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THE APPLICATION OF ROBUST CALIBRATION TO RADIOIMMUNOASSAY

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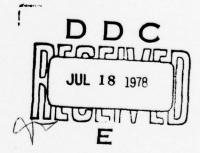
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TECHNICAL REPORT NO. 52

June 1977

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REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER Technical Report No. 52	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) The Application of Robust Calibration to Radioimmunoassay.		5. TYPE OF REPORT & PERIOD COVERED Technical
		6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) Marcello Pagano & James J. Tiede		8. CONTRACT OR GRANT NUMBER(s) N00014-75-C-0734
9. PERFORMING ORGANIZATION NAME AND ADDRESS Statistical Science Division State University of New York at Buffalo Amherst, New York 14226		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS NR 042-234
11. CONTROLLING OFFICE NAME AND ADDRESS Office of Naval Research Statistics and Probability Program Code 436 Arlington, Virginia 22217		12. REPORT DATE June 1977
		13. NUMBER OF PAGES 36 p.
14. MONITORING AGENCY NAME & ADDRESS(if different from Controlling Office)		15. SECURITY CLASS. (of this report) Unclassified
		15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

Approved for public release; distribution unlimited.

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

NA

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Robust Estimation
Radioimmunoassay

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

In this paper, a new approach to the analysis of RIA data is discussed. An algorithm is presented for obtaining the M-estimates of nonlinear calibration curves. The curves to be fitted are modified hyperbolae based on 12 to 16 observations. A procedure, based on the application of the Bonferroni Inequality, is presented for obtaining tolerance-like interval estimates of the concentration of the hormone of interest in the patients. Results of simulations are cited to support the method of construction of confidence bands for the

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SUMMARY

The minute concentrations of many biochemically and clinically
important substances are currently estimated by radioimmunoassay (RIA).
Traditionally, the most popular approaches to the statistical analysis of
RIA data have been to linearize the data through transformation and fit
the calibration curve using least squares, or to directly fit a nonlinear
calibration curve using least squares. Estimates of the hormone concentra-
tion in patients are then obtained using this curve. Unfortunately, the
transformation is frequently unsuccessful in linearizing the data. Further-
more, the least squares fit can lead to erroneous results in both approaches
since the many sources of error which exist in the RIA process often result
in outlier observations.

In this paper, a new approach to the analysis of RIA data is dis-
cussed. An algorithm is presented for obtaining the M-estimates of nonlinear
calibration curves. The curves to be fitted are modified hyperbolae based
on 12 to 16 observations. A procedure, based on the application of the
Bonferroni Inequality, is presented for obtaining tolerance-like interval
estimates of the concentration of the hormone of interest in the patients.
Results of simulations are cited to support the method of construction of
confidence bands for the fitted calibration curve. Data obtained from the
Veteran's Hospital, Buffalo, New York are used for examples throughout the
paper.

Keywords: Statistical Calibration, Radioimmunoassay, M-Estimate,
          Nonlinear Estimation

1. Introduction

Traditionally, bioassay techniques have been used to estimate the concentration of various biological substances found in man. However, for many hormones, bioassay does not have the sensitivity needed to determine the very low concentrations at which these hormones exist. This is the case, for example, with polypeptide hormones involved in many protein-binding reactions. In 1959, Yalow and Berson [1968] developed a biological technique called radioimmunoassay (RIA) which is capable of measuring hormones existing at concentrations as small as 1 picogram per milliliter. Since its inception, the principles of RIA have been extended so that many substances including enzymes and plasma tissue proteins can now be assayed. In addition to routine clinical use, RIA techniques are now widely used as a tool in pregnancy and cancer detection (Skelley, Brown and Besch [1973]).

The radioimmunoassay process can be described in two steps. In the first step, radioactive counts, y, corresponding to a known concentration of hormone, x, are recorded over a fixed period of time. The observed number of radioactive counts often referred to as counts bound, is inversely proportional to x. The set of (x,y) pairs comprise the standards curve. In the data analyzed in this paper, duplicate counts are recorded at 6, 7 or 8 distinct values of X. In the second step of the procedure, the counts bound are obtained for patients where the hormone concentration is not known. By comparing the counts bound with the standards curve, estimates of the hormone concentration in the patient can be made. A more complete description of the RIA technique can be found in Skelley, et al [1973].

