illustrated in Table I. A significant elevation of *free* phenol and cresol was found in only 2 instances in 20 patients with renal disease and azotemia; in one of these, no anemia was present. Conjugated phenols tended to increase with degree of anemia and azotemia, but the relationship was not consistent. This same relationship held between severity of anemia and level of serum creatinine.

Discussion. It would appear that uremic patients are able to conjugate potentially toxic free phenols despite severe derangement of kidney function. The site of this "detoxifying" process is unknown, but presumably it is mainly in the liver. It is unlikely that these conjugated phenols can be implicated *per se* in pathogenesis of anemia of renal insufficiency. Previous investigations have shown that larger amounts of free phenolic acids and conjugated phenols than those detected in our patients do not produce any significant deleterious effect on the blood(6,8).

It is still possible that minute amounts of circulating free phenol and cresol might accelerate naturally occurring plasma lysins, and evoke a hemolytic reaction. Such a mechanism has been postulated by Ponder (10) to explain the hemolytic effect of napthalene and other agents. However, it is difficult to accept this possibility in relation to free phenol and cresol in uremic patients inasmuch as the levels of these circulating compounds are not greater than in normal subjects.

Summary. Free and conjugated ether-soluble phenols in blood of uremic patients have been determined by a specific and reliable method. Free phenol and cresol are not elevated in the great majority of these patients. Reasons are given for discounting the role of elevated conjugated phenols and free phenolic acids in the pathogenesis of the anemia of chronic renal disease.

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Effect of a High Protein and High Nucleic Acid Diet on Occurrence of 2- Acetylaminofluorene-induced Cancer in Rats.* (24257)

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A previous report(1) showed an increased percentage of acetylaminofluorene-induced liver cancers in rats on a high protein diet. Fischer line 344 female rats on a synthetic diet containing 45% casein and 0.06% 2-acetylaminofluorene were compared with similar rats on a diet containing 26% casein. In the first group 11 of 12 rats died with liver cancers in an average of 303 days while only 6 out of 16 rats on the 26% casein diet developed liver cancers in an average of 283 days.

Harris(2) found no difference in incidence of induced liver cancers in Wistar rats on diets containing 0.04% acetylaminofluorene and a smaller difference in casein content, *i.e.* between 20 and 13% or with the addition of 3% liver extract. Morris, Wagner and Velat (3) reported a higher incidence of induced

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TABLE I. Composition of Diets.

Material	Diet 6	Diet 13	Diet 14	Diet 15	Diet 16	Diet 17
	%					
Casein	45.0	40.0	44.0	43.0	40.0	40.0
Brewers yeast	0	0	0	0	5.0	0
High nucleic acid yeast*	0	5.0	0	0	0	0
Desoxyribonucleic acid	0	0	1.0	2.0	0	5.0
Salt mixture	4.0	4.0	4.0	4.0	4.0	4.0
Cellu flour	2.0	2.0	2.0	2.0	2.0	2.0
Dextrin	34.0	34.0	34.0	34.0	34.0	34.0
Crisco	15.0	15.0	15.0	15.0	15.0	15.0
Halibut liver oil	.4	.4	.4	.4	.4	.4
2-Acetylaminofluorene	.06	.06	.06	.06	.06	.06

Vitamin supplement per kilo of diet†

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

* Supplied through the courtesy of Dr. Joel B. Peterson, Standard Brands, Inc., New York City.

† The crystalline B vitamins were dissolved in distilled water and added as a daily supplement to each food cup.

mammary gland and ear-duct cancers and no difference in incidence of induced liver cancers in rats fed equal amounts of acetylaminofluorene with 10 μg compared with 1 or 2 μg of riboflavin added per gram to a semi-synthetic diet containing 24% casein.

In a preliminary experiment in our laboratory the average survival of rats on a 45% casein diet was increased from 317 days to 359 days by substitution of a high nucleic acid (6%) yeast for 5% casein. The added yeast significantly delayed the occurrence of the liver cancers but did not prevent their development. It seemed of interest to determine whether or not the protection resulted from the increased vitamin or nucleic acid content of the diet, or whether added protection might result from increased proportions of dietary nucleic acid.

In the current experiment 1, 2 and 5% desoxyribonucleic acid and 5% yeast were substituted for casein in a 45% casein diet.

