Advances in

ENZYME REGULATION

Volume 11

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Proceedings of the eleventh symposium on Regulation of Enzyme Activity and Synthesis in Normal and Neoplastic Tissues held at Indiana University School of Medicine Indianapolis, Indiana October, 1972

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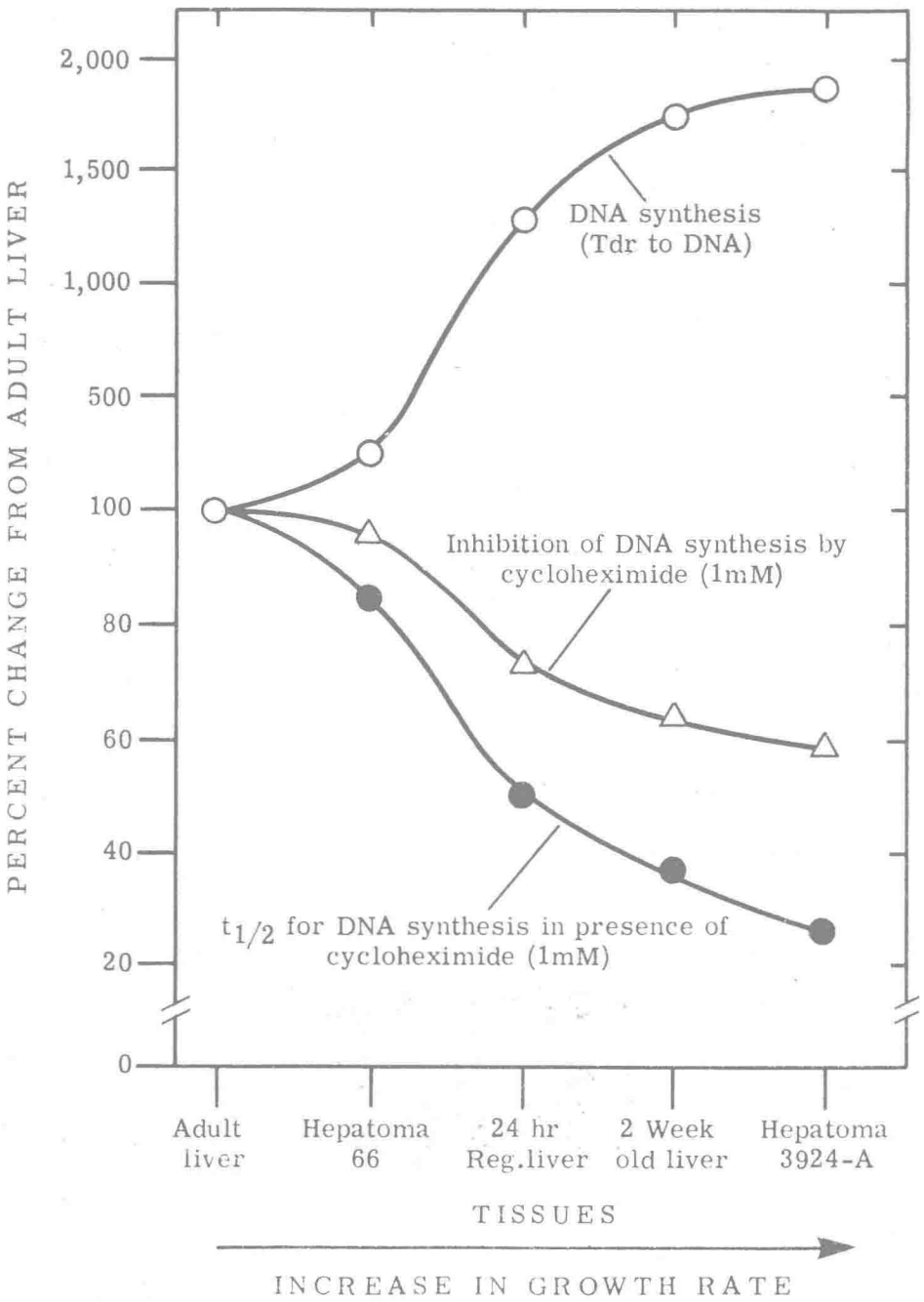
PERCENT CHANGE FROM ADULT LIVER
2,000
1,500
1,000
500
100
80
60
40
20
0
DNA synthesis
(Tdr to DNA)
Inhibition of DNA synthesis by
cycloheximide (1mM)
t1/2 for DNA synthesis in presence of
cycloheximide (1mM)
Adult
liver
Hepatoma
66
24 hr
Reg.liver
2 Week
old liver
Hepatoma
3924-A
TISSUES
INCREASE IN GROWTH RATE

FOREWORD

Advances in Enzyme Regulation is now in its eleventh volume. The appreciative reception of this series reflected the need for such a source of information, inspiration, and laboratory and teaching companion.

