COMPARISON OF VARIOUS ANALYTICAL METHODS FOR THE ESTIMATION OF HORMONE RECEPTOR PARAMETERS IN REPRODUCTIVE TISSUES*

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ABSTRACT

Because several analytical methods have been proposed in the literature for the estimation of the binding parameters (K_a and B_{max}), a comparative study was carried out to evaluate the practical usefulness of some of them. Two binding systems were used for this purpose: the cytoplasmic estradiol receptor from rat uterus and the particulate gonadotropin receptors from rat testis. Scatchard, Lineweaver-Burk, the direct linear plot, Rosenthal, Chamness-McGuire, and the simple extrapolation of the linear portion of Scatchard plot methods were utilized for the binding analyses. It was found that even though Scatchard, Lineweaver-Burk and the direct linear plot methods gave comparable K_a and B_{max} values to each other in the presence of a single class of binding sites (as in the case of estrogen receptors), the direct linear plot resulted in easier and the most rapid application. Therefore, its use was highly recommendable for this type of binding system. On the other hand, when more than one class of binding sites is involved (as in the case of gonadotropin receptors), then the curvilinear Scatchard plots should preferentially be analyzed by the method of Rosenthal for deriving the two components of the curve. This method implies a simple graphical procedure and represented an alternative of choice in such cases.

COMPARACION DE VARIOS METODOS ANALITICOS PARA LA ESTIMACION DE LOS PARAMETROS DEL RECEPTOR HORMONAL EN TEJIDOS REPRODUCTIVOS

RESUMEN

Debido a que en la literatura se han propuesto varios métodos analíticos para la estimación de los parámetros de la interacción hormona-receptor (K_a y B_{max}), se realizó un estudio comparativo para evaluar la utilidad práctica de algunos de ellos. Con este propósito, se emplearon dos sistemas de interacción: el del receptor citoplasmático al estradiol en el útero de rata, y el de los receptores gonadotrópicos particulados en el testículo de rata. Para los análisis de unión se ensayaron los métodos de Scartchard, Lineweaver-Burk, gráfico linear directo, Rosenthal, Chamness-McGuire, y el método de la simple extrapolación de la porción linear del gráfico de Scatchard. Se encontró que aunque los métodos de Scatchard, Lineweaver-Burk y el gráfico linear directo dieron valores de K_a y B_{max} comparables entre sí en la presencia de una sola clase de sitios receptores (como en el caso de los receptores estrogénicos), el gráfico linear directo resultó más fácil y el más rápido de emplear. Su uso es, por lo tanto, muy recomendable para el análisis de este tipo de sistema hormona-receptor. Por otro lado, cuando el sistema implicó más de una clase de sitios receptores (como en el caso de receptores gonadotrópicos), se encontró que los gráficos de Scatchard no lineales deberían analizarse preferencialmente por el método de Rosenthal, con el fin de derivar los dos componentes de la curva. Este método consiste en un simple procedimiento gráfico y constituyó una alternativa de elección en tales circunstancias.

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INTRODUCTION

A variety of methods have been applied to the analysis of binding data in an attempt to derive both affinity constants (K_a) for the hormone receptor interaction, and the number of binding sites (B_{max}).[19, 26, 25, 15, 5, 24, 13, 27] Most often, however, the use of a given analytical procedure appears to depend on a great knowledge about the specific system which in the majority of the studies is not feasible.[8, 27, 2, 20, 4] This fact makes the choice of the appropiate methodology for binding experiments a rather critical step, and points out the importance of easy access to most investigators of the various kinds of analyses. The present report has intented to compare some of the several analytical methods generally employed for hormone receptor interactions. For this purpose we have selected two different binding systems widely utilized in reproductive hormone receptor studies: the interaction of estradiol with the rat uterine cytoplasmatic estrogen receptors, and the hCG (human chorionic gonadotrophin) with the particulate gonadotropin receptors in rat testis. We applied the method most frequently used in each case to estimate the parameters of the binding data and compare their usefulness for rapid and routine determinations of the hormone receptor interactions in these tissues.

MATERIALS AND METHODS

Reagents:

(2, 4, 6, 7 - ^{3}H) 17β-estradiol (90Ci/mmol) was obtained from New England Nuclear Corporation. Its purity was periodically checked by thin layer chromatography on silica gel using ethyl acetate: hexane: ethanol (80:15:5, v/v) as the solvent system, and unlabeled estradiol as standard for identification. Progesterone, tetosterone, estrone, estriol and 17β-estradiol were purchased from Sigma Company, and used without further purification. Carrier free Na^{125}I was obtained from New England Nuclear and purified hCG (iodination grade) from Calbiochem-Behring Corporation. Other chemicals were of reagent or analytical grade.