Material and methods. Observations were completed on 6 groups of pedigreed Fischer line 344 female rats that received isocaloric portions of synthetic diets containing 0.06% 2-acetylaminofluorene. The diets varied only in proportion of casein, yeast or purified desoxyribonucleic acid as shown in Table I.

The rats were 4 to 5 months of age at start of the experiment. Each rat was housed in an individual cage with free access to water. The daily portion of 7 g 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.

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 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 results are summarized briefly in Tables II, III, and IV. Table II shows that average daily consumption for the 6 groups of rats varied from 5.9 to 6.4 g of diet and the average total dose of 2-acetylaminofluorene ingested per rat varied from 1.1 to 1.6 g for the rats of the 6 groups. In each case consumption was less for the rats that received 1% nucleic acid and higher for the rats

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

%	No. rats	Body wt (g)	Avg daily ration (g)	Avg total dose AAF (g)	Avg daily dose AAF (mg)
45 casein	16	145	6.3	1.3	3.8
5 nucleic acid yeast	18	147	6.4	1.6	3.8
1 nucleic acid	12	138	5.9	1.1	3.5
2 " "	12	146	6.2	1.2	3.7
5 " "	11	136	6.2	1.2	3.7
5 Brewer's yeast	12	138	6.3	1.2	3.8

that received the supplement of high nucleic acid yeast. Average daily consumption of acetylaminofluorene, however, varied only from 3.5 to 3.8 mg per rat per day.

From Table III it appears that average terminal body weight was considerably less than weight at the start of the experiment for rats of each group. An average weight loss of 14% was observed for the rats that received 5% Brewers yeast and more than twice that or 32% was observed for the rats on the diet containing 1% nucleic acid. The livers were from 2½ to 3 times larger than normal average liver weight and average kidney weights were slightly higher than normal for all groups. Otherwise there were no significant changes in the organs that were weighed.

Table IV shows average survival period for the rats of each group and the percentage of induced neoplasms that were observed. The rats with the 5% substitution of high nucleic acid yeast survived an average of 409 ± 9.4 days or significantly longer than the average survival (349 ± 11. days) for the group on the 45% casein diet or the average of 317 ± 6.5 days for the rats with 5% Brewers Yeast substitution. There were no significant differences between the groups that received different quantities of nucleic acid.

Malignant hepatoma were observed in from 67 to 92% of the rats of the 6 groups. There appeared to be no noteworthy differences in morphology or degree of malignancy except that lung metastases were observed in only 30% of the rats of the group that received 5% nucleic acid compared with 67% for the rats on the control or 45% casein diet.

Squamous cell carcinoma of the external auditory meatus were observed in 33 to 75% of the rats of the 6 groups. This type of neoplasm has been observed previously in about 20% of the Fischer line 344 rats on various dietary regimens containing 26% protein. Possibly the high protein diet accelerated occurrence of these tumors because the observed frequency was higher than expected in all 6 groups. In the rats with added nucleic acid the average latent period was only 280 to 290 days. The neoplasms were bilateral in 6 rats, 6 showed metastases to the lungs and one dissemination to the pituitary gland.

Adenocarcinomas of the mammary gland were observed in 4 or 25% of the rats on the control or 45% casein diet and in only one of the rats on the high nucleic acid yeast diet and in none of the rats of the other groups. In addition 3 rats, one each from the control, and 1, and 2% nucleic acid diets had squamous cell carcinoma of the bladder. These neoplasms had been observed previously only on

TABLE III. Number of Rats on Each Diet, Average Initial and Final Body Weight, and Percentage Weights of Organs.

		Wt (g)		Organ wt in % of body wt					
Group	No. rats	Initial	Final	Liver	Kidney	Adrenal	Pituitary	Ovary	Uterus
Diet 6	16	145	117	15.8	1.5	.05	.01	.05	.39
13	18	147	112	15.8	1.4	.04	.01	.04	.42
14	12	138	93	14.7	1.5	.05	.01	.05	.36
15	12	146	102	15.7	1.5	.05	.01	.04	.45
16	11	136	116	15.7	1.3	.05	.01	.05	.37
17	12	138	107	12.8	1.4	.05	.01	.04	.40

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.

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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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