Peter Dalgaard Søren Johansen

The Asymptotic Properties of the Cornish-Bowden-Eisenthal Median Estimator

Preprint
February
1986

2

Institute of Mathematical Statistics
University of Copenhagen

Peter Dalgaard* and Søren Johansen

THE ASYMPTOTIC PROPERTIES OF THE CORNISH-BOWDEN EISENTHAL MEDIAN ESTIMATOR

Preprint 1986 No. 2

INSTITUTE OF MATHEMATICAL STATISTICS
UNIVERSITY OF COPENHAGEN

February 1986

Abstract:

Conditions are found for the median estimator of Cornish-Bowden and Eisenthal to be asymptotically normally distributed, and expressions are found for the asymptotic bias and variance.

It is seen that the bias is in general of the order of the square root of the number of observation points times the standard deviation of a single measurement. The results are compared with published simulation results.

Key words:

Median estimator. Direct linear plot. Michaelis Menten parameters. Nonparametric estimation. Nonlinear regression.

1. Introduction and summary.

The purpose of this paper is to find the asymptotic properties of the median estimator of Cornish-Bowden and Eisenthal (1974), (1978) and Eisenthal and Cornish-Bowden (1974) for the Michaelis Menten parameters.

The Michaelis Menten relation is given by

$$v = \frac{V_{max}\, c}{K_m + c} \tag{1.1}$$

and expresses the relation between the velocity (v) of an enzyme reaction and the concentration (c) of the substrate. The parameters are V_{max}, the maximal reaction velocity, and K_m the chemical affinity.

We shall consider a design given by concentrations $c_1<c_2<\ldots<c_n$, where at each concentration an independent measurement of the velocity is taken, giving the data $v_1,\ldots,v_n$. The purpose is to estimate the parameters V_{max} and K_m.

In (1974) the following estimators were proposed by Eisenthal and Cornish-Bowden:

For each pair of points (c_i,v_i) and (c_j,v_j) we fit a curve of the form (1.1) and calculate

$$\tilde{K}_{mij} = \frac{v_j - v_i}{v_i/c_i - v_j/c_j}$$

and

$$\tilde{V}_{max\ ij} = \frac{c_i - c_j}{c_i/v_i - c_j/v_j} .$$

Note that (1.1) is equivalent to

$$\frac{1}{v} = \frac{1}{V_{max}} + \frac{K_m}{V_{max}} \cdot \frac{1}{c}$$

which gives a simple geometric interpretation of V_{max} and K_m. The estimates are then combined by taking the medians:

$$\tilde{K}_m = \operatorname*{med}_{i<j} \tilde{K}_{mij} \qquad (1.2)$$

$$\tilde{V}_{max} = \operatorname*{med}_{i<j} \tilde{V}_{maxij} \;. \qquad (1.3)$$

If $v_i/c_i < v_j/c_j$ then $v_j > v_i$ and the above construction yields negative values of $\tilde{K}_{mij}$ and a modified version was suggested by Eisenthal and Cornish-Bowden (1978) as follows:

$$K^*_{mij} = \begin{cases} \tilde{K}_{mij} & \text{if} \quad v_j/c_j < v_i/c_i \\ \infty & \text{if} \quad v_j/c_j > v_i/c_i \end{cases}$$

$$V^*_{maxij} = \begin{cases} \tilde{V}_{maxij} & \text{if} \quad v_j/c_j < v_i/c_i \\ \infty & \text{if} \quad v_j/c_j > v_i/c_i \end{cases}$$

and finally

$$K^*_m = \operatorname*{med}_{i<j} K^*_{mij} \qquad (1.4)$$

$$V^*_{max} = \operatorname*{med}_{i<j} V^*_{maxij} \qquad (1.5)$$

It was also suggested that one could estimate K_m/V_{max} and $1/V_{max}$ directly as follows:

$$\hat{K}_m/\hat{V}_{max} = \underset{i<j}{\text{med}} \frac{1/v_i - 1/v_j}{1/c_i - 1/c_j} \tag{1.6}$$

$$1/\hat{V}_{max} = \underset{i<j}{\text{med}} \frac{c_i/v_i - c_j/v_j}{c_i - c_j} \tag{1.7}$$

and then calculate

$$\hat{K}_m = (\hat{K}_m/\hat{V}_{max})/(1/\hat{V}_{max})$$

$$\hat{V}_{max} = 1/(1/\hat{V}_{max}) \ .$$

The estimators (1.6) and (1.7) have been investigated by simulation methods by Cornish-Bowden (1981), whereas Currie (1982) and Atkins and Nimmo (1975) investigate the unmodified estimators (1.2) and (1.3).

What we would like to do is to find the asymptotic distribution of these estimators. This would make the comparison with other estimators easier and supplement the simulation results.

The methods we use is a simple application of the theory of U-statistics. This method has been applied before by Sen (1968) to the estimation of the slope in a linear regression, and it is straightforward to apply the same technique to this more complicated situation.

The asymptotic theory of U-statistics was developed by Hoeffding (1948) and his results can be applied directly to the present situation.

The reason that the problems and results are somewhat more difficult in the non linear regression (1.1) is that different transformations of the data appear, depending on which parameter one wants to estimate, and these transformations of the data destroy the symmetry of the distributions, thus giving rise to a bias in the estimators.

We find that it is possible to put reasonable conditions on the design such that the estimators are asymptotically normally distributed, and we find expressions for the bias and the variance. It turns out that in general these estimators are asymptotically biased with a relative bias of the order of $\sqrt{n}\,\tau$, where τ^2 is the variance of a single measurement.

It thus appears dangerous to use these estimates without making a careful investigation of the error distributions, and if one can do that, it would appear more reasonable to apply the model based maximum likelihood estimator which has a relative bias of the order $\tau/\sqrt{n}$ and a smaller variance.

Finally some comments on the literature. The estimators of the type considered were suggested by Theil (1950) for linear regression and investigated by Sen (1968) using the technique described here.

Similar estimators have been investigated for linear regression by Johnstone and Velleman (1984) as well as by Bhattacharya, Chernoff and Yang (1983), who use a weighted U-statistic, in the case where the observations were truncated. In a paper by Scholz (1978) a weighted median regression estimator is investigated for linear regression. Finally Daniels (1954) used similar ideas to derive tests for the case of linear regression.

The paper is now organised as follows: In section 2 we give the relation between the asymptotic distribution of a class of median estimators and the theory of U-statistics.

In the next sections we apply the results of section 2 to the situation where the statistics are derived from i.i.d. random variables (section 3) or independent symmetric but not necessarily identically distributed variables (section 4). For the comparison with published results it is convenient to have a framework in which both K_m and V_m can be discussed and the most convenient one is that of the random variables being transformed Gaussian variables with a small variance. Thus the asymptotics is formulated as $n\to\infty$ and $\tau^2\to 0$, where τ^2 is the variance of single measurement. This is done in section 5 and finally the results are spelled out in section 6 for the estimators $K_m^*, V_{max}^*, \hat{K}_m/\hat{V}_{max}, 1/\hat{V}_{max}$ and $\tilde{K}_m$ and $\tilde{V}_{max}$.

Section 7 contains a few numerical examples where the results are compared with some previously published results.

2. Median estimators and U-statistics.

The basic idea is that results about median estimators of the form discussed in section 1 can be derived from results about U-statistics.

To illustrate the idea of the U-statistics, consider for instance the estimator (1.4). It is easily seen that if K_m^* is the lower median then

$$\{K_m^* \leq x\} = \{ \sum_{i<j} 1[\ln V_j - \ln\{V_{max}\, c_j/(c_j+x)\} \leq \ln V_i - \ln\{V_{max}\, c_i/(c_i+x)\}] \geq \frac{1}{2}\binom{n}{2}\} \tag{2.1}$$

Thus statements about the median can be converted into statements about sums of binary variables which are dependent.

The U-statistics we shall consider here have the form

$$U = \sum_{i<j} \phi(X_i, X_j)/\binom{n}{2}$$

where $X_1, \ldots, X_n$ are independent, $\phi(u,v)=\phi(v,u)$ and $E\phi(X_i, X_j)=0$.

