treated cells contain 0.7 and 3.4 nmole of cytochrome P-450 per milligram of microsomal protein, respectively. Microsomes also contain cytochrome b_5, cytochrome c reductase-NADPH (E.C. 1.6.2.3), and cytochrome b_5 reductase-NADH (E.C. 1.6.2.2) (*8*).

These results suggest that an important factor in the insensitivity of *T. cruzi* to chemotherapeutic agents may be its ability to metabolize foreign compounds at substantial rates and that this ability is increased by phenobarbital.

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Progesterone Binding to Hen Oviduct Genome: Specific versus Nonspecific Binding

Abstract. *Data are presented to explain discrepancies in the literature involving the in vitro binding of steroid receptor complexes to isolated nuclei and chromatin. The type of binding in vitro of the progesterone-receptor complex to nuclei, chromatin, or DNA of hen organs is largely determined by the ionic strength of the medium. Low ionic conditions (0.01 to 0.05 molar potassium chloride) result in a nonspecific, nonsaturable binding, while high ionic conditions (0.15 to 0.20 molar potassium chloride) create a tissue-specific, saturable binding. Pure DNA binds the steroid receptor complex extensively in low salt but very little in the higher salt conditions.*

There have been controversies in the literature concerning in vitro binding of steroid receptor complexes to cell nuclei. These controversies are concerned with the chemical identity of the nuclear binding sites (that is, acceptor sites) (*1–4, 5*) as well as with possible tissue-specific binding (*2, 3, 5–7*). This report explains some possible causes of these discrepancies and presents methods and conditions for assaying for tissue-specific, high-affinity binding.

Steroid hormones enter target cells and bind to receptor proteins with high affinity and stereospecificity. Part, if not all, of these complexes undergo an obscure "activation" process wherein they become capable of migrating and binding to the nuclei (*5, 8*). The result of this nuclear binding is an immediate alteration of nuclear transcription (*5, 9*). Nontarget tissues do not respond to physiological levels of the steroids because they do not contain "active" receptors. The presence in nontarget cells of inactive receptors not capable of binding steroids has not been investigated.

We assessed more carefully the binding that occurs in vivo as well as in vitro between isolated nuclei or chromatin and isolated progesterone-receptor complex (P-R) (*10*). More than one class of binding sites was observed in vivo and in vitro. The highest affinity class of binding sites was tissue specific, survived high ionic conditions (0.15 to 0.2*M* KCl), and represented the only type of nuclear binding in avian oviducts exposed to physiological levels of the hormone (1 to 17 ng per milliliter of serum). When this class was saturated in vivo with progesterone, the full responses of RNA polymerase I and II activities were observed (*10*). Although a higher level of binding to weaker sites could be achieved in vivo, there was no further alteration in polymerase activities. We feel that the highest affinity class of sites may represent the primary sites for steroid-induced alterations in transcription. We are thus able to selectively analyze (utilizing the high ionic conditions) binding in vitro only to the high affinity class of sites in isolated nuclei. Under low ionic conditions, the P-R binds in a nonsaturable, nonspecific fashion to many nuclear components. Under higher ionic conditions, however, a saturable binding to nuclear material is achieved which is tissue specific. Very little binding to DNA or other components occurs.

Figures 1 and 2 show the patterns of binding of P-R to the nuclear material of target tissue (oviduct) and nontarget tissue (spleen) under varying ionic condi-

