Indole-3-carbinol

A Novel Approach to Breast Cancer Prevention

H. LEON BRADLOW, DANIEL W. SEPKOVIC,
NITIN T. TELANG, AND MICHAEL P. OSBORNE
Strang-Cornell Cancer Research Laboratory
New York, New York 10021

Before reviewing the data, I would like to make two comments. The work that I will be describing, aside obviously from being a continuation of the elegant discussion by Fishman *et al.* on 16α-hydroxyestrone (see earlier in this volume), represents a long-term study that goes back to the 1950s when the late Tom Gallagher and Konrad Dobriner had the prescience to assemble a group at Memorial Sloan-Kettering to study hormone metabolism in human subjects using labeled precursors at physiological concentrations, which had never been done before. The present work has evolved out of those studies, with the aid of many past colleagues too numerous to mention, as well as my current colleagues at Strang-Cornell.

In FIGURE 1, the pathways of metabolism of estradiol to its principal metabolites are illustrated. At the top, we have the pathway leading to 16α-hydroxyestrone; in the middle, the pathway to 2-hydroxyestrone; and at the bottom, the pathway leading to 4-hydroxyestrone. As can be recalled, we have shown that 16α-hydroxyestrone is elevated in breast cancer,[1] is genotoxic,[2] and increases cell proliferation, anchorage-independent growth, and a variety of other basically risk-promoting events, whereas 2-hydroxyestrone does none of these.[3]

Before discussing the chemoprevention studies, I would like to point out some of the problems that any drug that is to be effective must deal with. If we look at the response of low-risk (LR) terminal duct lobular units (TDLU), that is, tissue taken from a mammoplasty specimen, versus high-risk (HR) TDLU taken from the breast of a woman with a tumor, but distal to the tumor, it can be seen that the two tissues respond very differently, both basally (FIGURE 2) and in response to stimuli (FIGURE 3).[4] There is a marked response to benzo[*a*]pyrene (BP) in the at-risk tissue from women with a tumor in the breast, whether one looks at Ras oncogene expression or at 16α-hydroxylation, relative to the responses observed in normal tissue (FIGURE 4). We were greatly surprised in carrying out these studies after we had previously studied indole-3-carbinol because, in theory, both benzo[*a*]pyrene and indole carbinol as its acid-generated trimer in the stomach should act by binding to the aryl hydrocarbon hydroxylase receptor and thereby stimulate P450-1A1. In actual fact, though, they have opposite responses. This remains a problem that has not been resolved, although the actions of P450-1B1 may explain these effects. Moreover, in examining normal mouse mammary epithelial cells, Nitin Telang[2] has shown that DMBA (7,12-dimethylbenz[*a*]anthracene) in this case also depresses 2-hydroxylation and increases 16α-hydroxylation (FIGURE 5). This again is contrary to what we would have anticipated would happen, but similar to the effect of BP in human tissue.

This same shift in estrogen metabolism is also induced by oncogenes. FIGURE 6 shows the results of a study in normal mammary cells transfected with the Ras on-

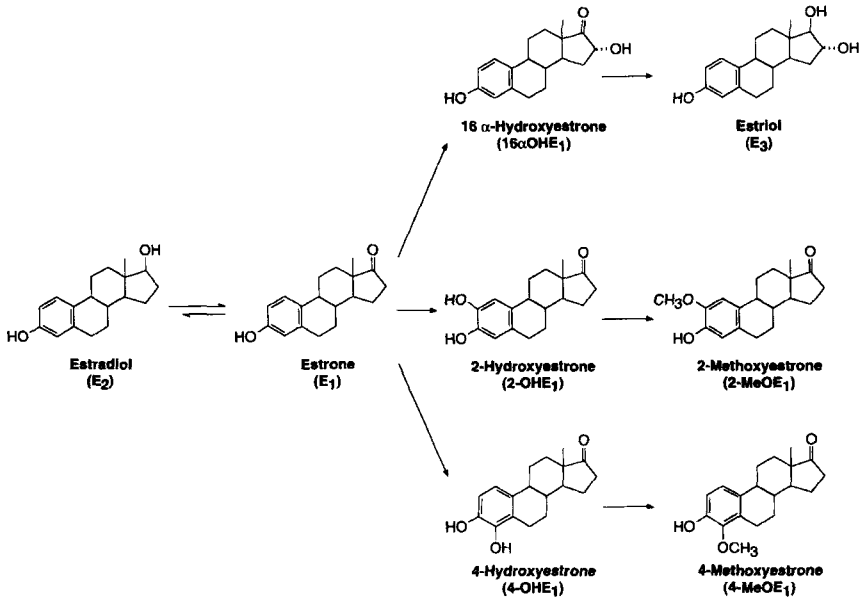

FIGURE 1. The principal pathways of estrogen metabolism at C-2, C-4, and C-16α.

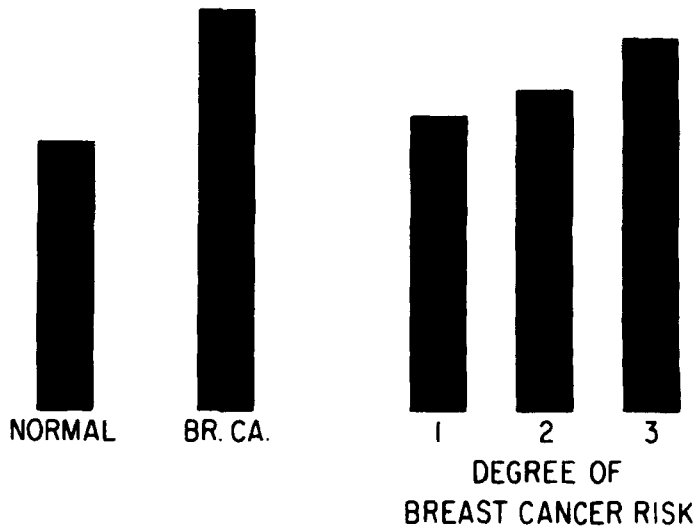

FIGURE 2. Increased 16α-hydroxylation in newly diagnosed breast cancer cases and in women at high risk for breast cancer. The two left-hand columns illustrate the increase in 16α-hydroxylation in women newly diagnosed for breast cancer. The three right-hand columns illustrate the increase in this reaction with increase in risk for cancer in women still free of disease.

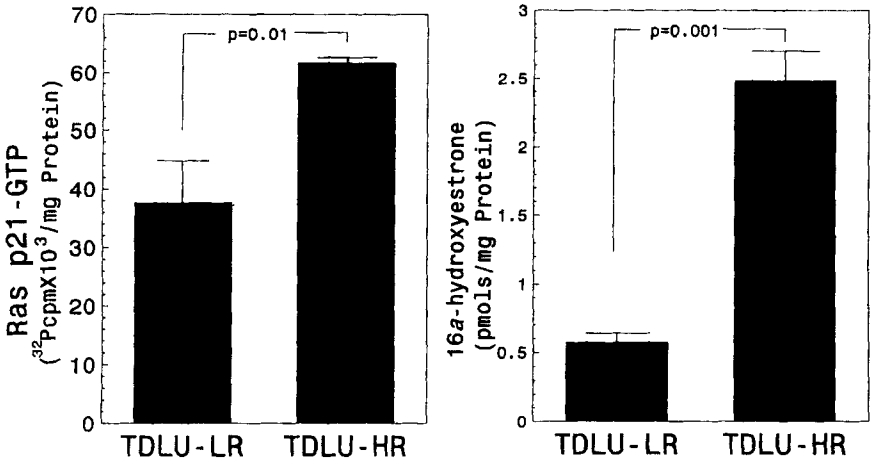

FIGURE 3. Metabolism of estradiol at C-16α (right) and Ras p21 expression (left) in normal [low-risk (LR)] TDLU (mammoplasty) and high-risk (HR) TDLU (from a breast containing a tumor, but distal to it).

cogene.[5] Whether one studies the transfected cells or the cells after they have been passed through a tumor to select the most responsive cells, one sees a substantial increase in 16α-hydroxylation relative to 2-hydroxylation. When we examine responses to the mouse mammary tumor virus, we see a similar change. When we deprive C3H/OuJ pups of the virus by foster-nurturing them on C57/BlJ mothers, which normally do not carry the mouse mammary tumor virus, 16α-hydroxylation goes down. When we add the virus

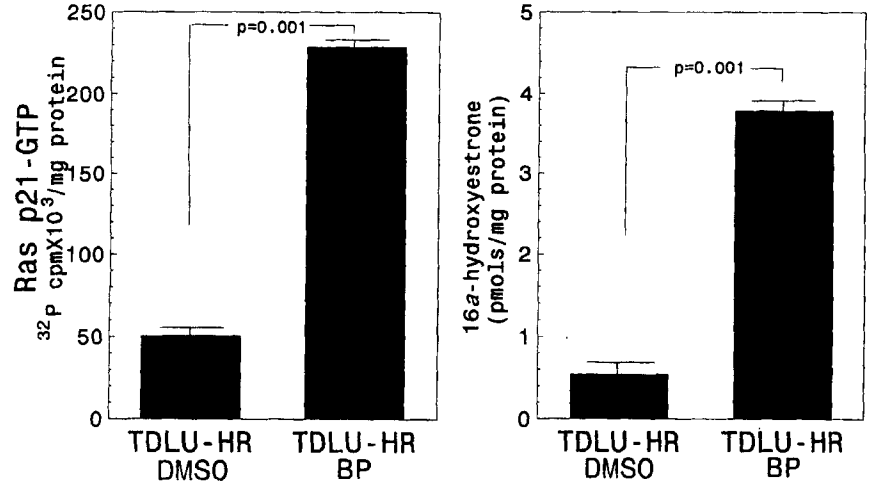

FIGURE 4. Modulation of estradiol 16α-hydroxylation (right) and Ras p21 expression (left) in high-risk TDLU by benzo[α]pyrene (versus DMSO).