From the description of the RIA process, it is apparent that the statistical techniques required for the analysis of RIA data are those of the classical calibration problem (Scheffe' [1973], Lieberman, Miller and Hamilton [1967]).   In the calibration problem the variable of interest, X , is difficult if not impossible to measure directly. However, a related variable Y , which is dependent on X , is relatively easy to measure. Given a known value x_i of X , y_i is assumed to be a random variable with mean $f(x_i, \beta)$ and dispersion σ . To determine the functional relationship between X and Y , n pairs $(x_1, y_1), \ldots, (x_n, y_n)$ are observed at known X , where it is assumed that the errors in measurement associated with X are negligible relative to the measurement errors on Y . Assuming that the form of $f(\cdot, \cdot)$ is known, β and σ can be estimated from these data. Subsequently, k independent observations y_j , $j = n+1, n+2, \ldots$ are made at unknown values x_j . The estimated calibration curve $f(x_i, \hat{\beta})$ is used to make inferences on the unknown x_j . In the context of RIA, X refers to the hormone of interest and Y the counts bound. Typically, k , the number of patients can be anywhere within range a range of 20 to 200 .

In this paper, we present a method for the analysis of RIA data using the concepts of statistical calibration. The analysis is broken into three parts; the specification of the calibration model, the estimation of the parameters of the calibration model and the estimation of hormone concentration in the patients. Robust techniques are used in the parameter estimation to minimize the effect of outliers which frequently occur in the standards data. Because of the small number of observations

available for the estimation of the parameters, less than 16 , simulations are used to determine the properties of the parameter estimates in Section 3 and the calibration curve confidence bands obtained in Section 4. The techniques developed are applied to 124 RIA data which were kindly supplied to us by Dr. J. Steinbach of the Veterans Administration Hospital, Buffalo, New York.

2.   Calibration Model

The proper identification of the calibration model is crucial to the analysis of RIA data. If the model does not adequately describe the standards data, there can be little confidence in the accuracy of the hormone concentration estimates for patients. Many models have been proposed for calibrating purposes. Among the models are the theoretical hyperbola of Yalow and Berson [1968] and Ekins, Newman and O'Riordan [1968], low order polynomials (Ekins, et al [1968], Taljedal and Wold [1970]), and a "statistical" model proposed by Meinert and McHugh [1968]. These models, as well as many others, have not been widely accepted either because they are too complicated for routine clinical use or because they are not applicable to a wide variety of hormones.

The calibration curve which has been most widely adapted for use in RIA, which we now explain, is the logit model of Rodbard and others [1968], [1970], [1971]. Let y_i be the counts bound corresponding to dose $x_i \neq 0$. Define Y_0 to be the zero dose counts and N to be the background counts. Y_0 and N , which are mean values of observed counts,

are assumed to be fixed constants. Let a new response variable y_i' be defined as

$$y_i' = (y_i - N)/(Y_0 - N) \qquad (1)$$

After making certain critical assumptions about the chemical reactions of the RIA process, Rodbard and Lewald [1970] show that

$$\text{logit } y_i' = \log\left(y_i'/(1 - y_i')\right) = \alpha' + \beta' \log x_i + \epsilon_i \qquad (2)$$

where the ϵ_i have zero mean and variance σ_i^2. A weighted least squares algorithm in which the weight for each observation is the reciprocal of its standard deviation is proposed for the estimation of α', β' and σ^2. Rodbard and Cooper [1970] suggest that the variance of logit y_i' can be expressed as a quadratic function of the observed counts. An example of the fit provided by this method is presented in Figure 1.

