TABLE IV. Average Survival in Days ± P.E. and % of Rats with Induced Cancer.

Group %	No. rats	Survival, days ± P.E.	Liver cancer %	Liver cancer % met.	Ear cancer %	Ear cancer Avg days	Mammary cancer %	Mammary cancer Avg days
45 casein	16	349 ±11.	75	67	44	323	25	327
5 high nucleic acid yeast	18	409 ± 9.4	83	47	33	338	6	406
1 nucleic acid	12	324 ±11.	67	62	67	290	0	
2 " "	12	322 ± 6.1	92	54	50	280	0	
5 " "	12	315 ± 6.1	83	30	75	286	0	
5 Brewer's yeast	11	317 ± 6.5	82	62	64	319	0	

diets containing added tryptophan(1) or indole(4). An adenocarcinoma of the lungs that was probably primary was observed in one rat on the 2% nucleic acid diet. Two rats that received the Brewers yeast supplement had mixed tumors (adenocarcinoma and osteosarcoma) of the illium. One rat on the high nucleic acid diet had a fibrosarcoma of the right fore leg with lung and lymph node metastases and another had an adenoma of the adrenal.

Summary. 1. The substitution of 5% high (6%) nucleic acid yeast for casein in a synthetic diet containing 45% casein and 0.06% 2-acetylaminofluorene significantly increased average survival of the rats but did not prevent development of the induced neoplasms. 2. Substitution of 1, 2, or 5% purified desoxyribonucleic acid or 5% Brewers yeast for casein in similar diets did not affect average survival period or frequency of occurrence of the induced neoplasms. 3. The high protein diet appeared to accelerate occurrence of the acetylaminofluorene-induced squamous cell carcinomas of the external auditory meatus.

1. Dunning, W. F., Curtis, M. R., Maun, M. E., *Cancer Research,* 1950, v10, 454.
2. Harris, P. N., *ibid.,* 1947, v7, 88.
3. Morris, H. P., Wagner, B. P., Velat, C. A., *J. Nat. Cancer Inst.,* 1955, v16, 489.
4. Dunning, W. F., Curtis, M. R., *Abst. Proc. Am. Assn. for Cancer Res.,* 1957, v1.

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The Role of Indole in Incidence of 2-Acetylaminofluorene-Induced Bladder Cancer in Rats.* (24258)

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Previous reports(1,3) have shown that addition of 1.4% DL-tryptophan to a synthetic diet containing 25% tryptophan-free casein hydrolysate and 0.06% 2-acetylaminofluorene increased from 37 to 75 the percentage of rats that developed liver cancer and appeared to be an etiological factor in the incidence of bladder cancer. A study by Glass and Plaine (4) of the enhancing effect of dietary tryptophan on appearance of melanotic tumors in the fruit fly *Drosophilia melanogaster* showed that indole was the effective promoting agent.

In rats indole-acetic acid is the natural metabolite of tryptophan and indole results from action of intestinal bacteria. It seemed therefore, of interest to determine whether or not either indole or indole acetic acid were responsible for the observed enhancing effect of dietary tryptophan on the incidence of bladder cancer in these rats.

Material and methods. Pedigreed Fischer line 344 female rats 4 or 5 months of age were divided into 4 groups and placed on the synthetic diets to be described. Each rat was housed in an individual cage with free access to water. A daily portion of 7 g of diet or ap-

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TABLE I. Composition of Diets.

Material	Diet 30	Diet 31	Diet 32	Diet 33
	%			
Casein	25.0	25.6	24.8	25.4
Salt mixture	4.0	4.0	4.0	4.0
Cellu flour	2.0	2.0	2.0	2.0
Dextrin	52.0	52.0	52.0	52.0
Crisco	15.0	15.0	15.0	15.0
Halibut liver oil	.4	.4	.4	.4
DL-tryptophan	1.4	0	0	0
Indole	0	.8	1.6	0
Indole acetic acid	0	0	0	1.0
2-acetylaminofluorene	.06	.06	.06	.06

proximately 33 calories was weighed out and presented in a food cup to which was added the daily supplement of crystalline vitamins.