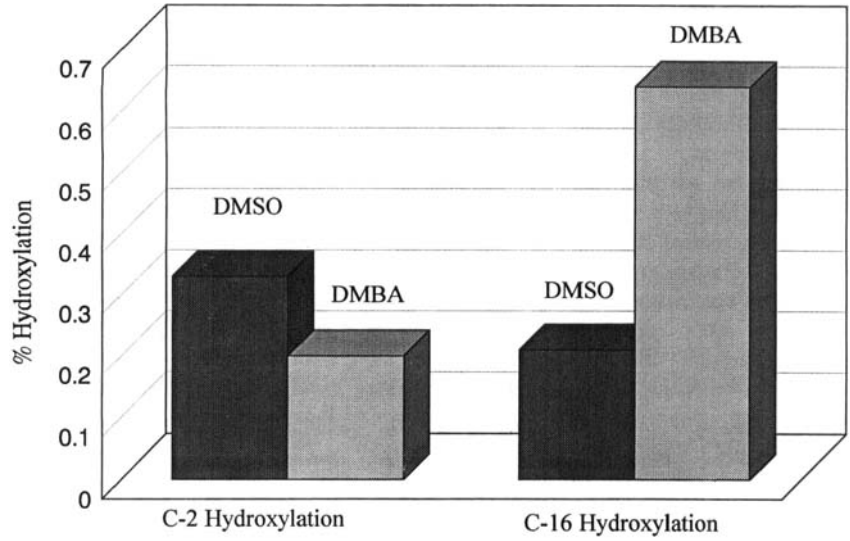

FIGURE 5. Modulation of estrogen metabolism in MCF-7 cells by DMBA: % reaction was determined radiometrically.

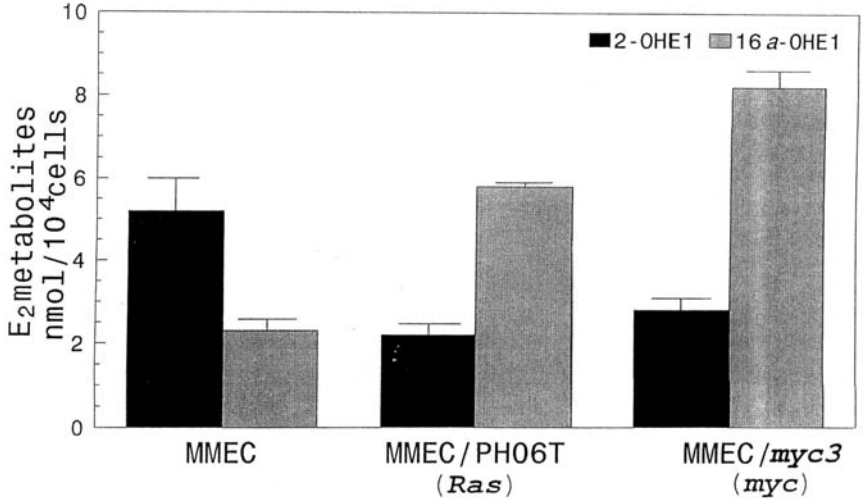

FIGURE 6. Effect of the Ras and Myc oncogenes on estradiol (E_2) metabolism in transfected murine mammary cells.

to C57/OuJ black pups by foster-nurturing them on C3H/OuJ foster-mothers, which carry the virus, 16α-hydroxylation goes up, nearly doubling (FIGURE 7). This seems to be a general response that is demonstrated in a number of mouse strains.[6] We believe that any drug that is to have protective effects must be able to reverse this ratio.

Another virus that affects 16α-hydroxylation is the human papilloma virus (HPV), which is responsible in part for cervical cancer as well as laryngeal papillomas. Here, again, one can see that this virus stimulates 16α-hydroxylation in different tissues (TABLE 1).[7] This is a reciprocal process in that adding 16α-hydroxyestrone to tissue fragments in culture containing the virus results in increased proliferation of the virus.

If we look again at all the factors that have been considered to change breast cancer risk, they all correlate with changes in the 2/16α ratio. The responses that increase risk decrease the ratio, whereas the factors that decrease risk raise the ratio (TABLE 2). As discussed earlier in this volume, attempts to directly decrease 16α-hydroxylation in human volunteers, a study on which we mounted a rather large effort, resulted in a complete lack of success, in that none of the drugs or dietary additives that we tried were capable of substantially decreasing 16α-hydroxylation directly. The enzyme appears to be constitutive and not readily altered.

At this point, we turned to consider that, instead of directly changing 16α-hydroxylation, we should attempt to increase 2-hydroxylation, thereby "bleeding" estradiol out of the system, which, after all, contains only a finite amount of this hormone, and thus decreasing 16α-hydroxylation indirectly. Concern has been expressed that if we increase 2-hydroxyestrone formation we will be generating a carcinogenic risk because it has been shown that 4-hydroxyestradiol, which is also a catechol, is tumorigenic in the Syrian hamster model. More recently, it has been suggested by Li that this compound

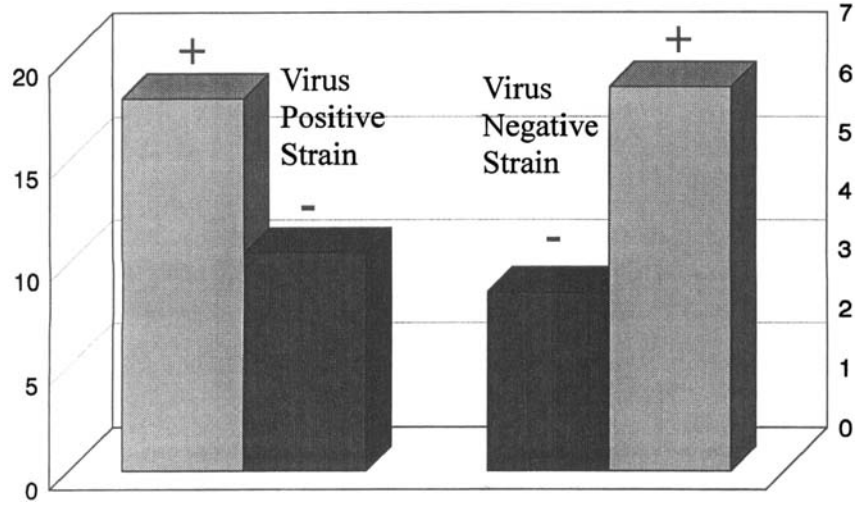

FIGURE 7. Effect of the mouse mammary tumor virus on estradiol 16α-hydroxylation: (left) pups from virus-positive mothers were foster-nurtured by virus-negative mothers; (right) pups from virus-negative mothers were foster-nurtured by virus-positive mothers. The ordinates represent % reaction in an *in vivo* study.

TABLE 1. Effect of HPV on the 16α-Hydroxylation of Estradiol in Genital Epithelial Cells

Cells	% 16α-Hydroxylation of E_2 (per 100 mg protein)	
	Normal	Immortalized with HPV
Foreskin	0.08 ± 0.08	1.6 ± 0.1
Endocervix	0.70	ND
Cervical transition zone	2.70 ± 0.7	16.3 ± 0.5
Cervical cancer	ND	8.6 ± 1.4

is active because of its enhanced estrogenicity.[8] In our studies, 2-hydroxyestrone is considerably less active than estradiol or the control medium alone in terms of inducing anchorage-independent growth. It seems to have an antiestrogenic effect (FIGURE 8). If we look at cell proliferation or unscheduled DNA synthesis (all work carried out by A. Suto in our laboratory), we see the same thing: 2-hydroxyestrone shows no signs of any tumorigenic or risk-promoting effects.[3] At least in animals and in human subjects, we believe the risk that has been imputed for 2-hydroxyestrone is misplaced—it just does not happen to do any of these things. Therefore, we set out to increase the formation of this compound.

