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.

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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.
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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

0.3*M*. Acetaldehyde was measured by gas-liquid chromatography (Perkin-Elmer F-40) (*4*). Mitochondrial respiration was assayed polarographically by determining oxygen consumption with a Clark oxygen electrode. The reaction was initiated at 30°C by the addition of acetaldehyde in a final concentration of 60 μM and 10 μl of 0.1*M* adenine 5′-diphosphate to a reaction mixture (2.5 ml) containing intact mitochondria (equivalent to 3 mg of protein) and the buffer described above. Mitochondrial NAD-dependent aldehyde dehydrogenase was assayed in a system containing an excess of NAD (0.5 m*M*) and sodium deoxycholate for disruption of mitochondrial membranes (*9*). Mitochondrial glutamate dehydrogenase was also measured (*9*). Each measurement was carried out at least in duplicate. The results were compared to the corresponding values obtained in the pair-fed control littermates, the means (± S.E.M.) and individual differences were calculated, and their significances were assessed by the paired Student's *t*-test.

Chronic ethanol consumption resulted in a significant reduction of the rate of acetaldehyde metabolism by intact rat liver mitochondria (Table 1). This was associated with decreased mitochondrial respiration with acetaldehyde as substrate (11.7 ± 1.2 nanoatoms of oxygen consumed per minute per milligram of mitochondrial protein in controls as compared to 8.1 ± 0.5 in ethanol-treated animals; six pairs; $P < .02$). The observed reduction of acetaldehyde metabolism can be ascribed, at least in part, to the decreased ability of NADH reoxidation in mitochondria of ethanol-fed animals, since prolonged intake of ethanol causes an impairment of the energy coupling site 1 of the mitochondrial respiratory chain (*3*), the level of NAD-linked dehydrogenases. Indeed, the addition of fatty acids and other substrates for NAD-linked dehydrogenases in mitochondrial respiration did decrease the rate of acetaldehyde oxidation (Table 1), probably by competing with the substrates for NAD. However, even in the presence of those substrates, acetaldehyde was again less metabolized in the mitochondria of ethanol-treated rats than in the controls (Table 1). By contrast, in disrupted mitochondria supplied with NAD, the activity of NAD-dependent aldehyde dehydrogenase (expressed as nanomoles of NADH produced per minute per milligram of mitochondrial protein) was found to be higher in ethanol-fed rats (37.1 ± 1.7) than in the controls (32.4 ± 1.6) (eight pairs; $P < .02$). Conversely, the activity of glutamate dehydrogenase remained unchanged (1.63 ± 0.07 μmole of NADH oxidized per minute per milligram of mitochondrial protein in the controls as compared to 1.76 ± 0.07 μmole in the ethanol-treated animals; 11 pairs). The discrepancy between the rate of acetaldehyde oxidation in intact mitochondria and the enzyme activity in disrupted organelles suggests that the rate-limiting step of acetaldehyde metabolism is the ability of mitochondria to reoxidize NADH rather than the activity of aldehyde dehydrogenase. The recent observation that 2,4-dinitrophenol, an uncoupler of oxidative phosphorylation in mitochondria, accelerated acetaldehyde metabolism in the perfused rat liver (*10*) supports the proposed mechanism.

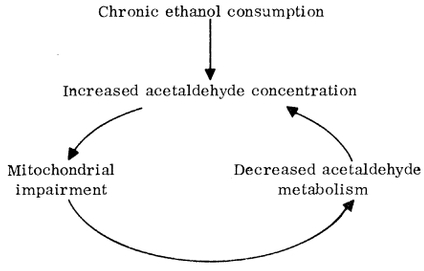

Fig. 1. Possible relation between ethanol consumption, altered acetaldehyde levels, and mitochondrial impairment.