The composition of the diets is shown in Table I. Each diet contained approximately 26% protein (casein), 52% carbohydrate (dextrin), 15% fat (crisco) plus adequate minerals and vitamins. The essential variants were 1.4% DL-tryptophan for diet 30; 0.8% indole for diet 31; 1.6% indole for diet 32; and 1.0% indole acetic acid for diet 33. Diet 30 served as the control diet for this experiment and was similar in composition to the previously reported(3) experimental Diet 4 except that the 1.4% DL-tryptophan was added to intact casein instead of the tryptophane-free casein hydrolysate. All of the diets contained the same quantity or 0.06% 2-acetylaminofluorene and the following vitamin supplement per kilo of diet:

	mg
Thiamin	4
Riboflavin	8
Pyridoxine HCL	4
Niacin	4
Calcium pantothenate	20
Choline HCL	2,000
α-tocopherol	150

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, bladder and mammary glands and inspection and weight of the liver, kidneys, adrenals, pituitary, thyroid and sex glands. Representative sections of each of these tissues and organs were preserved and examined microscopically.

Results. Table II shows the number of rats in each group, average initial body weights, daily food consumption, and daily and total dose of 2-acetylaminofluorene ingested. The 4 groups varied very little in either calorie or drug intake. Average daily caloric intake varied from 28 to 29 calories and average daily dose of 2-acetylaminofluorene ingested varied from 3.5 to 3.7 mg.

Table III shows average post-mortem body weight and percentage weights of some of the organs from the rats of each group. The rats that received 1.0% indole acetic acid and 1.6% indole lost more than 20% in total body weight. The group that received added DL-tryptophan lost an average of 15% in total body weight. Rats of the group that received 0.8% indole sustained an average loss of 8%. The livers were proportionately larger in the rats that received DL-tryptophan and indole acetic acid than they were in the other groups and the thyroids were larger in the 2 groups that received indole. Otherwise there appeared to be no consistent differences in the organs that were weighed.

Average survival period and percentage of induced neoplasms is shown in Table IV. A group of rats that received a synthetic diet deficient (0.14%) in tryptophan from a previous experiment(2) has been included for comparison with the currently reported groups. Average survival period varied from 334 days for rats on the diet containing 1.0% indole acetic acid to 436 days for the rats that

TABLE II. Number of Rats in Each Group, Average Initial Body Weight, Daily Food Consumption, and Dose of 2-Acetylaminofluorene.

%	Group	No. of rats	Body wt (g)	Daily ration (g)	Daily ration (cal.)	Total dose AAF (g)	Daily dose AAF (mg)
1.4	DL-tryptophan	12	124	5.9	28	1.3	3.5
1.0	Indole acetic acid	12	124	6.1	29	1.2	3.7
.8	Indole	12	126	6.0	28	1.5	3.6
1.6	"	12	131	6.2	29	1.6	3.7

TABLE III. Average Post Mortem Body Weight, and Percentage Weights of Some Organs.

%	Group	No. of rats	Body wt (g)	Organ wt in % of body wt: Liver	Kidney	Adrenal	Pituitary	Ovary	Uterus	Thyroid
1.4	DL-tryptophan	12	105	17.6	1.2	.04	.01	.05	.34	.01
1.0	Indole acetic acid	12	95	18.1	1.4	.04	.01	.05	.34	.01
.8	Indole	12	116	14.0	1.2	.03	.01	.04	.34	.02
1.6	"	12	97	10.3	1.2	.05	.01	.05	.36	.02

TABLE IV. Average Survival in Days, and Percentage of Rats with Induced Cancers.

%	Group	No. of rats	Days to death	% with cancer of: Liver	Ear	Breast	Bladder
1.4	DL-tryptophan	12	360	91	25	17	75
1.0	Indole acetic acid	12	334	83	25	0	58
.8	Indole	12	407	83	25	17	83
1.6	"	12	436	83	0	0	83
.14	DL-tryptophan	11	352	55	0	9	0

received 1.6% indole.

Liver cancers were observed in all groups. All but one of the rats on Diet 30 with the 1.4% tryptophan added to intact casein and all but 2 rats in each group that received indole and indole acetic acid developed liver cancers. This may be compared with 8 out of 11 or 73% that developed liver cancers in the previously reported group that received 1.4% added DL-tryptophan. As previously mentioned the livers were larger in the rats that received 1.4% tryptophan and 1.0% indole acetic acid than in the rats that received indole. The tumors also were notably larger in the rats of these 2 groups.