Animals:

The animals used in this investigation were adult male and female rats (200 to 300 g) of Sprague-Dawley Strain. They were housed in a 12 h light-12 h dark environment and fed rat chow *ad libitum*. The rats were sacrified by decapitation immediately prior to use. Female rats were used irrespective of cycle stage.

hCG binding to gonadal receptors:

Iodination of hCG was carried out by the chloramine T method[12] modified to allow the retention of biologic activity.[1] Separation of labeled hCG from other reagents was obtained by gel filtration through Sephadex G-100. The specific activity and the recovery of the labeled hormone were calculated using the method described by Greenwood et al.[12] The specific activity of ^{125}I-hCG was 15-18 uCi/ug. The labeled hCG was diluted to a final concentration of 7.5×10^{-14} moles (2.5 ng) of hCG per 50 ul of 0.01 M phosphate buffer pH 7.5, containing 5 mM $MgCl_2$, 0.1 M

sucrose and 0.1% (w/v) egg albumin (henceforth referred to as the albumin-phosphate buffer) and stored frozen in several vials.

Testicular homogenate as a source of particulate receptors was prepared as described previously.[23] Briefly, decapsulated testes were homogenized in a Teflon pestle tissue grinder (Arthur L. Thomas, Philadelphia, PA) in a ratio of 2 ml albumin-phosphate buffer per gram of tissue, and filtered through a single layer of cheesecloth. The filtrate was centrifuged at 1,500 xg for 10 min at 4°C and the supernatant was discarded. The pellet was recentrifuged at 20,000 xg for 10 min at 4°C to expel excess buffer, weighed and then resuspended in fresh albumin-phosphate buffer to a final concentration of 1 g pellet per 10 ml buffer.

Binding studies were performed using 2.5 ng of ^{125}I-hCG and 20 mg net weight of testicular homogenate in the absence and presence of 0, 1, 2.5, 5, 10, 20, 30, 50, 75, 100 ng of unlabeled hCG.[2, 23] Albumin-phosphate buffer was used to bring the reaction volume up to 1.0 ml. The reaction mixture was incubated at 37°C for two hours in a metabolic shaking water bath. At the end of the incubation period, the tubes were centrifuged at 1,500 xg for 10 min at 4°C and the supernatant containing free hormone was decanted. The tubes containing tissue pellets were inverted over adsorbent paper in a rack and kept at 4°C for 30 min prior to being counted for radioactivity in a In-V-tron 2000 gamma counter (Nuclear-Medical Laboratories Dallas, Texas).

For each set of experiments there were tubes which contained nothing but ^{125}I-hCG, and tubes which contained all reactants except homogenate which were replaced with an equivalent amount of supernatant (''Supernatant control'').[21, 33] The ''total count'' tubes yielded cpm per ng ^{125}I-hCG a value needed to convert binding data from units of radioactivity to ng. The ''Supernatant control'' was treated in the same way as the assay tubes, and the radioactivity value associated with them was substracted from those of the assay tubes to yield radioactivity due to binding by homogenate alone.[22] The data were also analyzed by substration of non-specific binding according to the method described previously.[2, 23]

The ''supernatant'' was prepared by suspending the tissue in buffer using a tissue to buffer ratio of 100 mg (wet tissue weight) per ml, and incubating the mixture for 1h at 37°C. At the end of the incubation period, the mixture was centrifuged at 20,000 xg for 1 h at 4°C and the tissue pellet discarded. Every 200 ul aliquot of this preparation contained the quantity of substance which would normally be released by 20 mg of the testicular homogenate at the end of the incubation period in the binding assays.[2] The amount of radioactivity bound to glass tubes containing supernatant, represented approximately 5-10% fo the tissue bound radioactivity, whereas the amount bound by the homogenate in the presence of 500-1000 ng/ml of unlabeled hCG was about 10%. Therefore, supernatant controls were used to assess the non-specific binding.[2, 23]

Estradiol binding to cytoplasmic estrogen receptors:

Uteri were removed, weighed and placed in an ice cold Tris-EDTA buffer (0.01 M Tris, 0.0015 M disodium EDTA, pH 8.0). A tissue: volume ratio of 50-60 mg/ml (1 uterus/ml or 4-5 mg protein/ml) was adjusted for homogenization which was carried out in a tissue grinder with Teflon pestle driven by motor. Short periods of homogenization were used with alternate cooling in an ice bath. The homogenate was then centrifuged in a Beckman model L5-65 B preparative ultracentrifuge (rotor 60Ti) at 105,000 xg for 60 min at 4°C. Following centrifugation cytosol fractions were decanted and used in binding experiments. Protein concentration of the cytosols were determined by the method of Lowry et a.[16] using bovine serum albumin as a standard.

Specific binding of 17β-estradiol by tissue cytosol was measured essentially according to the charcoal adsorption technique described by Cidlowsky and Muldoon.[7] Charcoal solution of 0.3% and 0.03% dextran was divided into 1-ml portions and pelleted by centrifugation for 10 min at 2,000 xg in glass tubes. Supernatants were discarded and the procedure repeated through decanting. Cytosol was then added to the charcoal pellet, mixed, incubated for 15 min at 4°C and centrifuged for 10 min at 2,000 xg. The supernatant was decanted into a scintillation vial to which 10 ml of scintillation mixture was added (Aquasol-2; New England Nuclear) and counted in a liquid scintillation spectrophotometer (Packard Tri-Carb, Model 3255). Counting efficiency was 49% for tritium.