The reduction of acetaldehyde metabolism observed in rats fed ethanol continuously over a long period might result in the accumulation of acetaldehyde in the liver as well as in the blood if the production rate of acetaldehyde is unchanged or increased. Indeed, human studies revealed that the acetaldehyde concentration in the blood after the same dose of ethanol was significantly higher in alcoholics than in nonalcoholics, although there was no difference in ethanol disappearance rates between these two groups (*4*). Since acetaldehyde itself has an inhibitory effect on mitochondrial respiration, especially in the segment of the electron transport chain prior to NADH-ubiquinone oxidoreductase (*11*), it is possible that the enhanced acetaldehyde observed in the blood of alcoholics may contribute to the mitochondrial damage commonly found after chronic ethanol consumption. Indeed, it was reported that there are similarities between the effects of prolonged ethanol intake on liver mitochondria and the effects of acetaldehyde (*3*).

These results suggest the existence of a "vicious cycle" shown in Fig. 1: Elevated blood acetaldehyde resulting from enhanced ethanol metabolism (*12*) or decreased disposition, as shown in Table 1, may impair mitochondrial functions including the capacity of mitochondria to oxidize acetaldehyde. This in turn will elevate the acetaldehyde concentration in the blood even further, which may then perpetuate injury not only in the liver, but possibly also in the heart and brain.

Table 1. Effect of chronic ethanol feeding on acetaldehyde oxidation. Isolated liver mitochondria obtained from 11 pairs of rats fed ethanol (36 percent of total calories) or isocaloric carbohydrate were incubated with various substrates. The rate of acetaldehyde disappearance was measured by gas-liquid chromatography and expressed as nanomoles of acetaldehyde oxidized per minute per milligram of protein (± S.E.M.).

Substrate	Control	Ethanol	*P*
Acetaldehyde (60 μM)	14.6 ± 0.7	11.8 ± 0.8	< .02
Plus α-ketoglutarate (10 m*M*)	5.5 ± 0.9	4.2 ± 0.8	< .01
Plus β-hydroxybutyrate (10 m*M*)	10.2 ± 1.3	7.1 ± 0.8	< .02
Plus palmytoyl coenzyme A (15 μM) plus carnitine (3 m*M*)	8.8 ± 0.5	7.6 ± 0.4	< .01

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References and Notes

1. V. E. Davis, M. J. Walsh, Y. Yamanaka, *J. Pharmacol. Exp. Ther.* **174**, 401 (1970).
2. S. S. Schreiber, M. Oratz, M. A. Rothschild, F. Reff, C. Evans, *J. Mol. Cell Cardiol.* **6**, 207 (1974).
3. A. I. Cederbaum, C. S. Lieber, E. Rubin, *Arch. Biochem. Biophys.* **165**, 560 (1974).
4. M. A. Korsten, S. Matsuzaki, L. Feinman, C. S. Lieber, *N. Engl. J. Med.* **292**, 386 (1975).
5. L. Marjanen, *Biochem. J.* **127**, 633 (1972); N. Grunnet, *Eur. J. Biochem.* **35**, 236 (1973); R. Parrilla, K. Ohkawa, K. O. Lindros, U. P. Zimmerman, K. Kobayashi, J. R. Williamson, *J. Biol. Chem.* **249**, 15 (1974).
6. G. Redmond and G. Cohen, *Science* **171**, 387 (1971); N. H. Raskin and L. Sokoloff, *Nature (Lond.)* **236**, 138 (1972).
7. R. M. Dajani, J. Danielski, J. M. Orten, *J. Nutr.* **80**, 196 (1963); A. A. Horton, *Biochim. Biophys. Acta* **253**, 514 (1971).
8. The technique of pair-feeding described by L. M. DeCarli and C. S. Lieber [*J. Nutr.* **91**, 331 (1967)] was used. The composition of the ethanol and control diets was as follows: casein (supplemented with methionine, 0.3 mg/kcal, and cystine, 0.5 mg/kcal) represented 18 percent of the total calories; fat, 35 percent of total calories; adequate vitamins and minerals; and, in the control diet, carbohydrate, 47 percent of the total calories. In the ethanol formula, carbohydrate was isocalorically replaced to the extent of 36 percent of the total calories. These diets were fed to 11 pairs of rats with body weights at the start of the experiment of 145 ± 4 g (controls) and 149 ± 3 g (alcohol fed). The daily food intake (expressed as calories per kilogram of body weight) was the same in both groups (299 ± 7 in controls and 299 ± 9 in ethanol-treated animals). The average daily weight gain was 2.9 ± 0.2 g in the controls and 2.6 ± 0.2 g in the ethanol-fed animals.
9. The methods of S. O. C. Tottmar, H. Pettersson, and K.-H. Kiessling [*Biochem. J.* **135**, 577 (1973)]

