tation and the abrupt extinction of a few plant species. The destruction of plant life produced vacant vegetal niches, or what may be termed a partial ecological desert. Plant destruction was not total; refugia remained, possibly in widely scattered localities providing nuclei for recolonization. Ferns were the first invaders or colonizers. These were rapidly crowded out as those angiosperms that survived the boundary event gradually reestablished dominance. The succeeding climax vegetation following any major ecological crisis such as a volcanic eruption, flood, or forest fire (*12, 13*) would not be the same as it was previously. Such a crisis also provides the opportunity for new biotic radiations; the resulting plant community can be vastly different from the preceding one, as was the case during the early Paleocene in the western interior.

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Inhibition of Human Estrogen Synthetase (Aromatase) by Flavones

Abstract. *Several naturally occurring and synthetic flavones were found to inhibit the aromatization of androstenedione and testosterone to estrogens catalyzed by human placental and ovarian microsomes. These flavones include (in order of decreasing potency) 7,8-benzoflavone, chrysin, apigenin, flavone, flavanone, and quercetin; 5,6-benzoflavone was not inhibitory. 7,8-Benzoflavone and chrysin were potent competitive inhibitors and induced spectral changes in the aromatase cytochrome P-450 indicative of substrate displacement. Flavones may thus compete with steroids in their interaction with certain monooxygenases and thereby alter steroid hormone metabolism.*

Flavonoids are 2-phenyl-1,4-benzopyrone derivatives that are found throughout the plant kingdom (*1*). They occur in many human foods and have been used as drugs or food supplements (*2*). The physiological role of the naturally occurring flavonoids is not known, but they have been reported to exhibit antioxidant, antibacterial, and antiviral properties.

Two synthetic flavones, 5,6-benzoflavone (β-naphthoflavone) and 7,8-benzoflavone (α-naphthoflavone), have also attracted considerable interest. These derivatives can modify the metabolism of polycyclic aromatic hydrocarbons and other xenobiotics and thereby alter their toxicity and carcinogenicity (*3*). 5,6-Benzoflavone appears to act indirectly, inducing synthesis of specific cytochrome P-450 isozymes (*4*). 7,8-Benzoflavone, on the other hand, directly affects the activity of hepatic P-450 enzymes, inhibiting some forms (*5*) and activating others (*6*); these effects are complex and vary with the concentration of 7,8-ben-

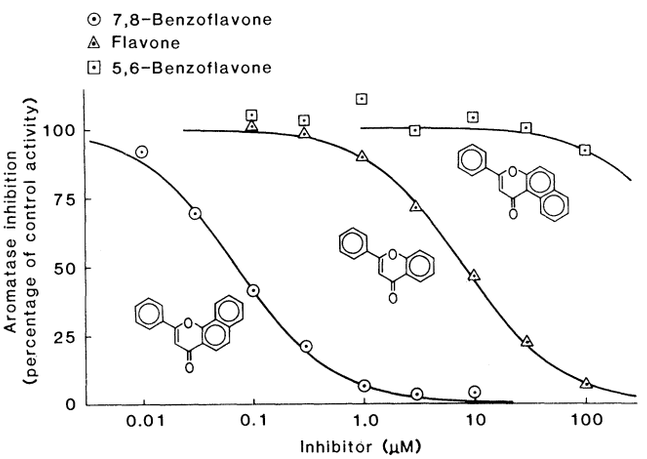

Fig. 1. Dose-response curves for flavone inhibition of androstenedione aromatization by human placental microsomes. The 1-ml reaction mixture contained 0.04 μ*M* androstenedione, 0.1 mg of protein, 2.5 m*M* glucose-6-phosphate, 0.25 unit of glucose-6-phosphate dehydrogenase, and 100 μ*M* reduced nicotinamide adenine dinucleotide phosphate in 0.05*M* sodium phosphate (*p*H 7.2). Incubations were for 6 minutes at 37°C; control activities (100 percent) averaged 16 pmol per milligram of protein per minute. Curves shown were calculated (*11*) by assuming I_{50} values of 0.07, 8, and 500 μ*M*.