To measure total binding (specific and non specific), aliquots of cytosol (generally 100 ul) were incubated in a final volume of 0.8 ml with varying amounts of (^{3}H)-estradiol (0.02 pmoles to 4.00 pmoles; 1 fmole of (^{3}H)-estradiol was equivalent to 202 dpm) for 18-20 h at 4°C. Non specific binding was determined by incubation with (^{3}H)-estradiol in the presence of 100-fold excess of unlabeled estradiol. Substration of the non-specific binding component from that of the total binding value resulted in the amount of the specific binding.[7] The amount of specific steroid bound per mg of cytosol protein was a constant within the range of 100-500 ug of cytosol protein.

Analysis of the binding data:

The data were analyzed by assuming a simple bimolecular reaction: (H) + (R)⇄(H.R.), where (H) is the concentration of free hormone, (R) is the concentration of unoccupied receptors, and (H.R.) is the concentrarion of hormone-receptor complex.[19] Since Scatchard[25] and Lineweaver-Burk[15] graphical methods are the most commonly used mathemathical transformations to estimate B_{max} (the total number of binding sites) and K_d (the equilibrium dissociation constant) in studies on hormone receptors,[19, 26, 25, 15, 5, 24, 13, 27] the binding data were preferentially analyzed through these equations. In some cases (gonadotropin receptors), the binding inhibition data were converted to hormone saturation curves and then subjected to Scatchard or Lineweaver-Burn analyses.[23] When Scatchard plots were normalized to mg of protein concentration, bound hormone fraction (B) as well as B/F ratio were considerated for corrections.

The method described by Chamness and McGuire[5] was used to make the calculating corrections for non-specific binding on curvilinear Scatchard plots. The limiting B/F ratio was calculated and the value multiplied by the free hormone concentration (F) at each point of the data. The resulting value was then substracted from the measured total binding to estimate the specific binding. Finally, the corrected binding line was obtained by plotting specific binding against specific binding/free hormone ratio.[5] Also, for analysis of non linear Scatchard curves, the graphical method reported by Rosenthal[24] was applied to the

experimental data. Accordingly, straight lines were drawn to resolve the two binding components of the plot and the parameter of the system was correlated on a single graph.[24]

The direct linear plot was assessed as described by Woosley and Muldoon.[27] The axes B_{max} and K_d constituted the rectangular coordinate system for construction of the plot. Lines for each data point were drawn between $K_d = -F$ on the K_d axis and $B_{max} = B$ on the B_{max} axis, and projected into the first quadrant of the coordinates. The estimated values of K_d and B_{max} were obtained by the intersection of the lines.[27]

The method of unweighed least squares were used to calculate the parameters for various lines of regression. The statistical confidence in the data was assessed by applying the student's t test.

RESULTS

Interaction of estradiol with the cytoplasmic estrogen receptors

The saturation binding studies were typically plotted according to Scatchard, Lineweaver-Burk and the direct linear plot methods as shown in fig. 1-3, respectively. Specific binding was calculated after subtraction of the non-specific binding value obtained by using an excess of unlabeled estradiol. Under the experimental conditions, a single class of binding sites was detected having a K_d value of $1.84 \pm 0.06 \times 10^{-10}$ M and a B_{max} value fo $14.53 \pm 1.02 \times 10^{-14}$ moles/mg cytosol protein (Table I; see Scatchard method). These values were in the range of the binding parameters reported by others.[8-7] Scatchard, Lineweaver-Burk and the direct linear plot methods were found to be equivalents for the estimation of the binding parameters through several binding experiments (Table I); they eventually provided very similar K_d and B_{max} values.

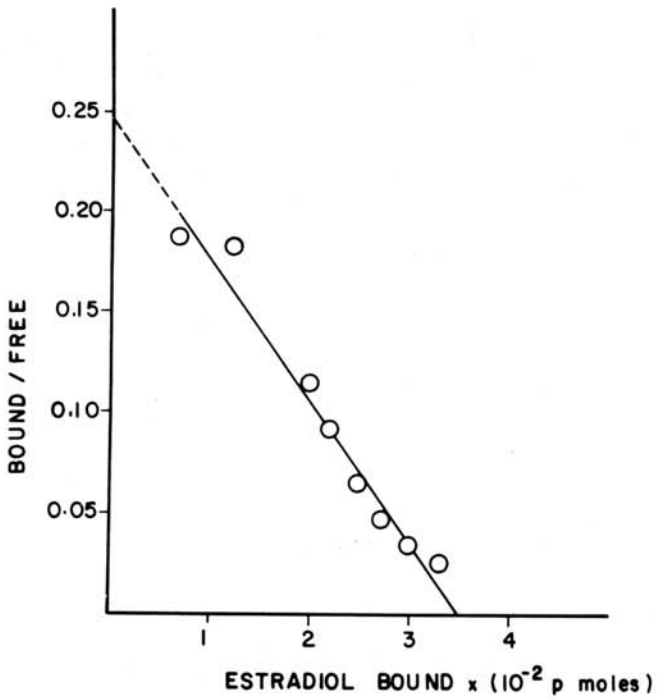

Fig. 1. Scatchard plot analysis of specific ^{3}H-estradiol binding to cytoplasmic estrogen receptor for rat uterus. Nonspecific binding was measured in presence of excess cold ligand.