were used for assays of total enzyme activity. To solubilize NAD-dependent aldehyde dehydrogenase from mitochondria, sonication, freezing and thawing, and treatment with detergent (sodium deoxycholate) were found to be equally effective. Sodium deoxycholate was chosen by us because it gave clear solutions for spectrophotometric assay.
10. C. J. P. Eriksson, K. O. Lindros, O. A. Forsander, *Biochem. Pharmacol.* **23**, 2193 (1974).
11. A. I. Cederbaum, C. S. Lieber, E. Rubin, *Arch. Biochem. Biophys.* **161**, 26 (1974).
12. P. S. Misra, A. Lefevre, H. Ishii, E. Rubin, C. S. Lieber, *Am. J. Med.* **51**, 346 (1971).
13. We thank G. Marcus for technical assistance. Supported in part by PHS grants AA 00224 and AM 12511 and by the Veterans Administration (project No. 5251-02).

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Phosphorus-31 as a Nuclear Probe for Malignant Tumors

Abstract. *Phosphorus-31 may prove useful as an additional nucleus for detecting malignancy by nuclear magnetic resonance. The spin-lattice relaxation times for phosphorus-31 determined by a saturation technique employing a 90°-τ-90°-τ-90° . . . pulse sequence were significantly higher for two rat malignancies, Novikoff hepatoma and Walker sarcoma, and the Crocker sarcoma of mice than for normal liver, muscle, brain, kidney, and intestine tissues. No individual measurement of malignant tissues overlapped any of the measurements of normal tissues, and the probabilities of insignificance ranged from .029 for Crocker sarcoma to .000184 for Novikoff hepatoma. The phosphorus-31 nucleus, because of its strategic placement in the nucleic acid molecule, may be useful as a new probe for exploring the mechanism of carcinogenesis. The results call attention to another nucleus that may prove valuable for nuclear magnetic resonance equipment aimed at the detection of internal malignancies in humans.*

Damadian and co-workers (*1*) suggested that nuclear magnetic resonance (NMR) might prove useful in the detection of internal cancers. Some experiments with animals provided evidence that it might be possible to make a noninvasive discrimination between normal and malignant tissue by this method. These authors also suggested that NMR might be used to diagnose and even to measure the extent of malignancy in surgical biopsy specimens. The initial observation relied mainly on the altered water signal of malignant tissue, and the technique has since been confirmed and refined at many laboratories, where new insights have been contributed (*2, 3*).

It seemed that both objectives, detection of internal tumors and tissue diagnosis, could best be served by the addition of other magnetic nuclei for diagnosing malignancy. Damadian and Cope (*4*) studied ^{39}K resonance in normal and malignant tissues, but did not find any significant differences in spin-lattice relaxation time, T_1. However, they did observe an oscillation in the T_1 plot for malignant tissue and for normal intestinal tissue; such oscillations had not previously been seen with biological tissue. In this report we discuss an investigation of ^{31}P as another possible nuclear probe of malignancy.

Because of its strategic placement in the nucleic acid molecule, ^{31}P seemed a particularly useful nucleus with which to probe malignancy. Boyd (*5*), in his *Textbook of Pathology*, has written that "the chief characteristics of the neoplastic cell as revealed by the light microscope are nuclear and chromosomal aberrations." Tumor cells characteristically have polyploid nuclei and elevated mitotic rates, and these nuclear changes permit the microscopic diagnosis of malignancy. We considered that the DNA changes implicit in these chromosomal aberrations might be reflected in the ^{31}P relaxations of the malignant cell.

