which give the exact formulæ for the bias.

An immediate consequence of these equations are the upper bounds for the biases

$$|\text{Bias in } \hat{y}| \leqslant \sigma_{y|x}\,\sigma_x \text{ and } |\text{Bias in } \tilde{y}| \leqslant \sigma_{\bar{y}|\bar{x}}\,\sigma_{\bar{x}} \qquad (3)$$

which are proved (but only as large-sample approximations) in most text-books. Next, we may try to estimate the exact bias in $\hat{y}$ from the sample. This leads to the unbiased ratio estimate

$$y' = \bar{X}\bar{r} + \frac{n(N-1)}{(n-1)N}(\bar{y} - \bar{r}\bar{x}). \qquad (4)$$

If variance formulæ are derived by the customary expansions in powers of $\Delta y_i = (y_i - \bar{Y})/\bar{Y}$ and $\Delta x_i = (x_i - \bar{X})/\bar{X}$, it can be shown that (for $N \to \infty$) up to and including cubic terms

$$\text{Var } y' = n^{-1}\bar{Y}^2(\sigma_y^2/\bar{Y}^2 + \sigma_x/\bar{X}^2 - 2\,\text{Cov}(x,y)/\bar{X}\bar{Y}),$$

which is also the approximate formula for Var $\hat{y}$ and Var $\tilde{y}$ if terms up to and including the quadratic are considered. The cubic terms in Var $\hat{y}$ or Var $\tilde{y}$ may be positive or negative (depending on certain product moments of the y, x distribution) and are 0 in Var y'. Within the framework of this approximate variance theory, therefore, no large price in variance increment is paid for bias elimination; indeed, for certain populations Var y' is less than Var $\hat{y}$ or Var $\tilde{y}$. Similar results are available for bias elimination in $\tilde{y}$, and exact variance formulæ have been derived. Since the approximate variance formula depends on the fact that the coefficients of variation of y and x are small, a useful modification consists of the device of splitting the sample of n y_ix_i into (say) K subsamples with means $\bar{y}_j\bar{x}_j$ and computing the unbiased ratio estimate $y'' = \bar{r}\bar{X} + \frac{K}{K-1}(\bar{y} - \bar{r}\bar{x})$, where $\bar{r} = \frac{1}{K}\Sigma\bar{y}_j/\bar{x}_j$ and N is assumed large.

Adaptations to stratified and multistage sampling have been developed, and it is hoped to publish applications of these estimators shortly.

H. O. Hartley
A. Ross

Statistical Laboratory,
Iowa State College, Ames.
March 24.

Solubility of Uroporphyrin I in Ethyl Acetate

According to Waldenström, Fink and Hoerburger[1], uroporphyrin I does not pass from aqueous solutions into ethyl acetate at any *p*H, thus differing from uroporphyrin III, which is readily extracted by ethyl acetate around *p*H 3·1. This statement has been widely accepted in the literature. We have found, however, that uroporphyrin I in the concentrations studied is readily extracted into ethyl acetate from aqueous solutions at *p*H 3·0–3·2, and, provided these are strongly buffered, quantitative recoveries can be obtained.

The results of some recovery experiments with uroporphyrin I and 'Waldenström porphyrin' are illustrated in Table 1. The experimental technique was as follows: a solution of the free porphyrin in 0·1 *N* sodium bicarbonate was added to 50 ml. of 10 per cent (w/v) hydrochloric acid, followed by 150 ml. saturated sodium acetate. The solution was rapidly brought to a *p*H between 3·0 and 3·2 with concentrated hydrochloric acid and the final volume of solution made up to 250 ml. The order of adding the reagents appeared to affect the recovery materially, and is therefore given in full. The buffered solution was extracted exhaustively with 100-ml. portions of ethyl acetate, readjusting the *p*H with concentrated hydrochloric acid and saturated sodium acetate between extractions. The last few ethyl acetate extracts are usually non-fluorescent; but extractions were continued until such ethyl acetate layers gave non-fluorescent aqueous layers when shaken with 2 per cent hydrochloric acid. The combined ethyl acetate extracts were then extracted to completion with 2 per cent hydrochloric acid, and this extract determined spectrophotometrically, using the correction factor of Rimington and Sveinsson[2]. It was found that the number of extractions required at each stage was not significantly different for the two isomers; in both cases about 50 per cent of the porphyrin present in the aqueous phase passed into the ethyl acetate during each extraction. The results in Table 1 show clearly that the recovery of uroporphyrin I is not appreciably less than that of 'Waldenström porphyrin'.

Table 1

μgm. Porphyrin added (range)	Percentage recoveries: Uroporphyrin I	Percentage recoveries: 'Waldenström porphyrin'
46–49	94, 95, 94, 100, 102	99
22–24	99, 99, 102, 99 100	
9–10	100, 89, 94, 95, 97, 97, 101, 99, 101	92, 93
4–5	90, 91, 89, 89 91, 91	95, 86

A suitable method for comparable extraction of both isomers is of value with respect to recent investigations of the porphyrins formed by tissues *in vitro* from precursors such as porphobilinogen and δ-amino-lævulinic acid[3]. A detailed investigation, which we hope to report elsewhere, has led us to conclude that equivalent recovery of each isomer from tissue preparations should also be possible.

The uroporphyrin I used in this investigation was a hydrolysed sample of fraction *A*(i) shown in Fig. 1 of the paper by Rimington and Miles[4]. 'Waldenström porphyrin' was taken from a bulked sample of Waldenström ester (m.p. 260°, corr.), which, when tested by the paper chromatographic method of Falk and Benson[5], behaved as uroporphyrin III only. Hydrolyses were carried out in 25 per cent hydrochloric acid at room temperature for 36 hr.

We thank Prof. C. Rimington for his support of this work and Miss H. James for technical assistance.

E. I. B. Dresel
B. E. Tooth

Nuffield Unit for the
Investigation of Pyrrole Pigment Metabolism,
Department of Chemical Pathology,
University College Hospital Medical School,
London, W.C.1.
June 11.

[1] Waldenström, J., Fink, H., and Hoerburger, W., *Z. physiol. Chem.*, **233**, 1 (1935).
[2] Rimington, C., and Sveinsson, S. L., *J. Clin. Lab. Invest.*, **2**, 209 (1950).
[3] Falk, J. E., Dresel, E. I. B., and Rimington, C., *Nature*, **172**, 292 (1953). Dresel, E. I. B., and Falk, J. E., *Nature*, **172**, 1185 (1953).
[4] Rimington, C., and Miles, P. A., *Biochem. J.*, **50**, 202 (1951).
[5] Falk, J. E., and Benson, A., *Biochem. J.*, **55**, 101 (1953).