TABLE I

Comparison of the binding constants for cytoplasmic estrogen receptor system determined by Scatchard, Lineweaver-Burk and direct linear plot analyses

Method	K_d (10^{-10}M)	B_{max} (10^{-14}moles/mg cytosol protein)
Scatchard	1.84±0.06	14.53±1.02
Lineweaver-Burk	1.84±0.10	14.47±1.20
Direct linear plot	1.77±0.13	13.97±1.28

TABLE II

Binding constants for cytoplasmic estrogen receptors calculated by Chamness-McGuire and Rosenthal analyses

Method	K_d (10^{-10}M)	B_{max} (10^{-14}moles/mg cytosol protein)
Chamness-McGuire	0.99±0.14*	8.49±2.06*
Rosenthal	1.50±0.21**	9.50±1.09**

* Values obtained from the linear portion of the data points.

** Values corresponding to the high affinity, low capacity-binding component of the curve.

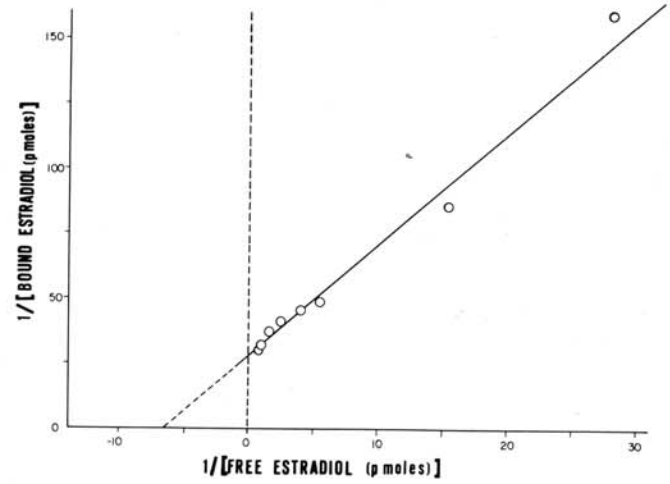

Fig. 2. Lineweaver-Burk plot analysis of the specific binding data of fig.1.

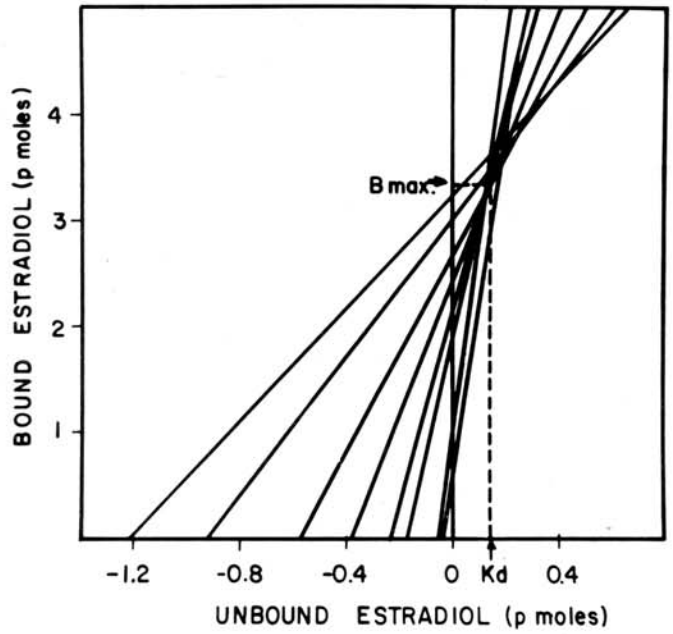

Fig. 3. Direct linear plot analysis of the specific ^{3}H-estradiol binding data of fig. 1 and 2.

