Table 1. Relationships between flocculation and bactericidal activities of three quaternaries.

| Quaternaries* | Flocculation concentration† | Bactericidal concentration‡ | Relative values | |
|---|---|---|---|---|
| | | | Flocculation values | Bactericidal activities |
| DAC | 1: 2380 | 1: 21,000 | 1 | 1 |
| ABC | 1: 2050 | 1: 24,000 | 0.86 | 1.14 |
| DBC | 1: 1300 | 1: 17,000 | 0.55 | 0.81 |

* DAC = p-diisobutyl phenoxy ethoxy ethyl dimethyl benzyl ammonium chloride; ABC = alkyl dimethyl benzyl ammonium chloride; DBC = dodecyl methyl benzyl ammonium chloride.

† All tubes contained 6.0 ml of washed *S. schottmuelleri* cells (Klett reading = 180), 0.75 ml of quaternary dilution and 0.75 ml of 1.0*M* NaCl. All tubes centrifuged 60 sec at 2200 rev/min on International Clinical Centrifuge to separate clumped cells and then read in Klett. The concentrations giving 50 percent flocculation of cells were arbitrarily selected as flocculation concentrations.

‡ Indicated concentrations kill in 10 min but not in 5.

cides, such as p-diisobutyl phenoxy ethoxy ethyl dimethyl benzyl ammonium chloride, to a constant density of washed *S. schottmuelleri* cells suspended in NaCl solution gives gradations of flocculation that can be estimated visually or read turbidimetrically. Controls have repeatedly demonstrated that the flocculation is the result of a recation between the cells and the quaternary ammonium germicides. Thus far, no quantitative relationship between bactericidal activity, as determined by the phenol coefficient method, and the degree of flocculation has been established. Table 1 illustrates the bactericidal activities and flocculation values for three structurally different quaternaries.

Kivella, *et al.* (*3*) and Dyar and Ordal (*4*) have shown that strongly adsorbed quaternary germicides lower the electrophoretic mobilities of cells and ultimately reverse their negative charge. In the former work (*3*), clumping was demonstrated microscopically in the ranges giving positive mobilities. Flocculation as reported in the present paper occurs in the same range of concentrations. From these observations, it appears that the flocculation is an expression of the alteration of the charge at cell surfaces due to adsorption of quaternary germicide. Quaternaries of diverse chemical structure show differences in hydrophobic, polar, and other properties affecting adsorption (*5*); these differences in properties might result in the setting up of different zeta potenials at cell surfaces with dissimilar quaternaries, and flocculation might then occur at different levels of germicide, as shown in Table 1. From this, it would follow that the property being measured by the flocculation is the relative adsorption of the germicides, and since adsorption may be only a part of the bactericidal mechanism, it might not be unexpected that this flocculation test does not measure bactericidal activity. This would seem to be in contrast to the work cited in the first paragraph (*2*) in which a correlation between adsorption on an amphoteric material (wool) and bactericidal activity was indicated. However, close scrutiny of the data presented by these workers shows that the correlation is not quantitative.

Work is now in progress to determine other physical aspects of flocculation. Details on all findings will be published at another time.

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## Experimental Dental Caries, IV. The Effect of Feeding Desiccated Thyroid and Thiouracil on Dental Caries in Rats[1]


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The most commonly accepted mechanism by which fluorine reduces dental caries is by means of ionic substitution of various components of the hydroxyapatite by fluorine during the calcification of the enamel. The result is the formation of enamel less soluble in the intraoral acids produced from ingested food. Unequivocal proof for such a mechanism, however, is lacking. Nevertheless, it is readily accepted that a marked diminution of salivary flow predisposes an increased incidence of dental caries. The corollary to this fact—that is, that a reduction in caries experience follows an increased salivary flow—also lacks experimental evidence but is an attractive hypothesis, since many investigators feel that caries resistance is associated with the ability of saliva to neutralize promptly intraoral acid formation.

Rathje (*1*) postulates further that the resistance to dental caries afforded by fluorine and the relationship of salivary flow to dental caries are intimately related. It is his opinion that the reduction in dental caries produced by fluorine may be mediated through the thyroid gland by increasing salivary flow. Other work also has indicated a relationship between the activity of fluorine and the thyroid gland.

