To 1 part of each 0.1 ml catalase solution (10 micrograms) was added. Catechol gave an intense yellow color, pyrogallol a brown color, adrenalin a pink color, and p-phenylenediamine gave a light-reddish-purple color.

Discussion and summary. Our experiments show that dilute solutions of crystalline catalase, in the presence of hydrogen peroxide, can peroxidize a variety of compounds. α-Naphthol and p-phenylenediamine are oxidized to indophenol purple; p-aminobenzoic acid, sulfathiazole, adrenalin, ephedrine sulphate, and tyrosine, are coupled with catechol by oxidation to form colored compounds. Pyrogallol, catechol, adrenalin and p-phenylenediamine are oxidized by catalase and hydrogen peroxide. Related compounds (m-aminobenzoic acid, sulfadiazine) are also oxidized. A commercial crystalline catalase preparation, as well as crude horse radish peroxidase gave similar results. A catalase solution which had been boiled for 5 minutes did not give the described reactions.

The difference between catalases and peroxidases is: catalases can act as peroxidases but peroxidases can not act as catalases (decompose hydrogen peroxide without the presence of a second substrate). The present experiments are in full harmony with Theorell's theory which states that catalases are a special group of peroxidases.

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Combination Chemotherapy of Cancer with 8-Azaguanine and a Riboflavin Analog.* (19833)

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Quantitative biochemical differences between normal and tumor tissues have been reported by many investigators(1-5), and reveal, for the most part, lower metabolite concentrations in neoplastic tissue. Metabolite inhibition by an antimetabolite depends upon the concentration ratio of metabolite to antagonist. Thus, the function of a metabolite in low concentration in cancer cells may be blocked by an antagonist while only minimally depressing the metabolic activities of the higher metabolite level in normal cells. The possibility that the utilization of combinations of drugs against experimental neoplasms may result in simultaneous damage to different metabolic pathways, achieving greater tumor damage, has been reported by a number of investigators (6-9). For example, the vit. B_6 antagonist, desoxypyridoxine, was chosen for chemotherapeutic trial because it had been demonstrated that pyridoxine is in lower concentration in many solid tumors than in most normal tissues (1,3,4). Desoxypyridoxine was found to be a weak carcinostat against a mammary adenocarcinoma, and, in combination with a guanine antagonist (8-azaguanine), produced a carcinostatic effect not obtainable with either drug alone(6).

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TABLE I. Effect of Flavotin Alone and in Combination with 8-Azaguanine on the 755 Carcinoma in C57 Mice.

Exp. No.	Group	Sex	Dose,* mg/kg	Mean tumor wt (mg)	No. of animals Dead/Total	% change in body wt
43A	Control	♂		2418	3/19	+12
	Flav.	♂	60	1687	1/18	+ 5
27B	Aza.	♂		954	0/20	+ 2
	Aza. + Flav.	♂	50	770	5/26	— 3
36A	Aza.	♂		969	0/20	0
	Aza. + Flav	♂	65	782	8/20	— 3
44	Control	♀		1795	1/20	+18
	Flav.	♀	60	1355	2/20	+ 9
49	Control	♀		1549	0/20	+12
	Flav.	♀	60	1160	1/20	+ 4
61	Control	♀		1831	0/20	+12
	Flav.	♀	60	1805	1/20	+ 8
29	Aza.	♀		736	0/20	+ 6
	Aza. + Flav.	♀	50	268	0/25	— 5
35B	Aza.	♀		1041	1/20	0
	Aza. + Flav.	♀	60	490	0/27	— 7
36B	Aza.	♀		699	0/20	+ 2
	Aza. + Flav.	♀	60	239	5/20	— 8
63	Aza.	♀		2496	2/20	+ 4
	Aza. + Flav.	♀	60	1310	0/20	— 6

* Refers to dose of Flavotin. 8-Azaguanine always administered at a dose of 50 mg/kg one hour after injection of Flavotin.