In experiments in which higher levels (2 pmole or higher) of the labeled hormone were used, the data points showed deviation from linearity (fig. 4). This deviation was also observed when binding experiments were carried out in the presence of 100-fold molar excess of diethylstilbestrol instead of unlabeled estradiol for estimation of non-specific binding (data not shown). This observation and the fact that adult animals were used in this study, would suggest that the deviation was not due to α-fetoprotein. It appears that a second binding site is present in the cytoplasmic estrogen receptor of the rat uterus. Such heterogenity of estrogen binding sites has been recently demostrated by Clark et al.[9]

When total binding rather than specific binding data were used to construct Scatchard plots, a biphasic curve was obtained (figs. 5, 6). In these cases, the resolution of the two binding components were then attempted by the procedure described by Chamness and McGuire (fig. 5) and by the graphical method of Rosenthal (fig. 6). We have found that the K_d parameter values

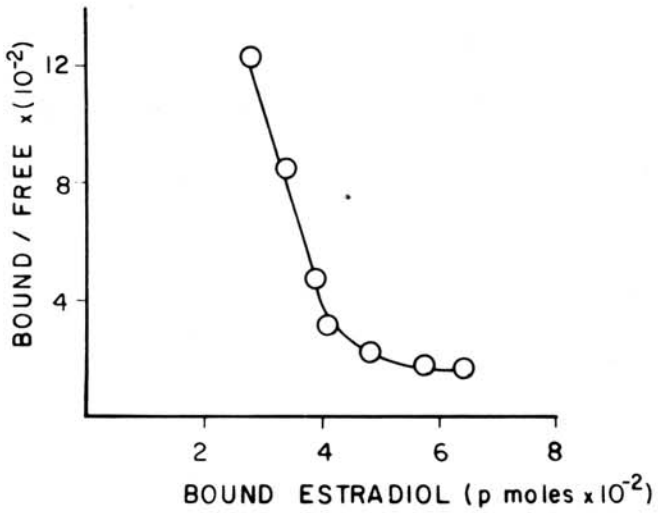

Fig. 4. Scatchard plot of specific ^{3}H-estradiol binding to cytoplasmic estrogen receptor of rat uterus. A higher concentration of ^{3}H-estradiol was used to detect a linear deviation. For details see text.

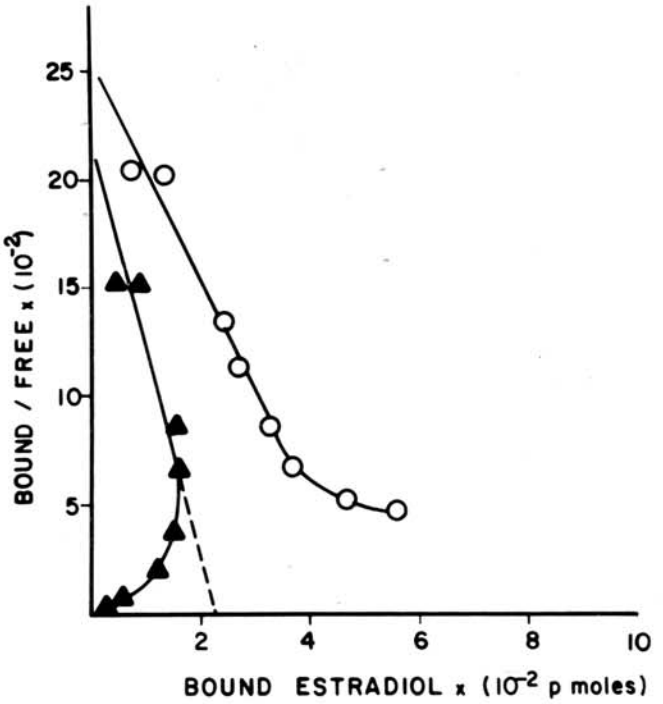

Fig. 5. Chamness-McGuire analysis of the curvilinear Scatchard plot obtained when total rather than specific binding of the data illustrated in fig. 1-3 is used. ○○, Scatchard analysys; ◆◆, Chamness-McGuire analysis.

obtained by each of the methods were not comparable (Table II); however, the method of Rosenthal appeared to be closer to the values found by the methods of Scatchard, Lineweaver-Burk or direct linear plot when specific binding had been plotted (compare Table I and II). It is possible that the problems in determining unequivocally the limiting B/F ratio in the method of Chamness-McGuire, or the asymptote to the second class of binding sites in the method of Rosenthal, can account for the differences reported. This can particularly be the case when the second binding component cannot clearly be defined along the non-linear plot, which makes its estimation unreliable for the graphical extrapolations required in the methods. Besides, the Chamness-McGuire analysis was also curvilinear; there was an