Volume 11 concentrates on subjects which have reached the stage of productive summarization and critical evaluation in the light of extensive new results. This book also lives up to its goal of advancing a few steps ahead of the general front of mammalian enzyme regulation studies.

It has been my editorial policy to impose as few restrictions as possible, emphasizing, however, the objectives of excellence of contribution, perfection in presentation, and penetration and scope in interpretation. This principle gives a wide range of freedom to the participants to express their concepts. Thus, the responsibility for detail—accuracy of reporting, preciseness of references, allocations of priority, expressions of judgement and evaluation—lies with the individual authors.

The Editor, who enjoyed the advice of leaders in the field, has been organizing the Symposia and selecting new topics and speakers on the basis of immediate and long-range significance of the scientific contributions. It is hoped that the comments and suggestions of investigators and teachers in this field will continue to come to the Editor's office and contribute to shaping the course of forthcoming conferences and volumes.

Indiana University 1972 GEORGE WEBER, *Editor*

ACKNOWLEDGEMENTS

THIS is the eleventh in a series of Symposia dedicated entirely to problems and advances in regulation of enzyme activity and synthesis in mammalian systems.

I take great pleasure in expressing appreciation for the support and assistance I received in organizing and conducting this Conference. I wish gratefully to acknowledge that Indiana University School of Medicine, Burroughs Wellcome and Co., Squibb Institute for Medical Research and Eli Lilly and Co. provided the financial support for this meeting.

In the planning of the program, selection of participants and arrangements for the Symposium the advice of the following was invaluable: O. Barnabei, H. Holzer, N. Katunuma, H. A. Krebs, H. P. Morris, C. A. Nichol, V. R. Potter, C. G. Smith, S. Weinhouse and H. G. Williams-Ashman.

I am obligated to Drs. Fuller, Jensen, Krahl, Krebs, Nichol, Pitot, Potter, Smith, Weinhouse and Williams-Ashman for serving as chairmen of the sessions, and to all contributing authors for their cooperation in the preparation of this volume.

At Indiana University School of Medicine in the local organization of the Symposium I had the kind assistance of Dean Glenn W. Irwin, Jr. The efficient and competent help of David M. Paul and Mrs. Reba Nix in accommodation arrangements and the expert assistance of James Glore in preparation of illustrations are very much appreciated.

Thanks are due to members of my secretarial staff who assisted in the local arrangements and in the typing of manuscripts.

My highest appreciation is due to my wife, Catherine E. Forrest Weber, whose contribution to the format and English style has been most valuable in the assembling of this volume.

GEORGE WEBER
Symposium Chairman

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SESSION I

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Session Chairman: H. G. WILLIAMS-ASHMAN

TRANSFORMED ESTROGEN RECEPTOR IN THE REGULATION OF RNA SYNTHESIS IN UTERINE NUCLEI

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INTRODUCTION

STUDIES from many laboratories (1) have demonstrated that the characteristic affinity of estrogen-responsive tissues for the hormone, both *in vivo* and *in vitro*, results from their content of specific estrogen-binding proteins called estrogen receptors or estrophiles. The interaction of estradiol with target tissues, such as uterus, takes place by a sequential mechanism in which the hormone first combines with an extranuclear receptor protein to form a complex which, by a temperature-dependent process, is translocated to the nucleus where most of the incorporated steroid becomes localized (Fig. 1).

During the course of the foregoing interaction, the receptor protein undergoes a change that can be recognized by physical as well as biochemical criteria. The first indication of this phenomenon came from observations of differences between the nuclear and the cytosol estradiol–receptor complexes on sucrose density gradient ultracentrifugation, an informative technique introduced by Toft and Gorski (2) to characterize the cytosol complex. In low salt sucrose gradients the cytosol complex sediments at about 8S, but, as first reported by Erdos (3) and by Korenman and Rao (4), the presence of 0.3 M to 0.4 M KCl in the gradient effects dissociation of the receptor to more slowly sedimenting units, subsequently shown (5) to be about 4S. It was found (6, 7) that the estradiol bound in the nucleus can be solubilized as a 5S steroid–receptor complex by extracting the nuclei with 0.3 M or, preferably, 0.4 M KCl and that, contrary to some reports, this product is clearly distinguishable (5, 8) from the 4S cytosol complex by ultracentrifugation in salt-containing gradients. After the nuclear complex was shown to originate from the cytosol complex (5, 9–11) it became evident that, during its translocation to the nucleus, the binding unit of the receptor protein undergoes some kind of change, a phenomenon we have called "receptor transformation".

