Further Studies on the Relation of Dietary Tryptophan to the Induction of Neoplasms in Rats*

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A previous report (1) showed that the addition of 1.4 per cent DL-tryptophan to a synthetic diet containing 25 per cent tryptophan-free casein hydrolysate increased the number and percentage of diethylstilbestrol-induced mammary cancers and significantly reduced the average latent period below that observed for rats of the control group on an isocaloric 26 per cent casein diet. A subsequent report (2) showed that the addition of 1.4 or 4.3 per cent DL-tryptophan to a similar diet to which 0.06 per cent 2-acetylaminofluorene was also added doubled the percentage of rats that died with liver cancer and appeared to be an etiological factor in the initiation of bladder cancers. No bladder cancers were observed in the rats on the control 26 per cent casein diet, while 92 and 100 per cent, respectively, of those on the diets with 4.3 and 1.4 per cent added DL-tryptophan died with gross papillary and squamous-cell cancers of the bladder.

These experiments indicated that an excess of dietary tryptophan increased the incidence of neoplasms that resulted from a continuous state of hyperestrinism and also from the ingestion of 2-acetylaminofluorene. The present experiments were undertaken, therefore, to determine whether or not diets similar to those used in the previous experiments but deficient in the essential amino acid tryptophan would have any protective action against neoplasms induced by these same carcinogens.

MATERIALS AND METHODS

As in the previously reported experiment the rats used in the study of the incidence of mammary cancers were pedigreed A × C line 9935 females that had been given subcutaneous implants of cholesterol pellets containing 4–6 mg. (average 4.8) of diethylstilbestrol. For the study of the incidence of liver and bladder cancers Fischer line 344 female rats were fed 2-acetylaminofluorene in the diet. The rats of both groups were between 4 and 5 months of age at the start of the experiment.

Each rat was housed in an individual cage with free access to water. The daily portion of 7 gm. of food was weighed out, and ½ cc. of distilled water containing the daily supplement of crystalline B vitamins was added to each cup which was shaken briefly to mix in the vitamins before it was placed in the cage. Attempts were made to recover, weigh, and record any food that was spilled. Each gram of diet was equivalent to 4.7 calories, and the daily portion was 33 calories or a little less than the ad libitum consumption.

The diets as shown in Table 1 differed only in the nature of the protein and the presence or absence of 2-acetylaminofluorene. Diets number 7

TABLE 1

COMPOSITION OF THE DIETS

Material	Diet no. 7	Diet no. 11	Diet no. 9	Diet no. 12
	(per cent)			
Casein	26.0	0	26.0	0
Tryptophan-free casein hydrolysate*	0	26.0	0	26.0
Salt mixture	4.0	4.0	4.0	4.0
Cellu flour	2.0	2.0	2.0	2.0
Dextrin	54.0	54.0	54.0	54.0
Crisco	15.0	15.0	15.0	15.0
Halibut liver oil	0.4	0.4	0.4	0.4
DL-Tryptophan	0	0.14	0	0.14
2-Acetylaminofluorene	0	0	0.06	0.06

Vitamin supplement per kilogram of diet†

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

* Vacuum oven-dried casein hydrolysate Batch Sah-A-79, tested and supplied through the courtesy of Dr. Charles F. Kade of the Sterling-Winthrop Inst., Rensselaer, N.Y.

† The crystalline B vitamins were dissolved in distilled water and stored in a brown bottle in the refrigerator and added as a daily supplement to each food cup. (Preliminary experiments indicated that some of the rats showed evidence of vitamin deficiency when an attempt was made to mix these vitamins in the diet. It was felt that the mass of diet was so great compared to the quantity of vitamin that an equal distribution was not always achieved. The method adopted proved satisfactory and has been used by the authors in all reported experiments.)

* Supported in part by a grant-in-aid from the United States Public Health Service and a grant from the Damon Runyon Memorial Fund for Cancer Research, Inc.