2-Hydroxylation, unlike 16α-hydroxylation, is relatively labile. It can be changed by body composition; for example, it is lower in obese subjects[9] and markedly increased in anorectics and athletes who exercise vigorously (FIGURE 9).[10] We are referring here particularly to marathon runners or women who go out for eight-oar crew. One of the side effects during the rowing season is the cessation of menstruation in many of these female athletes due to the fact that they no longer have enough active estrogen because it is largely going to 2-hydroxyestrone. The decrease in 2-hydroxylation observed in obesity appears to be due to the fact that the stromal cells in fat deposits secrete a

TABLE 2. Correlation of Breast Cancer Risk and the 2/16α Metabolite Ratio

Characteristic	Breast Cancer Risk	2/16α Metabolite Ratio
Heredity	↑	↓
Obesity	↑	↓
Thinness	↓	↑
Smoking	↓	↑
High fat diet	↑	↓
Fish oil diet	↓	↑
Exercise	↓	↑
Cruciferous vegetables	↓	↑
Indole-3-carbinol	↓	↑
Dioxin	↓	↑

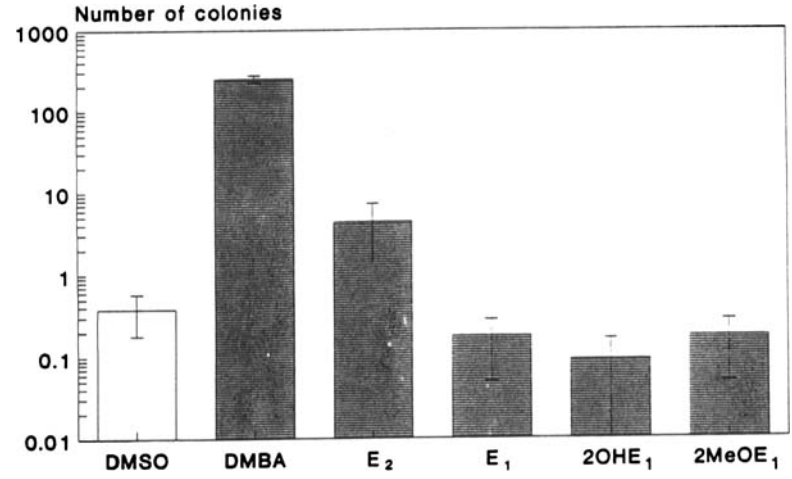

FIGURE 8. Inhibition of anchorage-independent growth of MMEC cells by 2-hydroxyestrone.

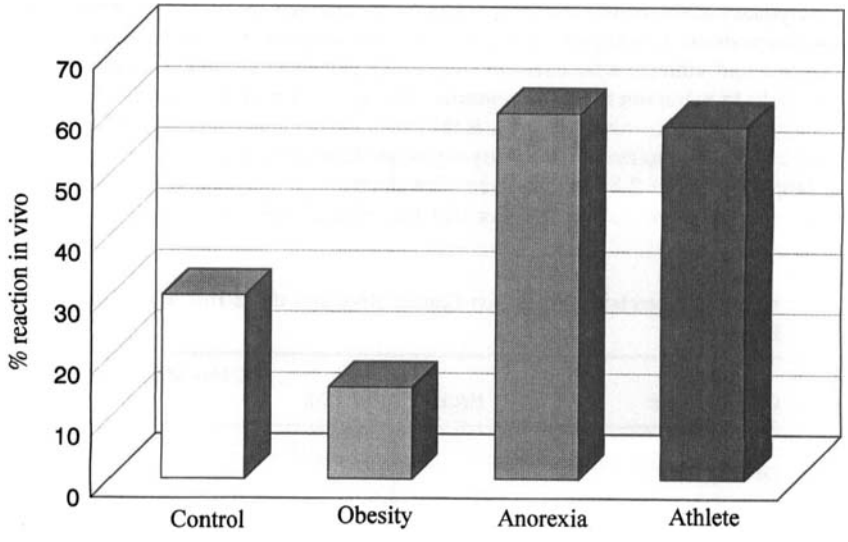

FIGURE 9. Effect of body composition on overall 2-hydroxylation in human subjects: % reaction was measured radiometrically using [2-^{3}H]estradiol as substrate.

compound that inhibits 2-hydroxylation (FIGURE 10). When people get very thin and lose their fat deposits, they stop secreting this compound and 2-hydroxylation goes up. To illustrate how labile 2-hydroxylation can be, FIGURE 11 shows the response to an ultimate basal diet. This is a study carried out in volunteers put on a liquid formula diet containing casein, corn oil, sugar, vitamins, and minerals at the Rockefeller University Hospital. Using an ELISA assay on urine samples obtained from these subjects at intervals during the study, we measured the 2 and 16α metabolites in these subjects. It can be seen that 16α-hydroxylation is essentially constant through all the periods. In the third, fourth, and fifth weeks, when the volunteers were put on the liquid formula diet, 2-hydroxylation dropped promptly to very low levels. This effect actually takes place in as little as two days after subjects are put on the liquid formula diet. These results point out the need to pay some attention to this lability in any study on estrogen metabolism.

Early on, we did some studies with J. F. Gierthy and his colleagues at the state laboratory in Albany with TCDD, which they had observed had antiestrogenic effects for reasons that were not immediately clear.[11] We were able to show that in receptor-positive cells, like the MCF-7 cell, TCDD powerfully increases 2-hydroxylation and has at best minimal effect on 16α-hydroxylation.[12] This does not happen in receptor-negative cells (MDA-MB-231) and we believe that this is due to the fact that the estrogen receptor must bind to an upstream ERE element on the P450-1A1 gene to effect this kind of induction (FIGURE 12). This correlates with the fact that following an explosion in Sevaso, where a number of women were exposed to TCDD, Bertazzi *et al.* subsequently reported that breast cancer rates were diminished in the women exposed to TCDD relative to women in the surrounding communes who were not exposed to TCDD.[13] This is not the kind of experiment that is likely to be repeated, unless another accident occurs,

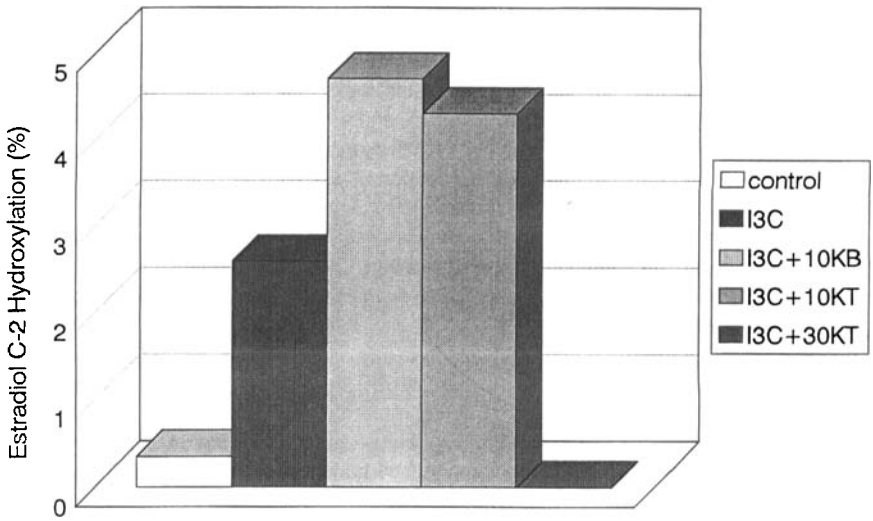

FIGURE 10. Effect of human fat cell secretions on estradiol 2-hydroxylation in MCF-7 cells. Stromal cells from body fat are incubated in Krebs-Ringer buffer and the media are shown to inhibit estradiol 2-hydroxylation. The effect is primarily in the 30-kDa fraction of the media.

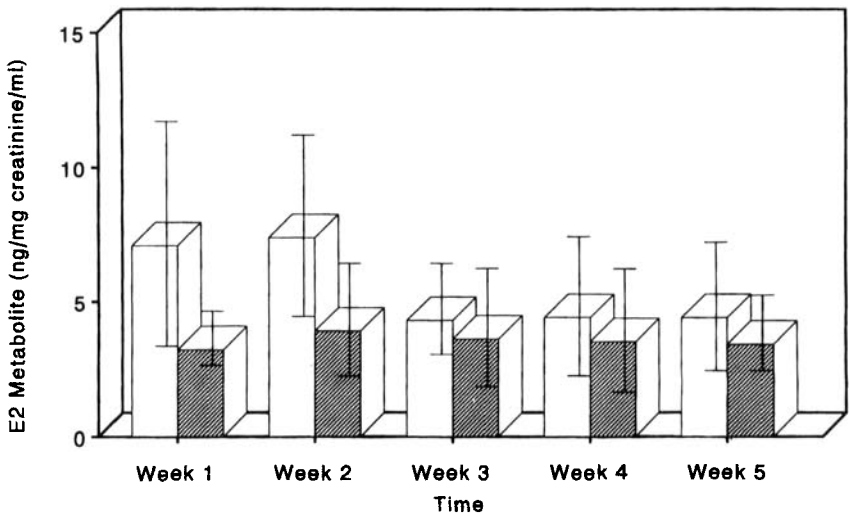

FIGURE 11. Effect of a liquid formula diet on 2- and 16α-hydroxylation in human subjects maintained at constant body weight. Urinary estradiol metabolites: ☐ 2-OHE_1; ▨ 16-OHE_1.

and one would hardly advocate TCDD lotions as a form of treatment. It is not likely to generate any popularity.