When fluorides are given in conjunction with the thyroid hormone, they appear to accentuate the effect on basal metabolic rate normally produced by thyroid hormone alone (*2*). Also, evidence indicates that the thyroid hormone enhances the bleaching of rat incisors normally produced by fluorine (*3*). However, the relationship between altered metabolism and salivary flow

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Table 1. Effect of fluorine, thiouracil, and desiccated thyroid on dental caries incidence in rats and the fluorine, calcium, and phosphorus levels in the skeleton.

| Supplement | No. of animals | Dental caries experience | | Skeletal analysis (ashed femur) | | | |
|---|---|---|---|---|---|---|---|
| | | No. of lesions | Extent | F conc. (ppm) | Total F (mg) | Ca (mg %) | P (mg %) |
| Thyroid + F* | 18 | 5.2 ± 0.31† | 1.2 | 3619 | 1.115 | 39.2 | 14.5 |
| Thyroid | 18 | 6.5 ± .33 | 1.5 | 131 | 0.029 | 40.0 | 14.2 |
| Thiouracil + F | 32 | 10.0 ± .59 | 2.7 | 5662 | .802 | 40.8 | 13.6 |
| Thiouracil | 33 | 10.1 ± .62 | 2.6 | 158 | .030 | 38.9 | 14.3 |
| Control + F | 31 | 6.4 ± .45 | 1.5 | 3992 | 1.107 | 39.3 | 14.5 |
| Control | 29 | 7.6 ± .71 | 1.9 | 265 | 0.050 | 39.5 | 14.4 |

* 20 ppm F as NaF.
† Standard deviation.

has not been defined, although it seems clear that, with increased metabolism, one might expect a greater ability to create flow from the salivary gland. It is known that there is an increased oxygen consumption (*4*) in salivary glands with increased blood flow. Thus, since no previous work has been reported on any correlation between the thyroid gland and experimental dental caries, it seemed important to investigate whether any relationship exists between the dental caries experience in rats and the activity of this gland.

Approximately 175 rats of the Sprague-Dawley strain were divided into six experimental groups. One received desiccated thyroid in the diet (in increasing amounts from 10 to 60 mg per day per animal, depending upon the growth of the animal) and another received the same amount of desiccated thyroid plus 20 μg fluorine per milliliter (as NaF) in their drinking water. A third group received thiouracil (0.1 percent in the diet), while a fourth group received the same amount of thiouracil plus 20 μg fluorine per milliliter (as NaF) in the drinking water. Another group received the same concentration of fluorine in the drinking water, while the last group received no added supplement in either food or water and served as controls. All the animals received a stock cariogenic diet (*5*) and were on the experimental supplements for 145 days. At the termination of the experiment, the animals were sacrificed by chloroform inhalation, and the heads were removed for caries evaluation and the femurs for fluorine, calcium, and phosphorus analyses (*5*).

The animals receiving thiouracil failed to gain as much weight as the controls; the final weight gain was about 80 g less in the males and 60 g less in the females. The growth of the animals in the groups that received desiccated thyroid or sodium fluoride was not affected to any significant degree, and the administration of thyroid, thiouracil, or sodium fluoride at the concentration used in this experiment did not affect the calcification of the skeleton as judged by the amount of calcium and phosphorus in the femurs. These data appear in Table 1.

The skeletal-fluorine concentration, however, varied markedly. The data indicate that the thiouracil group receiving sodium fluoride had a higher concentration of fluorine than control animals given a similar fluorine concentration but not thiouracil. That thiouracil is related to this increased storage is strengthened by the fact that the group receiving thiouracil alone had a higher fluorine concentration than similar control animals not given thiouracil. Apparently, the desiccated thyroid was without marked effect in altering the fluorine storage in the skeleton, since the fluorine concentrations in both the thyroid and the thyroid-fluoride groups are essentially similar to their controls not receiving thyroid. This is further strengthened by noting the total fluorine in the femurs of the thyroid groups and their respective controls. In the thyroid-NaF group, there was approximately 1.12 mg of F, and in the control-NaF group 1.1 mg. However, while the highest fluorine concentration is found in the thiouracil-NaF group, the total fluorine was considerably less than in the control-NaF group. This further corroborates the fact that fluorine concentration is intimately associated with skeleton growth, since the thiouracil animals' final weight was less than the controls.

The results shown in Table 1 indicate that desiccated thyroid reduces the incidence of dental caries to the same degree as sodium fluoride alone, while desiccated thyroid plus fluorine reduces the caries experience by approximately 55 percent more than either desiccated thyroid or fluoride alone. This agrees with previous work indicating a synergism between the activity of fluorine and the thyroid gland (*2, 3*). When the activity of the thyroid gland was markedly decreased through the administration of thiouracil, the incidence of dental caries in the rat was decidedly increased. The addition of 20 ppm fluorine to the drinking water of animals receiving thiouracil, a quantity of fluorine that previous work has indicated to reduce caries by approximately 20 percent (*6*), was without effect on caries. The extent or size of the tooth destruction also parallels the number of lesions found in each respective group and further indicates that decreased thyroid activity is related to increased caries susceptibility in the rat. This evidence appears convincing that the activity of the thyroid is related

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 $x$, $(e^{-\bar{x}} \cdot \bar{x}^{\bar{x}}/x!)$. 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.