and 1.37 for authentic guanine. When the enzyme guanase was added to the sample, the guanine peak and shoulder disappeared, and a single peak at 277 nm, characteristic of xanthine, was observed.

Additional proof that the compound extracted from the femoral head was guanine was obtained by comparing the fluorescence spectrum of the sample with that of authentic guanine. From 5 g of femoral head 100 μg of the guanine material was obtained. Similar sections that did not contain crystals were also extracted from the same animal, but no guanine was obtained. From a local meat-packing plant we obtained bones from healthy pigs of comparable age and processed the bones in an identical manner; no accretions of guanine were found. These data and observations are consistent with the hypothesis that Pb^{2+} inhibited the enzyme guanase and caused an increase of guanine concentration in vivo that resulted in the crystallization of this insoluble purine in the joints.

Finally, accretions of guanine have been described by Mendelson in swine tissue as a very rare occurrence. Investigators in the veterinary sciences who have attempted to repeat Mendelson's observation by examining large amounts of normal material obtained at slaughterhouses have been unsuccessful (*7*). It is possible that the meat products from animals in which guanine crystals have been observed were from animals suffering from lead poisoning, possibly due to ingestion of lead-based paint.

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References and Notes

1. A. B. Garrod, *Med. Chic. Tr.* **37**, 181 (1859); A. Magnus Levy, *Z. Klin. Med.* **36**, 353 (1898).
2. G. Lormier, *Br. Med. J.* **2**, 163 (1898).
3 .B. T. Emmerson, *Arthritis Rheum.* **11**, 623 (1968).
4. G. D. Ludwig, *Arch. Intern. Med.* **100**, 803 (1957); A. B. Gutman, *Ann. Intern. Med.* **39**, 1062 (1953).
5. G. V. Ball and L. B. Sorensen, *N. Engl. J. Med.* **280**, 1199 (1969).
6. W. Jones and C. R. Austrian, *Z. Physiol. Chem.* **48**, 110 (1906).
7. W. Mendelson, *Am. J. Med. Sci.* **95**, 109 (1888).
8. G. Gomori, in *Histochemistry Principles and Practice* (Univ. of Chicago Press, Chicago, 1972), vol. 40, pp. 120–136 and 162–165.
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Human Breast Cancer: Androgen Action Mediated by Estrogen Receptor

Abstract. *Growth of the human breast cancer cell line MCF-7 is enhanced by androgens, but only at pharmacological concentrations. Although physiological concentrations of androgens translocate the androgen receptor into the nucleus, no mitogenic effects are observed. By contrast, pharmacological androgens translocate not only the androgen receptor but also the estrogen receptor, and at these high doses significantly increase both DNA and estrogen-dependent protein synthesis. We therefore propose that androgens stimulate MCF-7 cell growth not through the androgen receptor but rather through the estrogen receptor.*

Lippman *et al.* (*1*) recently reported that the growth of MCF-7 human breast cancer cells is stimulated by androgens and concluded that this response is mediated by an androgen receptor. Since androgens are normally used to induce tumor regression in breast cancer patients, it is important to determine why the opposite effect is observed in human breast cancer cells in culture.

The requirement for very high pharmacological doses of androgen to stimulate MCF-7 cell growth provided a clue that some mechanism other than an androgen receptor might be responsible. It had been reported (*2*) that in the rat uterus pharmacological concentrations of androgens interact not only with androgen receptors but also with estrogen receptors, resulting in translocation of estrogen receptors into the nucleus. If pharmacological concentrations of androgens similarly affect the estrogen receptors in MCF-7 tumor cells this would provide a mechanism for the observed growth stimulation, since physiological doses of estrogen stimulate MCF-7 cell growth (*3*, *4*). We therefore in-

