles F. Kade of the Sterling-Winthrop Research Institute, Rensselaer, N.Y.

³ Supplied through the courtesy of Dr. R. C. Pogge of Merck & Co., Rahway, N. J.

shown in Table 1. The 12 rats on Diet 7, the control diet, consumed all the food that was offered them, while those on Diets 3 and 5, with the added tryptophane, averaged 32 calories per rat per day, or 1 calorie less per day than those on the control diet.

A decrease in body weight as shown by the average post-mortem weights in Table 2, is characteristic of diethylstilbestrol-treated rats. However, the rats on Diet 5 with the greatest increment of tryptophane lost proportionately more than the rats of the two other groups. Their caloric consumption was essentially similar, so they must have failed to utilize some of the ingested food. There were no significant differences in the organs which were weighed as shown by their percentage weights in Table 2, except in the case of the pituitary. The average pituitary weights were 70, 76, and 29 mg., respectively, for the rats on Diets 3, 7, and 5. The respective percentage weights were 0.06, 0.06, and 0.03. If size of organ is correlated with function or physiological demand, the rats on the high-tryptophane diet had less pituitary hormone stimulation than the rats of the two other groups.

The average survival in days and the number

and percentage of induced mammary cancers are given in Table 3. The individual survival record, time of observation of the first mammary cancer, and number of induced cancers for each rat are shown graphically in Figure 1. The twelve rats on Diet 3 all developed multiple mammary cancers in an average of 316 ± 5.7 days, while nine of the

The effect of the increased proportion of tryptophane seems to be analogous to that of caloric restriction, as shown in a previous report (1), but disproportionately great for the loss of approximately 3 per cent of unusable protein. The caloric intake was similar in the three groups of rats, but, obviously, there was a difference in assimilation,

TABLE 3

THE AVERAGE SURVIVAL IN DAYS, THE NUMBER AND PERCENTAGE OF RATS IN EACH GROUP WITH MULTIPLE MAMMARY CANCERS, THE NUMBER OF MACROSCOPIC CANCERS, AND THE TOTAL NUMBER OF CANCER FOCI OBSERVED, THE AVERAGE WEIGHT OF CANCER PER RAT, AND THE MINIMUM AND MEAN LATENT PERIOD IN DAYS

GROUP	NUMBER OF RATS	AVERAGE SURVIVAL (days)	RATS WITH CA. (per cent)		NUMBER OF CAS.		AVE. WT. OF CAS. (gm.)	MINIMUM LATENT PERIOD	MEAN LATENT PERIOD
			(No.)	(per cent)	Macroscopic	Total			
Diet 3	12	361	12	100	79	194	13.2	209	316 ± 5.7
Diet 7	12	433	9	75	51	133	10.7	163	363 ± 8.6
Diet 5	15	399	9	60	31	109	5.1	288	399 ± 7.5

twelve rats on the control or 26 per cent casein diet developed multiple mammary cancers in an average of 363 ± 8.6 days. The number of rats in each group is too small for the difference in per cent of induced tumors, as shown in Figure 2, to be significant; but the difference of 47 ± 10 days in the mean latent period is certainly significant. This is shown graphically in Figure 3. Table 3 also shows that 79 gross mammary cancers—including with these the cancers identified by microscopic examination of the mammary tissue, a total of 194 cancer foci—were observed in the rats on Diet 3; while 51 gross cancers, and a total of 133 cancer foci, were identified in the rats of Diet 7. The average weight of mammary cancers per rat was 13.2 in the former group and 10.7 in the latter group, while the individual tumors averaged 2.0 gm. in the rats on Diet 3 and 1.7 gm. in the rats on Diet 7. All these differences seem to indicate that the substitution of approximately 1 per cent of DL-tryptophane for casein, in an otherwise similar diet, increased the susceptibility of these rats in induced mammary cancer.

These same charts and Table 3 show, conversely, that the substitution of as much as 4 per cent DL-tryptophane for a similar amount of casein decreased the number and percentage of induced mammary cancers and significantly prolonged the average latent period. In this group 9, or 60 per cent, of 15 rats developed 31 macroscopic and a total of 109 macroscopic and microscopic induced mammary cancers in an average of 399 ± 7.5 days. The average weight of mammary cancer per cancer rat was only 5.1 gm. compared with 13 gm. for cancers in rats on Diet 3, and the individual tumors averaged 1.5 gm. compared with 2.0 gm.