Phosphorus-31 resonance has been used in determining the structure of relatively pure compounds that can be isolated in significant quantity (*6*). For NMR studies of biological molecules it has the advantages of large chemical shifts and narrow linewidths. The reduced NMR sensitivity of phosphorus compared to hydrogen and the poor solubility of many organic phosphorus compounds has limited its use. The use of ^{31}P in the NMR analysis of a biological tissue was reported by Moon and Richards (*7*) for red blood cells, where it was introduced as a new technique for monitoring intracellular *p*H. Kornberg and McConnell (*8*) and Berden *et al.* (*9*) used ^{31}P NMR to study phospholipid structure in membrane vesicles. To the best of our knowledge, it has not been applied to organ tissues where its placement in the nucleic acid molecule could prove useful.

Sprague-Dawley rats were used as the source of all normal and malignant tissue, with the exception of the Crocker sarcoma 180 of mice. Rats were killed by cervical dislocation; the desired tissue was then quickly dissected out of the animal, blotted, and placed in a test tube on ice. After 15 minutes at ice temperature, the chilled tissue was cut into small pieces and packed into NMR tubes (outer diameter, 5 mm), which were stored in crushed ice.

The NMR probe was maintained at a temperature of 7° ± 1°C to minimize tissue degeneration during the analysis. This was accomplished by circulating a stream of cold nitrogen gas through the probe at a rate of 12 cubic feet per hour (0.34 m^3/hour). The temperature of the probe was monitored continually by two thermistors, one over the probe body and one at the nitrogen outflow port of the probe. Temperatures of both thermistors were maintained within 0.5°C of each other.

All relaxation measurements were made with a Nuclear Magnetic Resonance Specialties Corporation PS-60 AW pulse spectrometer operating at 100 Mhz, a high Q probe at this frequency (SEIMCO, New Kensington, Pa.), and a Westinghouse superconducting magnet operating at 58,000 gauss. Because of the low concentrations of phosphorus in tissue samples, it was necessary to make use of a computer of average transients (Fabri-Tek Instruments, model 1072) for signal enhancement.

Spin-lattice relaxation times (T_1) were measured by the method of progressive saturation (*10*), in which the incident radiation consists of a train of 90° pulses at the resonant frequency. The pulses have the same amplitude and duration and are adjusted so that the height of the free induction decay (FID) obtained is maximal and corresponds to a rotation of the net magnetization from the Z-axis into the X-Y plane. Once the magnetization has been rotated, it tends to return to its equilibrium along the Z-axis at a rate proportional to T_1, in general taking approximately $5T_1$ to return to equilibrium. A second pulse at this time will produce the same height of the FID. If the time between pulses, τ, is less than T_1, the height of the FID, h, will be less than maximal and will diminish to a constant value in the course of the saturation train in accordance with the relation

$$h = h_0(1 - e^{-\tau/T_1})$$

where h_0 is the height of the FID for $\tau \geq 5T_1$. Therefore, when $\ln[(h_0 - h)/h_0]$ is plotted against τ, a straight line with a slope of $-1/T_1$ is obtained.

This method is more efficient for measuring T_1, particularly for samples where many repetitions of the experiment are required before a usable signal is obtained. In the more conventional methods using pulse sequences of 180°-τ-90° or 90°-τ-90°, it is necessary to wait for a time longer than $5T_1$ (10 to 25 seconds for tissue phosphorus) before a second pulse can be given. In addition, prior knowledge of T_1 is not required in the progressive saturation method, since τ is simply increased until h reaches a maximum, thereby specifying h_0.

We chose values of τ in the range of $0.1T_1$ to T_1—that is, 0.26 to 4.2 seconds for normal tissues and 0.524 to 8.4 seconds for malignant tissues. From 2^9 to 2^{12} repetitions were required in the signal averager