in some manner to the incidence of dental caries in the rat.

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Limitations of the "Zero Method" of Population Counts

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In counts of organisms per unit of area or substrate, such as are common in insect population surveys, if the distribution is fully random, numbers found will be expected to agree with the Poisson series. In practice, they usually show somewhat greater dispersion, with more zeros and high values than in a Poisson. The "negative binomial" often gives a fairly good fit to actual distributions. Sparse populations seem to diverge less than denser ones from the Poisson condition. I have discussed these situations (*1, 2*).

In a Poisson, the proportion of zeros or noninfested units is estimated as $e^{-\bar{x}}$, where $\bar{x}$ is the mean number per unit. This opens the way to estimation of the mean from the proportion not infested, as $\bar{x} = -\ln(q)$, where $\ln(q)$ is the natural logarithm of the proportion of zeros.

The method noted by Tippett (*3*) has occasionally been discussed or applied in entomology; Bowen (*4*) discusses it and some of its limitations. It is intended to save work, because it is easier to classify units as infested or noninfested than to count organisms per unit, although less information is gained. The relationship is used in bacterial estimation (*5, 6*) and in hemocytometer counting (*7*). Some of the articles referred to also discuss the additional use of proportion of ones, twos, . . . , which are readily deduced from the the Poisson expression for the expectation of x, $(e^{-x} \cdot \bar{x}^{x}/x!)$. Such an extension of the method may be expected to increase accuracy at the expense of more work and to reduce the limitations of the method as compared with complete counts. Only the use of proportion of zeros will be considered here, however.

Samples from several sources have been studied. Bowen's leafhopper counts (unpublished reports) and some Mormon cricket egg counts have been studied briefly. The most comprehensive study, however, has been of a series of citrus rust mite counts from the Florida fruit insect laboratory of the U.S. Bureau of Entomology. In all cases, the method seemed rather disappointing. In low population densities, it was fairly accurate but did not save much work, since careful examination was needed before discarding a unit as noninfested. In dense populations, it saved work, because a unit could be discarded without further work as soon as it was found to be infested; but accuracy was lower. Furthermore, the method consistently underestimated the population.

The citrus rust mite counts used a unit ½ in. square, covered by a lens placed over the leaf to reveal the minute mites. A sample consisted of 75 such units, with counts as well as determination of percentage of units infested. These results are summarized in Table 1, which shows the limitations of the method very well.

Because of the definite indication of loss of precision at high density and of bias, an examination of the theoretical consequences of the relationship is undertaken. It will be carried out (i) for the case in which actual distribution conforms to the Poisson, and (ii) for the case in which it tends to the negative binomial.

Table 1. Rust-mite counts, relationship of proportion infested (p) and $\bar{x}$.

Range of proportion infested	No. of samples	Av. proportion	Av. no. per unit	No. expected from Poisson
0.0– 9.9	83	0.048	0.31	0.05
10.0–19.9	46	.144	.91	.15
20.0–29.9	48	.242	1.85	.28
30.0–39.9	25	.344	3.55	.42
40.0–49.9	15	.441	3.88	.52
50.0–59.9	11	.556	4.43	.81
60.0–69.9	16	.658	11.30	1.07
70.0–79.9	11	.736	13.04	1.33
80.0–89.9	5	.838	28.56	1.82
90.0–99.9	4	.933	30.00	2.70

As already stated, where the Poisson condition occurs, $q = e^{-\bar{x}}$, where q is the proportion of units not infested; and the relationship is employed in estimating the mean, $\bar{x}$. Obviously no serious *bias* is to be expected where the Poisson holds; but the *variance* of the estimate of $\bar{x}$ as $-\ln(q)$ needs examination. If $\bar{x} = -\ln(q)$, $d\bar{x}/dq = -1/q$. The variance of q is pq/n. The variance V of $\bar{x}$ as a function of q is estimated as $V_{\bar{x}} = Vq(d\bar{x}/dq)^2 = (pq/n)\ (1/q^2) = p/nq$. (This can be shown to be identical with a formula given by Eisenhart and Wilson.) The variance of X estimated by direct counts is $\bar{x}$; hence, the variance of $\bar{x}$ directly determined is $\bar{x}/n$. Thus, the comparison of variances of estimation through q and by direct count is of $\bar{x}/n$ with p/nq, or of $\bar{x}$ with p/q. It is quite evident that as p approaches 1, $\bar{x}$ will rise moderately and p/q will increase greatly. As q approaches 1, on the other hand, p and p/q will come nearer and nearer to $\bar{x}$, as nearly all infested units will be ones.

Thus, the loss of information in denser populations, in using the indirect method rather than the direct counts, is shown to be expected from mathematical theory.