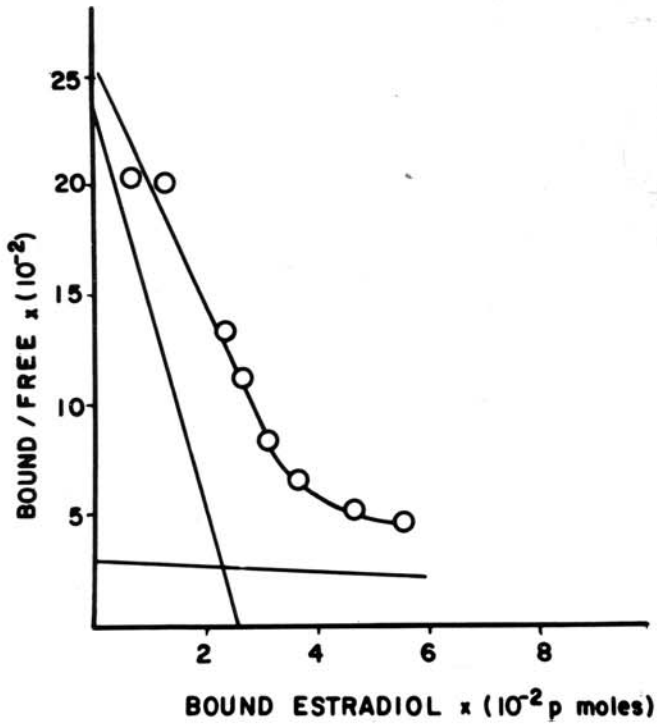

Fig. 6. Rosenthal analysis of the same data shown in fig. 5. For details see text.

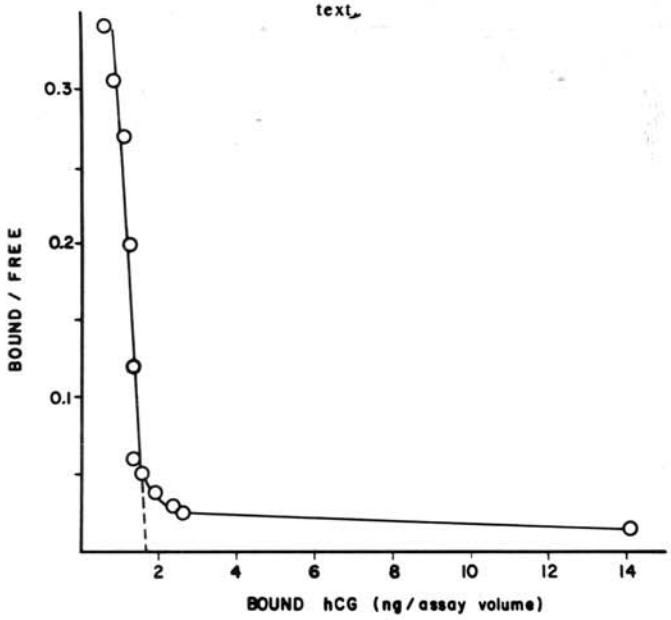

Fig. 7. **Scatchard plot analysis of specific ^{125}I-hCG binding to gonadotropin receptors of rat testicular tissue.**

evident deviation of the specific binding line at higher bound (B) values (fig. 5; see also fig. 9). Such situation led to the arbitrary selection of the data points corresponding to the linear portion of the curve needed to estimate the parameter by linear regression analysis.

Interaction of hCG to the particulate gonodatropin receptors

The binding data obtained from displacement experiments were first converted to hormone saturation curves and then analyzed by Scatchard and Lineweaver-Burk methods.[22, 23, 26] In these experiments supernatant controls were used to assess the non-specific binding.[2, 23] In this way the binding data were not subjected to arbitrary manipulations as occurs when the radioactivity bound by the tissue, in the presence of an "excess", unlabeled hormone concentration, is chosen as true non-specific binding.[22, 3, 2, 5, 8]

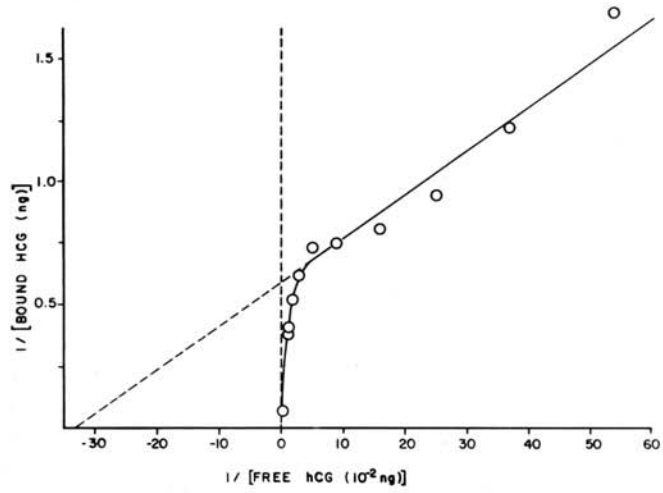

Fig. 8. Lineweaver-Burk plot analysis of binding data shown in fig. 7.

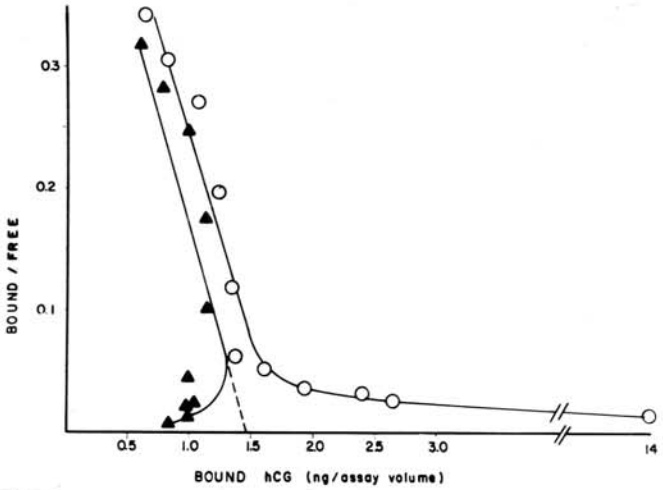

Fig. 9. Chamness-McGuire analysis of the Scatchard plot shown in fig. 7. ○○, Scatchard analysis; ◆◆, Chamness-McGuire analysis.