However, it has been pointed out that there are lots of other compounds around that are capable of effecting this induction. Following up on an early work of Wattenberg[14] on indole carbinol and vegetables containing it, as well as some observations from Bruce Ames, we decided to see if these compounds would successfully induce 2-hydroxylation of estradiol. Our first study was carried out in MCF-7 cell cultures by adding a variety of indole derivatives (10^{-7} M) to cell cultures and looking at the change in the extent of 2-hydroxylation (FIGURE 13). As one can see, indole-3-carbinol (I3C) was the most potent of these compounds in this assay. Subsequently, we found that I3C rapidly dimerizes in tissue culture media to diindolylmethane, which is simply two indole carbinols joined together. This is the actual active agent.[15]

This same induction is also readily demonstrated in human subjects. FIGURE 14 illustrates a study carried out at the Rockefeller University Hospital in which Jon Michnovicz and I gave 2-tritiated estradiol to both male and female volunteers. As is seen, in every subject, there is a significant increase in 2-hydroxylation after only five days on indole-3-carbinol. The effect is obviously very promptly induced.[16] Animal studies, in which rats were fed a single dose of I3C by gavage, showed that there was a prompt increase in 2-hydroxylation that remained elevated for 24 hours (FIGURE 15).

As seen in FIGURE 16, the effect is persistent in a long-term examination. This is a three-month study carried out on 60 women who volunteered and were divided into three groups: 20 and 20 and 20, who received a placebo, 400 mg of indole carbinol daily, or 20 g of cellulose to serve as a fiber supplement. Note that we picked 400 mg because it calculated out to about what one would eat in a third of a head of cabbage and this seemed to be about as big an amount as we could ever get people to reasonably eat. As

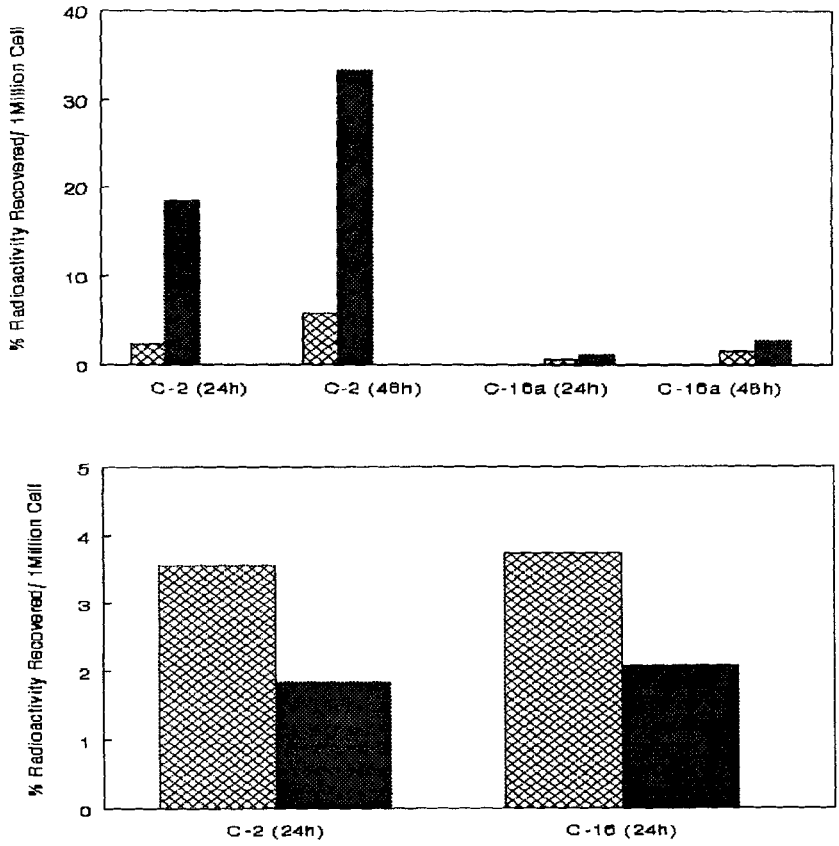

FIGURE 12. Effect of TCDD on estradiol metabolism in MCF-7 (top) and MDA-MB-231 (bottom) cells: ▩ control; ▩ TCDD.

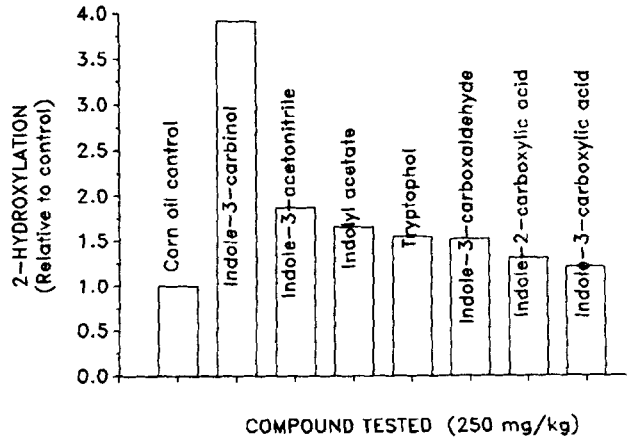

FIGURE 13. Induction of estradiol 2-hydroxylation by indole derivatives in MCF-7 cells.

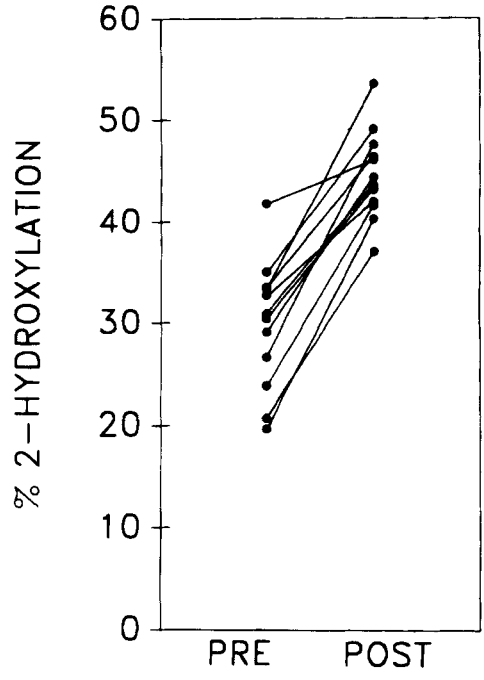

FIGURE 14. Induction of estradiol 2-hydroxylation by oral indole-3-carbinol (400 mg/day) in human subjects.

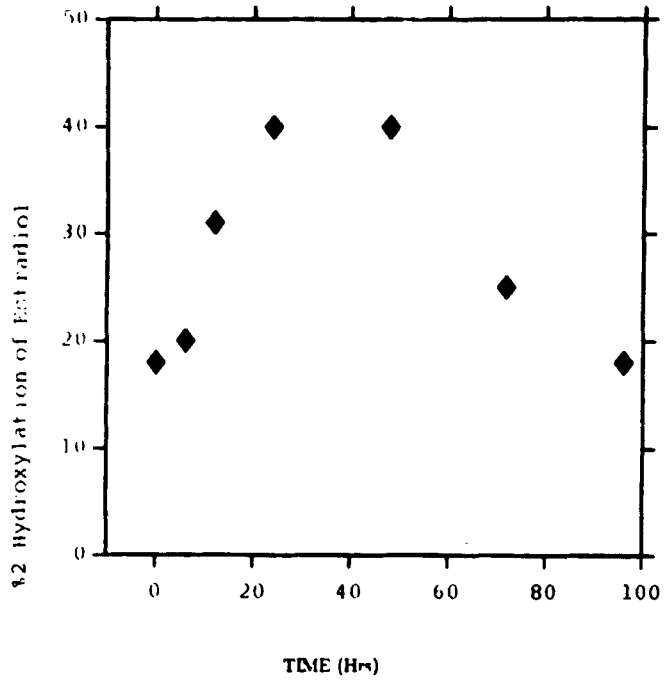

FIGURE 15. Induction of estradiol 2-hydroxylation by a single dose of indole-3-carbinol given by gavage to a female rat. Induction occurred rapidly and lasted for 24 hours.