Received for publication November 16, 1953.

and 9 were the control diets each containing 26 per cent casein, and 9 had in addition 0.06 per cent 2-acetylaminofluorene. Diets number 11 and 12 were similar, except that 26 per cent tryptophan-free casein hydrolysate was substituted for the casein and 0.14 per cent DL-tryptophan was added. This was somewhat less tryptophan than the 0.2 per cent considered by Rose (3) as sufficient to support normal growth in the rat. A previous attempt to reduce the added tryptophan to 0.10 per cent had proved unsuccessful for diethylstilbestrol-treated rats, because none of them survived the average latent period for the induction of mammary cancers.

Each rat was weighed and inspected for tumors once a week. At death, post mortem examination included description of all visible tumors, 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, and prepared sections were examined microscopically.

RESULTS

The two groups of diethylstilbestrol-treated rats ate nearly all the food that was offered to them, or an average of 6.9 gm. or 32 calories daily. The diets to which the 2-acetylaminofluorene was added were somewhat less palatable, and the control and experimental group consumed an average of 6.1 and 6.2 gm., respectively, or only 29 calories daily. The average daily dose of 2-acetylaminofluorene ingested was 3.7 mg. The results are summarized briefly in Tables 2 and 3.

From Table 2 it appears that the diethylstilbestrol-treated rats of both groups lost considerable body weight and that the loss was somewhat greater in the rats on the tryptophan-deficient diet than on the control diet. At death the rats on diet number 7 weighed an average of 119 gm., compared to an average of 92 gm. for the rats on the deficient diet number 11. The rats on diet number 9 (the control diet with added acetylaminofluorene) showed very little change in body weight, while those on the tryptophan-deficient diet number 12 decreased from an average of 159 gm. to 100 gm.

Organ weights except for the kidneys reflected the type of treatment rather than differences due to diet. The percentage weight of the kidneys was the same in both tryptophan-deficient groups and was slightly but probably not significantly higher than in either group on the control diet. Both groups of diethylstilbestrol-treated rats had proportionately larger pituitaries, adrenals, ovaries, and uteri than the acetylaminofluorene-fed rats, while both of the latter groups showed proportionately larger livers. The pituitaries of the diethylstilbestrol-treated rats on the control diet averaged 110 mg., while those on the low tryptophan diet averaged considerably less or 36 mg., but both were significantly higher than the average of 12 and 10 mg. observed for the two groups of acetylaminofluorene-fed rats.

TABLE 2

THE AVERAGE INITIAL AND FINAL BODY WEIGHT AND PERCENTAGE WEIGHTS OF SOME OF THE ORGANS

Group	No. rats	Initial body wt. (gm.)	Final body wt. (gm.)	Organ weight in per cent of body weight: Liver	Kidney	Adrenal	Pituitary	Ovary	Uterus
Diet No. 7	13	163	119	4.8	1.3	0.05	0.09	0.05	0.78
Diet No. 11	16	141	92	4.9	1.5	0.06	0.04	0.06	0.72
Diet No. 9	16	144	136	11.7	1.2	0.04	0.01	0.04	0.28
Diet No. 12	11	159	100	11.1	1.5	0.04	0.01	0.04	0.30

TABLE 3

DATA ON INDUCED MAMMARY GLAND AND LIVER CANCERS IN RATS ON DIETS ADEQUATE AND DEFICIENT IN TRYPTOPHAN

Group	No. rats	Av. survival (days)	Mammary cancers: Per cent with cancer	No. cancers	Av. latent period (days)	Liver neoplasms: Per cent with neoplasms	Av. latent period (days)
Stilbestrol:							
Control diet No. 7	13	416	85	93	346	0	
Deficient diet No. 11	16	316	56	83	273	0	
2-Acetylaminofluorene:							
Control diet No. 9	16	283	0			81	302
Deficient diet No. 12	11	352	9	1	253	55	400

Table 3 and Chart 1 show that 85 per cent of the diethylstilbestrol-treated rats on the control diet developed mammary cancers in an average of 346 days. There were a total of 93 probably independent mammary cancers or an average of more than eight for each of the eleven rats with cancer. One