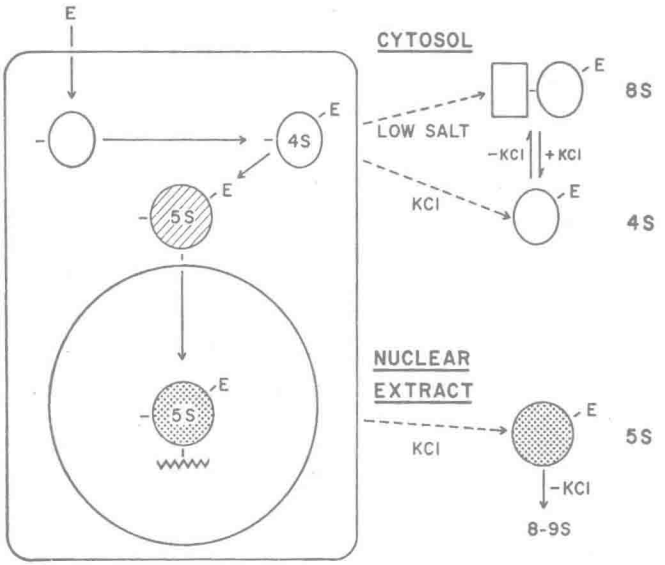

FIG. 1. Schematic representation of interaction pathway of estradiol (E) in uterine cells.

A number of observations (12–15) indicate that the conversion of the receptor binding unit from the native (4S) to the nuclear (5S) form is a key step in the biological action of estrogens and the induction of this process may well be the principal function of the hormone. This paper describes two procedures by which the estradiol–receptor complex can be converted to a transformed state in the absence of nuclei, presents two criteria, in addition to increased sedimentation rate, by which the transformed complex can be distinguished from the native one, and summarizes the evidence for the importance of receptor transformation in estrogen action.

RECEPTOR TRANSFORMATION

After it has been found (9, 16, 17) that incubation of rat or calf uterine nuclei with uterine cytosol containing estradiol gives rise to an extractable 5S hormone–receptor complex, indistinguishable from that formed in whole tissue, it was observed (12, 18) that conversion of the receptor to the 5S form does not require the presence of nuclei. Warming uterine cytosol in the presence but not the absence of estradiol yields a hormone–receptor complex that sediments in high salt sucrose gradients at approximately the same rate as the nuclear complex (Fig. 2). Receptor transformation in cytosol shows the same temperature dependence and pH characteristics as those associated with nuclear incubations. The reaction, which takes place only slowly in the