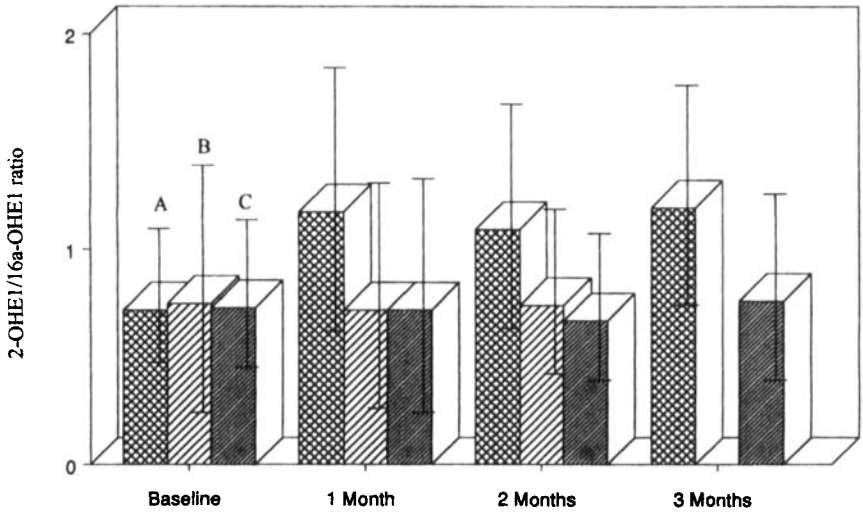

FIGURE 16. Steady-state response in estradiol 2-hydroxylation to a three-month trial. Human subjects were given (A) indole-3-carbinol (400 mg/day), (B) cellulose (20 g/day), or (C) placebo.

one can see, the 2/16α ratio goes up promptly on the indole-3-carbinol arm of the study and stays up. There are no changes in the volunteers who are on either cellulose or the placebo arms. We measured a variety of biochemical parameters in these volunteers—the usual tests in an SMAC assay, cholesterol, triglycerides, and a variety of other items. There were no significant changes in any of the measurements that we performed, showing that there do not appear to be any harmful side effects that are apparent over a three-month period (TABLES 3a–c).[17]

Encouraged by this, we decided to see if indole-3-carbinol could have any activities in terms of carcinogenicity. The results show that indole-3-carbinol, like HPR, will oppose the effects of DMBA on anchorage-independent growth in MCF-7 cells (TABLE 4). It also reverses the effect of DMBA on 2- and 16α-hydroxylation, increasing the reaction at 2 and decreasing that at 16α, showing that these compounds, although they theoretically work the same way, really have opposite effects in actual organ systems (TABLE 5).[18]

We went on from this to see if we could prevent tumors by feeding indole-3-carbinol to mice with high mammary tumor incidence. The first thing that we checked was to show that body weight did not change, to make sure that any tumor prevention we were seeing was not due to a decrease in body weight. This is done because it is well known that animals who lose weight have a decreased rate of tumor incidence. FIGURE 17 shows the effect on tumor incidence of a control diet and a diet containing 2000 parts per million of indole-3-carbinol. It can be seen that there is a substantial increase in latency on a 2000 parts per million diet, as well as a decrease in the number of animals having tumors, out at 250 days; there is also a decrease in multiplicity. This study was done in the C3H/OuJ mouse, which normally has a very high tumor incidence.[19] A study using

TABLE 3a. Biochemical Measurements in Patients Receiving Indole-3-carbinol for a Three-Month Period[a]

Variable	Baseline	Month 1	Month 2	Month 3
Blood Profile				
Hemoglobin	12.9 ± 0.7	12.7 ± 0.8	12.5 ± 0.7	12.7 ± 0.6
Platelets	290.5 ± 56.4	287.1 ± 40.6	293.4 ± 63.5	287.8 ± 55.0
BUN	14.0 ± 3.3	12.9 ± 2.4	13.3 ± 2.1	12.9 ± 2.0
Bilirubin	0.6 ± 0.5	0.5 ± 0.3	0.5 ± 0.3	0.4 ± 0.2
Uric acid	4.8 ± 0.8	4.2 ± 0.7	4.3 ± 0.9	4.3 ± 1.1
Total prot.	6.6 ± 0.3	6.6 ± 0.4	6.5 ± 0.4	6.6 ± 0.3
Albumin	4.5 ± 0.2	4.5 ± 0.2	4.4 ± 0.2	4.4 ± 0.2
Calcium	9.2 ± 0.3	9.2 ± 0.4	9.1 ± 0.4	9.1 ± 0.4
Phosphate	3.7 ± 0.5	3.7 ± 0.5	3.6 ± 0.5	3.6 ± 0.5
SGOT	18.4 ± 4.2	19.8 ± 4.2	20.3 ± 5.2	20.8 ± 6.1
LDH	156.2 ± 14.7	155.8 ± 20.5	153.1 ± 19.9	51.9 ± 24.5
Alk. phos.	49.8 ± 13.6	47.6 ± 12.9	44.6 ± 12.1	46.8 ± 13.2
Cholesterol Profile				
Total chol.	186.9 ± 39.4	191.1 ± 36.2	188.3 ± 37.2	198.1 ± 40.4
HDL	59.7 ± 12.4	57.3 ± 11.8	55.7 ± 10.9	58.3 ± 13.4
LDL	112.1 ± 37.2	18.3 ± 36.1	116.0 ± 35.5	123.4 ± 39.1
TC/HDL	3.2 ± 0.9	3.5 ± 1.0	3.5 ± 1.1	3.6 ± 1.3
Endocrinological Parameters				
TSH	2.0 ± 2.3	1.5 ± 1.8	1.5 ± 1.5	1.6 ± 1.8
Estradiol	109.5 ± 74.6	112.9 ± 57.3	95.2 ± 81.3	80.9 ± 40.8
SHBG	65.4 ± 34.0	71.5 ± 36.9	68.9 ± 34.5	70.4 ± 37.4
Urinary Estrogens				
2OHE1	9.6 ± 6.1	12.5 ± 8.1	10.4 ± 5.0	13.3 ± 9.4
E3	13.9 ± 7.1	11.3 ± 5.3	10.3 ± 3.9	12.2 ± 7.7
E1	13.6 ± 10.1	13.7 ± 10.0	12.1 ± 9.5	11.9 ± 7.4
Sum (E)	37.0 ± 20.8	37.7 ± 17.5	32.8 ± 12.2	37.4 ± 20.6
2OHE1/E3	0.72 ± 0.31	1.17 ± 0.61	1.09 ± 0.52	1.19 ± 0.57
Menses (days)	27.2 ± 3.2	27.1 ± 2.6	27.3 ± 2.6	27.5 ± 2.4

[a]Mean ± SD.

the oncomouse was carried out with colleagues at the Orentreich Medical Foundation. It can be seen that there is a substantial difference in tumor incidence out at 54 weeks, contrary to some of the claims made for the oncomouse (FIGURE 18). It does not really get as many tumors in the control animals as has been reported. However, there is a substantial difference. For FIGURE 19, we are indebted to investigators at the Chemoprevention Branch at the NCI, namely, G. J. Kelloff, V. E. Steele, and G. Lubet (see reference 20). This is the result of a study that they presented at the AACR meeting in the spring of 1994. They showed that giving indole-3-carbinol to rats blocks DMBA-induced tumors. The treatment was successful whether the I3C treatment was started before, during, or after the DMBA treatment. In another study, which will not be shown here for space considerations, they also showed that the same thing is true for NMU-

TABLE 3b. Biochemical Measurements in Patients Receiving Cellulose for a Three-Month Period[a]

Variable	Baseline	Month 1	Month 2	Month 3
Blood Profile				
Hemoglobin	12.8 ± 0.7	12.7 ± 0.8	12.4 ± 0.8	11.4 ± 0.8
Platelets	264.1 ± 72.1	265.0 ± 65.6	279.6 ± 84.2	277.4 ± 89.5
BUN	12.4 ± 2.7	11.9 ± 2.5	12.6 ± 2.9	13.2 ± 3.4
Bilirubin	0.6 ± 0.4	0.6 ± 0.4	0.6 ± 0.4	0.5 ± 0.3
Uric acid	4.1 ± 0.7	3.9 ± 0.8	4.0 ± 0.8	4.0 ± 1.0
Total prot.	6.8 ± 0.4	6.7 ± 0.4	6.1 ± 0.4	6.6 ± 0.4
Albumin	4.6 ± 0.2	4.5 ± 0.2	4.5 ± 0.3	4.5 ± 0.2
Calcium	9.3 ± 0.3	9.3 ± 0.4	9.0 ± 0.4	9.3 ± 0.3
Phosphate	3.6 ± 0.4	3.7 ± 0.4	3.6 ± 0.5	3.6 ± 0.5
SGOT	21.5 ± 5.4	22.0 ± 3.9	22.0 ± 3.7	20.9 ± 4.3
LDH	168.3 ± 34.0	164.3 ± 28.1	157.8 ± 29.6	159.6 ± 28.3
Alk. phos.	53.2 ± 11.8	51.6 ± 11.7	52.9 ± 12.0	50.9 ± 8.9
Cholesterol Profile				
Total chol.	189.1 ± 33.0	186.8 ± 31.8	187.7 ± 33.2	190.6 ± 35.8
HDL	59.9 ± 10.6	59.8 ± 9.6	59.0 ± 9.7	61.6 ± 11.7
LDL	109.5 ± 32.1	106.7 ± 30.2	110.5 ± 34.4	113.3 ± 36.1
TC/HDL	3.3 ± 0.8	3.2 ± 0.7	3.3 ± 0.8	3.2 ± 1.0
Endocrinological Parameters				
TSH	1.8 ± 1.2	1.5 ± 1.5	1.9 ± 2.1	1.4 ± 1.0
Estradiol	70.1 ± 36.9	75.2 ± 44.9	79.2 ± 43.0	95.9 ± 53.7
SHBG	65.4 ± 17.8	64.4 ± 17.7	59.7 ± 18.0	58.9 ± 15.9
Urinary Estrogens				
2OHE1	8.6 ± 5.5	7.8 ± 4.9	8.6 ± 4.4	9.4 ± 5.6
E3	13.1 ± 6.9	12.5 ± 7.4	13.2 ± 7.9	13.8 ± 7.5
E1	9.4 ± 3.9	9.7 ± 5.5	9.8 ± 4.5	11.9 ± 6.5
Sum (E)	31.3 ± 10.1	30.0 ± 13.0	31.6 ± 11.6	35.1 ± 15.4
2OHE1/E3	0.87 ± 0.71	0.80 ± 0.64	0.86 ± 0.67	0.79 ± 0.42
Menses (days)	29.1 ± 6.1	28.1 ± 3.5	27.6 ± 2.9	29.5 ± 6.6

