may pass across the flowers before the dangers themselves (*14*).

It is noteworthy that the flowers, *Dryas* at least, rely, in part, on the services of the basking insects (as well as others) for pollination and hence reproduction, dispersal, and colonization (*8*) and display the attributes of entomophily (*14, 15*).

Flower power, generated by the combined effects of heliotropism and radiant heat focusing, in the high arctic is important to reproduction of the plants and to the insects which visit flowers and may pollinate them. Insolation may play a role in the distribution of the high arctic fauna and flora (*8, 16*).

PETER G. KEVAN

Research Unit on Vector Pathology, Memorial University of Newfoundland, St. John's, Newfoundland, Canada A1C 5S7

References and Notes

1. P. G. Kevan, *Can. Field-Nat.* **86**, 41 (1972).
2. B. Hocking and C. D. Sharplin, *Nature (Lond.)* **206**, 215 (1965).
3. B. Hocking, *Oikos* **19**, 359 (1968).
4. Symbols used in this report are defined as follows:
   F_c, plane of foci for spherical section
   F_p, principal focus of paraboloid
   C, center of sphere
   N, number of observations
   O, origin of paraboloids and spheres
   T_a, ambient air temperature
   T_c, temperature in corolla of whole flower
   T_e, temperature excess above ambient
   T_{ea}, T_e in ambient air
   T_{ec}, T_e in decorollate flower
   T_{en}, T_e in whole, unmutilated flower
   T_{es}, T_e in desporophyllate flower
   $T_{e\alpha}$, T_{en} in wind of speed α (m/sec)
   P, probability determined by t-test
5. P. S. Corbet, *Can. Def. Res. Board Dir. Phys. Res. Oper. Hazen No. 29* (1966).
6. M. K. Gavrilova, *Radiation Climate of the Arctic* (translated from Russian) (National Science Foundation, Washington, D.C., 1966); C. I. Jackson, *Can. Def. Res. Board Dir. Phys. Res. Oper. Hazen Nos. 8 and 9* (1959).
7. J. M. Powell, *Can. Def. Res. Board Dir. Phys. Res. Oper. Hazen No. 14* (1961).
8. P. G. Kevan, *J. Ecol.* **60**, 831 (1972).
9. Calculations are based on the probability of sunshine ($P_s > .5$) (*5, 6*), the probability of heliotropism operating ($P_h = .3$ for *Dryas* and 1.0 for *Papaver*), and the approximation that under sunny conditions the ambient air temperature at flower height is between 10° and 16°C (*7, 10*). From Eq. 3 *Dryas* flowers heat to about 1.5 times the ambient temperature, so that $T_e \sim 0.5\ T_a$. Hence for *Dryas* $P_s \times P_h = .15$ is the probability of temperature excess being generated; multiplying this by $0.5\ T_a$ gives about 8 percent for the growing degree-days above 0°C. For *Papaver*, $P_s \times P_h = .5$, and with $T_e = 0.5\ T_a$, the number of growing degree-days above 0°C is 25 percent.
10. P. S. Corbet, *Can. Def. Res. Board Dir. Phys. Res. Oper. Hazen No. 31* (1967); *Acta Arct. No. 18* (1973).
11. Phototropism and heliotropism in flowers has remained almost unstudied since the time of J. Weisner [*Denkschr. Akad. Wiss. Wien* **39**, 143 (1879); *ibid.* **43**, 1 (1882)] despite the wealth of information on coleoptiles of oats.
12. P. S. B. Digby, *J. Exp. Biol.* **32**, 279 (1955).
13. Birds such as ptarmigan (*Lagopus mutus rupestris*), hoary redpoll (*Acanthis h. hornemanni*), knot (*Calidris c. canutus*), and ruddy turnstone (*Arenaria i. interpres*) have been observed eating flower visitors inside and outside flowers. One insect, *Rhamphomyia filicauda*, may prey on other flower visitors inside the flowers [J. A. Downes, *Can. Entomol.* **102**, 769 (1970)].
14. P. G. Kevan, *Polar Rec.* **16**, 667 (1973); thesis, University of Alberta (1970).
15. ———, *Can. J. Bot.* **50**, 2289 (1972).
16. ——— and J. D. Shorthouse, *Arctic* **23**, 968 (1970).
17. I am grateful to the late B. Hocking, who supported and encouraged my research through the National Research Council of Canada (grant NRC A-2560). I thank the Canada Defence Research Board for financial support and for the use of Hazen Camp. I also thank my colleagues at Hazen Camp in 1966 to 1968 for their help and enthusiasm. A. Wilkinson and P. Wilkinson, at Nuffield Radio Astronomy Laboratories, University of Manchester, Jodrell Bank, England, helped through the productive exchange of ideas.