The statistic on the right hand side of (2.1) is then a linear function of a U-statistic if we define

$$X_i = \begin{Bmatrix} \ln V_i \\ \ln\{c_i/(c_i+K_m)\} \end{Bmatrix}$$

and

$$\phi\left\{\binom{u}{a}, \binom{v}{b}\right\} = \text{sign}(u-a-v+b)\,\text{sign}(a-b)$$

where sign $(x) = 21\{x \geq 0\}-1$. We use here the property of the design that if $i<j$ then $c_i<c_j$ and $\ln\{c_i/(c_i+K_m)\} <$ $\ln\{c_j/(c_j+K_m)\}$.

A linear function of a U-statistic will also be called a U-statistic. For later reference we give the relations for the other estimators as well; see (1.5),(1.6) and (1.7).

$$\{V^*_{max} \leq x\} = \{\sum_{i<j} 1\{c_i/V_i - (c_i+K_m)/x \leq c_j/V_j - (c_j+K_m)/x\} \geq \tfrac{1}{2}\binom{n}{2}\} \tag{2.2}$$

$$\{\hat{K}_m/\hat{V}_{max} \leq x\} = \{\sum_{i<j} 1\{1/V_i - x/c_i - 1/V_{max} \leq 1/V_j - x/c_j - 1/V_{max}\} \geq \tfrac{1}{2}\binom{n}{2}\} \tag{2.3}$$

$$\{1/\hat{V}_{max} \leq x\} = \{\sum_{i<j} 1\{c_i/V_i - xc_i - K_m/V_{max} \geq c_j/V_j - xc_j - K_m/V_{max}\} \geq \tfrac{1}{2}\binom{n}{2}\} \tag{2.4}$$

Note that the relations (2.2) and (2.4) are equivalent.

We shall throughout work with lower medians, and since only asymptotic results are considered, the same results will hold for the upper median.

The statistics on the right hand side of (2.1)-(2.4) are U-statistics of the form

$$U_n(x) = \sum_{i<j} 1\{U_i-u_i(x) \geqq U_j-u_j(x)\} \tag{2.5}$$

where $U_1,\ldots,U_n$ are independent random variables and the functions $u_1(\cdot),\ldots,u_n(\cdot)$ are smooth functions, such that $u_i(x)-u_j(x)$ is decreasing in x, and such that $u_i(x)$ is monotone in i.

Note that U_i is a suitable transform of c_i and V_i and that $u_i(\cdot)$ only depends on c_i, thus $(U_i,u_i(\cdot))$ are related to the i'th experiment only. Note also that different transformations are needed to bring the estimates for K_m and V_{max} into the standard form (2.5).

Thus the results we obtain under the assumption of symmetry, say, of the distribution of $U_i = \ln V_i$ (section 4) can be applied to the estimator K_m^*, but not to V_{max}^*, since then c_i/V_i would not have a symmetric distribution.

This is the reason for working in section 5 with the assumption that U_i is transformed Gaussian, since if $\ell n\, V_i$ is transformed Gaussian then so is c_i/V_i, and the results obtained can be applied to both the estimator V_{max}^* and K_m^*.

In this section, however, we shall first give a theorem which is a special case of a result of Hoeffding (1948) on the asymptotic distribution of U-statistics, and then we shall give conditions on the moments of $U_n(x)$ which will guarantee that the median estimate K'_n defined by

$$\{K'_n \leq x\} = \{U_n(x) \geq \frac{1}{2}\binom{n}{2}\} \qquad (2.6)$$

will be asymptotically normally distributed.

Note that if $u_i(x) - u_j(x)$ is decreasing in x, then $U_n(x)$ is right continuous and increasing and

$$K'_n = \sup\{x \mid U_n(x) < \frac{1}{2}\binom{n}{2}\}. \qquad (2.7)$$

Theorem 2.1 Let $X_1,\ldots,X_n$ be independent random variables, and let $\phi(u,v)$ be symmetric and bounded, then

$$U_n = \sum_{i<j} \phi(X_i,X_j)$$

is asymptotically normally distributed with parameters $\{E(U_n),V(U_n)\}$ provided

$$\lim_{n\to\infty} V(U_n)/n^3 = a > 0. \qquad (2.8)$$

Proof. The result is a corollary of Theorem 8.1 of Hoeffding (1948), since the variables

$$\bar{\psi}_{1(i)}(X_i) = \frac{1}{n-1}\sum_{j\neq i} E\{\phi(X_i,X_j)\mid X_i\} - E\{\phi(X_i,X_j)\}$$

are bounded uniformly in n and i and hence satisfy the Ljapunov condition.

To apply the result to $U_n(x)$ and K'_n we introduce $\mu_n(x) = E\{U_n(x)\}$ and $\sigma_n^2(x) = V\{U_n(x)\}$.

Note that $\mu_n(x)$ is increasing, and we shall assume further, that $\mu_n(x)$ is continuously differentiable, that $\sigma_n^2(x)$ is continuous, and that there exists a unique point κ_n, such that $\mu_n(\kappa_n) = \frac{1}{2}\binom{n}{2}$. Further let $\mu_n'(\kappa_n)>0$.

Theorem 2.2.

If the conditions

$$\sigma_n^2(\kappa_n)/n^3 \to a>0, \quad n\to\infty \tag{2.9}$$

$$\sigma_n^2(\kappa_n+x\delta_n)/\sigma_n^2(\kappa_n)\to 1, \quad n\to\infty \tag{2.10}$$

$$(\mu_n(\kappa_n+x\delta_n)-\mu_n(\kappa_n))/x\delta_n\mu_n'(\kappa_n)\to 1, \quad n\to\infty \tag{2.11}$$

are satisfied, with $\delta_n=\sigma_n(\kappa_n)/\mu_n'(\kappa_n)$ then K_n' defined by (2.7) is asymptotically normal with parameters (κ_n,δ_n^2).

Proof. We find

$$P\{(K_n'-\kappa_n)/\delta_n \leq x\} = P\{K_n' \leq \kappa_n+x\delta_n\} = P\{U_n(\kappa_n+x\delta_n) \geq \tfrac{1}{2}\binom{n}{2}\}$$

$$= P\left\{\frac{U_n(\kappa_n+x\delta_n)-\mu_n(\kappa_n+x\delta_n)}{\sigma_n(\kappa_n+x\delta_n)} \geqq \frac{\mu_n(\kappa_n)-\mu_n(\kappa_n+x\delta_n)}{\sigma_n(\kappa_n+x\delta_n)}\right\}$$

We want to apply Theorem 2.1 to the left hand side, and define

$$\phi_{ij}(u,v) = 1\{u-u_i(x) \geq v-u_j(x)\}-P\{U_i-u_i(x) \geq U_j-u_j(x)\}.$$

Then $|\phi_{ij}(u,v)| \leq 1$, $E\{\phi_{ij}(U_i,U_j)\} = 0$ and conditions (2.9) and (2.10) ensures that the variance condition (2.8) is satisfied.

The right hand side converges to $-x$ by condition (2.10), (2.11) and the definition of δ_n. This shows that

$$P\{(K'_n-\kappa_n)/\delta_n \le x\} \to P\{W \ge -x\} = 1-\phi(-x) = \phi(x),\ n\to\infty$$

where W is normally distributed, and ϕ is its distribution function. This completes the proof of Theorem 2.2 and the next sections describe some situations, where the conditions of Theorem 2.2 can be verified.

3. The asymptotic properties of the median estimators for independent identically distributed variables.

We shall consider the estimates K'_n given by (2.7) where $U_n(x)$ is given by (2.5). Now assume that there exists a κ, such that the distribution function of $U_i-u_i(\kappa)$ is given by F, which has density f with continuous derivative f',where both f and f'are bounded. We shall call such an F smooth.

In order to prove the results in this and the following sections we need the quantities:

$$\pi_{ij}(x) = P\{U_i-u_i(x) \ge U_j-u_j(x)\} = \int F(u+a_{ij}(x))F(du) \qquad (3.1)$$

$$\pi_{ijk}(x) = P\{U_i-u_i(x) \ge U_j-u_j(x) \ge U_k-u_k(x)\}$$

$$= \int [1-F\{u-a_{ij}(x)\}]F\{u-a_{kj}(x)\}F(du) \qquad (3.2)$$

where $a_{ij}(x) = u_j(x)-u_j(\kappa)-u_i(x)+u_i(\kappa)$.