[a]Mean ± SD.

induced mammary tumors, although not so completely. Hence, following the administration of two chemical carcinogens, indole-3-carbinol also worked to decrease the number of tumors. FIGURE 20 taken from this same presentation shows the induction of CPY1A1 in the indole-3-carbinol-treated animals, as seen in the second column across. A parallel response in the glutathione sulfotransferase enzyme is shown in the next-to-last column. Thus, indole-3-carbinol is clearly acting both as a Phase I inducer and as a Phase II inducer, which means that we are getting activity by at least two different methods. A similar preventive effect on endometrial tumors was reported by Kojima *et al.* in the Donryku rat.[21]

Now, recalling that the human papilloma virus was sensitive to 16α-hydroxyestrone,[7] we have carried out animal experiments in which we implanted laryngeal

TABLE 3c. Biochemical Measurements in Patients Receiving Placebo for a Three-Month Period[a]

Variable	Baseline	Month 1	Month 2	Month 3
Blood Profile				
Hemoglobin	12.6 ± 1.2	12.7 ± 0.6	12.9 ± 0.6	12.4 ± 0.9
Platelets	259.0 ± 74.4	276.7 ± 66.1	277.5 ± 71.1	260.8 ± 72.5
BUN	12.6 ± 2.3	12.4 ± 2.7	12.0 ± 2.8	12.0 ± 3.5
Bilirubin	0.4 ± 0.2	0.5 ± 0.2	0.5 ± 0.2	0.4 ± 0.2
Uric acid	4.0 ± 1.1	4.2 ± 1.2	4.1 ± 0.9	4.2 ± 0.9
Total prot.	6.7 ± 0.4	6.7 ± 0.3	6.8 ± 0.4	6.7 ± 0.5
Albumin	4.5 ± 0.3	4.5 ± 0.2	4.5 ± 0.3	4.5 ± 0.3
Calcium	9.2 ± 0.3	9.2 ± 0.3	9.2 ± 0.3	9.2 ± 0.3
Phosphate	4.1 ± 0.6	3.9 ± 0.5	4.0 ± 0.5	4.1 ± 0.4
SGOT	21.2 ± 5.0	21.4 ± 8.6	24.5 ± 16.8	21.4 ± 6.6
LDH	208.9 ± 219.9	147.5 ± 13.3	156.5 ± 24.6	154.1 ± 18.8
Alk. phos.	50.3 ± 13.7	47.7 ± 13.8	47.9 ± 13.0	47.9 ± 13.3
Cholesterol Profile				
Total chol.	183.8 ± 35.9	184.5 ± 33.3	133.8 ± 28.8	178.2 ± 31.9
HDL	56.5 ± 11.4	59.2 ± 12.9	56.9 ± 13.0	56.4 ± 12.3
LDL	110.9 ± 29.0	110.5 ± 32.0	111.7 ± 22.9	106.4 ± 24.0
TC/HDL	3.3 ± 0.7	3.2 ± 0.8	3.3 ± 0.7	3.2 ± 0.6
Endocrinological Parameters				
TSH	1.6 ± 0.7	1.6 ± 1.3	1.7 ± 1.0	1.7 ± 1.0
Estradiol	77.1 ± 35.7	82.9 ± 43.8	64.8 ± 34.8	81.8 ± 50.3
SHBG	68.2 ± 26.2	62.9 ± 19.2	69.2 ± 20.2	74.4 ± 22.5
Urinary Estrogens				
2OHE1	9.2 ± 5.4	9.1 ± 4.1	8.4 ± 3.4	10.3 ± 5.3
E3	14.3 ± 11.4	13.0 ± 6.3	15.0 ± 9.1	16.2 ± 9.5
E1	11.6 ± 7.6	11.6 ± 5.0	10.6 ± 4.6	11.4 ± 5.0
Sum (E)	35.6 ± 19.3	33.8 ± 10.2	34.1 ± 12.2	37.8 ± 14.5
2OHE1/E3	0.75 ± 0.36	0.78 ± 0.32	0.68 ± 0.34	0.77 ± 0.40
Menses (days)	29.3 ± 3.3	27.7 ± 4.5	23.9 ± 4.4	29.9 ± 5.3

[a]Mean ± SD.

TABLE 4. Effect of Chemopreventive Agents on Anchorage-independent Growth Induced by DMBA in MCF-7 Cells

Agent	Number of Colonies	Cloning Efficiency (%)
None	153.2 ± 15.7	15.3
Indole-3-carbinol	40.2 ± 14.3	4.0
Tamoxifen	42.0 ± 17.7	4.2
4-Hydroxytamoxifen	15.4 ± 4.2	1.5

TABLE 5. Altered Metabolism of Estradiol by Retinoid, Indole-3-carbinol, and Tamoxifen in DMBA-treated C57/MG Cells[a]

Treatment	% Estradiol Metabolism		
	2-Hydroxylation[b]	16α-Hydroxylation[b]	2/16α Ratio
DMBA	0.17 ± 0.03[c]	0.37 ± 0.02[d]	0.45
DMBA + HPR	1.40 ± 0.04[e]	0.23 ± 0.002[f]	6.08
DMBA + I3C	0.68 ± 0.02[g]	0.19 ± 0.005[h]	3.57
DMBA + TAM	0.17 ± 0.03[i]	0.33 ± 0.01[j]	0.51

[a]Determined after a 48-h incubation with [2-^{3}H]E_2 and [16α-^{3}H]E_2.
[b]Mean ± SEM, N = 12, normalized per 10^6 cells.
[c–j]*c-e, c-g, d-f, d-h*: $p < 0.0001$; *c-i, d-j*: NS.

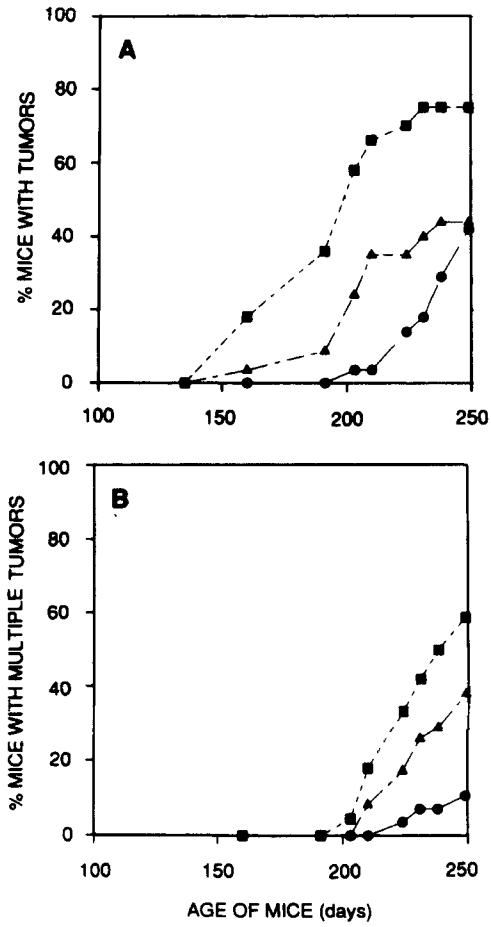

FIGURE 17. Decrease in mammary tumor formation following induction of 2-hydroxyestrone by feeding indole-3-carbinol to C3H/OuJ mice. Panel A illustrates tumor incidence and latency. Panel B represents tumor multiplicity in the same animals. Symbols: ■ = control; ▲ = 500 ppm of indole-3-carbinol in the diet; ● = 2000 ppm of indole-3-carbinol in the diet.