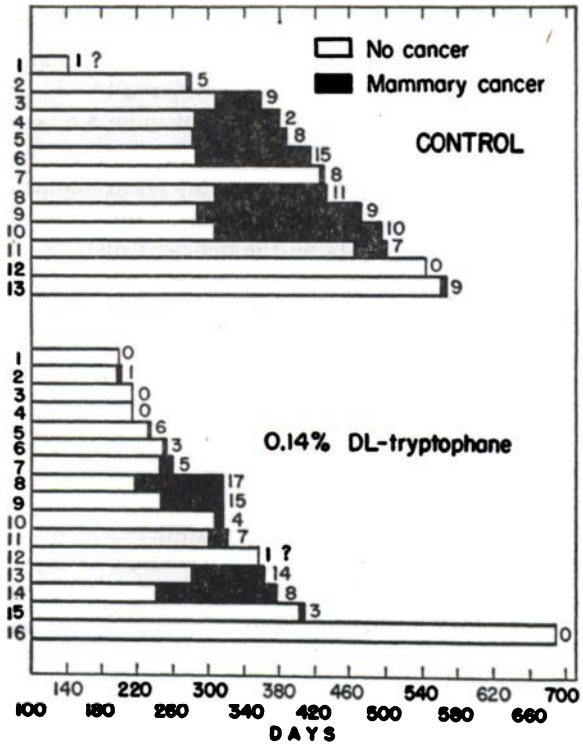

CHART 1.—Survival period and mammary cancer history of each group of diethylstilbestrol-treated rats. (Each rat is represented by a bar, the length of which indicates the period of survival; the blackened area represents the time elapsed after observation of the first mammary cancer; the number at the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

rat, number 12 in Chart 1, survived for 544 days and died with no evidence of neoplasia. Number 3, in addition to nine mammary cancers, had a papilloma in the vagina and number five with eight mammary cancers had also an adenocarcinoma of the left adrenal. Of the sixteen rats on the deficient diet only 56 per cent died with mammary cancers, but these nine rats had a total of 83 probably independent cancers or an average of more than nine for each cancer-bearing rat, and the average latent period was only 273 days. The average survival period for these rats was 100 days less than for the ones on the control diet, and with one exception the noncancer rats died relatively early, as shown in Chart 1. The exceptional rat survived for 689 days and died with ample evidence of excessive estrogenic stimulation but no evidence of mammary neoplasia and with gross and microscopic evidence of lymphatic leukemia. Rat number 8 of this group had a fibrosarcoma in the right ovary, and number 12 had an adenocarcinoma of the left adrenal with lung metastases.

Among the acetylaminofluorene-fed rats on the control diet five or 31 per cent of the sixteen controls had multiple malignant hepatomas, and eight additional rats had liver neoplasms that were considered probably benign. One of the latter, however,