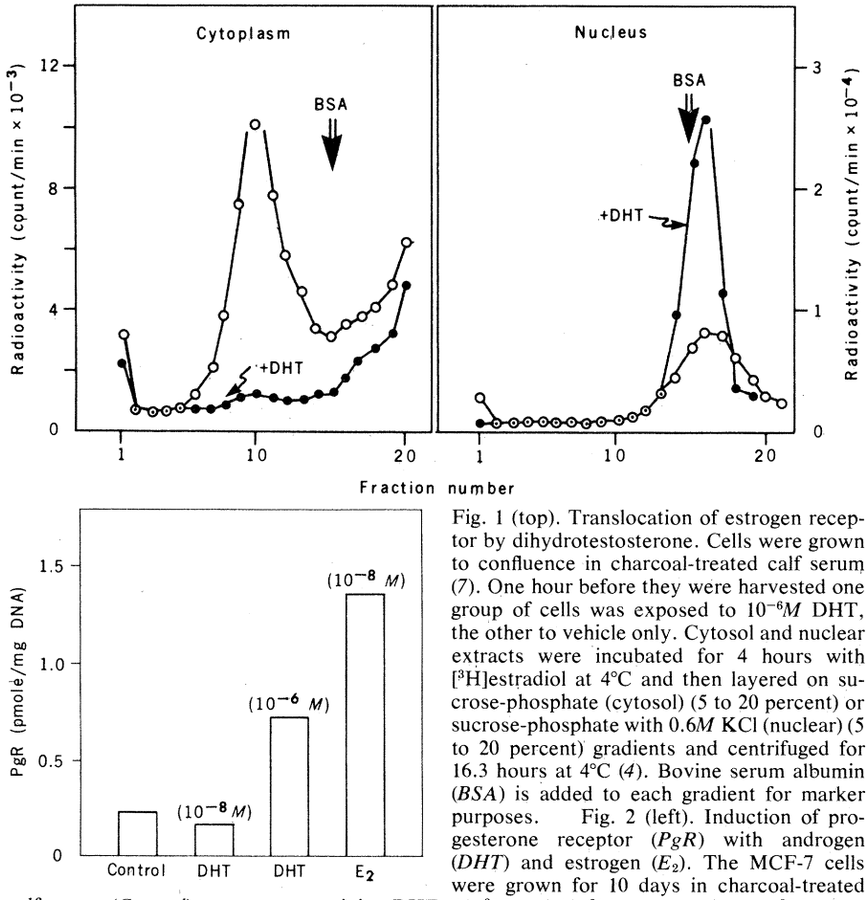

Fig. 1 (top). Translocation of estrogen receptor by dihydrotestosterone. Cells were grown to confluence in charcoal-treated calf serum (*7*). One hour before they were harvested one group of cells was exposed to $10^{-6}M$ DHT, the other to vehicle only. Cytosol and nuclear extracts were incubated for 4 hours with [^{3}H]estradiol at 4°C and then layered on sucrose-phosphate (cytosol) (5 to 20 percent) or sucrose-phosphate with 0.6*M* KCl (nuclear) (5 to 20 percent) gradients and centrifuged for 16.3 hours at 4°C (*4*). Bovine serum albumin (*BSA*) is added to each gradient for marker purposes. Fig. 2 (left). Induction of progesterone receptor (*PgR*) with androgen (*DHT*) and estrogen (E_2). The MCF-7 cells were grown for 10 days in charcoal-treated calf serum (*Control*) or serum containing DHT ($10^{-8}M$ and $10^{-6}M$) or estradiol ($10^{-8}M$). Progesterone receptor was measured by means of [^{3}H]R5020 (a synthetic steroid) and both the protamine sulfate and dextran charcoal methods previously described (*7*, *8*). Briefly, cytosol was incubated for 4 hours with [^{3}H]R5020 at 4°C and then treated either with protamine sulfate or dextran charcoal. No quantitative differences were noted between the two assays. Values represent the mean of duplicate determinations for the dextran charcoal assay.

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cubated intact MCF-7 cells with physiological ($10^{-8}M$) and pharmacological ($10^{-6}M$) concentrations of dihydrotestosterone (DHT) and then examined the effects on receptors for estrogen, androgen, progesterone, and glucocorticoids. With $10^{-8}M$ concentrations of DHT, cytoplasmic androgen receptor was translocated to the nucleus while the other receptors remained unaltered. The higher dose of DHT, however, depleted not only the androgen receptor but also a significant portion of the estrogen receptor; neither progesterone nor glucocorticoid receptor levels were affected. Figure 1 shows that after the cytoplasmic depletion of estrogen receptor with $10^{-6}M$ DHT a near equal number of estrogen receptors entered the nucleus.

The cytoplasmic depletion and translocation of the MCF-7 estrogen receptor was not limited to DHT; other androgens such as testosterone and androstanediol were equally effective, whereas high concentrations or progesterone or hydrocortisone did not bind this receptor. Thus, androgens appear to have a specific low affinity for the estrogen receptor.

If the DHT-translocated nuclear estrogen receptor is functional, then specific products of estrogen action should be induced. Progesterone receptor is such a product of estrogen action. Low and high concentrations of DHT were therefore compared for their ability to induce progesterone receptor. When intact cells were exposed to $10^{-8}M$ DHT, progesterone receptor levels remained unaltered, whereas in cells exposed to $10^{-6}M$ DHT progesterone receptors were significantly stimulated (Fig. 2). Thus the androgen-translocated nuclear estrogen receptor must be active at specific gene acceptor sites, inducing products normally considered to be restricted to the action of estrogen.

For these data to be clinically relevant, it must be shown that pharmacological concentrations of androgens are capable of stimulating breast tumor growth in vivo under certain conditions. Heise and Gorlich (*5*) studied the effect of various doses of androgen on breast tumor regression in rats treated with dimethylbenzanthracene. They found that very high concentrations of androgen were much less effective in causing tumor regression than lower concentrations of androgen; in some instances, the very high concentrations of androgen even appeared to stimulate tumor growth. Similar results were reported by Segaloff (*6*).