It then follows that

$$\mu_n(x) = E\{U_n(x)\} = \sum_{i<j} \pi_{ij}(x) \qquad (3.3)$$

$$\sigma_n^2(x) = V\{U_n(x)\} = \sum_{i<j} \pi_{ij}(x)(1-\pi_{ij}(x))+4 \sum_{i<j<k} \pi_{ijk}(x)+$$

$$2 \sum_{i<j<k} \{\pi_{ik}(x)-\pi_{ij}(x)\pi_{jk}(x)-\pi_{ij}(x)\pi_{ik}(x)-\pi_{ik}(x)\pi_{jk}(x)\} \quad (3.4)$$

and further

$$\mu_n'(x) = \sum_{i<j} \int f\{u+a_{ij}(x)\}f(u)du \; a_{ij}'(x) \quad (3.5)$$

$$\mu_n''(x) = \sum_{i<j} \int f'\{u+a_{ij}(x)\}f(u)du \; (a_{ij}'(x))^2 \quad (3.6)$$

$$+ \sum_{i<j} \int f\{u+a_{ij}(x)\}f(u)du \; a_{ij}''(x)$$

$$\pi_{ijk}'(x) = \int f\{u-a_{ij}(x)\}F\{u-a_{kj}(x)\}F(du) \; a_{ij}'(x) \quad (3.7)$$

$$-\int [1-F\{u-a_{ij}(x)\}]f\{u-a_{kj}(x)\}F(du)a_{kj}'(x).$$

Notice, that the assumption, that $u_i(x)-u_j(x)$ is decreasing in x implies, that $a_{ij}(x)$ is increasing in x.

With these results we can now formulate and prove the main result of this section:

Theorem 3.1.

If

$$\frac{1}{n} \sum_i \{u_i'(x)^2+|u_i''(x)|\} < a_1 \quad (3.8)$$

uniformly in x a neighbourhood of κ and uniformly in n, and if

$$\sum_i u_i'(\kappa)(n+1-2i) \geq a_2 n^2 > 0 \qquad (3.9)$$

then, as $n \to \infty$, K_n' is asymptotically normal with parameters κ and

$$\delta_n^2 = \frac{\frac{1}{6}\binom{n}{3} + \frac{1}{4}\binom{n}{2}}{[\sum_i u_i'(\kappa)(n+1-2i)\int f^2(u)du]^2} \qquad (3.10)$$

Proof. Note that for $x = \kappa$ we get from (3.1) that $\pi_{ij}(\kappa) = \frac{1}{2}$, hence, see (3.3), $\mu_n(\kappa) = \frac{1}{2}\binom{n}{2}$, and since $a_{ij}(\kappa) = 0$, we have $\mu_n'(\kappa) =$

$$\int f(u)^2 du \sum_{i<j} a_{ij}'(\kappa) = \int f(u)^2 du \sum_{i<j} (u_j'(\kappa) - u_i'(\kappa))$$

$$= \int f^2(u) du \sum_i u_i'(\kappa)(2i-1-n) \geq n^2 a_2 > 0.$$

Similarly we find from (3.2) that $\pi_{ijk}(\kappa) = 1/6$ and hence that $\sigma_n^2(\kappa) = \frac{1}{6}\binom{n}{3} + \frac{1}{4}\binom{n}{2}$ which gives the result for δ_n stated in (3.10).

We now have to verify the conditions of Theorem 2.2. The condition (2.9) follows directly from the explicit form for $\sigma_n^2(\kappa)$. To check (2.11) note that

$$\gamma_n = \{\mu_n(\kappa + x\delta_n) - x\delta_n\mu_n'(\kappa) - \mu_n(\kappa)\}/x\delta_n\mu_n'(\kappa)$$

$$= \tfrac{1}{2} x\delta_n \mu_n''(\kappa + \tilde{x}\delta_n)/\mu_n'(\kappa) \text{ for some } |\tilde{x}| < |x|.$$

From (3.6) it follows that $\mu_n''(x)$ is bounded by

$$|f'| \sum_{i<j} (a_{ij}'(x))^2 + |f| \sum_{i<j} |a_{ij}''(x)|.$$

In a neighbourhood of κ this is of the order of n^2 by assumption (3.8). Since $\mu_n'(\kappa)$ is also of the order of n^2, and since $\delta_n \to 0$, we have verified condition (2.11).

To check condition (2.10) let

$$\phi_n = \sigma_n^2(\kappa + x\delta_n)/\sigma_n^2(\kappa) - 1 = x\delta_n \sigma_n^{2'}(\kappa + \tilde{x}\delta_n)/\sigma_n^2(\kappa)$$

for some $|\tilde{x}| < |x|$. Now $\sigma_n^2(\kappa)$ is of the order of n^3, and from (3.4), (3.7) and (3.8) we find that $|\sigma_n^{2'}(x)|$ is of the order of n^3 and again δ_n takes ϕ_n to zero, which proves condition (2.10) and hence Theorem 3.1.

Note that in the variance we clearly could do without the term $\frac{1}{4}\binom{n}{2}$, but the term is retained because it is simple to calculate and improves the approximation of the asymptotic distribution to the exact distribution as given by the simulation results. This also holds for the results below.

As an application of Theorem 3.1 let us consider the original problem of estimating K_m in the Michaelis Menten relation. That is, we consider a design $c_1 < \ldots < c_n$, the function $u_i(x) = \ln\{c_i V_{max}/(c_i + x)\}$, and assume that $\ln V_i = \ln\{c_i V_{max}/(c_i + K_m)\} + Z_i$, where $Z_1, \ldots, Z_n$ are i.i.d. with a smooth distribution.

<u>Corollary 3.2.</u>

The Cornish-Bowden Eisenthal median estimate K_m^* given by (1.4) is asymptotically normally distributed with parameters K_m and

$$\delta_n^2 = \frac{\frac{1}{6}\binom{n}{3} + \frac{1}{4}\binom{n}{2}}{[\sum_i (n+1-2i)/(c_i + K_m) \int f^2(u)\,du]^2}$$

provided $n \to \infty$, and the design measure converges to a nondegenerate measure.

Proof. It is easy to check condition (3.8) with $u_i(x) = \ln\{V_{max} c_i/(c_i+x)\}$. Now since c_i is increasing and $1/(c_i+K_m)$ is decreasing we have

$$\sum_i (n+1-2i)/(c_i+K_m) = \sum_{i<n/2} (n+1-2i)\{1/(c_i+K_m)-1/(c_{n+1-i}+K_m)\}$$

$$\geq \sum_{i<[np]} (n+1-2i)\{1/(c_{[np]}+K_m)-1/(c_{[nq]}+K_m)\}$$

$$\simeq n^2 pq\{1/(c_{[np]}+K_m)-1/(c_{[nq]}+K_m)\}$$

where $p+q=1$ and $0<p<\frac{1}{2}$.

If the design measure for $c_1,\ldots,c_n$ does not converge to a one point measure, then one can choose a value of $p \in]0,\frac{1}{2}[$, such that $1/(c_{[np]}+K_m)-1/(c_{[nq]}+K_m) \nrightarrow 0$, and this verifies condition (3.9).

4. The asymptotic distribution of the median estimators for symmetric distributions.

We still consider the estimator K_n' given by (2.7) but now we let $U_i - u_i(\kappa)$ have distribution function F_i, which is assumed to be smooth with a symmetric density $f_i(u) = f_i(-u)$. We define $w_{ij} = \int f_i(u) f_j(u)\,du$ and $\pi_{ijk} = \int\{1-F_i(u)\}F_k(u)F_j(du)$.

Theorem 4.1.