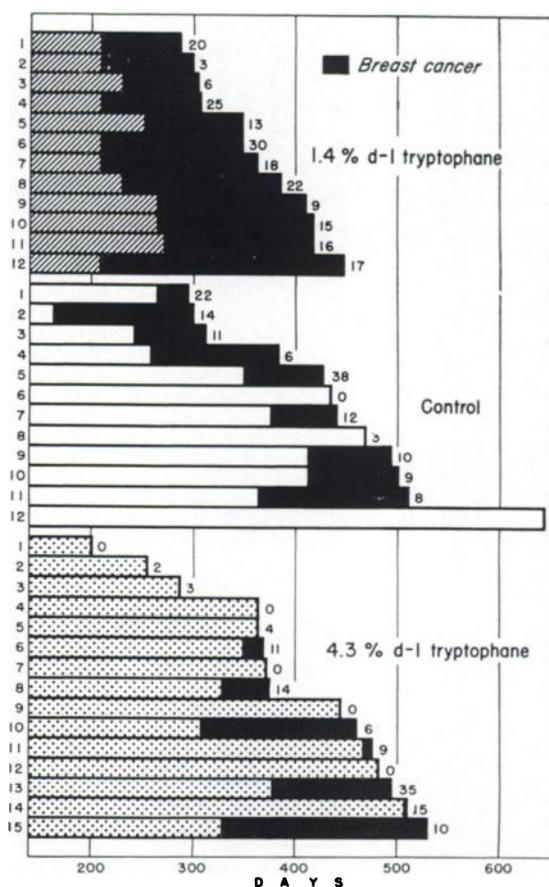


FIG. 1.—Survival period and tumor history of rats of each group. (Each rat is represented by a bar, the length of which indicates the period of survival; the blackened area represents the time elapsed after observation of the first mammary cancer; and the number at the right of the bar represents the total number of mammary cancer foci identified in the post-mortem study.)

for the rats on Diet 5 weighed nearly 20 per cent less than the rats of the two other groups.

Tannenbaum and Silverstone (4, 5) have recently concluded, on the basis of experiments with spontaneous mammary and lung cancers and induced skin cancers in mice, that the effect brought about by caloric restriction is a function of the low body weight of the animals. That is, the actual caloric intake or metabolic turnover is not as important in the genesis of these tumors as the bal-

mas, and 2 with areas of squamous-cell cancer. The gross tumors in the rats on the high-tryptophane diet were classified as 29 papillary adenocarcinomas, 1 squamous carcinoma, and 1 early carcinoma. Axillary lymph node metastases were observed in one rat of the control group and lung metastases in one of the rats on Diet 3. One of the rats in the latter group had, in addition, a fibrosarcoma of the stomach, and one of the rats on the high-tryptophane diet had a mixed tumor of the left ovary.

SUMMARY

1. Thirty-nine A×C line 9935 female rats with cholesterol pellets containing 4–6 mg. of diethylstilbestrol implanted in their scapular region were distributed into three groups and placed

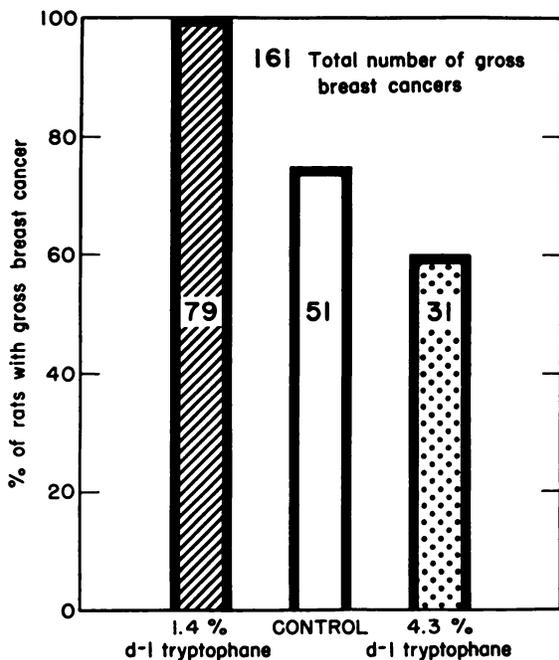


FIG. 2.—The number and per cent of mammary cancers in each group.