Table V shows that malignant hepatoma (Fig. 1) were identified in all 11 of the rats that received 1.4% tryptophan and in 10 of the rats that received 1.0% indole acetic acid. Lung metastases (Fig. 2) composed of neoplastic primary liver cells were identified in 63 and 90%, respectively, of the rats of these 2 groups. In addition, 4 of the former and 6 of the latter had areas of cholangioma and adenocarcinoma. No metastases resembling these neoplasms were identified. Of the rats that received 0.8% and 1.6% indole 7 and 6, respectively, had neoplasms identified as malignant hepatoma with lung metastases identified in 4 and 2, respectively. In addition, 8 rats in each of the indole-fed groups had liver tumors that were classified as cholangioma or adenocarcinoma (Fig. 3) probably of bile duct origin. Metastases (Fig. 4) from these tumors were identified in the lungs of 3 rats that received 0.8% indole and in one rat that received 1.6% indole. In general the liver tumors in the indole-fed rats were smaller and more discreet than in the rats of the other groups so that the apparent difference in morphology may have resulted from errors in sampling. Only representative sections from each liver and lung were examined microscopically and in the larger livers and tumors of the rats that received 1.4% tryptophan and 1.0% indole acetic acid the tumors of bile duct origin may have been missed.

In previous studies tumors of the external auditory meatus have been observed in about 20% of Fischer line 344 female rats that have

TABLE V. Classification of Induced Liver Neoplasms, and Percentage with Metastases.

%	Group	Hepatoma	% with met.	Cholangioma and adeno-carcinoma	% with met.
1.4	DL-tryptophan	11	63	4	0
1.0	Indole acetic acid	10	90	6	0
.8	Indole	7	57	8	38
1.6	"	6	33	8	12