If

$$\frac{1}{n}\sum_i \{|u_i'(x)^2| + |u_i''(x)|\} \qquad (4.1)$$

is bounded uniformly in n and in x in a neighbourhood of κ and

$$\max_i |f_i| \leq a_1 \tag{4.2}$$

$$\lim_{n\to\infty} \frac{1}{n^3} \sum_{i<j<k} (\pi_{ijk} - 1/8) = a_2 > 0 \tag{4.3}$$

$$\sum_{i<j} w_{ij} \{u_j'(\kappa) - u_i'(\kappa)\} \geq a_3 n^2 > 0 \tag{4.4}$$

then K_n' will be asymptotically normally distributed with parameters κ and δ_n^2 defined by

$$\delta_n^2 = \frac{4 \sum_{i<j<k} (\pi_{ijk} - 1/8) + 1/4 \binom{n}{2}}{[\sum_{i<j} w_{ij}(u_j'(\kappa) - u_i'(\kappa))]^2}$$

Proof. We shall first calculate $\mu_n(x)$ and $\sigma_n^2(x)$ in this case and therefore evaluate $\pi_{ij}(x)$ and $\pi_{ijk}(x)$ given by expressions, similar to (3.1) and (3.2).

We find

$$\pi_{ij}(x) = \int F_j\{u + a_{ij}(x)\} F_i(du)$$

which shows that the symmetry of F_i implies that $\pi_{ij}(\kappa)$ $= \int F_j(u) F_i(du) = \frac{1}{2}$, since $a_{ij}(\kappa) = 0$, hence $\mu_n(\kappa) = \frac{1}{2}\binom{n}{2}$. Similarly $\pi_{ijk}(x) = \pi_{ijk}$ for $x = \kappa$, and hence

$$\sigma_n^2(\kappa) = 1/4\binom{n}{2} + 4 \sum_{i<j<k} (\pi_{ijk} - 1/8)$$

This shows that condition (4.3) implies condition (2.9) of Theorem 2.2.

To check the other conditions of Theorem 2.2, we first evaluate, see (3.5),

$$\mu_n'(\kappa) = \sum_{i<j} \int f_j(u) f_i(u)du\, a_{ij}'(\kappa)$$

$$= \sum_{i<j} w_{ij}\{u_j'(\kappa) - u_i'(\kappa)\},$$

which by condition (4.4) is of the order of at least n^2, and hence $\delta_n = \sigma_n(\kappa)/\mu_n'(\kappa) \to 0$.

As in the proof of Theorem 3.1 we define

$$\gamma_n = \tfrac{1}{2}\, x\delta_n \mu_n''(\kappa + \tilde{x}\delta_n)/\mu_n'(\kappa) \text{ for some } |\tilde{x}| < |x|.$$

One finds, see (3.6), that $\mu_n''(x)$ is bounded by

$$\max_i |f_i| \sum_{i<j} \{a_{ij}'(x)^2 + |a_{ij}''(x)|\}$$

which by condition (4.2) and (4.1) is of the order of n^2.

Thus δ_n makes γ_n tend to zero which verifies condition (2.11) of Theorem 2.2.

In a similar way one can check condition (2.10) which shows that the result of Theorem 2.2. implies that of Theorem 4.1.

We shall now apply this result to the case of a Gaussian distribution where a more explicit expression can be derived.

Corollary 4.2.

If U_i is Gaussian with mean $u_i(\kappa)$ and variance σ_i^2, and if $0<b<\sigma_1^2 \leq \ldots \leq \sigma_n^2 < B < \infty$ then conditions (4.1), (4.3), and (4.4) suffice to ensure that K_n' is asymptotically normal with parameters κ and

$$\delta_n^2 = \frac{4 \sum\limits_{i<j<k} [\{(\text{Arccos}\ \rho_{ijk})/2\pi\}-1/8] + 1/4\binom{n}{2}}{[(2\pi)^{-\frac{1}{2}} \sum\limits_{i<j} (\sigma_i^2 + \sigma_j^2)^{-\frac{1}{2}}(u_j'(\kappa) - u_i'(\kappa))]^2}$$

where

$$\rho_{ijk} = \sigma_j^2\{(\sigma_i^2 + \sigma_j^2)(\sigma_j^2 + \sigma_k^2)\}^{-\frac{1}{2}} .$$

<u>Proof</u>.

We have to calculate

$$\pi_{ijk} = P\{U_i - u_i(\kappa) \geq U_j - u_j(\kappa) \geq U_k - u_k(\kappa)\}$$

$$= P\{U_i - u_i(\kappa) - U_j + u_j(\kappa) \geq 0 \quad \text{and}$$

$$U_j - u_j(\kappa) - U_k + u_k(\kappa) \geq 0\} = P\{X \geq 0 \text{ and } Y \geq 0\}$$

where

$$\begin{pmatrix} X \\ Y \end{pmatrix} \sim N\left\{\begin{pmatrix} 0 \\ 0 \end{pmatrix}, \begin{pmatrix} \sigma_i^2+\sigma_j^2 & -\sigma_j^2 \\ -\sigma_j^2 & \sigma_j^2+\sigma_k^2 \end{pmatrix}\right\} .$$

Now the correlation between X and Y is just $-\rho_{ijk}$ and then it is known that $P(X \geq 0, Y \geq 0) = (\text{Arccos}\ \rho_{ijk})/2\pi$. Note that

$$|\rho_{ijk}| \leq \sigma_j^2/\{(b\sigma_j^2/B+\sigma_j^2)(\sigma_j^2+\sigma_j^2)\}^{-\frac{1}{2}}$$

$$= \{\tfrac{1}{2}B/(B+b)\}^{-\frac{1}{2}} < 2^{-\frac{1}{2}}$$

and hence that

$$\sum_{i<j<k} (\pi_{ijk} - 1/8) > \pi\varepsilon \binom{n}{3}/8$$

which shows that in condition (4.3) the boundedness of the variances imply that $a_2 > 0$.

If further $\sigma_1^2 = \ldots = \sigma_n^2$ one could have obtained this result from Theorem 3.1 since then the errors would have had the same distribution. Thus we have:

Corollary 4.3

If U_i is Gaussian with mean $u_i(\kappa)$ and variance σ^2, then conditions (3.8) and (3.9) imply that K_n' is asymptotically normal with parameters κ and

$$\delta_n^2 = \frac{\frac{1}{6}\binom{n}{3} + \frac{1}{4}\binom{n}{2}}{\left[\frac{1}{\sigma\sqrt{4\pi}} \sum_{i<j} \{u_j'(\kappa) - u_i'(\kappa)\}\right]^2}$$

It is tempting at this point to compare with the maximum likelihood estimator which, under similar conditions on the design, see Jennrich (1969), is asymptotically normal with parameters κ and

$$\delta_{ML}^2 = \sigma^2 / \{\sum_i (u_i'(\kappa) - \bar{u}'(\kappa)\}^2$$

where $\bar{u}'(\kappa) = \frac{1}{n} \sum_i u_i'(\kappa)$.

From the inequality

$$\{\sum_i (n+1-2i)(u_i'(\kappa) - \bar{u}'(\kappa))\}^2 < \sum_i (n+1-2i)^2 \sum_i \{u_i'(\kappa) - \bar{u}'(\kappa)\}^2$$

it follows that $\lim \delta_{ML}^2/\delta_n^2 \leq 3/\pi = 0.95$.

Thus the efficiency of the median estimator is at most 95 % if the underlying distribution is Gaussian. How large the efficiency is depends on the inner product of the vectors $\{n+1-2i\}$ and $\{u_i'(\kappa)\}$.

5. The asymptotic distribution of the median estimators when the distribution is a transformed Gaussian variable with a small variance.

In order to derive properties of the median estimates of the Michaelis Menten parameters K_m and V_{max}, it will be apparent from (2.1)-(2.4) that different transformations of the observations are needed. The assumptions made in section 3 and 4 are not invariant under transformation. Thus for instance if we assume, as in section 4, that $\ln V_i - \ln\{c_i V_{max}/(c_i+K_m)\}$ has a symmetric distribution, then $1/V_i-(c_i+K_m)/c_i V_{max}$ will not have a symmetric distribution. Hence Theorem 4.1 can be applied to K_m^* but not to V_{max}^*.

In order to find a framework in which the asymptotic properties of both estimators can be found, we shall assume that the measurements are smooth transformations of some Gaussian variables with a small variance. This class of models is clearly invariant under smooth transformations of the observations and thus allows both estimators to be investigated.

Unfortunately the analysis shows that a bias may appear, and this will be discussed further below.