ance struck between caloric intake and utilization. This might explain the effect observed here and the previously reported lack of effect from the increased consumption of a high-fat diet (1), but this theory is not substantiated by the results of a forthcoming study of acetylaminofluorene-induced liver and bladder cancer in rats on similar diets. In this study, rats with low body weights do not show a proportionate reduction in either type of induced cancer.

The tumors in the three groups were of the same general morphology, with a larger proportion of the more malignant types occurring in the rats on the 1.4 per cent tryptophane diet. The gross, i.e., macroscopic, tumors in the rats of this group included 37 papillary adenocarcinomas, 36 adenocarcinomas and solid type carcinomas, and 6 with varying amounts of squamous-cell cancer. The tumors in the control group comprised 41 papillary adenocarcinomas, 8 solid type and adenocarcino-

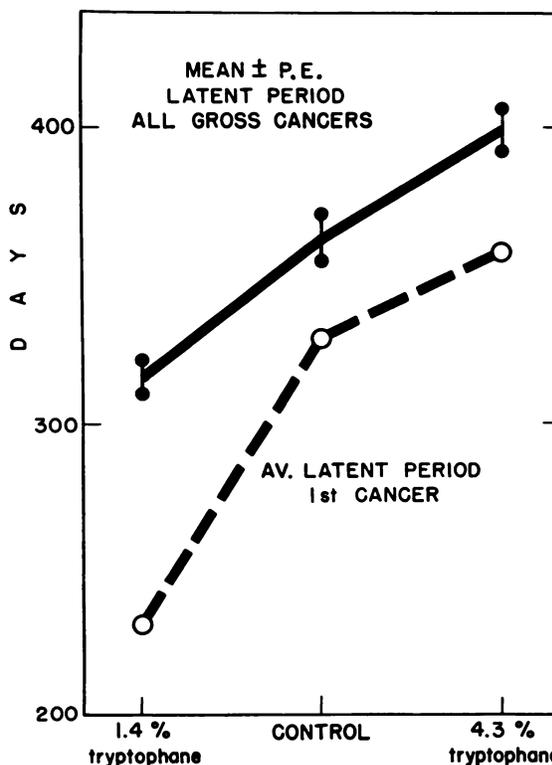


FIG. 3.—The average latent period for the development of first mammary cancers in each group and the mean latent period ± P.E.

on isocaloric synthetic rations containing 26 per cent protein with substitutions of 1.4 and 4.3 per cent of DL-tryptophane for tryptophane-free casein hydrolysate in two of the groups.

2. All 39 rats survived the minimum latent period of 163 days, and 30, or 77 per cent, developed 161 macroscopic mammary cancers and a total of 436 independent mammary cancer foci.

3. The largest proportion of tumors (79 macro-

scopic or 194 total) was observed in the rats on the diet containing 1.4 per cent DL-tryptophane and the smallest number (31 macroscopic and 109 total) in the rats on the diet containing 4.3 per cent DL-tryptophane.

4. The average latent period of 316 ± 5.7 days for the tumors in rats on the 1.4 per cent tryptophane diet was significantly shorter than that for the tumors in rats on the control diet (363 ± 8.6 days), or the average latent period of 399 ± 7.5 days for the tumors in the rats on the high-tryptophane diets.

5. The addition of approximately 1 per cent of dietary tryptophane appeared to enhance the development of diethylstilbestrol-induced mammary cancer, while the greater increase of 4 per cent in dietary tryptophane inhibited the formation of these tumors. Depletion in body weight or the consequent inanition may explain the latter result.

6. The 161 macroscopic tumors included 107 papillary cyst adenocarcinomas, 44 adenocarcinomas and solid type carcinomas, 9 with varying amounts of squamous-cell cancer and 1 unclassified.

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