(Figure 1 about here)

Although the logit transformation appears to provide an adequate model for calibrating purposes, it is not without serious deficiencies. Since Y_0 and N are measured quantities, they are subject to the same errors as the other standards data. To assume these quantities to be constant is an oversimplification of the problem. In addition, by assuming Y_0 fixed and taking logarithms of dose, important information about the

zero dose region of the curve is lost. This becomes particularly critical in instances such as pregnancy detection when the low dose region of the calibration curve is of primary importance. More disturbing, is the fact that too frequently the transformed data are not linear as in Figure 2, so that the fitted straight line does not properly represent the data. Furthermore, the y_i' may not be between 0 and 1. The possible ramification of the poor fit are indeed serious since the standard curve often provides the basis for the treatment of patients.

(Figure 2 about here)

Since the logit transformation, as well as other linearization methods, do not consistently provide acceptable fits of the standard data, we examined scatter plots of Y (counts bound) vs. X (known concentration) from 124 data sets. These data sets include standards data from the assay of Digoxin, Folic Acid, Gastrin, Insulin, Renin, TSH, T3 and Vitamin B-12. The scatter plots suggest, as a calibration model, the modified hyperbola

$$y_i = \alpha + \beta/(1 + \gamma x^\delta) + e_i \tag{3}$$

where the e_i have zero mean and dispersion σ^2. Figure 3 illustrates the excellent fit of equation (3) to Insulin standards data. The dashed curve, which is the fitted logit, is included for comparison.

(Figure 3 about here)

Modelling the RIA standard data by the modified hyperbola has a number of advantages. Since the curve is based on empirical observations about the data and not on the chemical reactions underlying RIA, equation (3) is not restricted by the unreasonable assumptions (Feldman [1972]) imposed on other "theoretical" curves. The key assumption we make is that the mean response Y decreases monotonically as hormone concentration increases. This assumption seems reasonable and assures a 1 to 1 relationship between counts and hormone concentration. Equation (3) also allows Y_0 to be included in the data set used to estimate the parameters of the model. As we have already indicated, this is of particular importance to the clinician concerned with the low dose region of the calibration curve.

Another feature of the modified hyperbola is that this model yields parameters which have physical interpretations. $\hat{\alpha}$, the estimated value of α, is an estimate of background counts or noise. $\hat{\beta}$ estimates the zero dose counts. The midrange of the assay, also referred to as the effective dose for 50% response (Rodbard and Hutt [1974]), is estimated by $\hat{\gamma}$. The estimated value of δ, which is in the neighborhood of 1.0, provides an indication of the sharpness in the bend of the curve. The larger the value of $\hat{\delta}$, the more sharp is the bend in the curve. It is important to note that for some hormones such as Gastrin and Vitamin B-12, the parameter δ can be set to 1.0 without altering the fit significantly. The resulting model is the rectangular hyperbola proposed by Bliss [1970]

and Taljedal and Wold [1970].

If one assumes zero error in measurement of Y , it is easily shown that equation (2) and equation (3) are mathematically identical. Taking anti-logarithms of equation (2),

$$y_i' / (1 - y_i') = e^{\alpha'} x_i^{\beta'} \tag{4}$$

Solving for y_i ,

$$y_i = N + \left(Y_0 - N\right) / (1 + e^{\alpha'} x^{-\beta'}) \tag{5}$$

which is identical to equation (3) where $N = \alpha$, $(Y_0 - N) = \beta$, $\alpha' = -\log \gamma$ and $\beta' = -\delta$. The difference between the two models is the error structure which is assumed and the added flexibility of equation (3) which is gained by estimating zero counts and background noise.

We are not alone in using the modified hyperbola to model the calibration curve. Independent of our research, Rodbard and Hutt [1974] have proposed the same calibration model. In addition, Finney [1976] observes that equation (3) can be expressed in terms of the logistic model which he uses as a calibration model.

Having selected a calibration model for RIA, an algorithm for parameter estimation is required. In most instances, the ordinary least squares fit is adequate. (Tests for equality of variance suggest that the assumption of homoscedacity is valid for the 8 hormones considered (Tiede [1976])). However, too often, observations which are obviously in

gross error are found in the standard data. This is illustrated in Figure 4. The least squares fit of the data (dashed line) is unacceptable since it is overly influenced by the outlier observations. Thus, a procedure which will not be overly influenced by outliers is desirable. As Finney [1976] states, "A computer program for routine analysis of RIA in a clinical environment must be designed for use by non-statisticians, with protection against gross errors arising from uncritical acceptance of data on output." For these reasons, we propose the following method for the estimation of the parameters of equation (3).