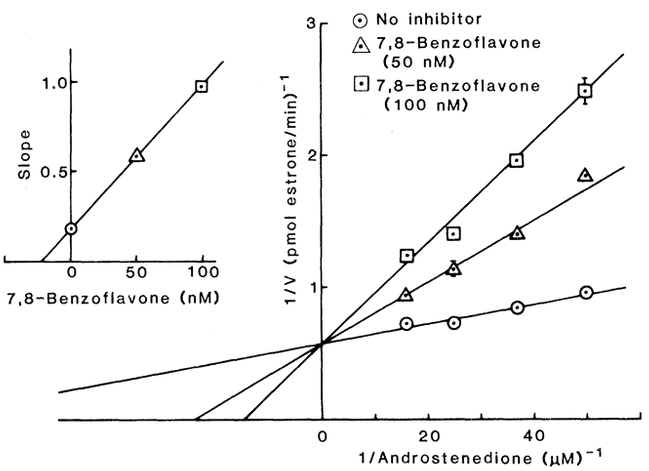

Fig. 2. Kinetic analysis of the mechanism of inhibition of aromatase by 7,8-benzoflavone. (Right) Double-reciprocal plot of reaction velocity (*V*) versus the substrate concentration. (Left) Slope of lines at right versus inhibitor concentration. Assay conditions are as described in the legend to Fig. 1.

zoflavone used, the animal source of the enzyme, and the isozyme tested. Similar effects of several of the naturally occurring flavones on hepatic monooxygenases have also been reported recently (*7*), but the underlying biological basis for the activities of the flavones and the reason for the selectivity for a particular isozyme are unclear.

We recently tested the effects of a number of flavonoids on estrogen synthetase, a cytochrome P-450 enzyme that catalyzes the conversion of androgens to estrogens. We report here that several flavone derivatives, including synthetic and natural forms, inhibit the aromatization of androstenedione to estrone and of testosterone to estradiol.

Aromatase activity was determined in microsomal preparations from placentas obtained after normal deliveries and from ovaries removed from premenopausal women during elective surgery (*8*). Activity was measured by the release of tritium from [1,2-^{3}H]androstenedione or from [1,2-^{3}H]testosterone (*8, 9*); formation of product was confirmed by radioimmunoassay (*8*). Substrates and inhibitors were added from stock solutions in ethanol (*10*). Control reaction rates were linear for at least 8 minutes and were directly proportional to the amount of added microsomal protein; time courses observed in the presence of flavonoid inhibitors were also linear, indicating that no substantial metabolism of the inhibitors to more active or less active forms occurred during the assay period.

Figure 1 shows dose-response curves obtained with the placental enzyme system for three flavones. When 40 n*M* androstenedione was used as the substrate, flavone caused 50 percent inhibition (I_{50}) at a concentration of 8 μ*M* and 7,8-benzoflavone at 0.07 μ*M*; 5,6-benzoflavone was almost without effect up to 100 μ*M*. For flavone and 7,8-benzoflavone the data show a good fit to curves calculated under the assumption of reversible, noncooperative binding to form an inhibitor-enzyme complex (*11*). These derivatives were also tested with microsomes from human ovaries; similar ranges of potencies were observed, indicating that the effects are not unique to the placental enzyme.

Several other flavones were also assayed with placental microsomes, and aromatization of testosterone and androstenedione was measured. The potencies of the different derivatives, relative to flavone, were similar with either substrate (Table 1). No derivatives were found to stimulate activity (concentrations tested ranged from 0.01 to 100 μ*M*); in this respect the effects of flavones on aromatase are less complex than those observed with hepatic monooxygenases, where mixed effects are found (*6, 7*).

Table 1. Inhibition of human placental aromatase by flavones.