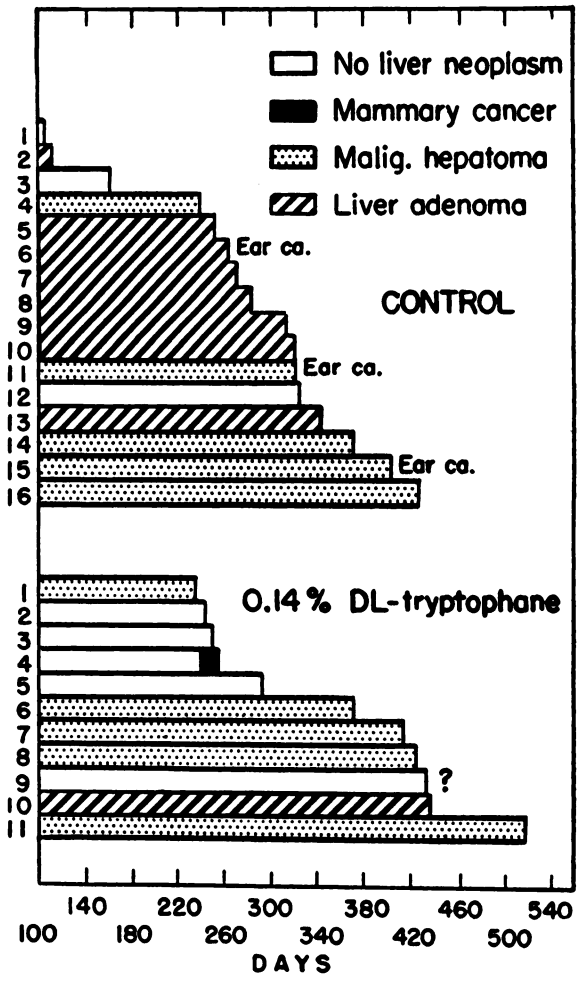

CHART 2.—Survival period and tumor history of each group of 2-acetylamino-fluorene-fed rats. (Each rat is represented by a bar, the length of which indicates the period of survival; the pattern as shown in the key indicates the character of the neoplasm identified in the post mortem study.)

and two with recognized malignant hepatomas had extensive lung metastases. All the rats showed extreme cirrhosis of the liver. Three rats of the control group as shown in Chart 2 also had squamous-cell carcinoma of the external auditory meatus, and the neoplasms were bilateral in one of them.

Five or 46 per cent of the eleven rats on diet 12

with deficient tryptophan had malignant hepatomas. One additional rat had several neoplastic nodules that were probably benign, and rat number 9 had an area of reduplicated bile ducts containing an area of possible early adenocarcinoma. Rat number 4 had a large squamous-cell carcinoma in the right groin that was probably of mammary gland origin. No tumors of the external ear were observed in the rats of this group, and no evidence of metastases was found in the lungs of the rats with liver cancers.

No gross papillomas or squamous-cell cancers were found in the urinary bladders of the rats of either group. Slight hyperplasia of the bladder mucosa was found in four rats of the control group and in six rats on the deficient diet. An area that was suspicious of early cancer but not definite enough for a positive diagnosis was found in one rat on the deficient diet. These data contrast notably with the previously reported (2) findings of 100 and 92 per cent bladder cancers, respectively, in the groups fed 1.4 and 4.3 per cent DL-tryptophan in a similar diet. The average survival period for the group that received 0.14 per cent DL-tryptophan was 352 days or intermediate between the average of 400 and 320 days for the survival of the previously reported groups, and was therefore not a factor in the observed difference in the incidence of bladder cancers. The same batch of casein hydrolysate was used as the source of protein for all three of these diets, so the difference in tryptophan content of the diet must be a real factor in the etiology of the bladder neoplasms. A tolerable chronic deficiency in dietary tryptophan, however, proved inadequate protection against the incidence of either induced liver or mammary gland neoplasms.

SUMMARY

1. Sixteen diethylstilbestrol-treated A×C line 9935 female rats on a synthetic diet containing 0.14 per cent DL-tryptophan and 26 per cent tryptophan-free casein hydrolysate were compared to thirteen similar rats on an isocaloric synthetic diet containing 26 per cent casein.

2. The rats on the deficient diet lost more weight and showed a smaller percentage increase in pituitary weight than the control rats.

3. Eighty-five per cent of the rats on the control diet died with multiple mammary gland cancers in an average of 416 days compared to 56 per cent of the rats on the deficient diet in an average of 316 days.

4. A total of 93 mammary adenocarcinomas were observed in the rats of the control group compared to 83 in the rats on the deficient diet.

5. Sixteen Fischer line 344 female rats on the same control diet plus 0.06 per cent 2-acetylaminofluorene were compared to eleven similar rats on a diet containing 0.14 per cent DL-tryptophan and 0.06 per cent 2-acetylaminofluorene.

6. Eighty-one per cent of the rats on the control diet with 2-acetylaminofluorene had benign or malignant liver neoplasms, compared to 55 per cent of the rats on the tryptophan-deficient diet. The average latent periods were 302 and 400 days, respectively.

7. No bladder cancers were observed in the 2-acetylaminofluorene-fed rats of either group.

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