order kinetics reasonably well during the initial phase of the hydrolysis. Rates are calculated from the slopes of the straight lines fitted to the points and, when compared, show that the invertase oxidized by active tyrosinase has only 57% of the activity of the control invertase which had been treated with boiled tyrosinase.

In more than 100 experiments using purified preparations of tyrosinase the invertase was inactivated 10–40% when compared with the control invertase treated with boiled tyrosinase. While all yeast invertase preparations, whether crude or highly active, can be inactivated by tyrosinase, it is found that not all tyrosinase preparations are effective. Crude tyrosinase does not inactivate invertase, and purified tyrosinase of high "cresolase" activity has little effect, while purified tyrosinase of high "catecholase" activity[3] (*3*) is most effective.

The question of whether the tyrosinase directly inactivates the invertase, or converts by oxidation impurities (or products of protein autolysis) to invertase inhibitors, cannot be satisfactorily answered at this time. Since the phenomenon is equally apparent if continuous dialysis of the solutions of tyrosinase plus invertase occurs during the inactivation, it is clear that the results cannot be accounted for by an action of tyrosinase on dialyzable potential inhibitors of invertase. The fact that invertase in many different stages of purification may be inactivated by tyrosinase makes it more likely that invertase is inactivated directly rather than by a high-molecular-weight impurity, which becomes an inhibitor after the oxidation of its phenolic groups by tyrosinase.

The results might conceivably be explained on the hypothesis that the tyrosinase preparations merely contain an invertase inhibitor which is destroyed by boiling. To test this idea it is necessary to employ some means other than boiling to prevent the action of tyrosinase in the control experiment. This can be done by excluding oxygen from the control by bubbling nitrogen through the control solution and then evacuating it at the water pump. An equally effective method is to bubble continuously through the control solution hydrogen activated with platinized asbestos.[4] Results with both techniques are comparable to those with the usual method using boiled tyrosinase and show that the inactivation of invertase by tyrosinase is not explained by the tyrosinase preparation acting as an inhibitor. These experiments also show the dependence upon oxygen of the inactivation reaction. Experiments are now in progress to correlate the loss in activity of the invertase oxidized by tyrosinase with chemical and physical changes in the invertase molecule.

These preliminary results show that yeast invertase can be partially inactivated by incubation with mushroom tyrosinase. This inactivation is best explained on the basis of an oxidation of essential tyrosyl groups in the invertase molecule by tyrosinase.

[3] Both the "cresolase" and the "catechlase" preparations of tyrosinase were generously supplied by C. R. Dawson, of Columbia University.

[4] This technique is not applicable to highly purified Dieu invertase, which is partially inactivated by activated hydrogen.

References

1. Dieu, I. H. *Bull. Soc. Chim. Belg.*, 1947, **55**, 306.
2. Edman, P. *J. biol. Chem.*, 1947, **168**, 367.
3. Mallette, M. F., Lewis, S., Ames, S. R., Nelson, J. M., and Dawson, C. R. *Arch. Biochem.*, 1948, **16**, 283.
4. Olcott, H. S., and Fraenkel-Conrat, H. *Chem. Rev.*, 1947, **41**, 141.
5. Sizer, I. W. *J. biol. Chem.*, 1946, **163**, 145; 1947, **169**, 303.
6. Sumner, J. B. *J. biol. Chem.*, 1925, **63**, 393.

Mammary Carcinoma in Female Rats Fed 2-Acetylaminofluorene[1]

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In connection with studies concerning the relation of nutrition to cancer it was observed that mammary tumors developed consistently in from 3 to 6 months in all female rats receiving diets containing .03% 2-acetylaminofluorene. This observation has been confirmed in several experiments, results of which are reported in this paper.

Piebald female rats of the Alabama Experiment Station (AES) strain were used for these studies. The animals were placed on the experimental diets at 23 days of age, at weights of 35 to 50 gm. They were caged individually on screen floors and fed daily *ad libitum*.

The composition of the diets used in these studies is given in Table 1. Two dietary modifications—omission of choline and the addition of iodinated casein—were tried, but these did not influence the results, as can be seen from the summary data in Table 2.

A period of about 16 weeks was required for the animals to attain a body weight of approximately 200 gm. This amount of gain is normally made in 6–8 weeks when these diets are fed without carcinogen.

Twenty-four of the 25 female rats fed the carcinogenic diets developed palpably detectable mammary tumors between the 95th and 181st day of the experiment. One animal died of unknown causes during the third month of the experiment, at which time no tumors were detectable in any of the animals. The animals were killed and autopsies were made, usually within one or two weeks

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[2] The authors are indebted to H. L. Stewart, senior pathologist, National Cancer Institute, for assistance in the interpretation of the microscopic material.

after a mammary tumor was detected by palpation. At autopsy, multiple mammary tumors were observed in 14 and single tumors in each of the remaining 10 animals.