Hence let us again consider the estimate K_n' given by (2.7), and let us assume that W_i are independent Gaussian variables with mean $w_i(\kappa)$ and variance τ_i^2/λ_n^2, and that

$U_i = h(W_i)$, and $u_i(x) = h\{w_i(x)\}$, where h is twice continuously differentiable in a neighbourhood of $w_i(\kappa)$ for all i.

We introduce the shorthand notation $h_i = h\{w_i(\kappa)\}$, $h_i' = h'\{w_i(\kappa)\}$, and $h_i'' = h''\{w_i(\kappa)\}$.

For large λ_n we have that U_i is approximately normally distributed with parameters h_i and σ_i^2/λ_n^2, where $\sigma_i^2=\tau_i^2(h_i')^2$. Hence $\tilde{\mu}_{ij}(\kappa)$ given by (3.1) will be only approximately equal to ½, and in the following we shall apply an Edgeworth expansion to show that this may introduce an asymptotic bias in the median estimators.

We can then formulate the main result:

Theorem 5.1.

Assume that $\lambda_n \to \infty$ such that $n^{\frac{1}{2}}\lambda_n^{-2} \to 0$ and that $0<b<\sigma_1^2 \leq \ldots \leq \sigma_n^2 < B < \infty$ and $u_i(.)$ satisfy conditions (4.1), (4.3), and (4.4), with $w_{ij} = \{2\pi(\sigma_i^2+\sigma_j^2)\}^{-\frac{1}{2}}$. Further we want h_i' and h_i'' to be bounded and $|h_i'| \geq a > 0$

Then K_n' is asymptotically normally distributed with parameters

$$\kappa_n = \kappa - \frac{\sum_{i<j} \left(\frac{h_i'}{(h_i')^2} - \frac{h_j''}{(h_j')^2}\right)\sigma_i^2\sigma_j^2(\sigma_i^2+\sigma_j^2)^{-\frac{3}{2}}}{2\lambda_n^2 \sum_{i<j} (u_j'(\kappa)-u_i'(\kappa))(\sigma_i^2+\sigma_j^2)^{-\frac{1}{2}}} \tag{5.1}$$

and

$$\delta_n^2 = \frac{4\sum_{i<j<k}\{(\text{Arccos}\,\rho_{ijk})/2\pi - 1/8\} + \frac{1}{4}\binom{n}{2}}{[\lambda_n(2\pi)^{-\frac{1}{2}}\sum_{i<j}\{u_j'(\kappa)-u_i'(\kappa)\}(\sigma_i^2+\sigma_j^2)^{-\frac{1}{2}}]^2} \tag{5.2}$$

where $\rho_{ijk} = \sigma_j^2/\{(\sigma_i^2+\sigma_j^2)(\sigma_j^2+\sigma_k^2)\}^{-\frac{1}{2}}$.

Thus the same results hold as for the Gaussian distribution, as given in Corollary 4.2, but for the bias term in (5.1).

Proof. The technique for proving this result is the same as before except for the bias. Let us consider

$$\pi_{ij}(\kappa) = P\{U_i - u_i(\kappa) \geq U_j - u_j(\kappa)\}$$

$$= P\{h(W_i) - h(w_i(\kappa)) \geq h(W_j) - h(w_j(\kappa))\}$$

An Edgeworth expansion, see for instance Bhattacharya and Ghosh (1978) shows that

$$\pi_{ij}(\kappa) = \tfrac{1}{2} + \frac{1}{2\sqrt{2\pi}\,\lambda_n}\left(\frac{h_i''}{(h_i')^2} - \frac{h_j''}{(h_j)^2}\right)\sigma_i^2\sigma_j^2(\sigma_i^2+\sigma_j^2)^{-3/2} + 0(\lambda_n^{-2})$$

Thus

$$\mu_n(\kappa) = \tfrac{1}{2}\binom{n}{2} + n^2 c_n/\lambda_n + 0(n^2/\lambda_n^2)$$

where c_n, given by

$$c_n = \frac{1}{2\sqrt{2\pi}n^2}\sum_{i<j}\left(\frac{h_i''}{(h_i')^2} - \frac{h_j''}{(h_j)^2}\right)\sigma_i^2\sigma_j^2(\sigma_i^2+\sigma_j^2)^{-3/2},$$

is bounded in n.

Similarly we can expand the expression for $\mu_n'(x)$, $\mu_n''(x)$, $\sigma_n^2(x)$, and $\sigma_n^{2\prime}(x)$ and keep the term corresponding to the Gaussian approximation.

We find, that $\mu_n'(\kappa)$ and $\mu_n''(\kappa) \in 0(\lambda_n n^2)$, $\sigma_n^2(\kappa) \in 0(n^3)$ and $\sigma_n^{2\prime}(\kappa) \in 0(\lambda_n n^3)$. Now κ_n is defined by

$$\tfrac{1}{2}\binom{n}{2} = \mu_n(\kappa_n) = \mu_n(\kappa)+(\kappa_n-\kappa)\mu_n'(\kappa) + 0(n^2\lambda_n(\kappa_n-\kappa)^2)$$

which gives the bias

$$\kappa_n-\kappa = -c_n n^2/\lambda_n\mu_n'(\kappa)+0(\lambda_n^{-3}).$$

An expansion of $\delta_n^2(\kappa_n)$ shows that $\delta_n^2(\kappa_n)=\delta_n^2(\kappa)(1+0(\lambda_n^{-1}))$ which shows that κ can be used in the expression for the asymptotic variance.

The actual proof follows the same lines as the proofs for Theorem 3.1 and 4.1.

Note that the bias is of the order of λ_n^{-2}, i.e. the order of the variance of a single measurement, and that the relative bias is

$$(\kappa_n-\kappa)/\delta_n \simeq n^{\frac{1}{2}}/\lambda_n \simeq (nV(U))^{\frac{1}{2}}$$

which in general will be large. Thus an appreciable bias can be expected in the median estimators, even under the usual assumption of Gaussian errors.

The assumption $\lambda_n^{-2}n^{\frac{1}{2}}\to 0$ ensures that the remainder term in the expansion of κ_n can be neglected, since $\lambda_n^{-3}/\delta_n\in 0(\lambda_n^{-2}n^{\frac{1}{2}})\in o(1)$.

6. Application of the asymptotic results to the median estimators of Cornish-Bowden and Eisenthal.

We shall now return to the estimators given in section 1. and find the asymptotic distribution under the assumption that is usually assumed in the simulation results:

$$V_i \sim N_\varepsilon\{V_{max} c_i/(c_i+K_m), \tau_i^2/\lambda_n^2\}$$

where $\lambda_n \to \infty$ such that $\lambda_n^{-2} n^{\frac{1}{2}} \to o$ and where N_ε denotes the normal distribution censored at $\varepsilon > 0$, so as to give strictly positive values of V_i. Note that the function $x \to \max(x,\varepsilon)$ is a smooth transformation of x in the interval $]\varepsilon,\infty[$ and so is $\ln(\max(x,\varepsilon))$ and $\max(x,\varepsilon)^{-1}$

The relations (2.1)-(2.4) express the statistics $K_m^*, V_{max}^*, \hat{K}_m/\hat{V}_{max}$ and $1/\hat{V}_{max}$ in the form (2.6) for suitable choices of U_i and $u_i(.)$. In all cases we can apply the results of section 5, since we have a smooth transformation of the underlying Gaussian variables with a small variance.