The Scatchard plot constructed from the data showed a non linear curve with upward concavity (fig. 7). This deviation from linearity indicated the presence of more than one class of binding sites; one class of higher affinity for the hormone but lower in number, and another class of lower affinity and higher in number. The same data plotted according to Lineaweaver-Burk equation were also curvilinear (fig. 8). It was interesting to observe that the two graphical analyses showed lines which consistently deviated at an unlabeled hormone concentration of approximately 30 ng/ml (fig. 7 and 8). The apparent dissociation constant (K_d) for hCG and the high-affinity class of binding sites were estimated to be $1.51 \pm 0.08 \times 10^{-10}$ M, and the binding site concentration was estimated to be 0.13 ± 0.02 ng/mg tissue (Table III, see Rosenthal method) assuming that hormone and receptor interacted with each other on a one to one basis.[23, 20, 2] The values for the lower-affinity binding sites were difficult to determine due to the non saturability of the binding sites.

TABLE III

Binding parameters of hCG interaction to testicular receptors calculated by Chamness-McGuire, Rosenthal, Scatchard and Lineweaver-Burk analyses

Method	K_d (10^{-10}M)	B_{max} (ng/mg tissue)
Chamness-McGuire	1.43±0.03*	0.12±0.02*
Rosenthal	1.51±0.08**	0.13±0.02**
Scatchard	1.68±0.07*	0.15±0.02*
Lineweaver-Burk	1.83±0.11*	0.16±0.03**

* Values obtained from the linear portion of the data points.

** Values corresponding to the high affinity, low capacity-binding component of the curve.

The estimation of the binding parameters by simple extrapolation of the linear portion of the Scatchard plot, a method used by some investigators,[3, 14] resulted in values close to those obtained by the linear portion of the Lineweaver-Burk plot (Table III). However, this procedure consistently had the tendency to overstimate the number of binding sites.

The biphasic line resulting from Scatchard graphs was also reevaluated by the method of Chamness-McGuire and by that of Rosenthal (fig. 9 and 10, respectively). In both cases the shape of the curve allowed an easy resolution of the two binding components. The K_d and B_{max} values calculated from any of these two analysis were similar and comparable to those obtained through Scatchard and/or Lineweaver-Burk plots (Table III).

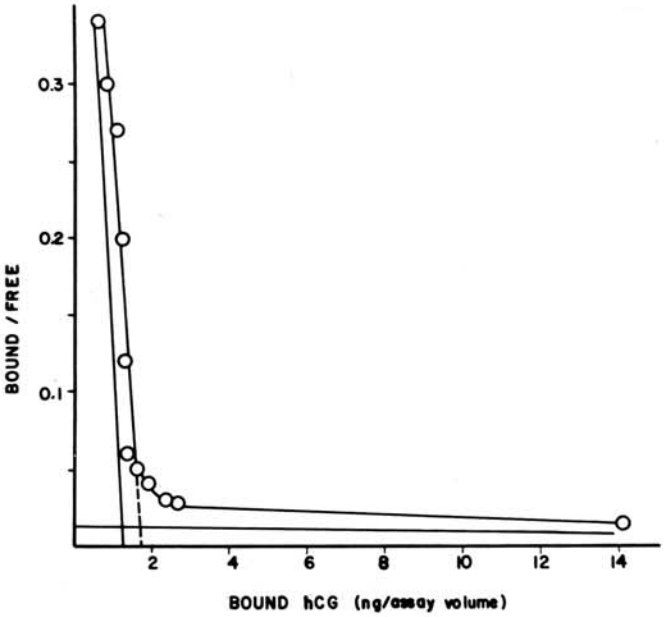

Fig. 10. Rosenthal analysis of the Scatchard plot shown in fig. 7. For details see text.