Intraperitoneal therapy was given depending upon the daily weight of the treated animals, injections usually being given daily. Therapy was begun upon well established tumors varying between experiments from 3 to 13 days old. The duration of tumor growth varied between experiments from 20 to 34 days.

Inasmuch as riboflavin has also been reported in lower concentration in many tumors than in most normal tissues(1,4), the effect of a riboflavin analog, 6-chloro-9-(1'-D-sorbityl)-isoalloxazine, or flavotin, was evaluated on a mouse breast cancer, alone and in combination with 8-azaguanine.

Methods. A transplantable mammary adenocarcinoma, the 755 tumor in C57 black mice was the neoplasm employed in this study. The C57 mice were maintained in plastic cages in an air-conditioned, constant-temperature room (74°F), and had access to Rockland pellets and water *ad libitum*. Mice 2 to 3 months old and weighing 18 to 25 g were inoculated with tumor fragments into the axillary region by the usual trocar method. As noted in the tables, treatment was begun upon well established tumors. At the termination of an experiment the tumors were removed and the wet weights were determined to the nearest milligram. Statistical analysis of the significance of the difference between two mean tumor weights (M_1 and M_2) was made and the results were considered beyond chance variation when $\frac{M_1 - M_2}{\sqrt{(\sigma_{M_1})^2 + (\sigma_{M_2})^2}} = 2.5$ or greater. The guanine analog, 8-azaguanine, was dissolved in 0.1 N NaOH as previously described(10). Flavotin, a water insoluble compound, was dissolved by heating in 100% propylene glycol, and diluted with distilled water to a 10% propylene glycol solution. Ampules, containing a solution of riboflavin-5-phosphate, were used as the source of the vitamin in the reversal experiments.

Results. The lack of inhibitory effect of flavotin, alone or in combination with 8-azaguanine, on the 755 carcinoma grown in male animals is summarized in Table I. In addition, Table I records the results of similar experiments with flavotin in females. It is to be noted that in no experiment did flavotin alone produce significant inhibition of tumor growth. However, increased carcinostasis was obtained when flavotin was administered one hour prior to the injection of 8-azaguanine to female mice bearing the 755 carcinoma as

TABLE II. Reversal of Carcinostatic Effect of Flavotin on the 755 Carcinoma in Female Mice by Riboflavin.

Exp. No.	Group	Dose,* mg/kg	Mean tumor wt (mg)	No. of animals Dead/Total	% change in body wt
60	Aza.	50	366	0/20	+1
	Aza. + Flav.	50 + 60	148	1/20	—3
	Aza. + Ribo.	50 + 100	270	2/20	—2
	Aza. + Flav. + Ribo.	50 + 60 + 100†	288	4/19	—1
68B	Aza.	50	564	0/20	—1
	Aza. + Flav.	50 + 60	254	0/20	—7
	Aza. + Ribo.	50 + 100	324	2/19	—7
	Aza. + Flav. + Ribo.	50 + 60 + 100†	523	6/20	—6
72	Aza.	50	578	0/19	+1
	Aza. + Flav.	50 + 60	272	1/19	—6
	Aza. + Ribo. + Flav.	50 + 100 + 60†	351	1/18	—6

* 8-Azaguanine injected one hour after administration of Flavotin or Riboflavin-5-phosphate.
† Flavotin injected 15 min. after administration of Riboflavin-5-phosphate; 8-Azaguanine injected one hour after administration of Flavotin.
Daily intraperitoneal therapy was begun upon well established tumors varying between experiments from 5 to 7 days old. The duration of tumor growth varied between experiments from 21 to 24 days.

compared to the tumor growth obtained when the 8-azaguanine was administered alone. 8-azaguanine's consistent inhibitory effect upon the growth of the 755 tumor has been previously recorded(6,10). The results of all experiments with this drug combination in female animals are statistically significant. Further analysis of the data reveals a minimal mortality rate and slight to moderate weight loss. The degree of weight loss present in the treated mice is far below a level which in itself would inhibit tumor growth.

