4. Myrvik, Q. N., Ann. N. Y. Acad. Sci., 1956, v66, 391.
5. Peters, T., Giovanniello, T. J., Apt, L., Ross, J. F., J. Lab. Clin. Med., 1956, v48, 274.
6. Schade, A. L., Biochem. Z., 1963, v338, 140.
7. Wood, D. C., Ono, J., Bessey, H. J., Lab. Invest., 1958, v7, 174.
8. Yotis, W. W., J. Bact., 1963, v85, 911.
9. Yotis, W. W., Ekstedt, R. D., ibid., 1960, v80, 719.

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Comparison of Ratio and Covariance Analysis of TSH Assays. (31907)

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Sakiz and Guillemin(1) examined the calculation of the results of the McKenzie(2) assay for TSH activity. They recommended: a) transformation of both the initial and the response radioactivity counts to logarithms; b) analysis of the log response counts using the log initial count as a covariate. They demonstrated that the transformation removes the heterogeneity of variance inherent in the original (untransformed) counts. Levy *et al* (3) analyzed the logarithm of the ratio of the response count to the initial count, remarking that they used this procedure because in prior work they had found only minor gains in precision by using covariance analysis. It is the purpose of this paper to examine the consequences of using as the response metameter the logarithm of the ratio of the response count to the initial count as compared with the technique of covariance analysis.

The mathematical model underlying the covariance analysis may be written

$$y = a + b \log \text{dose} + c\,z + e$$

where y is the logarithm of the response count,
z is the logarithm of the initial count
a,b,c are (unknown) constants and
e is a normally distributed random variable with mean zero and variance s^2

The mathematical model for the "ratio analysis" is similar to the above, with the a priori assumption that $c = 1$. We may then write

$$y' = a' + b' \log \text{dose} + e'$$

where $y' = \log$ (response count/initial count) $= y - z$
a', b' are constants, and e' is a random variable.

From a strictly statistical point of view, the question at issue is the appropriateness of either of these models to the biological system; the only 'proper' analysis is the one based on the 'right' model. However, the experimenter's interest is in the usefulness of the results, so rather than investigate the appropriateness of the models, we ask, instead, what (if any) difference does it make which analysis is performed?

Since the error of the estimated relative potency is affected by the experimental design used (*e.g.*, number of levels and actual levels of dosage for standard and unknown, number of animals allocated to the different dosage levels of standard and unknown, etc.), as well as the form of model assumed, we avoid confusion due to effects of design by considering responses to known dosages of standard TSH. The data examined were 6 assays using mice (Veterans Research Hospital, Chicago) and 9 assays using chicks (VA Hospital, Indianapolis). These dose-response curves were calculated according to both models with results as tabulated.

Using the Wilcoxon signed rank test for the hypothesis that the parameter estimates from the two methods of calculation do not differ (for the 13 assays giving legitimate estimates of all parameters) we have the following p-values:

slopes (on log dose),	$p > .60$
standard deviation,	$p > .31$
lambda,	$p > .86$

TABLE I. Estimates of Parameters

	Covariance analysis				Ratio analysis		
	c	b	s	λ=s/b	b′	s′	λ′=s′/b′
Mouse assays							
2-22	1.16	.48	.11	.24	.47	.10	.22
3-08	.72†	.47	.08	.17	.49	.09	.19
3-29	*				.50	.17	.35
4-12	.45†	.55	.13	.24	.57	.16	.28
5-11	.95	.37	.11	.29	.37	.10	.27
6-10	1.07	.42	.11	.26	.42	.11	.25
Chick assays							
12-17	.78	.33	.10	.31	.33	.11	.33
12-20	.95	.32	.15	.46	.33	.14	.43
12-21	.86	.28	.14	.51	.26	.15	.57
12-22	.67†	.23	.15	.64	.27	.16	.59
12-23	.58†	.35	.18	.51	.39	.20	.51
1-10	1.00	.40	.10	.26	.40	.10	.25
1-11	1.01	.37	.11	.31	.37	.11	.30
1-12	.79	.51	.12	.23	.47	.12	.26
1-18	*				.39	.11	.29

* Regression on log initial count not homogeneous across dosage groups ($p < .05$).
† Significantly different from 1.00 ($p < .05$).
s = standard deviation.

There seems to be little evidence of any gross difference between the results of the two methods of calculation.

If the covariance model is assumed to be correct and

$$\text{Variance } (y) = \varsigma^2 = \text{Variance } (z)$$

then $y' = a + b \log \text{dose} + (c-1)\, z + e$

so $\text{Variance } (y') = [(c-1)^2 + 1]\, \varsigma^2$

The width of confidence intervals is, in general, proportional to the standard deviation. The average value of $[(c-1)^2 + 1]$ from these data is 1.06, so that confidence limits (as, for instance, for relative potency) would be expected, on the average, to be about 3% too wide.

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1. Sakiz, E., Guillemin, R., Proc. Soc. Exp. Biol. and Med., 1964, v115, 856.
2. McKenzie, J. M., Endocrinology, 1958, v63, 372.
3. Levy, Richard, P., McGuire, William L., Shaw, Richard K., Bartsch, Glenn E., ibid., 1965, v76, 890.

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