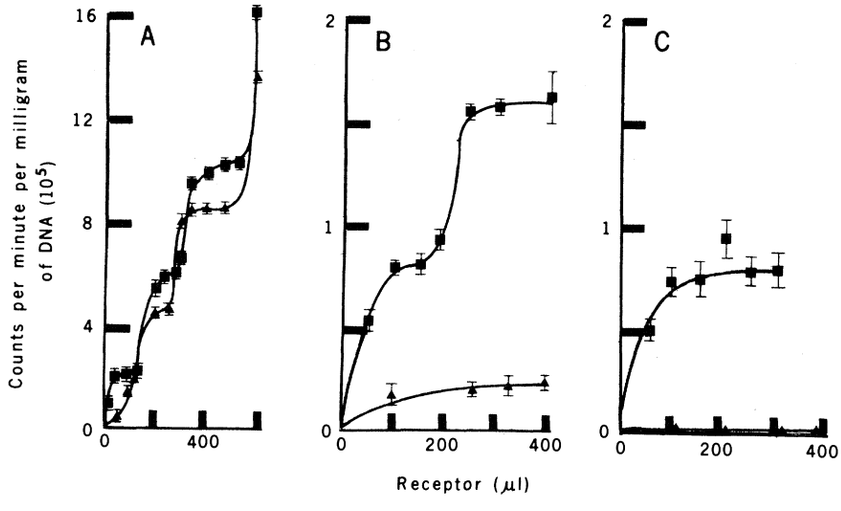

Fig. 1. Binding of isolated [^{3}H]progesterone-receptor complex (P-R) to (■) hen oviduct and (▲) spleen nuclei in (A) 0.05*M* KCl, (B) 0.10*M* KCl, and (C) 0.18*M* KCl. The isolation of partially purified P-R is described elsewhere (*10, 11*). The binding of the P-R to nuclei was performed according to the "standard method" in (A) and (C) and the "streptomycin method" in (B) (*10*). Briefly, 25 to 50 μg of DNA (as nuclei) was incubated at 4°C for 90 minutes with increasing levels of P-R. The incubation period was selected since equilibrium binding is achieved at the ratio of receptor to DNA used in these experiments (*10*). Tubes containing the binding assays were centrifuged, the pellets were washed several times in dilute buffers, and the nuclear material was collected on membrane filters and counted in a scintillation spectrometer. The micrograms of DNA per filter were then estimated by diphenylamine assay and the counts per minute per milligram of DNA were calculated as described (*10*).

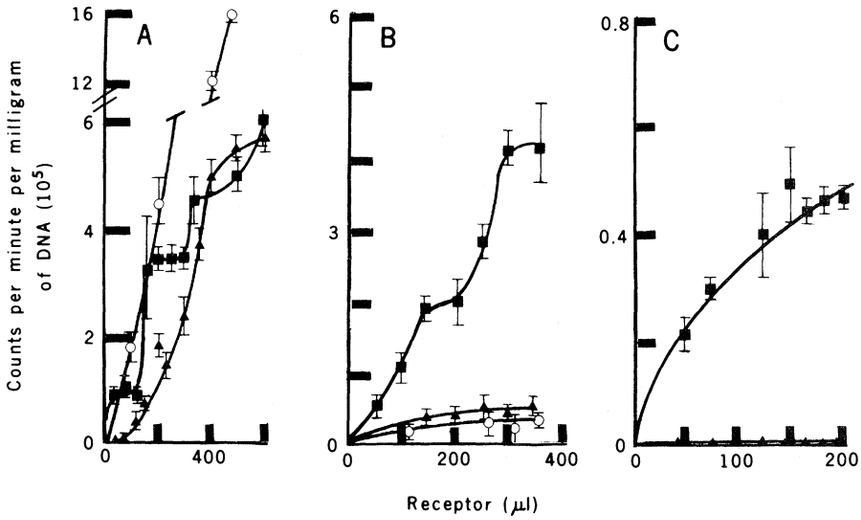

Fig. 2. Binding of the isolated [^{3}H]progesterone-receptor complex (P-R) to (■) hen oviduct chromatin, (▲) spleen chromatin, and (○) DNA in (A) 0.05*M* KCl, (B) 0.10*M* KCl, and (C) 0.18*M* KCl. The methods are described in the legend of Fig. 1. The chromatin binding in (A) and (C) were performed according to the "standard method" (*10*); chromatin binding in (B), as well as the DNA binding in (A) and (B), was performed according to the "streptomycin method" (*10*). The streptomycin assay fails to sufficiently precipitate pure DNA under the 0.18*M* KCl conditions as performed in (C). Consequently, results of DNA binding under these conditions are not obtainable.