The results of experiments designed to determine whether the carcinostatic action of flavotin is due to interference with riboflavin metabolism are presented in Table II. The data demonstrate that the addition of riboflavin-5-phosphate to the 8-azaguanine-flavotin drug combination completely nullified the increased carcinostatic effects produced by flavotin in two experiments (Exp. 60 and 68, Table II). and partially blocked flavotin's action in a third experiment (Exp. 72, Table II), converting its usual carcinostatic effect into a statistically insignificant result. The results achieved by the combination of 8-azaguanine and flavotin in the three experiments recorded in Table II are statistically significant.

Discussion. As recorded in the tables, there is a difference in the effect of the flavotin-8-azaguanine drug combination upon the tumor depending upon the sex of the host. In every experiment in which the 755 carcinoma was carried by female mice, significant inhibition of tumor growth over that produced by 8-azaguanine alone occurred, whereas none of the experiments on male mice revealed this increased carcinostatic effect.

This variation of response in the two sexes is not understood, but it is not without precedent. Taylor and Carmichael(11) reported that folic acid was more toxic to female than male mice of the DBA strain. Goldin *et al.* (12) reported a 2-fold sex difference in lethal toxicity to aminopterin in adult mice. Folic acid was found to augment the carcinostatic activity of 8-azaguanine upon the 755 carcinoma in male mice only(6). Female mice bearing a transplantable lymphosarcoma demonstrated greater regression of the tumor than males after treatment with compound E(13). Okey *et al.* noted sex differences in biotin deficiency symptoms in rats(14). There are many other examples of sex differences in metabolic response, but the mechanisms through which these variations are mediated have yet to be elucidated.

The structural similarity of flavotin to riboflavin, together with the positive results obtained in the reversal experiments, permit the conclusion that flavotin's mechanism of action in producing carcinostasis (when used in combination with 8-azaguanine) is due to interference with riboflavin metabolism. Moreover, the lack of carcinostatic activity when flavotin

is used alone in female mice presents the interesting possibility of a specific relationship between 8-azaguanine and flavotin. Studies are in progress to clarify the suspected relationship. It is conceivable, for example, that flavotin prevents the degradation of 8-azaguanine into a molecule lacking carcinostatic activity. Folic acid was found to potentiate 8-azaguanine's carcinostatic activity against the 755 tumor in this manner(6,15).

The positive results obtained with the riboflavin analog, flavotin, together with the demonstration that flavotin acts as a riboflavin antagonist in this tumor-host system, furnish additional support for the hypothesis presented at the beginning of this report.

Summary. Flavotin, a riboflavin analog, had no effect on the growth of a transplantable mammary carcinoma. However, the combination of flavotin and 8-azaguanine was carcinostatic, but only in female mice. The sex difference in response is not understood. Reversal experiments utilizing riboflavin-5-phosphate reveal the mechanism of increased carcinostatic activity afforded by the drug combination to be due to interference with riboflavin metabolism.

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Surface Activities of Biotin and Biocytin. (19834)

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Evidence has been presented by numerous workers that biotin functions in a variety of systems concerned with a) decarboxylation of, or carbon dioxide fixation in, acetoacetate, aspartate, malate, oxalacetate or succinate, b) aspartic acid, serine, and threonine deamination, c) succinic acid dehydrogenation, and d) the synthesis of adenine, arginine, aspartic acid, bone carbonate, citric acid, citrulline, guanine, and unsaturated fatty acids. One explanation for the apparent multiplicity of functions of biotin is that the compound is involved in only one primary system whose failure in biotin deficiency manifests itself by a variety of secondary metabolic derangements. Williams and Williams have shown (1) that biotin is highly "surface active" as inferred from its remarkable effect in eliminating the diffusion current maximum encountered during the polarographic reduction of Cu^{++} at the dropping mercury electrode. If biotin is highly "surface active" and does indeed function in this way, for example, as the prosthetic group of a larger surface active molecule, or as a factor regulating cell permeability, then it is apparent that biotin-