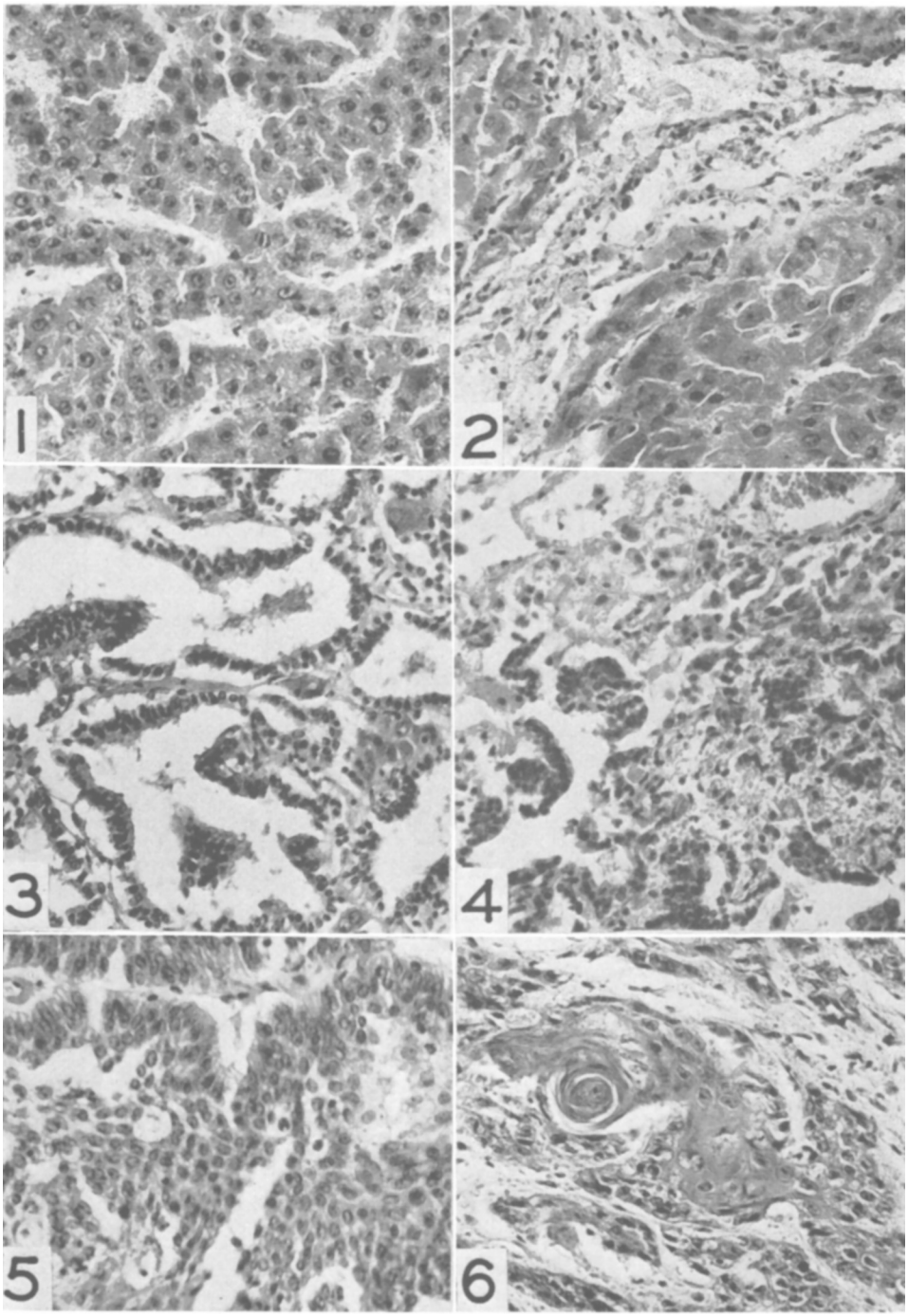

FIG. 1. Malignant hepatoma from rat 333 days after being placed on diet containing 1.0% indole acetic acid and 0.06% acetylaminofluorene. × 272.

FIG. 2. Multiple metastases in lungs of rat with malignant hepatoma shown in Fig. 1. × 272.

FIG. 3. Adenocarcinoma of liver in rat 553 days after being placed on diet containing 0.8% indole and 0.06% acetylaminofluorene. × 272.

FIG. 4. Metastases in lungs of rat with adenocarcinoma of liver shown in Fig. 3. × 272.

FIG. 5. Papillary carcinoma of bladder in rat 384 days after being placed on diet containing 0.8% indole and 0.06% acetylaminofluorene. × 272.

FIG. 6. Squamous cell carcinoma of the bladder from rat 477 days after being placed on diet containing 1.6% indole and 0.06% acetylaminofluorene. × 272.

been fed 0.6% 2-acetylaminofluorene. In this study 3 rats or 25% in each of 3 groups had tumors of the external ear at death. All 9 were squamous cell carcinoma.

Adenocarcinomas of the mammary gland were found in 2 rats that received 1.4% tryptophan and in 2 rats that received 0.8% indole.

Cancers of the urinary bladder were present in rats of all 4 groups. They were observed in 75% of the rats that received 1.4% DL-tryptophan; 58% of the rats that received 1.0% indole acetic acid; and in 83% of the rats of the 2 groups that received indole.

Previously bladder cancers were observed in 100 and 92%, respectively, of the rats that received 1.4 and 4.3% DL-tryptophan added to a tryptophane-free casein hydrolysate but were not found in rats on a 26% casein diet or in rats on a synthetic diet deficient in tryptophan that survived as long or longer than the rats that received added tryptophan or indole. The induced cancers were classified as papillary (Fig. 5) or squamous cell carcinoma (Fig. 6) or a combination of both types. In many instances the cancerous growth filled and distended the lumen of the bladder. From these results it is apparent that indole is as effective as tryptophan in the initiation of these neoplasms.

Summary. 1. Fischer line 344 female rats were fed 2-acetylaminofluorene in synthetic diets containing 25% casein and supplements of 1.4% DL-tryptophan; 1.0% indole acetic acid; 0.8% indole and 1.6% indole. Liver cancers developed in 91% of the rats of the first group and in 83% of the rats of the other 3 groups. 2. The majority of the liver neoplasms in the rats that received 1.4% DL-tryptophan and 1.0% indole acetic acid were malignant hepatoma and lung metastases were present in 63% and 90% respectively. 3. The majority of the liver neoplasms in the rats that received 0.8% and 1.6% indole were cholangioma or adenocarcinoma and lung metastases from these tumors were identified in 38 and 12%, respectively. 4. Cancers of the urinary bladder were observed in 75% of the rats that received 1.4% DL-tryptophan; 58% of the rats that received indole acetic acid and in 83% of the rats that received indole. Indole appeared to be as effective as dietary tryptophane in the initiation of the bladder neoplasms. 5. The cancers of the urinary bladder were papillary or squamous cell carcinoma.

1. Dunning, W. F., Curtis, M. R., *Acta,* 1955, v11, 654.

2. ———, *Cancer Research,* 1954, v14, 299.

3. Dunning, W. F., Curtis, M. R., Maun, M. E., *ibid.,* 1950, v10, 454.

4. Glass, B., Plaine, H. E., *Proc. 9th Internat. Congress of Genetics II. Carylogia VI* suppl. 1954, 1163.

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Effect of Age at Ovariectomy on Mammary Gland Development in C3H/He Crgl Mice.* (24259)

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It is well recognized that in certain strains of susceptible mice, including the C3H strain (1,2), mammary tumors develop despite ovariectomy. The hormonal stimulus for genesis of these tumors has been ascribed to the adrenal cortex which, in several strains, undergoes a tumorous change leading to so-called nodular hyperplasia or to carcinoma(1-6).

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