DISCUSSION

It is well recognized now that a decisive step through which a hormone carries out its biological effect is the interaction with a high affinity receptor protein; membrane-bound receptors being involved in polypeptide hormone action and cytoplasmatic and nuclear receptors in the action of steroid hormones. In a number of target tissues, such hormone receptors have been studies by their binding properties and high specificity for the biologically active forms of the hormone molecule. If the hormone is present in radioactively labeled form, then the measurable concentrations of the hormone-receptor complex and the free hormone at equilibrium are preferentially used as variables in saturation or displacement experiments. In most of these analyses, reaction is depicted as a simple reversible bimolecular equilibrium from which several mathematical transformations have been derived in order to estimate the K_d and B_{max} values of the binding system (for review see 19). Most frequently used are made of the methods reported by Scatchard,[25] and by double-reciprocal plot of Lineweaver-Burk.[15] However, in many events these analyses become difficult to interpret and can lead to large errors in the estimation of receptor parameters.[21, 22, 5, 26, 8, 2] To resolve some of the problems in the interpretation of binding experiments, several studies have been conducted. Among them, Chamness and McGuire[5] introduced calculating corrections to determine the specific binding in Scatchard plots, and both graphical[13] and computer[19, 13] analyses were proposed for resolving the two binding components of the non linear Scatchard curves. Also, Woosley and Muldoon[27] recently reported that the direct linear plot method was a better alternative for estimating the binding constants of steroid receptor interactions. In the present report, we have accomplished a comparative study of these methods using two of the binding systems we currently utilize in our laboratory for the hormone receptor investigations in reproduction. Since we particularly wanted to evaluate their practical application and easy accessibility to most laboratories, we did not include in this work, the somehow costly methodologies such as the computer analysis.[19, 13]

The results we have obtained show that in the case of the estradiol binding to the rat uterine cytoplasmic receptors, the data can be readily analyzed by either Scatchard, Lineweaver-Burk or direct linear plot methods for estimating the binding parameters; they appear to be equivalents (Table I). Because Scatchard and Linearweaver-Burk methods have been previously analyzed in view of the implications that the experimental errors in the bound (B) and free (F) measurements could have upon the plots, we have been cautious in their evaluation. It is considered, for example, that one of the limitations of a

Lineweaver-Burk plot is that a slight error in the determination of bound radioactivity can lead to a significant change in the slope of the line when reciprocals are taken.[26] Also, a criticism to Scatchard analysis on the other hand, is that B appears on both sides of the equation[25] therefore, a plot of B/F versus B would inevitable show some degree of correlation.[19, 26] However, in spite of these considerations, we have found that under the experimental conditions of this study, the use of Scatchard or Lineweaver-Burk methods gave in practice comparable B_{max} and K_d values. Regarding the usefulness of Scatchard, Lineweaver-Burk and the direct linear plot methods for the analysis of estrogen receptor binding, it was most relevant the simplicity showed by the direct linear plot.[27] This method has several advantages over Scatchard and Lineweaver-Burk graphs: it is easy to construct, requires no transformation of data, and the binding parameters can be read directly from the plot, all of which confirms an earlier report by Woosley and Muldoon.[27] The use of the direct linear plot, therefore, provides a good and reliable alternative for the analysis of the hormone-receptor system involving a single class of binding sites.

The plotting of total binding rather than specific binding for Scatchard graph leads to a non linear curve. The analysis of the cytoplasmic receptors in this case, can then be achieved by the methods of Chamness-McGuire[5] and Rosenthal[24] (fig. 5 and 6). We found that the utility of these methods depends upon the practical feasibility to identify easily the second binding component of the curve, a condition observed in the case of gonadotropin receptor system (see for example fig. 7-10). We observed that the method of Rosenthal generally appeared to be more consistent and to provide closer values to those obtained by the analysis of linear data points (Table I and II).

It became evident that the most advantageous applicability of the method of Rosenthal or of the Chamness-McGuire should be for systems showing a definite biphasic binding data, such as for gonadotropin receptors (fig. 7 and 8) or for estrogen receptors when the hormone concentrations used during the binding experiments were higher enough to allow the detection of a second class of binding sites (fig. 6). These methods were eventually more reliable than the simple extrapolation[3, 14] of the linear portion of the Scatchard plot which tends to overestimate the number of binding sites (Table III). For hCG particulate receptors, both Rosenthal and Chamness-McGuire methods gave comparable binding parameter values (Table III).

We feel that the method of Chamness-McGuire was somehow cumbersome due to the calculations involved. Furthermore, the method was also curvilinear, a fact unexpected since this possibility was not even mentioned by the authors in their study.[5] This deviation was observed in the two binding reactions studied herein, i.e., the cytoplasmic estrogen receptor and gonadotropic receptor systems (fig. 5 and 9). Therefore, taken into account all the data, we strongly support the use of the Rosenthal method as the graphical method of choice for the analysis of non linear Scatchard plots such as gonadotropin receptors, or in the case of cytoplasmic estrogen receptors, when more than a single population of binding sites are being detected (fig. 6). The method is simple and implies no extra calculations.

Accurate and reproducible determinations of the physicochemical parameters of hormone-receptor interactions have become of primary interest for many laboratories. In clinical analysis for example, the decision as to whether endocrine treatment is likely to benefit patients with certain metastatic cancers is generally made on the basis of estimation of the hormone receptor levels in the tumor cells.[10, 17, 18, 6] The comparative study of the analytical methods discussed in this manuscript can provide some practical evaluations for being applied in most of the clinical and routine binding assays.

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