30 December 1974; revised 7 April 1975

Predicting Response to Endocrine Therapy in Human Breast Cancer: A Hypothesis

Abstract. *We hypothesize that the presence of progesterone receptors in human breast tumors may be a sensitive marker for predicting response to endocrine therapy. Progesterone receptors were found in 56 percent of tumors with estrogen receptors, but were absent in tumors without estrogen receptors. Preliminary clinical correlations show that only those breast tumors with progesterone receptors regressed after endocrine therapy.*

Metastatic human breast cancer has long been treated by surgical ablation of endocrine glands or by pharmacologic hormone therapy. Unfortunately, endocrine responsive tumors constitute only 20 to 40 percent of cases. The success rate can be improved to 55 to 60 percent by selecting for endocrine therapy only those patients whose tumors contain estrogen receptor (ER) (*1*). Our concern now is to identify the 40 percent of ER+ but endocrine resistant tumors, whose growth cannot be inhibited by either ablative or additive endocrine manipulation. To this end we hypothesize that when progesterone receptors (PgR) are present, tumors will be endocrine responsive and that in their absence tumors will be resistant to endocrine manipulation.

Table 1. Comparison of ER and PgR in 50 human breast tumors. More than 3 fmole per milligram of cytosol protein constitutes a positive assay.

	PgR+
ER+	20/36 (56 percent)
ER−	0/14 (0 percent)

Normal target tissues for any hormone contain specific receptors for that hormone, and when malignant transformation occurs, the cell may retain all or part of its normal complement of receptors. The assumption has been implicit that when ER is present in malignant mammary cells, tumor growth is regulated by the hormonal environment and that a change in this environment will cause tumor regression. However, since binding to receptors is only an early step in hormone action, it is possible that in ER+ tumors where endocrine manipulations fail, the lesion is at a later step. An ideal marker of an endocrine responsive tumor would, therefore, be a measurable product of hormone action rather than the initial binding step.

Because in estrogen target tissues the synthesis of PgR depends on the action of estrogen (*2*), we investigated the possibility that PgR might be such a marker. If so, it would be expected that PgR would be rare in tumors which lack ER. The presence of PgR in tumors containing ER would indicate that the tumor is capable of synthesizing at least one end product under estrogen regulation, and that the tumor remains endocrine responsive. Tumors with ER but no PgR would be resistant to endocrine therapy.

PgR has been found in the reproductive tracts of several species (*3*), but its demonstration in rats and humans has been difficult because of progesterone's predilection for binding to glucocorticoid receptors and to corticosteroid binding globulin (*4*). One indirect approach to this problem has been to mask the competing sites with unlabeled hydrocortisone (*5*).

Recently the synthetic progestational compound R5020 has been found to bind specifically to PgR in immature rat and mouse uteri (*6*). With this progestin, the receptor sediments in the 8*S* region of sucrose gradients, where serum contaminants do not interfere.

We have used 8*S* binding of [^{3}H]R5020 to identify PgR in human breast cancer tissue (*7*). Excess unlabeled progesterone or R5020 completely inhibits the binding, whereas hydrocortisone, dexamethasone, or estradiol do not compete effectively (*8*). We have now determined PgR and ER (*9*) in 50 human mammary tumors and the response to endocrine therapy.

Table 2. Comparison of ER, PgR, and tumor response to endocrine therapies in nine patients. Abbreviations: Hx, hypophysectomy; Ov, oophorectomy; A, androgen (fluoxymesterone).

Patient	ER	PgR	Response*	Therapy
1	+	+	+	Hx
2	+	+	+	Ov
3	+	+	+	A
4	+	0	0	Ov
5	+	0	0	Ov
6	0	0	0	Ov
7	0	0	0	Ov
8	0	0	0	Hx
9	0	0	0	Hx

*Objective remission defined as in (*10*).

sults are summarized in Table 1. PgR is absent in all of the 14 tumors lacking ER. Of the 36 ER+ tumors, 56 percent also had PgR. This approximates the percentage expected to respond to endocrine therapies, so that this result is consistent with the hypothesis that PgR is a marker of endocrine responsive tumors.

Confirmation of our hypothesis requires direct correlation of the presence of PgR with objectively defined clinical remission. Only 9 of the 50 patients have been completely evaluated to date, and the results appear in Table 2. Objective remissions occurred only in those patients whose tumors contained PgR. We should emphasize that this very preliminary correlation is not conclusive, but is offered only as initial support of our hypothesis.