3. Robust Nonlinear Regression

Robust estimation techniques provide an alternative means of minimizing the influence of outlier observations in a set of data. These methods also provide least squares-type estimates in the absence of outlier observations. Andrews [1974a] and Relles [1968] report success in obtaining M-estimates (Huber [1964, 1972]) of parameters in the linear model. In this section, we present an algorithm for estimating parameters in the nonlinear model such as equation (3).

Consider a continuous function $y = f(x, \theta)$ which is nonlinear in the parameter vector θ. The Gauss-Newton algorithm can be used to obtain the least squares estimate of θ which satisfies

$$\sum_{i=1}^{n} f'(x_i, \theta) \; \varphi\left(y_i - f(x_i, \theta)\right) = 0 \qquad j = 1, \ldots, p \qquad (6)$$

where $f'(\cdot, \cdot)$ is the derivative of f with respect to $\underset{\sim}{\theta}$;

p is the length of the vector $\underset{\sim}{\theta}$

and $\varphi(z) = z$.

The M-estimate of $\underset{\sim}{\theta}$ is obtained by replacing $\varphi(z) = z$ with an alternative function of z . Many functional forms for φ have been proposed (Hampel [1974], Huber [1964], Tukey [1960]). In this paper, we concentrate on the SINE function (Andrews, et al [1972])

$$\varphi(z) = \begin{cases} \sin(z/sc) & |z| \leq \pi sc \\ 0 & |z| > \pi sc \end{cases} \tag{7}$$

where s is an estimate of scale and c is a constant which influences the robustness of the resulting estimates. Assuming an underlying Gaussian distribution, Andrews [1974b] indicates that the efficiency of the SINE estimate with $c = 2.1$ relative to the least squares estimate is 96%.

The SINE estimate of $\underset{\sim}{\theta}$ is easily obtained in an iteratively reweighted algorithm similar to the Gauss-Newton least squares algorithm. Denote the result of the kth iteration by $\underset{\sim}{\theta}^{(k)}$. Expanding $f(\underset{\sim}{x}, \underset{\sim}{\theta})$ in a Taylor Series and assuming the remainder term is negligible, $\underset{\sim}{\theta}^{(k+1)}$ is the solution to the set of equations

$$\sum_{i=1}^{n} \left(F(\underset{\sim}{\theta}^{(k)}) \right)_{ij} w_i^{(k)} \left(v_i - \sum_{j=1}^{p} F(\underset{\sim}{\theta}^{(k)})_{ij} \theta_j^{(k+1)} \right) = 0 \tag{8}$$

where

$$F(\underset{\sim}{\theta}) = \frac{\partial f(x_i, \underset{\sim}{\theta})}{\partial \theta_j}$$

$$r_i^{(k)} = \left(y_i - f(t_i, \underset{\sim}{\theta}^{(k)})\right)/s^{(k)}$$

$$w_j^{(k)} = \varphi(r_i^{(k)})/r_j^{(k)}$$

$$v_i^{(k)} = s^{(k)} r^{(k)} + \sum_{j=1}^{p} \left(F(\underset{\sim}{\theta}^{(k)})\right)_{ij} \hat{\theta}_j^{(k)}$$

and $s^{(k)}$ is an appropriate estimate of scale.

Iterations continue until a convergence criterion is satisfied.

The covariance matrix of $\underset{\sim}{\theta}$ is estimated by

$$\hat{\underset{\sim}{\chi}} = \left(ns/(n-p)\right)^2 \left[\sum_{i=1}^{n} \varphi^2(z_i) / \left(\sum_{i=1}^{n} \varphi'(z_i)\right)^2\right] K(\underset{\sim}{F}^T \underset{\sim}{W} \underset{\sim}{F})^{-1} \qquad (9)$$

where

$$F_{ij} = \frac{\partial f(x_i, \hat{\underset{\sim}{\theta}})}{\partial \theta_j}$$

$$z_i = y_i - f(x_i, \hat{\underset{\sim}{\theta}})$$

$$K = s/n\left(\sum_{i=1}^{n} \varphi(z_i)/z_i\right)$$

and

$$w_{ij} = \begin{cases} \text{final "weight" of } i\underline{th} \text{ observation} & j = i \\ 0 & j \neq i \end{cases}$$