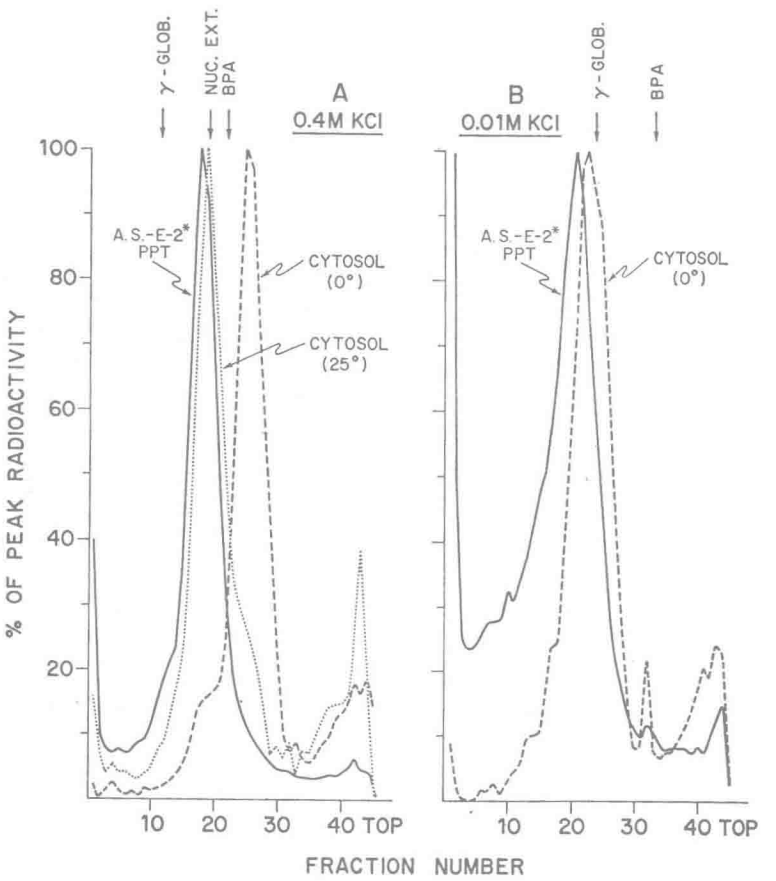

FIG. 2. Sedimentation patterns of radioactive estradiol–receptor complexes in high salt (A: 5 to 20% sucrose, 400 mM KCl) and low salt (B: 10 to 30% sucrose, 10 mM KCl) gradients containing 1 mM EDTA and 10 mM Tris, pH 7.4. Calf uterine cytosol containing 3.8 nM estradiol-6, 7-^{3}H(E-2*, spec. act. 46.6 Ci/mmol) and redissolved ammonium sulfate (25% saturation) precipitate of estradiol–receptor complex (A.S.–E-2* ppt), 17.5 nM in E-2*, were prepared in 0.32 M sucrose-10 mM Tris, pH 7.4 (buffer I), as described previously (15). The cytosol–estradiol mixture was incubated 1.5 hr at 0° or 25°C. All preparations were diluted 1:2.75 with 10 mM Tris before layering 100 μl samples on gradients and centrifuging at 2°C for: A, 16 hr at 308,000 × g or B, 15 hr at 246,000 × g. Successive 100 μl fractions were collected and counted as described elsewhere (36). BPA and γ-GLOB. indicate respective positions of bovine plasma albumin and γ-globulin markers and NUC. EXT. that of complex extracted from nuclei of calf uteri previously incubated with E-2* *in vitro*. Total dpm on each gradient: cytosol (0°) 12, 240, (25°) 9, 510; A.S.-E-2* ppt. 50, 860. From De Sombre *et al.* (15).

cold, proceeds readily at 25° to 37°C and is accelerated with increasing pH over the range 6.5–8.5, as well as the presence of salt. It is retarded slightly by Ca^{++}, Mg^{++} or Mn^{++} and more strongly by EDTA. Under the same conditions that estradiol is effective, estrone does not induce formation of 5S complex, either in the presence or absence of nuclei (12, 14), although it has been found to do so when present in higher concentrations.*

Recently it was observed that ammonium sulfate precipitation of the estradiol–receptor complex of calf uterine cytosol, prepared in the absence of EDTA, is accompanied by conversion of the complex to what appears to be the transformed state (15). The complex from the redissolved precipitate sediments in high salt sucrose gradients slightly faster than that obtained by warming uterine cytosol with estradiol (Fig. 2). By careful comparison with bovine plasma albumin (4.6S) and γ-globulin (7.0S) markers (Fig. 3), the

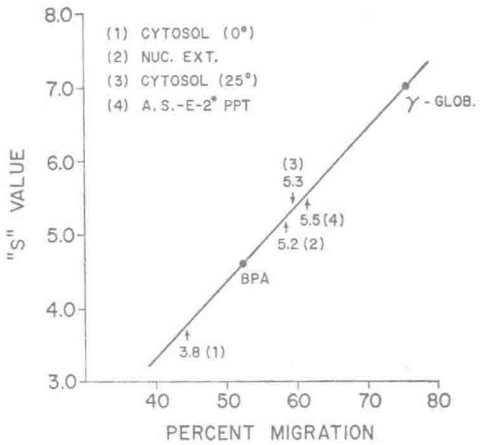

FIG. 3. Estimate of sedimentation coefficients of estradiol–receptor complexes in high salt gradients, based on values of 4.6S for bovine plasma albumin and 7.0S for bovine γ-globulin.

following sedimentation coefficients can be calculated: native cytosol, 3.8; transformed (25°C) cytosol, 5.3; nuclear extract, 5.2; and ammonium sulfate precipitate, 5.5. Whether these small but reproducible differences in sedimentation behavior among the various preparations are due to variations in the molecular milieu or whether they represent subtle differences in the hormone–receptor complexes themselves is difficult to ascertain. But all three transformed complexes have two characteristics in common in addition to increased sedimentation rate; unlike the native complex, they bind to isolated uterine nuclei and they alleviate a deficiency in RNA synthesizing capacity characteristic of these nuclei.

* H. Rochefort, personal communication.