tions. Figure 2 includes binding to pure DNA. The binding assays were performed for 90 minutes at 4°C, which was previously shown to give equilibrium conditions at the receptor to DNA ratios used in these experiments (*10*). Under low ionic conditions (0.01 to 0.05*M* KCl), the binding to nuclei and chromatin of both oviduct and spleen is nonsaturable and nonspecific. Pure DNA displays an extensive, nonsaturable binding; even cellulose, glass beads, and so forth, bind the P-R extensively under these conditions. At an intermediate ionic condition (0.10*M* KCl), saturation is achieved and a tissue specificity with respect to levels of binding to the nuclei and chromatin becomes apparent; DNA binding is greatly reduced. At high ionic conditions (0.15 to 0.2*M* KCl), binding to the oviduct nuclei and chromatin is extensive relative to that of spleen nuclei and chromatin which display no binding. The nuclei of some nontarget tissues, for example, lung and erythrocyte, do show some binding at high ionic strength. We believe this binding is due to our inability to obtain clean nuclear preparations since the chromatins of all nontarget tissues (for example, spleen, liver, erythrocyte, and lung) display no binding under the high ionic conditions. Binding of P-R to DNA is not readily measurable at the high ionic conditions because of the methodology (see legend to Fig. 2). However, considering the greatly reduced binding in the intermediate, compared to the low, ionic conditions, such DNA binding must be a minor component of the nuclear binding under the high ionic conditions.

There has been controversy in the literature concerning a lack of tissue-specific, saturable binding of receptors to nuclear components. However, many of these studies used conditions (low ionic strength) which permit extensive nonspecific binding. Of course, other parameters must be considered, such as sufficient receptor levels to saturate the sites as well as sufficient time of incubation to achieve equilibrium. There has also been controversy in the literature concerning the chemical identity of the nuclear "acceptor" to which the steroid receptor is bound. Other laboratories have strongly supported DNA as the nuclear "acceptor" for steroids even though many observed a nonsaturable or nonspecific binding, or both (*3, 4, 7*). The studies presented here show that under low ionic conditions, DNA does markedly bind P-R, probably due either to adsorption or to a unique DNA binding receptor (*11*). In any event, this binding is greatly reduced even at intermediate ionic conditions, and certainly does not represent the highest affinity class of nuclear binding sites.

Early work in this laboratory with the immature chick system demonstrated a tissue-specific, saturable binding to chick organ nuclei and chromatin (*2, 5, 12*). Our work with the hen system supports these early studies and also explains the reasons for differences in binding patterns reported in the literature (*10*). In short, there are multiple classes of binding sites in the cell nuclei of all organs with the highest affinity class found only in the target tissue (oviduct). While the higher ionic conditions allow selective analysis of these high affinity sites, the low ionic conditions do not. Consequently, only binding under high ionic conditions reveals a tissue-specific, saturable nuclear binding. It is recommended that future studies of steroid binding to nuclear material in vitro utilize the higher ionic conditions together with the other parameters of sufficient P-R levels, equilibrium conditions, and elimination of receptor aggregation. Although the interactions between steroid-receptor complexes and nuclei of different systems may have different characteristics, the effects of ionic conditions should at least be investigated with each system. More importantly, titrations with multiple levels of the steroid in in vivo studies and of the steroid-receptor complex in in vitro studies should be performed in attempts to identify whether different classes of nuclear binding sites exist.

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Hyaluronidase-Induced Reductions in Myocardial Infarct Size

Abstract. *The size of myocardial infarctions following coronary artery occlusion in the rat was determined directly by measurement of creatine phosphokinase activity in homogenized whole left ventricles and by planimetric measurement of the area of the infarctions in histologic sections of serial slices of the left ventricles. Hyaluronidase was shown to produce significant reductions in expected infarct size both 48 hours and 3 weeks after occlusion without impairing fibrosis during the healing phase. Thus, the amount of myocardial necrosis that follows a coronary artery occlusion has been shown directly to be amenable to reduction with a pharmacological intervention.*

Acute myocardial infarction is the condition that occurs when interruption of blood flow in a coronary artery results in the necrosis of the myocardium supplied by that artery. In hospitalized patients with actue myocardial infarction the two most frequent causes of death are disturbances of heart rhythm and mechanical failure of the left ventricle. The mortality caused by the former has been reduced by the application of monitoring techniques and the use of antiarrhythmic drugs. Since mechanical failure of the left ventricle depends on the quantity of viable, contractile myocardium, reduction in the infarcted (nonviable) tissues should also improve prognosis. Indirect evidence now exists that various interventions modify the expected size of the myocardial infarct following experimental occlusion of a coronary artery (*1*). Based on the application of indirect criteria, hyaluronidase is thought to decrease the size of an infarction (*2*). This enzyme is known to depolymerize the mucopolysaccharides that are abundant in the interstitium of the myocardium. Consequently, it has been postulated that hyaluronidase may reduce infarct size by three mechanisms—(i) improved transport of nutrients to the ischemic myocardium, (ii) enhanced "washout" of noxious metabolic waste products, and (iii) increased collateral blood flow to the ischemic area (*3*), probably secondary to a decrease in myocardial edema (*4*). However, direct evidence that hyaluronidase (or other interventions) actually reduce infarct size is lacking. The goal of this investigation was to describe two independent methods that permit the direct measurement of the size of the myocardial infarct after experimental coronary artery occlusion in the rat and to determine whether these methods can be used to demonstrate directly the effects of interventions that may decrease infarct size.