Although our data provide evidence that $10^{-6}M$ DHT stimulates MCF-7 cell growth by acting through the estrogen receptor, and that this may also account for the breast tumor stimulation in vivo in the rat, they do not provide direct evidence that these events occur in a clinical setting in human breast cancer. Nevertheless, the possibility remains that the failure of androgen to induce tumor regression in certain breast cancer patients may be related to the paradoxical estrogenic effects of pharmacological concentrations of androgens.

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References and Notes

1. M. Lippman, G. Bolan, K. Huff, *Cancer Res.* **36**, 4610 (1976).
2. H. Rochefort and M. Garcia, *Steroids* **28**, 549 (1976).
3. M. Lippman, G. Bolan, K. Huff, *Cancer Res.* **36**, 4595 (1976).
4. D. T. Zava, G. C. Chamness, K. B. Horwitz, W. L. McGuire, *Science* **196**, 663 (1977).
5. E. Heise and M. Gorlich, *Br. J. Cancer* **20**, 539 (1966).
6. A. Segaloff, *Recent Prog. Horm. Res.* **22**, 351 (1966).
7. D. T. Zava and W. L. McGuire, *J. Biol. Chem.* **252**, 3703 (1977).
8. K. B. Horwitz, M. E. Costlow, W. L. McGuire, *Steroids* **26**, 785 (1975).
9. This work was supported by the American Cancer Society. D.T.Z. is an NCI postdoctoral fellow (CA 05357). We thank J. P. Raynaud of Roussel Uclaf for providing the [^{3}H]R5020.
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Amantadine: Neuromuscular Blockade by Suppression of Ionic Conductance of the Acetylcholine Receptor

Abstract. *Amantadine hydrochloride decreases the sensitivity of denervated mammalian muscle to iontophoretically applied acetylcholine. The drug depresses the amplitude of the end-plate current and reverses the slope of the relation between half-decay time and membrane potential suggesting that it alters the ionic conductance that is mediated by the acetylcholine receptor. Binding studies confirm that amantadine acts on the ion conductance modulator rather than the acetylcholine receptor.*

At neuromuscular junctions, the acetylcholine (ACh) receptor serves as the recognition site for ACh released from motoneurons. Binding of ACh to its receptor induces permeability changes in the postjunctional membrane through activation of the ion conductance modulator (ICM). Our recent studies suggest that the ACh receptor and ICM are separate membrane proteins that are coupled in the membrane (*1*). This ICM may consist of a channel and structures that link the channel to the receptor. The ionic conductances that are mediated at neuromuscular junctions by the ACh receptor may be blocked by drugs or toxins that act directly on the receptor (for example, the receptor antagonists *d*-tubocurarine and α-bungarotoxin) or on the ICM (for example, local anesthetics and histrionicotoxin) (*1*-*3*).

Amantadine hydrochloride (1-adamantanamine hydrochloride, Symmetrel) is an antiviral drug known for its prophylactic effect against A_2 (Asian) influenza in animals and man (*4*). Its antiviral activity is suggested to result from the ability of the drug to prevent viral penetration into the host cell (*5*). This drug is also effective in the treatment of human Parkinsonism (*6*). Though it is considered likely that amantadine exerts its anti-Parkinson effect through an action similar to that of L-dopa (*6*), the drug has recently been shown to block neuromuscular transmission by reducing the response of muscle postjunctional membrane to ACh (*7*). Since such an effect may result from actions on the ACh receptor or its ICM, we utilized biochemical and electrophysiological techniques to distinguish between these possibilities and to elucidate the molecular target of amantadine.

For the biochemical study, membranes were prepared from a tissue extremely rich in nicotinic neuromuscular-type ACh receptors and ICM's, namely, the electric organ of the electric ray, *Torpedo ocellata* (*8*). The ACh receptor was identified by its binding of [^{3}H]ACh (49.5 Ci/mole, New England Nuclear) after inhibition of all the acetylcholinesterase present with $10^{-4}M$ diisopropylfluorophosphate. The ICM was identified by its binding of [^{3}H]perhydrohistrionicotoxin ([^{3}H]H_{12}-HTX; 4800 Ci/mole). The effect of this radioactively labeled toxin on end-plate current (EPC) was checked, and its binding (dissociation constant, $K_d = 4 \times 10^{-7}M$) was saturable, specific, and blocked only by drugs and toxins shown to affect the ionic conductance of the end plate (*1*). A membrane preparation was made from the electric organ (600 pmole of receptor sites per milligram of protein), and binding was studied by equilibrium dialysis at 22°C for 4 hours as previously described (*1*).

Amantadine significantly reduced the

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