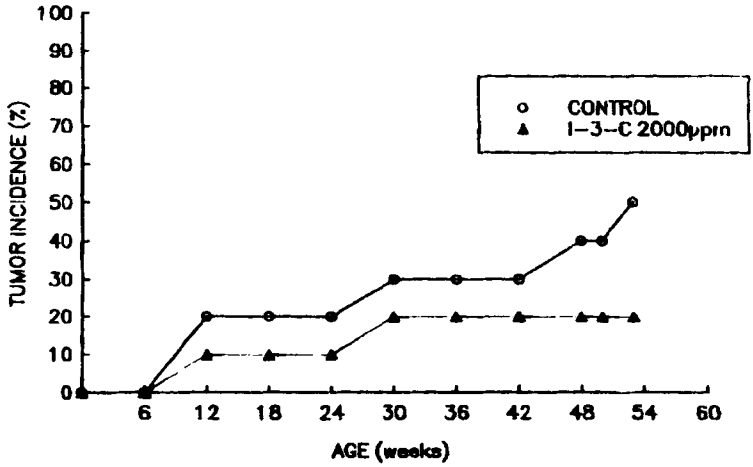

FIGURE 18. Effect of an indole-3-carbinol diet on prevention of spontaneous tumors in the oncomouse. Treatment was started at six weeks of age.

fragments infected with HPV into the kidney capsule of nude mice. The animals were then put on either a control diet or an indole-carbinol-containing diet. As seen in FIGURE 21, there is a dramatic drop in the formation of papillomatous cysts in the animals given indole carbinol. Thus, cysts do not develop when we deprive the virus of 16α-hydroxyestrone in this manner. This study was carried out with a colleague at Long

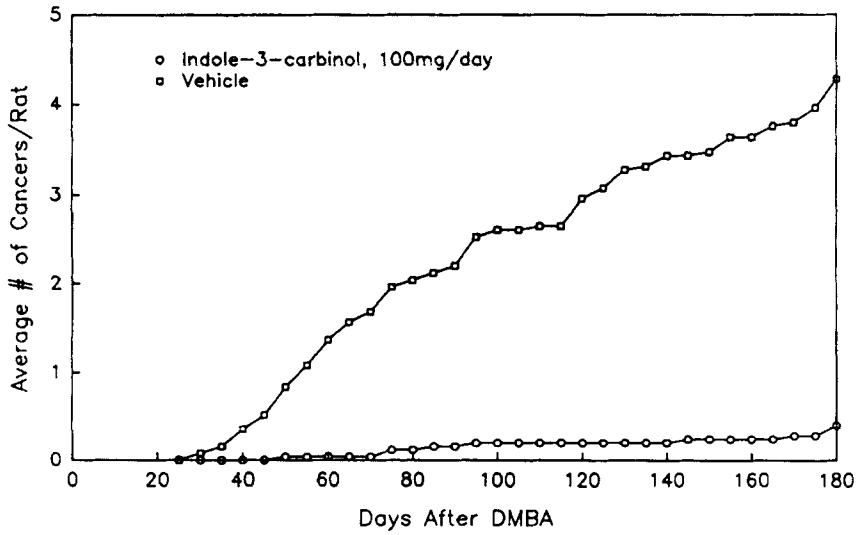

FIGURE 19. Inhibition of DMBA-induced mammary tumors in rats by administration of indole-3-carbinol in the diet. (See Grubbs *et al.*[20])

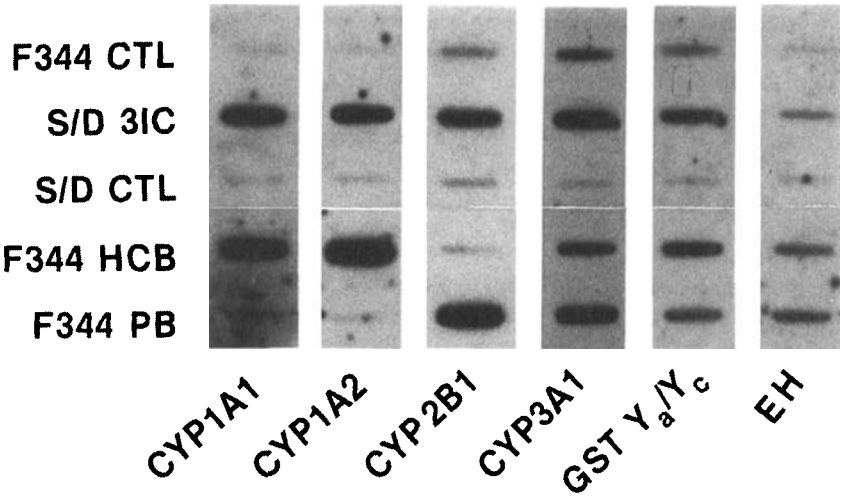

FIGURE 20. Induction of Phase I and Phase II enzymes in the rat by indole-3-carbinol. (See Grubbs *et al.*[20])

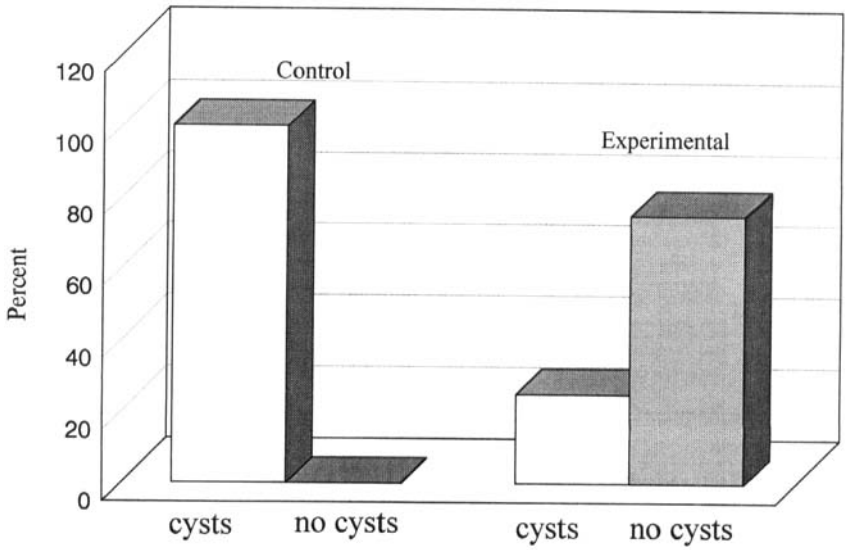

FIGURE 21. Inhibition of the growth of papillomatous cysts in nude mice by indole-3-carbinol in the diet. Fragments of laryngeal tissue infected with HPV were implanted under the renal capsule of nude mice, who were fed AIN76a or AIN76a+I3C.

Island Jewish Hospital, Karen Auborn, and her collaborators.[22] She told the clinicians there about these results, who in turn told some of the patients about it. This resulted in a large number of families putting their children and other relatives with laryngeal papillomas on cabbage juice or cabbage extract diets. The results of this study are shown in FIGURE 22. If we measure the 2-hydroxyestrone/16α-hydroxyestrone ratio by the same ELISA procedure as described earlier, it can be seen that there is a nice inverse correlation: as the 2/16α ratio goes down, the rate of growth of the papillomas increases. Patients who have been maintained on high 2/16α ratios, either by feeding cabbage or directly on indole-3-carbinol, have gone for periods of up to two years now without requiring further surgery. This is, as far as we know, the only truly successful treatment in which this kind of remission in laryngeal papillomas has been observed. It is also evidence that therapeutic alteration of estrogen metabolism can serve to decrease tumorous growths.

SUMMARY

The results show that all of the carcinogens, oncogenes, and tumor-associated viruses that we have studied profoundly affect the extent of 2- and 16α-hydroxylation in a prorisk direction. All of the dietary and biological responses associated with increased cancer risk decrease 2-hydroxylation and increase 16α-hydroxylation.

Remarkably, although PAHs are reported to induce P450-1A1, we have found them to decrease 2-hydroxylation. Finally, using indole-3-carbinol to induce 2-hydroxylation results in the chemoprevention of mammary tumors in rodents and recurrences of laryngeal papillomas in humans. Also correlating with these studies in HPV is the decrease in the C-2/C-16α metabolite ratio observed in women with CIN relative to

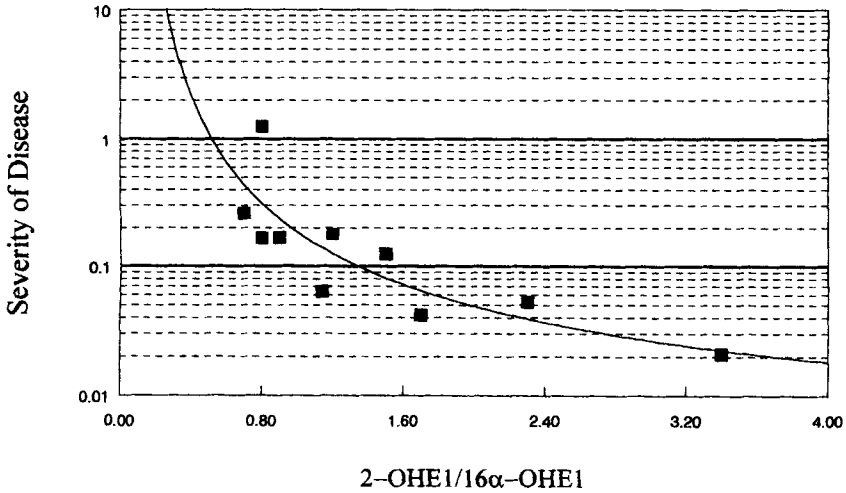

FIGURE 22. Inverse relationship between the urinary 2/16α metabolite ratio and the rate of growth of laryngeal papillomas (■ = series 1).