In summary, it is already well established that absence of ER in a breast tumor almost always indicates that the tumor will be resistant to endocrine therapy. Yet some malignant cells may retain ER but also be endocrine resistant. If further correlations support our hypothesis, the presence of PgR will show that the tumor remains under at least partial endocrine control and may be classified as endocrine responsive. Patients with such tumors would be likely to benefit from endocrine therapies.

KATHRYN B. HORWITZ
WILLIAM L. MCGUIRE*
Department of Medicine, University of Texas Health Science Center, San Antonio 78284
OLOF H. PEARSON
Department of Medicine, Case Western Reserve University, Cleveland, Ohio 44106
ALBERT SEGALOFF
Alton Ochsner Medical Foundation, 1520 Jefferson Highway, New Orleans, Louisiana 70121

References and Notes

1. W. L. McGuire, O. H. Pearson, A. Segaloff, in *Estrogen Receptors in Human Breast Cancer*, W. L. McGuire, P. P. Carbone, E. P. Vollmer, Eds. (Raven, New York, 1975), p. 17.
2. M. L. Freifeld, P. D. Feil, C. W. Bardin, *Steroids* **23**, 93 (1974).
3. W. W. Leavitt, D. O. Toft, C. A. Strott, B. W. O'Malley, *Endocrinology* **94**, 1041 (1974); E. Milgrom, L. Thi, M. Atger, E.-E. Baulieu, *J. Biol. Chem.* **248**, 6366 (1973); L. E. Faber, H. L. Sandmann, H. E. Stavely, *Endocrinology* **93**, 74 (1973).
4. P. D. Feil, S. R. Glasser, D. O. Toft, B. W. O'Malley, *Endocrinology* **91**, 783 (1972).
5. L. Terenius, *Eur. J. Cancer* **9**, 291 (1973).
6. D. Philibert and J.-P. Raynaud, *Steroids* **22**, 89 (1973).
7. Human mammary carcinoma primary breast or metastatic tissue biopsies were quick frozen in liquid nitrogen, shipped to San Antonio in Dry Ice, and stored at −70°C in a Revco freezer (Revco, West Columbia, S.C.). At assay the tissue was crushed with a Thermovac frozen tissue pulverizer; the powder was weighed, thawed to 4°C, and homogenized in two volumes of buffer with three 10-second bursts of a Polytron PT 10-ST homogenizer set at low speed. The homogenization buffer was $5 \times 10^{-3}M$ sodium phosphate, *p*H 7.4, at 4°C, containing $10^{-3}M$ thioglycerol and 10 percent glycerol [L. E. Faber, H. L. Sandmann, H. E. Stavely, *Fed. Proc.* **22**, 229 (1973)]. The cytosol was harvested by a 50-minute centrifugation at 105,000*g*, 4°C, in a Beckman 75 Ti rotor. Protein concentration was measured by the method of Lowry *et al.* [O. H. Lowry, N. J. Rosebrough, A. L. Farr, R. J. Randall, *J. Biochem.* **193**, 265 1951)]. Five picomoles of [^{3}H]R5020 (^{3}H-labeled 17,21-dimethyl-19-nor-4,9-pregnadiene-3,20-dione-6,7; 51.4 c/mmole) obtained from Roussel-Uclaf were added to 250 μl of cytosol and incubated for 4 hours at 4°C. Parallel samples were incubated for 15 minutes with 100-fold excess of unlabeled R5020. Pellets were prepared from a 1-ml suspension of dextran-coated charcoal (0.25 percent Norit A, 0.0025 percent dextran in 0.01*M* tris-HCl, *p*H 8.0, at 4°C) by a 10-minute centrifugation at 2000*g*. The charged cytosol was transferred onto the pellet, mixed, and incubated for 15 minutes at 4°C to adsorb unbound radioactivity. After recentrifugation for 10 minutes at 2000*g*, a 200-μl portion of the supernatant was layered onto a 5 to 20 percent sucrose gradient prepared in the homogenization buffer. ^{14}C-Labeled bovine serum albumin [R. H. Rice and G. E. Means, *J. Biol. Chem.* **246**, 831 (1971)], 1500 count/min per 10 μl of buffer, was added to each gradient as an internal marker. Gradients were centrifuged in a Beckman SW 56 rotor at 50,000 rev/min (246,000*g*, average) for 17 hours. Four-drop fractions were collected and counted in 5 ml of modified Bray's solution [G. A. Bray, *Anal. Biochem.* **1**, 279 (1960)].
8. K. B. Horwitz and W. L. McGuire, *Steroids* **25**, 497 (1975).
9. W. L. McGuire and M. De La Garza, *J. Clin. Endocrinol. Metab.* **37**, 986 (1973).
10. Objective remissions follow the criteria of the Cooperative Breast Cancer Group [*Cancer Chemother. Rep.* **11**, 130 (1961)]: a 50 percent decrease in size of at least 50 percent of measurable lesions, while other lesions are unchanged and no new lesions appear. In osteolytic metastases, roentgenographic evidence of healing is required, again without increase in size or number of destructive lesions. Osteoblastic metastases as the only measurable lesions are not acceptable criteria. Evaluations were performed without knowledge of receptor assay results.
11. Tumor specimens were obtained from Wilford Hall Air Force Hospital, San Antonio, Texas (Dr. W. Kemmerer and Dr. J. McCulloch); Bexar County Hospital, San Antonio, Texas (Dr. A. Cruz); Methodist Hospital, San Antonio, Texas (Dr. E. Gregory); Alton Ochsner Medical Foundation, New Orleans, La. (Dr. A. Segaloff); Henry Ford Hospital, Detroit, Mich. (Dr. R. Talley); University Hospitals, Cleveland, Ohio (Dr. O. Pearson and Dr. C. Hubay); University Hospitals, Madison, Wis. (Dr. F. Ansfield); Duke University Medical Center, Durham, N.C. (Dr. W. Shingleton and Dr. L. Stocks). We thank Drs. D. Philibert and J.-P. Raynaud for providing the R5020. This work has been aided by PHS grants CA-11378, CA-05197-16, CB-23862, and CB-23859; American Cancer Society grants BC-23D and BC-61P; and the Robert A. Welch Foundation.
* Send reprint requests to W.L.M., Department of Medicine, University of Texas Health Science Center, 7703 Floyd Curl Drive, San Antonio 78284.