<u>Theorem 6.1</u>. Let $V_i \sim N_\varepsilon\{V_{max} c_i/(c_i+K_m), \tau_i^2/\lambda_n^2\}$ where $0 < b \leq \tau_1^2 < \ldots \leq \tau_n^2 < B < \infty$ and $o \leq a < c_1 \leq \ldots \leq c_n < A < \infty$ are chosen such that the limiting design measure is not equal to a one point measure, then if (4.3) holds, K_m^* is asymptotically normal with parameters K_m and

$$\delta_n^2 = \frac{4 \sum_{i<j<k} \{(\mathrm{Arccos}\, \rho_{ijk})/2\pi - 1/8\} + \frac{1}{4}\binom{n}{2}}{[\lambda_n (2\pi)^{-\frac{1}{2}} \sum_{i<j} \{1/c_i+K_m) - 1/(c_j+K_m)\}(\sigma_i^2+\sigma_j^2)^{-\frac{1}{2}}]^2} \tag{6.1}$$

where $\sigma_i^2 = \tau_i^2(1+K_m/c_i)^2/V_{max}{}^2$

and

$$\rho_{ijk} = \sigma_j^2/\{(\sigma_i^2+\sigma_j^2)(\sigma_j^2+\sigma_k^2)\}^{\frac{1}{2}}$$

Proof. The result follows directly from Theorem 5.1 by the choice $h(x) = \ln\{\max(x,\varepsilon)\}$ which gives $u_i(x) = \ln\{V_{max} c_i/(c_i+x)\}$ which is seen to satisfy the conditions (4.1) and (4.4). The bias term disappears in this case, since $h_i''/(h_i')^2 = -1$ for all i.

Theorem 6.2. Under the same assumptions as in Theorem 6.1 it holds that V^*_{max} is asymptotically normally distributed with parameters

$$V_{max} - \frac{V^3_{max} \sum_{i<j} \{1/(c_i+K_m)-1/(c_j+K_m)\}\sigma_i^2\sigma_j^2(\sigma_i^2+\sigma_j^2)^{-3/2}}{[\lambda_n^2 \sum_{i<j} (c_i-c_j)(\sigma_i^2+\sigma_j^2)^{-\frac{1}{2}}} \qquad (6.2)$$

and

$$\delta_n^2 = \frac{V^4_{max}\,[4 \sum_{i<j<k} \{(\text{Arccos } \rho_{ijk})/2\pi - 1/8\} + \frac{1}{4}\binom{n}{2}]}{\lambda_n (2\pi)^{-\frac{1}{2}} \sum_{i<j} (c_j-c_i)(\sigma_i^2+\sigma_j^2)^{-\frac{1}{2}}]^2} \qquad 6.3)$$

where now $\sigma_i^2 = (\tau_i^2/c_i^2)\{(c_i+K_m)/V_{max}\}^4$

Proof. In this case

$$V_i/c_i \sim N_\varepsilon\{V_{max}/(c_i+K_m),\ \tau_i^2/(c_i\lambda_n)^2\}$$

and we then take $h(x) = \{\max(x,\varepsilon)\}^{-1}$ and hence $u_i(x)=(c_i+K_m)/x$. It is easily seen that $u_i(.)$ satisfies conditions (4.1) and (4.4), and since

$$h_i''/(h_i')^2 = 2V_{max}/(c_i+K_m)$$

we find the expression for the bias and variance.

Thus one would expect to find a relative bias in the distribution of V_{max} which is not negligible.

For the usual assumption that V_i is (censored) Gaussian one finds a bias for V_{max} but not for K_m. This is not due to the censoring but to the lack of symmetry in the distribution of $1/V_i$.

If instead we assume that $\ln V_i$ is Gaussian then again K_m^* will have no bias, but V_{max}^* will be biased. If, however, we assume that $1/V_i$ is Gaussian, then both K_m^* and V_{max}^* will be asymptotically unbiased. Thus the bias properties depend crucially on the underlying distribution.

For later use we give the results for $\hat{K}_m/\hat{V}_{max}$ and $\hat{V}_{max}$.

<u>Theorem 6.3.</u> Under the assumptions of Theorem 6.1 it holds that $\hat{K}_m/\hat{V}_{max}$ is asymptotically normally distributed with parameters

$$\frac{K_m}{V_{max}} - \frac{V_{max} \sum\limits_{i<j} \{c_i/(c_i+K_m)-c_j/(c_j+K_m)\}\sigma_i^2\sigma_j^2(\sigma_i^2+\sigma_j^2)^{-3/2}}{\lambda_n^2 \sum\limits_{i<j} (1/c_j-1/c_i)(\sigma_i^2+\sigma_j^2)^{-\frac{1}{2}}}$$

and

$$\delta_n^2 = \frac{4 \sum\limits_{i<j<k} \{(\text{Arccos}\ \rho_{ijk})/2\pi-1/8\}+\frac{1}{4}\binom{n}{2}}{[\lambda_n(2\pi)^{-\frac{1}{2}} \sum\limits_{i<j} (1/c_i-1/c_j)(\sigma_i^2+\sigma_j^2)^{-\frac{1}{2}}]^2}$$

where $\sigma_i^2 = \tau_i^2\{(K_m+c_i)/(c_iV_{max})\}^4$.

<u>Theorem 6.4.</u> Under the assumptions of Theorem 6.1 it holds that $1/\hat{V}_{max}$ is asymptotically normally distributed with parameters

$$\frac{1}{V_{max}} - \frac{V_{max} \sum\limits_{i<j} \{1/(c_i+K_m)-1/(c_j+K_m)\}\sigma_i^2\sigma_j^2(\sigma_i^2+\sigma_j^2)^{-3/2}}{\lambda_n^2 \sum\limits_{i<j} (c_j-c_i)(\sigma_i^2+\sigma_j^2)^{-\frac{1}{2}}}$$

and

$$\delta_n^2 = \frac{4 \sum\limits_{i<j<k} \{(\text{Arccos}\ \rho_{ijk})/2\pi-1/8\}+\frac{1}{4}\binom{n}{2}}{[\lambda_n(2\pi)^{-\frac{1}{2}} \sum\limits_{i<j} (c_j-c_i)(\sigma_i^2+\sigma_j^2)^{-\frac{1}{2}}]^2}$$

where $\sigma_i^2 = \tau_i^2 c_i^2\{(K_m+c_i)/(c_i V_{max})\}^4$.
This last result can of course be derived from Theorem 6.2, but is given here because of the numerical illustrations in section 7.

It is seen that both $\hat{K}_m/\hat{V}_{max}$ and $1/\hat{V}_{max}$ are biased, and a multivariate version of this theory, which we shall not go into details with, shows, that for $\hat{K}_m$ the bias still remains. Thus from the point of view of asymptotic bias the estimator K_m^* is to be preferred to $\hat{K}_m$ in the case of an underlying Gaussian distribution. See also the comment by Currie (1982) p. 915. This property still depends on the error distribution, and if $1/V_i$ has a Gaussian distribution then all the estimates will be unbiased. A comparison of the variances of $\hat{K}_m$ and K_m^* was too complicated to give a simple answer and will not be reported here.

We shall finally turn to the estimates $\tilde{K}_m$ and $\tilde{V}_{max}$ proposed by Cornish-Bowden and Eisenthal in (1974). These estimators were later given up because it was felt that their bias was too big. The simulations of Currie (1982) indicate that this is sometimes the case, depending on the design.

We shall give here the asymptotic properties of the two unmodified estimators.

<u>Theorem 6.5</u>. Under the conditions of Theorem 6.1 and the following extra condition on the design:

$$c_{i+1}-c_i \geq a/n, \quad i=1,\ldots,n, \qquad (6.4)$$

it holds that K_m is asymptotically normal with parameters

$$K_m - \frac{\sum_{i<j} (1-\phi[\lambda_n V_{max}\{1/(c_i+K_m)-1/(c_j+K_m)\}\{\tau_i^2/c_i^2+\tau_j^2/c_j^2\}^{-\frac{1}{2}}])}{\lambda_n (2\pi)^{-\frac{1}{2}} \sum_{i<j} \{1/(c_i+K_m)-1/(c_j+K_m)\}(\sigma_i^2+\sigma_j^2)^{-\frac{1}{2}}}$$

and a δ_n^2 given by (6.1) and $\sigma_i^2 = \tau_i^2(1+K_m/c_i)^2/V_{max}^2$

Similarly $\tilde{V}_{max}$ is asymptotically normal with parameters (6.2) and (6.3) except that an extra bias term appears, which is equal to

$$-\frac{\sum_{i<j} (1-\phi[\lambda_n V_{max}\{1/(c_i+K_m)-1/(c_j+K_m)\}\{\tau_i^2/c_i^2+\tau_j^2/c_j^2\}^{-\frac{1}{2}}])}{\lambda_n (2\pi)^{-\frac{1}{2}} \sum_{i<j} (c_i-c_j)(\sigma_i^2+\sigma_j^2)^{-\frac{1}{2}}}$$

here $\sigma_i^2 = \tau_i^2 c_i^2\{(c_i+K_m)/c_i V_{max}\}^4$

Proof. The estimate $\tilde{K}_m = \underset{i<j}{\text{med}}(V_i-V_j)/(V_j/c_j-V_i/c_i)$ is investigated as follows:

$$\{\tilde{K}_m<x\} = \{\sum_{i<j} 1\{(V_i-V_j)/(V_j/c_j-V_i/c_i) \leq x\} \geq \tfrac{1}{2}\tbinom{n}{2}\}$$

$$= \{\tilde{U}_n(x) \geq \tfrac{1}{2}\tbinom{n}{2}\}$$

where $$\tilde{U}_n(x) = \tfrac{1}{2} \sum_{i<j} [\phi\{\binom{V_j}{c_j}, \binom{V_i}{c_i}\}+1]$$

and

$$\phi\{\binom{v}{a},\binom{u}{b}\} = \text{sign}(v/a-u/b)\,\text{sign}\{v(1+x/a)-u(1+x/b)\}.$$