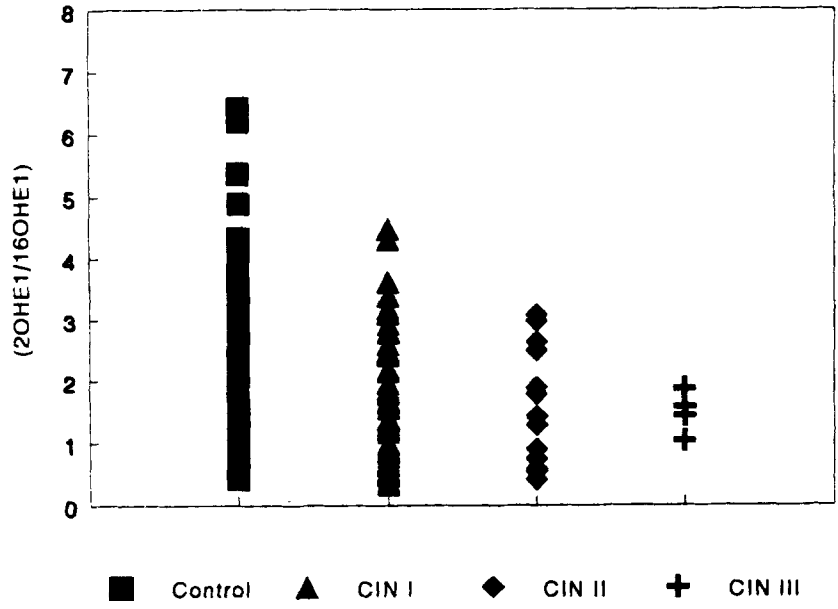

FIGURE 23. Inverse relationship between CIN status in women patients and their urinary 2/16α metabolite ratio.

control subjects. The greatest decrease was observed in women with the most severe form, CIN3 (FIGURE 23). These findings are under further investigation.

REFERENCES

1. SCHNEIDER, J., D. KINNE, A. FRACCHIA, V. PIERCE, K. E. ANDERSON, H. L. BRADLOW & J. FISHMAN. 1982. Abnormal oxidative metabolism of estradiol in women with breast cancer. Proc. Natl. Acad. Sci. U.S.A. **79:** 3047–3051.
2. TELANG, N. T., A. SUTO, G. Y. WONG *et al.* 1992. Estrogen metabolite, 16α-hydroxyestrone, induces genotoxic damage and aberrant cell proliferation in mouse mammary epithelial cells in culture. J. Natl. Cancer Inst. **84:** 634–638.
3. SUTO, A., H. L. BRADLOW, G. Y. WONG *et al.* 1993. Experimental down-regulation of intermediate biomarkers of carcinogenesis in mouse mammary epithelial cells. Breast Cancer Res. Treat. **27:** 193–202.
4. OSBORNE, M. P., H. L. BRADLOW, G. Y. WONG & N. T. TELANG. 1993. Increase in the extent of estradiol 16α-hydroxylation in human breast tissue: a potential biomarker of breast cancer risk. J. Natl. Cancer Inst. **85:** 1917–1920.
5. TELANG, N. T., R. NARAYANAN, H. L. BRADLOW & M. P. OSBORNE. 1991. Coordinated expression of intermediate biomarkers for tumorigenic transformation in RAS transfected mouse mammary epithelial cells. Breast Cancer Res. Treat. **13:** 155–163.
6. BRADLOW, H. L., R. J. HERSHCOPF, C. P. MARTUCCI & J. FISHMAN. 1985. Estradiol 16α-hydroxylation in the mouse correlates with mammary tumor incidence and presence of murine mammary tumor virus: a possible model for the hormonal etiology of breast cancer in humans. Proc. Natl. Acad. Sci. U.S.A. **82:** 6295–6299.

7. AUBORN, K. J., C. WOODWORTH, J. DIPAOLO & H. L. BRADLOW. 1991. The interaction between *HPV infection and estrogen metabolism in cervical carcinogenesis*. Int. J. Cancer **49:** 867–869.
8. LI, S. A. & J. J. LI. 1992. Metabolism of moxestrol in the hamster kidney: significance for estrogen carcinogenesis. *In* Hormonal Carcinogenesis. J. J. Li, S. Nandi & S. A. Li, Eds.: 110. Springer-Verlag. New York/Berlin.
9. SCHNEIDER, J., H. L. BRADLOW, G. STRAIN, J. LEVIN, K. ANDERSON & J. FISHMAN. 1983. Effects of obesity on estradiol metabolism: decreased formation of nonuterotropic metabolites. J. Clin. Endocrinol. Metab. **56:** 973–978.
10. SNOW, R., R. BARBIERI & R. FRISCH. *1991. Estrogen 2-hydroxylase oxidation and* menstrual function among elite oarswomen. J. Clin. Endocrinol. Metab. **69:** 369–376.
11. GIERTHY, J. F., D. W. LINCOLN, M. B. GILLESPIE, J. I. SEEGAR, H. L. MARTINEZ, H. DICKERMAN & S. A. KUMAR. 1987. Suppression of estrogen-regulated extracellular tissue plasminogen–activated activity of MCF-7 cells by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Cancer Res. **47:** 6198–6203.
12. GIERTHY, J. F., D. W. LINCOLN II, S. J. KAMPCIK, H. W. DICKERMAN, H. L. BRADLOW, T. NIWA & G. E. SWANECK. 1988. Enhancement of 2- and 16α-estradiol hydroxylation in MCF-7 human breast cancer cells by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Biochem. Biophys. Res. Commun. **157:** 515–520.
13. BERTAZZI, P. A., C. ZOCCHETTI, A. C. PESATORI, S. GUERCILENA, M. SANARICO & L. RADICE. 1989. Ten year mortality study of the population involved in the Sevaso incident in 1976. Am. J. Epidemiol. **129:** 1187–1200.
14. WATTENBERG, L. W. & L. W. LOUB. 1978. Inhibition of polycyclic aromatic hydrocarbon–induced neoplasia by naturally occurring indoles. Cancer Res. **38:** 1410–1413.
15. NIWA, T., G. SWANECK & H. L. BRADLOW. 1994. Alterations in estradiol metabolism in MCF-7 cells induced by treatment with indole-3-carbinol and related compounds. Steroids **59:** 523–527.
16. MICHNOVICZ, J. J. & H. L. BRADLOW. 1990. Induction of estradiol metabolism by dietary indole-3-carbinol in humans. J. Natl. Cancer Inst. **50:** 947–950.
17. BRADLOW, H. L., J. J. MICHNOVICZ, G. Y. C. WONG, M. P. HALPER, D. MILLER & M. P. OSBORNE. 1994. Long-term responses of women to indole-3-carbinol or a high fiber diet. Cancer Epidemiol. Biomarkers Prev. **3:** 591–595.
18. BRADLOW, H. L., J. J. MICHNOVICZ, N. T. TELANG & M. P. OSBORNE. 1991. Effect of dietary indole-3-carbinol on estradiol metabolism and spontaneous mammary tumors in mice. Carcinogenesis **12:** 1571–1574.
19. MALLOY, V., H. L. BRADLOW, J. MATIAS & N. ORENTREICH. 1991. Further studies on chemoprevention of mammary tumors with indole-3-carbinol. Abstract no. 24. Terra Symposium on Estrogens. Mohonk, New York.
20. GRUBBS, C., V. E. STEELE, T. CASEBOLT *et al.* 1995. Chemoprevention of chemically induced mammary carcinogenesis by indole-3-carbinol. Anticancer Res. In press.
21. KOJIMA, T., T. TANAKA & M. MORI. 1994. Chemoprevention of spontaneous endometrial cancer in female Donryku rats by dietary indole-3-carbinol. Cancer Res. **54:** 1446–1449.
22. NEWFIELD, L., A. GOLDSMITH, H. L. BRADLOW & K. AUBORN. 1993. Estrogen metabolism and human papilloma virus–induced tumors of the larynx: chemo-prophylaxis with indole-3-carbinol. Anticancer Res. **13:** 227–242.