A standard size infarct was produced in the left ventricles of albino Charles River male rats (weight, 250 to 300 g) by occluding the left coronary artery 2 to 3 mm from its origin, as described (*5*). In order to assess the effect of an intervention on expected infarct size, three groups of rats were studied: sham-operated (S-rats), rats with an occlusion but no intervention (O-rats), and rats in which an intervention was added to the occlusion (OI-rats). The intervention consisted of the intravenous administration of 1500 units of hyaluronidase (Alidase, Searle) per kilogram of body weight 5 minutes and 24 hours after coronary artery occlusion. The S-rats were not given the interventions, since we had previously found that in S-rats this dose of hyaluronidase does not affect the mean creatine phosphokinase (CPK) activity of the left ventricle when determined 48 hours later. Animals were killed either 48 hours after occlusion, that is, when the necrotic process was at its peak, or 21 days after occlusion, when the process of repair was complete. Just prior to death, and with the heart again exposed, 1 ml per kilogram of body weight of 10 percent carbon black (particle size, 300 Å) was injected intravenously. If the anterior surface of the left ventricle was not discolored by the carbon, a complete coronary artery occlusion was deemed to be present. The heart was then excised and either homogenized for enzymatic study or placed in 10 percent phosphate-buffered formalin for histologic processing and examination.

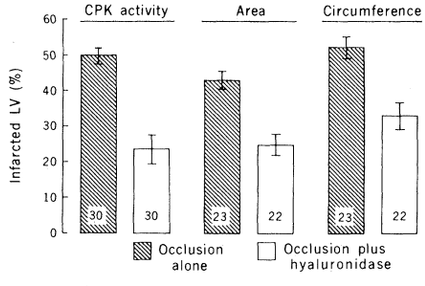

Fig. 1. Percentage of the left ventricle that was infarcted 48 hours after occlusion of the main left coronary artery in the untreated (O) rats (hatched columns) and hyaluronidase-treated rats (OI) (nonhatched columns). The extent of the infarcts was assessed by left ventricular (*LV*) creatine phosphokinase activity (*CPK*) and by two histologic methods based on the analysis of serial slices of the ventricle, that is, the infarcted area and the circumference overlying it. Bars indicate ± 1 S.E.M. The numbers in the columns are the numbers of rats studied in each group.

For the enzymatic studies, CPK activity of the homogenized whole left ventricle (free wall plus interventricular septum) was measured as described (*6*). In order to permit calculation of "enzymatic" infarct size, the minimum CPK activity in the infarcted tissue 48 hours after occlusion was also determined and was found to be 2.6 international units per milligram of protein. For histologic studies the left ventricles were sectioned into four slices (2 to 2.5 mm thick) from the apex to the base of the heart in a plane parallel to the atrioventricular groove. Paraffin-embedded sections (5 μm in thickness) were prepared from each slice, stained with hematoxylin and eosin, projected onto a screen, and planimetered to determine the cross-sectional area of the left ventricle and of the infarcted myocardium (*7*). The fraction of the left ventricle that was infarcted was calculated as a mean of this value in each of the four slices, then expressed as a percentage. Both the thickness of the interventricular septum and the ratio of the thickness of the infarcted portion of the left ventricle to that of the septum (representing normal left ventricular wall thickness) were also measured. The former would be expected to increase if compensatory hypertrophy developed in the remaining viable myocardium, and the latter is a useful index of the degree of thinning of the infarcted part of the free wall of the left ventricle. When the calculation of infarct size was based on the planimetered area, the thinning of the in-