Thus $\tilde{U}_n(x)$ is a U-statistic and its moments will determine the asymptotic properties of $\tilde{K}_m$. We want to compare these moments to those of $U_n^*(x) = \sum_{i<j} 1\{K_{mij}^* \leq x\}$ which were investigated in Theorem 6.1.

We now find

$$1\{\tilde{K}_{mij}\leq x\} = 1\{V_i - V_j \leq x(V_j/c_j - V_i/c_i) \text{ and } V_j/c_j > V_i/c_i\}$$

$$+ 1\{V_i - V_j \geq x(V_j/c_j - V_i/c_i) \text{ and } V_j/c_j < V_i/c_i\}$$

$$= 1\{V_i(c_i+x)/c_i \leq V_j(c_j+x)/c_j \text{ and } V_j/c_j > V_i/c_i\}$$

$$+ 1\{V_i(c_i+x)/c_i \geq V_j(c_j+x)/c_j \text{ and } V_j/c_j < V_i/c_i\}$$

Now

$$V_i/c_i < V_j/c_j \Rightarrow V_i(c_i+x)/c_i < V_j(c_j+x)/c_j$$

hence the first indicator function equals

$$1\{V_i/c_i < V_j/c_j\} = Z_{ij} \text{ say}$$

and the second becomes

$$1\{V_i(c_i+x)/c_i \geq V_j(c_j+x)/c_j\} = 1\{K^*_{mij}\leq x\}$$

Thus $\tilde{U}_n(x) = U^*_n(x)+Z_n$, where $Z_n = \sum_{i<j} Z_{ij}$.

Hence

$$\tilde{\mu}_n(x) = \mu^*_n(x)+E(Z_n)$$

$$\tilde{\sigma}^2_n(x) = \sigma^{*2}_n(x)+V(Z_n)+2V(Z_n,\tilde{U}_n(x)).$$

Now we shall prove below that $E(Z_n) \in O(n^2/\lambda_n)$ and $V(Z_n) \in O(n^3/\lambda_n^2)$ which shows that $V(Z_n)$ and $V(Z_n, \tilde{U}_n(x))$ are $o(\sigma_n^{*2}(x))$. Thus for the calculation of the asymptotic variance we can use the result for K_m^* given in (6.1), since $\tilde{\mu}_n'(\kappa) = \mu_n^{*\prime}(\kappa)$. The term $E(Z_n)$ induces a bias however. The bias term due to the transformation $h(x) = \ln(x)$ becomes zero, since $h_i''/(h_i')^2 = -1$ for all i, hence

$$\tilde{\mu}_n(\kappa) = \tfrac{1}{2}\binom{n}{2} + E(Z_n) = \tilde{\mu}_n(\kappa_n) + E(Z_n)$$

giving, as in section 5,

$$\tilde{\mu}_n(\kappa) + (\kappa_n - \kappa)\tilde{\mu}_n'(\kappa) + E(Z_n) + O(n^2\lambda_n(\kappa_n-\kappa)^2),$$

i.e.

$$\kappa_n - \kappa = -E(Z_n)/\tilde{\mu}_n'(\kappa)$$

as the leading term in the bias.

We then find

$$E(Z_n) = \sum_{i<j} P(V_i/c_i < V_j/c_j)$$

$$\simeq \sum_{i<j} (1-\phi[\lambda_n\{V_{max}/(c_i+K_m) - V_{max}/(c_j+K_m)\}\{\tau_i^2/c_i^2 + \tau_j^2/c_j^2\}^{-\frac{1}{2}}])$$

where only the leading term corresponding to neglecting ε has been kept. Combining these results we get the bias for $\tilde{K}_m$. To complete the proof for $\tilde{K}_m$ we have to prove that $E(Z_n) \in O(n^2/\lambda_n)$. Under the assumption that τ_i^2 and c_i are bounded away from 0 and ∞ we find that

$$\{V_{max}/(K_m+c_i)(K_m+c_j)\}\{\tau_i^2/c_i^2+\tau_j^2/c_j^2\}^{-\frac{1}{2}}$$

is bounded below by $d>0$, and from (6.4) we get that $c_j-c_i\geq(j-i)a/n$. Thus

$$E(Z_n)\leq \sum_{i<j}[1-\phi\{\lambda_n d(c_j-c_i)\}]$$

$$\leq \sum_{i<j}[1-\phi\{\lambda_n da(j-i)/n\}]$$

Now let $m = \lambda_n da/n$, then we get

$$E(Z_n)\leq\binom{n}{2}\{1-\phi(mn)\} + \sum_{i<j}\ \sum_{j-i\leq k<n}[\phi\{m(k+1)\}-\phi(mk)].$$

The first term is bounded by $\binom{n}{2}/nm\in 0(n^2/\lambda_n)$ for large values of λ_n.

The second term equals

$$\sum_s(n-s)\sum_{s\leq k<n}[\phi\{m(k+1)\} - \phi(mk)] \leq n\sum_{k<n}k[\phi\{m(k+1)\} - \phi(mk)]$$
$$\leq \frac{n}{m}\sum_{k<n}\int_{mk}^{m(k+1)} u\phi(u)du \in 0(n^2/\lambda_n)$$

This proves that $E(Z_n)\in 0(n^2/\lambda_n)$. The result about $V(Z_n)$ can be proved via a relation similar to (3.4), and we have to evaluate terms like $P(V_i/c_i < V_j/c_j < V_k/c_k)$. Now let $X = V_j/c_j - V_i/c_i$ and $Y = V_k/c_k - V_j/c_j$, then X and Y are jointly Gaussian with a negative correlation ρ. It is then easily seen that $P(X>0, Y>0)$ is increasing in ρ and hence that $P(X>0, Y>0) \leq P(X>0)P(Y>0)$. Thus with $m = \lambda_n da/n$, we have

$$p(V_i/c_i < V_j/c_j < V_k/c_k)$$

$$\leq \sum_{i<j<k} [1-\phi\{m(j-i)\}][1-\phi\{m(k-j)\}]$$

$$\leq n \sum_i \sum_j \{1-\phi(mi)\}\{1-\phi(mj)\} \in 0(n^3/\lambda_n^2) .$$

This completes that part of Theorem 6.4 which concerns $\tilde{K}_m$. As for $\tilde{V}_{max} = \operatorname{med}_{i<j}(c_i-c_j)/(c_i/V_i-c_j/V_j)$ we find by an argument similar to that for $\tilde{K}_m$, that

$$1\{\tilde{V}_{maxij} \leq x\} = 1\{V^*_{maxij} \leq x\} + 1\{V_i/c_i < V_j/c_j\}$$

Thus the analysis proceeds as before, giving exactly the same extra contribution to the bias as for $\tilde{K}_m$.

7. Numerical examples.

We shall give a few examples to show that some of the simulation results obtained by others are in accordance with the formulation given here.

Cornish-Bowden (1981) considers the following situation. Let $c_i = 0.2i$, $i=1,\ldots,10$ and take $K_m = V_{max} = 1$, and $\tau_i = 0.025$. Then, with V_i distributed as $N\{c_i/(c_i+1), \tau_i^2\}$, the following values are reported $V(\hat{K}_m/\hat{V}_{max}) = 11.06\times10^{-3}$ and $V(1/\hat{V}_{max}) = 5.62\times10^{-3}$.