This is the nonlinear analog of the parameter covariance matrix in the linear model proposed by Huber [1973] and Welsch [1975] where the design matrix $\underset{\sim}{X}$ is replaced by the Jacobean $\underset{\sim}{F}$. The factor K is an adjustment factor to account for the asymptotic bias in the estimate (Mallows [1975], Gross [1975].

Estimates of the parameter vector in the RIA calibration model are obtained using the robust algorithm with $c = 2.1$ and $s = \text{median} \{\text{largest } (n - p + 1) |y_i - f(x_i, \hat{\underset{\sim}{\theta}})|\}$. In only 9 of the 124 cases did the parameters fail to converge. Inspection of the 9 data sets revealed that the data contained too many outlier observations. Time for convergence for the other data sets took about 1.5 times as much CPU time on a CDC-6400 as that required to obtain the least squares estimates using Gauss-Newton. Most of the excess time is due to the need to compute the sine function for each observation at each iteration.

(Figures 4 & 5 about here)

Figures 4 and 5 are typical of the results for the data sets examined. When outliers are present in the data, as in Figure 4 , the robust fit is unaffected by their presence. This is indicated by the zero "weight" assigned to the observations in Table 1 . The least squares

fit (dashed line), on the other hand, is severly influenced by the out-liers.   In the case where there are no apparent outliers in the data, the robust and least squares fits are virtually indistinguishable, as indicated by the single solid line in Figure 5. In this case, all "weights" are relatively large (Table 2).

(Tables 1 and 2 about here)

A simulation was conducted to determine the distribution of $\hat{\underset{\sim}{\theta}}$, the M-estimate of $\underset{\sim}{\theta}$. Typical RIA standard data containing 0, 1 or 2 outliers were generated using known values for the parameters in equation (3). $\hat{\theta}$ was then estimated using the algorithm defined above. The results of the simulation indicate that the parameter estimates are normally distributed about the true parameter value.

As in all nonlinear regression problems, initial estimates of the parameters are required for the parameter estimation algorithm. In RIA the initial estimates of α , β and γ are conveniently obtained from the following relationships:

$$f(\infty,\beta) = \alpha \text{ if } \delta \geq 0 \Rightarrow \alpha_0 = \text{mean background count}$$

$$f(0,\beta) = \alpha + \beta \Rightarrow \hat{\beta}_0 = \left(\text{mean 0 count}\right) - \alpha_0$$

$$\frac{\partial f}{\partial x} (0,\beta) = -\beta\gamma \text{ if } \delta = 1 \Rightarrow \gamma_0 = -\beta_0^{-1} \text{ x (slope of curve near x = 0) .}$$

For the data analyzed, we have found $0.5 \leq \delta \leq 2.5$. Thus, we suggest that $\delta_0 = 1$ provides an adequate initial estimate of δ .

4.   Estimation of Hormone Concentration

Having addressed two of the three aspects of RIA data analysis, we turn to the final and most important, the estimation of hormone concentration in the patients. Point estimates are easily obtained by solving

$$y_{obs} = \hat{\alpha} + \hat{\beta}/(1 + \hat{\gamma}x^{\hat{\delta}}) \tag{10}$$

for x . If $y_{obs} > \hat{\alpha} + \hat{\beta}$ or $y_{obs} < \hat{\alpha}$, we suggest that $\hat{x}$ be set to 0 or ∞ , respectively, as y_{obs} is beyond the calibration limits. One means of correcting for $y_{obs} < \hat{\alpha}$ is to dilute the X sample by same known amount, and then redetermine y_{obs} . This should produce a $y_{obs} > \alpha$.