TABLE 1

PERCENTAGE COMPOSITION OF BASAL DIETS*

Ingredient	Diet No.	
	C–5	C–20
Water-extracted casein	9.00	6.00
Alcohol-extracted peanut meal	0.00	30.00
Degerminated corn grits	20.00	0.00
Sucrose	51.70	39.90
Salt mixture	4.00	4.00
Lard	14.00	19.00
Cod-liver oil	1.00	1.00
L-Cystine	0.30	0.10
2-Acetylaminofluorene	.03	.03

* Each kg of basal diet was fortified with the following (mg): α-tocopherol, 50; thiamine, 2; pyridoxine, 2; riboflavin, 4; pantothenate, 10; niacin, 20; i-inositol, 200; and choline, 2,000. The authors are indebted to Merck & Co., Rahway, New Jersey, for these vitamins.

A total of 82 mammary tumors were removed and prepared for microscopic examination. These ranged in size from small, barely palpable knots of firm tissue to growths up to 3 or 4 cm in diameter and were distributed in all areas of the mammary region. On the basis of microscopic examination, the tumors were diagnosed as mammary carcinoma in all cases. Metastases to the lungs were observed in a few cases, and in one case a primary carcinoma was also present in the external auditory canal.

TABLE 2

A 100% INCIDENCE OF MAMMARY TUMORS IN FEMALE RATS FED 2-ACETYLAMINOFLUORENE

Diet No.	Litter No.	No. of animals	Average body weight (gm)		No. of animals with mammary tumor*	Tumor induction period (days)	
			Initial	16 weeks		Mean	Range
C–5	24–14	2	44	156	2	141	136–145
C–5†	24–14	2	47	193	2	151	136–165
C–5	24–35	4	36	198	4	128	123–132
C–5	25–11	5‡	47	203	4	115	95–121
C–20	25–20	5	47	221	5	128	121–148
C–20	26–32	4	42	223	4	151	121–181
C–20§	25–29	1	49	218	1	152	.....
C–20§	25–18	1	45	233	1	146	.....
C–20§	24–25	1	35	190	1	152	.....

* The total number of mammary tumors distinctly visible at autopsy in the 24 animals was 82, the largest number appearing in any one animal was 10, and a single mammary tumor was present in each of 10 animals.

† Choline was omitted from the diet.

‡ One of these animals died after 86 days on experiment. No tumors were observed and the cause of death was undetermined.

§ The diet was supplemented with iodinated casein (.2 gm/kg of diet). The authors are indebted to the Cerophyl Laboratories, Inc., Kansas City, Missouri, for this material.

So far as is known, a uniform production of mammary tumors in the rat has not been previously reported. Whether this unusual result is related to the age or strain of animal or the type of diet used is not known. No significant litter differences were observed in this strain.

Wilson and associates (*6*), who first demonstrated the carcinogenicity of 2-acetylaminofluorene, observed mammary tumors in 40% of female rats fed the compound at a level of .031% of the diet. Bielschowsky (*1*) noted a much higher incidence (60%) of mammary tumors with this compound in female rats of a Wistar substrain than in piebald females (4%). Harris (*5*) likewise observed a high incidence (40–75%) of mammary tumors in Wistar female rats fed this compound. Cantarow (*3*), working with the Sherman strain, and Dunning (*4*), comparing 5 other strains, both observed a low incidence of mammary tumors. In summarizing the reported investigations, it is apparent that a relatively high incidence of mammary tumors in female rats was observed only in animals descendant of the Wistar strain. It should be pointed out, however, that a comparison of reported results hardly seems valid until the relation of nutritional factors to the carcinogenicity of 2-acetylaminofluorene has been more thoroughly investigated. With the exception of the work of Harris (*5*), in which purified diets were used, the previously reported investigations with this compound have involved diets composed of natural foodstuffs. In the present study, semipurified diets were used. Bielschowsky (*2*) has reported that a yeast supplement in a diet of bread and skim-milk powder markedly reduced the mammary tumor incidence. On the other hand, Wilson and associates (*7*) have recently reported no change in the incidence or time of development of tissue changes induced by 2-acetylaminofluorene by enriching the diet with cod-liver oil, yeast, and wheat germ.

Investigations are under way to evaluate more carefully the relation of specific dietary factors to the carcinogenicity of this compound. A more detailed report of the pathologic tissue changes observed in these studies is being prepared. The ease with which the mammary tumor can be detected and the assurance herein demonstrated that this type of tumor will appear consistently under controlled dietary conditions should make this a reliable experimental approach to the problem.

References

1. BEILSCHOWSKY, F. *Brit. J. exp. Path.*, 1946, **XXVII**, 135.
2. BIELSCHOWSKY, F. *Brit. J. Cancer*, 1947, **1**, 146.
3. CANTAROW, A., PASCHKIS, K. E., STASNEY, J., and ROTHENBERG, M. S. *Cancer Res.*, 1946, **6**, 610.
4. DUNNING, W. F., CURTIS, M. R., and MADSEN, M. E. *Cancer Res.*, 1947, **7**, 134.
5. HARRIS, P. N. *Cancer Res.*, 1947, **7**, 88.
6. WILSON, R. H., DEEDS, F., and COX, A. J. *Cancer Res.*, 1941, **1**, 595.
7. WILSON, R. H., DEEDS, F., and COX, A. J. *Cancer Res.*, 1947, **7**, 450.