29 March 1975

Acetaldehyde Oxidation by Hepatic Mitochondria: Decrease After Chronic Ethanol Consumption

Abstract. *Prolonged consumption of ethanol significantly reduces the capacity of rat liver mitochondria to oxidize acetaldehyde. This is associated with decreased mitochondrial respiration with acetaldehyde as substrate. The reduced ability of mitochondria to metabolize acetaldehyde may explain the high levels of acetaldehyde in the blood of alcoholics, which in turn could promote the perpetuation of liver injury.*

Acetaldehyde, the first product of ethanol oxidation in the liver, has been incriminated in the development of ethanol dependence, on the basis of the findings that acetaldehyde competitively inhibits the oxidation of other aldehydes, resulting in the formation of biologically active alkaloid derivatives (*1*). Acetaldehyde is also considered a possible cause for the cardiotoxic (*2*) and hepatotoxic (*3*) complications of alcoholism. The significance of this mechanism has been enhanced by the observation that alcoholics display significantly higher blood levels of acetaldehyde than nonalcoholics given the same dose of ethanol (*4*), but the cause for this difference is unknown. Since acetaldehyde is metabolized by hepatic mitochondria in a nicotinamide adenine dinucleotide (NAD)–dependent process (*5*), and since chronic ethanol consumption primarily impairs the ability of hepatic mitochondria to reoxidize reduced NAD (NADH) (*3*), we considered the possibility that the mitochondrial damage caused by prolonged intake of ethanol might result in a reduced capacity to metabolize acetaldehyde. It was reported, however, that chronic consumption of ethanol in animals did not change (*6*) or increased (*7*) the activity of hepatic NAD-dependent aldehyde dehydrogenase. Therefore, we compared the effects of chronic ethanol feeding on both the capacity of intact mitochondria to oxidize acetaldehyde and the activity of aldehyde dehydrogenase in disrupted mitochondria.

Female Sprague-Dawley rat littermates were pair-fed nutritionally adequate liquid diets with 36 percent of the total calories as ethanol or isocaloric carbohydrates (*8*). After 4 to 5 weeks, liver mitochondria were obtained as described (*3*), and the capacity of mitochondria to oxidize acetaldehyde was determined by acetaldehyde disappearance as follows. Intact mitochondria (0.5 mg of protein per 25-ml flask) were incubated with 60 μM acetaldehyde in a buffer consisting of 0.3*M* mannitol, 75 m*M* sucrose, 10 m*M* magnesium chloride, 10 m*M* potassium phosphate (*p*H 7.4), 0.5 m*M* ethylenediaminetetraacetic acid (EDTA), and 10 m*M* potassium chloride, in a final incubation volume of 0.5 ml. After acetaldehyde was added to the system, the flask was closed and incubated with shaking (120 strokes per minute) for 0, 3, or 6 minutes at 37°C. The reaction was stopped by the addition of 0.1 ml of perchloric acid in a final concentration of