Because of the large number of hormone predictions per assay; construction of confidence intervals for $\hat{x}$ is not easily accomplished. To illustrate this, consider the case of simple linear regression. The $100(1 - \alpha)\%$ confidence interval for $\hat{x}$, in this case, is obtained by solving for x_i^*

$$(y_{obs} - a - bx_i^*) \leq (kF_{k,n-2}) \; s^2 \; (1 + \frac{1}{n} + (x_i^* - \bar{x})/\sum_j (X_j - \bar{x})^2) \; ; \tag{11}$$
$$i = 1,\ldots,k$$

where k is the total number of predictions to be made. This procedure is adequate for small k . However, when k is large, as in RIA, the critical constant $kF_{k,n-2}$ becomes so large, the resulting confidence intervals are noninformative. Thus, an alternative approach, independent of k , is required.

Scheffé [1973] and Lieberman, Miller and Hamilton [1967] present procedures for obtaining tolerance like interval estimates on $\hat{x}$ when k is large. Although the Scheffé procedure produces narrower intervals than does the method of Lieberman, et al (Scheffé [1973]), we pursue the latter method because of the ease with which it is extended to the non-linear problem and the fact that the application of this method does not require too much decision making by the clinician.

To extend the results of Lieberman, et al to RIA, it is first necessary to calculate confidence bands for the fitted calibration curve. In the multiple linear model, Miller [1966] shows that the $100(1-\alpha)\%$ confidence bands for the fitted line are defined by

$$\underset{\sim}{X}^T\underset{\sim}{\beta} \in \underset{\sim}{X}^T\hat{\underset{\sim}{\beta}} \pm (pF_{p,n-p}^{\alpha})^{\frac{1}{2}}(\underset{\sim}{X}^T\text{cov}(\hat{\underset{\sim}{\beta}})\ X)^{\frac{1}{2}} \tag{12}$$

where $\underset{\sim}{X}$ is the n x p design matrix and $\hat{\underset{\sim}{\beta}}$ is the p-vector of coefficients. Replacing $\underset{\sim}{X}$ by the Jacobean $\underset{\sim}{F}$, we propose that the $100(1-\alpha)\%$ confidence bands for the RIA calibration curve are defined by

$$f(\underset{\sim}{x},\underset{\sim}{\theta}) \in f(\underset{\sim}{x},\hat{\underset{\sim}{\theta}}) \pm (pF_{p,n-p}^{\alpha})(\underset{\sim}{F}^T\hat{\underset{\sim}{V}}\underset{\sim}{F}) \tag{13}$$

where $\hat{\underset{\sim}{V}}$ and $\underset{\sim}{F}$ are as defined in equation (9). Confidence bands constructed in this fashion are presented in Figure 6.

(Figure 6 about here)

The validity of equation (13) is supported through simulation (Tiede [1976]). Confidence bands with α set to .05 and .10 are obtained for simulated RIA standard data containing 0, 1 and 2 outliers. The simulation revealed no abnormalities which would result in the rejection of the hypothesis that the confidence bands for the RIA calibration curve are defined by equation (13).

To complete the construction of interval estimates on $\hat{x}$, define

$$\tilde{s} = (n - p - m)^{-1} \sum_{i \ni w_i \pm 0} (r_i - \bar{r})^2 \tag{14}$$

where

$$\bar{r} = (n - m)^{-1} \sum_{i \ni w_i \neq 0} (y_i - \hat{y}_i) = (n - m)^{-1} \sum_{i \ni w_i \neq 0} r_i$$

and m is the number of zero weights. Assuming the $100\gamma\%$ confidence interval on the mean response $\epsilon(y_i) = \alpha + \beta/(1 + \gamma x^\delta)$ is contained in the interval

$$y \pm Z_\gamma \tilde{s} \left((n - p - m) / \chi^2_{n-p-m, \alpha/2} \right)^{\frac{1}{2}} \tag{15}$$

with probability $1 - \alpha/2$, equations (13) and (15) can be combined to produce interval estimates of the unknown hormone. The lower limit, $\hat{x}_L$, is the solution to