Where populations tend toward a negative binomial form, as is often true with insects, serious *bias*, as well as higher variance, must be expected from the indirect estimation of $\bar{x}$. The negative binomial is more complex in its algebraic expression than the Poisson, the usual equation for fitting involving both mean and variance (*2*). Sufficient to show bias in the indirect method is the fact that the proportion of zeros is always higher for a given mean in the negative binomial than in the Poisson. Hence, estimation of the mean from the proportion of zeros, in negative binomial material mistakenly regarded as Poisson, will give too low a value. The expression $\bar{x} = -\ln(q)$ can readily be seen to have this tendency if q is higher than expected in proportion to $\bar{x}$. This will give a definite negative bias to such population estimates in many populations.

The tendencies that variance would show, if unbiased estimation of the mean from the proportion of zeros in negative binomial material could be carried out, may be studied briefly. The expression used for fitting the negative binomial is complex and does not lend itself to such a study. However, for selected levels of excess of variance over mean (for example, variance equal to twice the mean), the expression is simplified and can easily be shown to have the same tendencies as with the Poisson.

Thus, it is shown that in populations agreeing with the Poisson, the method of estimating mean density from the proportion of zeros loses much information at higher densities as compared with direct counts, although it is practically unbiased. With populations tending toward the negative binomial conditions, a strong bias also appears. These factors should be considered in appraising this method.

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Histochemistry of Ketoenolic Substances (Hamazaki)

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Ketoenolic substance was first demonstrated in 1934 (*1*) by Y. Hamazaki, who completed the ordinary morphologic research in 1938 (*2*). This substance was disclosed as fine granules in the normal cytoplasm and rarely in the nucleus in tissues treated with the special fixation and staining method described here. Whether or not any substance containing desoxyribonucleic acid exists in cytoplasm outside the nucleus of normal cells is still unknown both morphologically and chemically (*3*). Though the significance of this substance in the living organism is still unknown, it has a function almost similar to folic acid and, moreover, it can be a carrier of molecular oxygen. When any chronic disturbance of metabolism of this substance occurs, wear and tear pigments are formed (*4*).

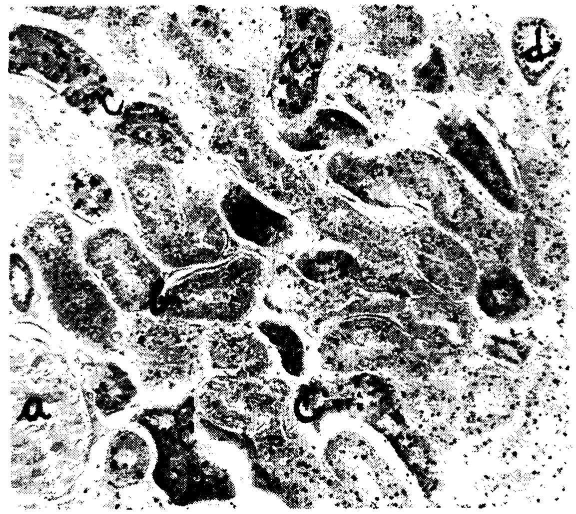

Fig. 1. Ketoenolic substance in the kidney of albino rat. Fixation with chromate mixture; carbol-fuchsin-iodine method. (*a*) Glomerulus; (*b*) proximal convoluted tubules; (*c*) intermediate portion; (*d*) intercalary portion.

For fixation, a chromate mixture originated by Hamazaki is applied; other fixation methods previously used were not successful. A mixture of 2.5 g of potassium bichromate, 1.0 g of sodium sulfate, 100.0 ml of distilled water, and 6.0 ml of glacial acetic acid is prepared, in which tissue is fixed for 48 hr. Paraffin or carbowax methods should be employed for making tissue sections. The carbol-fuchsin-iodine method invented by Hamazaki is applied as the staining method, because with other routine methods heretofore used the ketoenolic substance is not stained. Five-tenths gram of crystalline basic fuchsin is dissolved in 5.0 ml of absolute alcohol, into which 95 ml of 3-percent aqueous solution of carbolic acid is added. Tissue sections are stained in this solution for 1 hr and, after being washed in water, they are placed in 1-percent HCl for 10 min. After being washed in water, they are immersed in Lugol's solution for 30 min and later immersed in 1-percent sodium hyposulfite for 1 to 2 min to remove the iodine color. After being washed in water for several minutes, they are again placed in 3-percent HCl for 15 min. They are thoroughly washed in tap water, dehydrated with alcohol and xylol, and sealed with balsamum. This staining is a chemical color reaction.

When tissues are fixed with the chromate-mixture, the desoxypentose of nucleic acid is oxidized into ketone and then changed into enol, the OH of which reacts with fuchsin and iodine to produce a new kind of acidproof dye that is violet in color. Thus, the same