Compound	Substrate			
	Androstenedione (40 n*M*)		Testosterone (80 n*M*)	
	I_{50}	Relative potency	I_{50}	Relative potency
7,8-Benzoflavone	0.07	114	0.06	83
Chrysin	0.5	16	0.4	13
Apigenin	1.2	6.7	1	5
Flavone	8	1	5	1
Flavanone	8	1	5	1
Quercetin	12	0.67	10	0.5
5,6-Benzoflavone	>200	<0.04	>200	<0.04

7,8-Benzoflavone was the most effective compound tested; it is over ten times more potent than aminoglutethimide (*8*) and is the most potent nonsteroidal inhibitor of aromatase known to us. The 7,8-benzoflavone-to-substrate ratio required for half-inhibition indicates that 7,8-benzoflavone binds to the placental aromatase half as tightly as androstenedione and with a slightly higher affinity than testosterone. It also binds with an affinity comparable to that of two of the most potent steroidal inhibitors of aromatase, 1,4,6-androstatriene-3,17-dione and 4-hydroxy-4-androstene-3,17-dione (*12*); under our assay conditions, these steroids inhibited androstenedione aromatization 50 percent at 0.08 and 0.05 μ*M*, respectively.

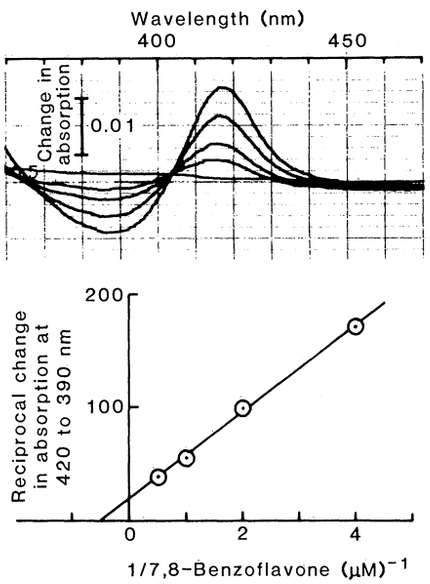

Fig. 3. Difference absorption spectra produced by binding of 7,8-benzoflavone to aromatase. The effects of successive additions of 0.25, 0.5, 1.0, and 2.0 μ*M* 7,8-benzoflavone are shown. Human placental microsomes were extracted with sodium cholate (1.0 mg per milligram of protein) in the presence of 1 μ*M* androstenedione. The detergent-solubilized enzyme was collected by precipitation with 60 percent saturated ammonium sulfate and dialyzed against 0.05*M* sodium phosphate (*p*H 7.2) (*8*). A baseline spectrum was recorded with enzyme in the sample and reference compartments, and 3-μl portions of 7,8-benzoflavone were added to the sample (solvent ethanol alone was added to the reference). The protein concentration was 7.5 mg/ml and the concentration of cytochrome P-450 (*19*) was 0.6 μ*M*. Spectra were recorded with a Cary 17D spectrophotometer.

The mechanism of inhibition was investigated by analysis of the kinetics obtained at various concentrations of substrate and inhibitor. Figure 2 shows that 7,8-benzoflavone is a competitive inhibitor with respect to androstenedione. The inhibition constant (K_i) (21 n*M* in this experiment and 19 and 22 n*M* in two others) is approximately twice the Michaelis constant (K_m) for androstenedione (10 to 15 n*M* in three experiments). In an experiment in which testosterone was used as the substrate (K_m = 80 n*M*), 7,8-benzoflavone was also found to be a competitive inhibitor, and a K_i of 23 n*M* was observed (*13*). The second most potent flavone inhibitor, chrysin, was also assayed with androstenedione as the substrate, and it too was found to inhibit competitively with respect to the substrate; the K_i was 0.26 μ*M*.