From Theorem 6.3 we find the values for the bias and variance for $\hat{K}_m/\hat{V}_{max}$ to be $.96\times10^{-3}$ and 10.64×10^{-3} in accordance with the simulation results. From Theorem 6.4 we find $V(1/\hat{V}_{max}) = 5.7\times10^{-3}$ with a bias of -1.67×10^{-3}. Note that in both cases the bias is insignificant. The relative bias, however, increases with $n^{\frac{1}{2}}$, thus taking more observations will make the estimator worse.

Currie (1982) considers among other situations the following design: $c_i = ai$, $i=1,\ldots,7$, $\tau_i^2 = 0.01$, $K_m = .75$, $V_{max} = 1$, and finds the asymptotic variance to be $V(\tilde{K}_m) = .24$ (Fig. 5D, a=1) and the bias of $\tilde{K}_m = -.04$ (Fig. 5C, a=1).

Using the results of Theorem 6.5 we find the bias to be -.09 and the variance .2059 giving a relative bias of -21%.

Tabulating these functions for various values of a we obtain a bias curve corresponding to Fig. 5C of Currie (1982). The curve for the variance looks different, however, especially for small values of a

Table 6.1

a	.2	.4	.6	.8	1.0
$V(\tilde{K}_m)$	.408	.210	.186	.190	.206
$\text{Bias}(\tilde{K}_m)$	-.750	-.238	-.141	-.109	-.095
Rel. bias $(\tilde{K}_m)$ (%)	- 118	- 52	- 33	- 25	- 21

Asymptotic parameters for $\tilde{K}_m$ for $n=7$, $K_m = .75$, $V_{max} = 1$ and $c_i = ai$, $i=1,\ldots,7$, $\tau_i^2 = 0.01$.

If n is increased by a factor of 4 to 28 then we get

Table 6.2

a	.2	.4	.6	.8	1.0
$V(\tilde{K}_m)$	.040	.044	.051	.068	.084
$\text{Bias}(\tilde{K}_m)$	-.215	.161	-.163	-.174	-.189
Rel. bias $(\tilde{K}_m)$ (%)	- 108	- 77	- 70	- 67	- 65

Asymptotic parameters for $\tilde{K}_m$ for $n=28$, $K_m = 0.75$, $V_{max} = 1$ and $c_i = ai$, $i=1,\ldots,28$, $\tau_i^2 = .01$.

Note that by increasing n we decrease $V(\tilde{K}_m)$ and $\text{bias}(\tilde{K}_m)$, but the relative bias is increased in most cases.

If we calculate the results for $\tilde{V}_{max}$ we find

Table 6.3

a	.2	.4	.6	.8	1.0
$V(\tilde{V}_{max})$	.145	.040	.024	.018	.015
Bias $(\tilde{V}_{max})$	.529	.144	.078	.055	.044
Rel. bias $(\tilde{V}_{max})$ (%)	139	72	51	41	35

Asymptotic parameters for $\tilde{V}_{max}$ for $n=7$, $K_m = 0.75$, $V_{max} = 1$ and $c_i = ai$, $i=1,\ldots,7$, $\tau_i^2 = 0.01$.

The bias is here due to two facts. Firstly that a median estimator is used, and secondly that the unmodified version is used.

If the modified version is used we find the bias of K_m^* to be zero whereas V_{max}^* still has a bias:

Table 6.4

a	.2	.4	.6	.8	1.0
$V(V^*_{max})$	.145	.040	.024	.018	.015
$Bias(V^*_{max})$	.066	.036	.026	.021	.018
Rel. bias (V^*_{max}) (%)	17	18	17	15	14

Finally Atkins and Nimmo (1975) choose among other situations the following $c_i = 0.25i$, $i=1,\ldots,7$, $K_m = V_{max} = 1$, and $\tau_i^2 = .01(c_i/(c_i+1))^2$ corresponding to a relative variance of 0.01. They report a value for $\tilde{K}_m$ of 0.94 ± 0.29 (Table 1). If we apply Theorem 6.5 we find a bias of -.12 and a variance .0898 corresponding to a standard deviation of .30, and a relative bias of -40%.

8. References.

ATKINS, G.L. & NIMMO, I.A. (1975). A comparison of seven methods for fitting the Michaelis Menten equation. Biochemical Journal 149, 775-7.

BHATTACHARYA, P.K., CHERNOFF, H. & YANG, S.S. (1983). Nonparametric estimation of the slope of a truncated regression. Ann. Statist. 11, 505-14.

BHATTACHARYA, R.N. & GHOSH, J.K. (1978). On the validity of the formal Edgeworth expansion. Ann. Statist. 6, 434-51.

CORNISH-BOWDEN, A. & EISENTHAL, R. (1974). Statistical considerations in the estimation of enzyme kinetic parameters by direct linear plot and other methods. Biochemical Journal 139, 721-30.

CORNISH-BOWDEN, A. & EISENTHAL, R. (1978). Estimation of Michaelis Menten kinetic constants from various linear transformations. Journal of Biological Chemistry 240, 863-69.

CORNISH-BOWDEN, A. (1981). Robust estimation in enzyme kinetics. In: Kinetic data analysis: Design and analysis of enzyme and pharmacokinetic experiments. Ed. Endrenyi. 105-19. New York: Plenum Press.

CURRIE, D.J. (1982). Estimating Michaelis-Menten parameters: Bias, variance and experimental design. Biometrics 38, 907-19.

DANIELS, H.E. (1954). A distribution free test for regression parameters. Ann. Math. Statist. 25, 499-513.

EISENTHAL, R. & CORNISH-BOWDEN, A. (1974). A new procedure for estimating enzyme kinetic parameters. Biochemical Journal 139, 715-20.

JENNRICH, R.I. (1969). Asymptotic properties of non linear least squares estimators. Ann. Math. Statist. 40, 633-43.

JOHNSTONE, I. & VELLEMAN, P. (1984). The resistent line and related regression methods. Tech. Rep. Stanford University 52 pp.

SCHOLZ, F.W. (1978). Weighted median regression estimates. Ann. Statist. 6, 603-9.

SEN, P.K. (1968). Estimates of the regression coefficient based on Kendall's tau. J. Amer. Stat. Assoc. 63, 1379-89.

THEIL, J. (1950). A rank-invariant method of linear and polynomial regression analysis, I, II and III. Koninklijke Nederlandse Akademi Proc. 53, 386-92, 521-5, 1397-412.

PREPRINTS 1985

COPIES OF PREPRINTS ARE OBTAINABLE FROM THE AUTHOR OR FROM THE INSTITUTE OF MATHEMATICAL STATISTICS, UNIVERSITETSPARKEN 5, 2100 COPENHAGEN Ø, DENMARK.

No. 1 Cohn, Harry: Almost Sure Convergence for Stochastically Monotone Temporally Homogeneous Markov Processes and Applications.

No. 2 Rootzén, Holger: Maxima and Exceedances of Stationary Markov Chains.

No. 3 Asmussen, Søren und Johansen, Helle: Über eine Stetigkeitsfrage betreffend das Bedienungssystem GI/GI/s.

No. 4 Asmussen, Søren and Thorisson, Hermann: A Markov Chain Approach to Periodic Queues.

No. 5 Hald, Anders: Galileo as Statistician.

No. 6 Johansen, Søren: The Mathematical Structure of Error Correction Models.

No. 7 Johansen, Søren and Johnstone, Iain: Some Uses of Spherical Geometry in Simultaneous Inference and Data Analysis.

PREPRINTS 1986

COPIES OF PREPRINTS ARE OBTAINABLE FROM THE AUTHOR OR FROM THE INSTITUTE OF MATHEMATICAL STATISTICS, UNIVERSITETSPARKEN 5, 2100 COPENHAGEN Ø, DENMARK.

No. 1 Jespersen, N.C.B.: On the Structure of Simple Transformation Models.

No. 2 Dalgaard, Peter and Johansen, Søren: The Asymptotic Properties of the Cornish-Bowden-Eisenthal Median Estimator.