$$f(\hat{x}_L, \hat{\theta}) - (pF_{p,n-p})^{\frac{1}{2}} (\underset{\sim}{F}^T \underset{\sim}{V} \underset{\sim}{F})^{\frac{1}{2}} = y + Z_\gamma \tilde{s} \left((n - p - m) / \chi^2_{n-p-m, \alpha/2} \right)^{\frac{1}{2}} \tag{16}$$

and the upper limit is the solution to

$$f(\hat{x}_y, \hat{\underline{\theta}}) + (pF_{p,n-p}^{\alpha/2})^{\frac{1}{2}}(\underline{F}\,\underline{V}\,\underline{F})^{\frac{1}{2}} = y - Z_\gamma \tilde{s}\left((n-p-m)/\chi_{n-p-m,\alpha/2}^2\right)^{\frac{1}{2}} \qquad (17)$$

By virture of the Bonferroni inequality, intervals constructed in this manner are such that at least $100\gamma\%$ of the intervals will contain the true concentration with confidence $1 - \alpha$. The bracketed interval in Figure 7 typifies intervals constructed by this algorithm.

(Figures 7 and 8 about here)

In the calculation of the interval estimates for $\hat{x}$, care must be taken, as in the point estimation of x, to insure that estimates are not made beyond the limits established by the calibration curve. Figure 8, in which the calibration curve and corresponding confidence bands are divided into five distinct sections, illustrates this concept. Hormone estimates which can be made in each section are presented in Table 3. In section 3, for example, estimates of x and the upper limit on $\hat{x}$, $\hat{x}_L$, can be obtained, but the lower limit on $\hat{x}, \hat{x}_L$, cannot be estimated. Since x cannot be less than zero, $\hat{x}_L$ is set equal to zero in this instance. Each calibration curve uniquely defines the estimation limits.

(Table 3 about here)

5.   <u>Conclusion</u>

In this paper, we have thus presented a procedure for the analysis of radioimmunoassay data. In addition to presenting a family of curves to model RIA standards data, as many others have done, we also pursue the construction of confidence interval for estimates of hormone concentration in patients. Unlike Finney [1976] who views the data analysis problems in terms of bioassay data analysis, we apply concepts of statistical calibration to radioimmunoassay. Because of the frequency at which outlier observations appear in clinical data, we recommend fitting the calibration curve by a robust nonlinear regression algorithm. The algorithm here has the virtue of being easily adopted to a standard nonlinear least squares program.

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TABLE 1

Hormone concentration, X, observed counts, Y, fitted counts, $\hat{Y}$, and final "weights," W, for TSH standards curve in Figure 4.

X	Y	$\hat{Y}$	W
0.0	5422.0	5357.1	.94
0.0	5311.0		.97
2.0	4034.0	4224.7	.57
2.0	4257.0		.99
5.0	3033.0	2992.7	.98
5.0	3138.0		.73
10.0	1864.0	1918.8	.96
10.0	1849.0		.93
20.0	1108.0	1055.9	.96
20.0	1012.0		.97
50.0	412.0	406.6	1.00
50.0	450.0		.97
100.0	123.0	182.4	.95
100.0	214.0		.99

TABLE 2

Hormone concentration, X , observed counts, Y , fitted counts, $\hat{Y}$, and final "weights," W , for TSH standards curve in Figure 5 .

X	Y	$\hat{Y}$	W
0.0	7720.0	8123.9	.00
0.0	8113.0		1.00
2.0	6664.0	6720.8	.96
2.0	6804.0		.91
5.0	4994.0	4962.3	.99
5.0	4948.0		1.00
10.0	3410.0	3397.1	1.00
10.0	3208.0		.57
20.0	4478.0	2184.2	.00
20.0	2396.0		.48
50.0	1302.0	1341.1	.98
50.0	1377.0		.98
100.0	1025.0	1076.9	.96
100.0	1096.0		.99

TABLE 3

Estimates of unknown hormone concentration and corresponding lower and upper limits which can be obtained in each section of Figure 8.

Section	Lower	Hormone Concentration Estimate	Upper
1	0	0	0
2	0	0	$\hat{x}_u$
3	0	$\hat{x}$	$\hat{x}_u$
4	$\hat{x}_\ell$	$\hat{x}$	$\hat{x}_u$
5	∞	∞	∞