The kinetic analyses suggest that flavones may inhibit aromatization by competing with androstenedione (and testosterone) for the substrate binding site on the enzyme. This possibility was further investigated by monitoring the effects of the flavones on the absorption spectral properties of the enzyme. Previous studies of the effect of 7,8-benzoflavone on liver microsomal cytochrome P-450 revealed that binding induced a "type 1" difference spectrum characteristic of the conversion of the heme-iron from a low-spin to a high-spin state (*14*). When 7,8-benzoflavone was added to placental microsomes or to a solubilized preparation of aromatase, no spectral changes could be detected. However, if the sample was first equilibrated with androstenedione,

addition of 7,8-benzoflavone produced the difference spectra shown in Fig. 3. These are opposite in sign to type I curves obtained with aromatase (*8, 15*) and reflect conversion of the heme-iron from a high- to a low-spin state, that is, displacement of androstenedione (*16*). The fact that a low-spin complex is produced indicates that the interaction of 7,8-benzoflavone with the aromatase P-450 is different than that with the hepatic microsomal cytochromes. In the case of aromatase the low-spin nature of the complex suggests the presence of an additional axial ligand to the heme-iron; this ligand could be contributed by an amino acid residue of the protein or by the ether or ketone oxygen atom of 7,8-benzoflavone (*17*).

Graphical analysis of the titration shows that only one class of binding site is detected and that it exhibits an apparent binding constant of 2.0 μM. This spectral dissociation constant is higher than the true dissociation constant for 7,8-benzoflavone because of competition with androstenedione for binding to the enzyme. We interpret this reverse type I spectral change as evidence for the formation of an aromatase–7,8-benzoflavone complex; it is probable that formation of this complex causes inhibition of the enzyme. Similar difference spectra were obtained when enzyme samples were equilibrated with testosterone or when chrysin was used in place of 7,8-benzoflavone.

Two types of interaction of the flavones with the aromatase could result in a competitive binding with respect to the androgen substrates. Flavones might simply compete directly for the substrate binding site on the aromatase; while flavones appear structurally dissimilar from steroids, certain isoflavones have been found to exhibit estrogenic activity (*18*), presumably as a result of binding to the estrogen receptor. Alternatively, there may be a distinct site on the aromatase that accommodates certain flavones and, when occupied, favors an enzyme conformation with decreased substrate affinity; in principle, such a "regulatory" site could also act differently to enhance enzyme activity, as observed with some of the hepatic monooxygenases. It is not yet possible to distinguish between these mechanisms, but additional structure-activity studies may provide further insight.

Estrogens have profound influences on reproduction, development, and behavior. Because of the presence of flavones in human diets and their therapeutic use and because of the many whole-animal experiments in which the effects of flavones have been studied, it is important to learn whether they affect estrogen biosynthesis in vivo.

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Small Cell Carcinoma of the Lung: Macrophage-Specific Antigens Suggest Hemopoietic Stem Cell Origin

Abstract. *Four surface antigens previously recognized only in macrophages are present on human small cell lung carcinoma cells and tumors. Cancerous cells may arise from macrophage precursors in bone marrow, and these precursors migrate to lung to participate in the repair of damaged tissue produced by continuous heavy smoking. The characteristic presence of neuropeptides such as bombesin in small cell carcinoma, when considered along with these findings, presents new possibilities for the role of such peptides in nervous, endocrine, and immune system function.*

Lung cancer is the leading cause of cancer death in the United States, and its incidence is increasing rapidly with an anticipated 100,000 new cases this year (*1*). Approximately 25 percent of all lung carcinomas are of the small cell (SCCL) or oat cell type, and epidemiological studies indicate that the occurrence of SCCL is associated with heavy smoking (*2*). As seen clinically, SCCL is a rapidly progressing disease with widespread and early metastases; the mean survival time for patients with untreated SCCL is 5 to 7 weeks after diagnosis. The treatment consists almost exclusively of high-dose combined chemotherapy which results in an extension of life expectancy to 10 months, but the treatment may result in considerable patient morbidity and occasional mortality (*3*).

Because SCCL is almost always associated with heavy smoking, some investigators have assumed that the primary tumor is of lung origin. Even though SCCL cell lines have been established (*4*) and various biochemical characteristics shared by these cell lines and by tumors obtained at autopsy have been observed, the origin of SCCL remains elusive. The concept that SCCL arises from lung epithelial stem cells of a neuroendocrine nature (*5*) has yet to be substantiated with direct experimental evidence. Moreover, the notion of a lung origin for SCCL is not only incompatible with the observation that 5 percent of