Table 1. Frequency of dicentric bridges 30 hr after irradiation in different high- and low-intensity experiments with 80 r of gamma rays.

Expt	High intensity			Low intensity		
	First anaphase cells	Bridges	Percentage	First anaphase cells	Bridges	Percentage
I	384	15	3.90	830	34	4.09
II	1175	58	4.94	474	23	4.85
III	492	23	4.67	502	20	3.98
IV	2025	85	4.20	2073	80	3.85
V	919	40	4.35	805	30	3.72
Total	4995	221		4684	187	
Mean %			4.42			3.99

strengthened the conclusion that the bridges originated not through two independent breaks in a chromosome but largely as a result of a single break in an unsplit chromosome, caused by a single ionization track.

The sensitivity of the chromosomes of the primary spermatocyte resting cells of *Gesonula* to x-ray breakages does not alter appreciably for a relatively long period, inasmuch as Ray-Chaudhuri and Sarkar (*3*) have demonstrated the constancy of the frequency of first anaphase bridges recorded at different hours after irradiation up to a period of 148 hr. It is also likely that there is a similar stable period in the meiotic resting cells of plants. It has recently been shown by Darlington and LaCour (*5*) that in *Tradescantia bracteata* a constant frequency of first anaphase bridges is found between 16 and 48 hr after irradiation with x-rays. They, however, interpreted these bridges as originating from sister union of broken ends of chromatids (physiological effect) and not from chromosome breakage.

But the dicentric bridges in the meiotic cells of *Gesonula* are undoubtedly due to breakage in the chromosomes and not to any kind of physiological disturbance. This can be substantiated by the following evidence. An examination of the numerous bridges obtained by us during the course of our experiments reveals that the length of the dicentric portion of the chromatid between the two centromeres varies in different nuclei, in such wise that the length of the accompanying fragment (Fig. 2*a*, *m*) has a negative correlation with the length of the dicentric portion of the bridge (Fig. 2*a*, *l*). From the size of the monocentric free arms at the two ends of the bridge (Fig. 2*a*, *n*), we can get an idea about the size of the bivalent. A comparatively short dicentric portion relative to the size of the unaffected chromatid (free arm) indicates a break near the centromere. A bridge resulting from such a proximal break will be accompanied by a comparatively large fragment (Fig. 2*a–d*). On the other hand, the bridge originating from a distal break (Fig. 2*e–h*) will have a large dicentric portion and a small fragment. These conditions are always satisfied in all the bridges examined by us from this point of view.

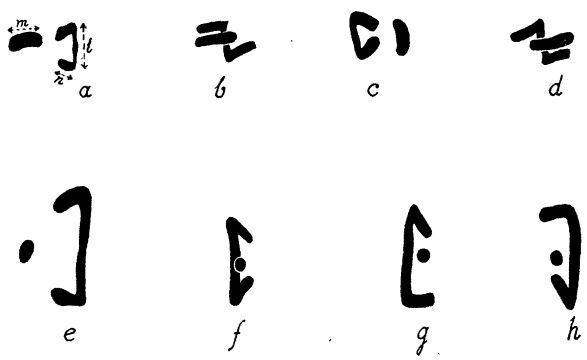

Fig. 2. *a–d*, dicentric bridges resulting from proximal breaks; *e–h*, bridges resulting from distal breaks.

References and Notes

1. B. P. Uvarov of the British Museum (Natural History) has recently informed us that the correct name for this species should be *Gesonula* (and not *Gesonia*) *punctifrons* as used by Ray-Chaudhuri and Manna (*2*) and Ray-Chaudhuri and Sarkar (*3*).
2. S. P. Ray-Chaudhuri and G. K. Manna, *J. Expt. Zool.* **114**, 421 (1950).
3. ——— and I. Sarkar, *Science*, **116**, 479 (1952).

4. We are deeply grateful to H. J. Muller, of Indiana University, for valuable suggestions incorporated in this paper. Thanks are also extended to S. Mitra, Director, Chittaranjan Cancer Hospital, for providing facilities for irradiation in his hospital and to A. Bose, physicist of the same hospital, for his help in arranging the irradiations.

5. C. D. Darlington and L. F. LaCour, *Heredity Suppl.* **6**, 41 (1952).

Received February 19, 1954.

Quantitative Flocculation of *S. schottmuelleri* Cells by Quaternary Ammonium Germicides[1]

R. L. Stedman, E. Kravitz, and H. Bell

Industrial Test Laboratory, Philadelphia
Naval Shipyard, Naval Base, Philadelphia

In recent years, relationships have been shown to exist between bactericidal properties and certain physico-chemical properties of quaternary ammonium germicides, for example, the release of conducting material by quaternary-treated cells (*1*) and the adsorption of quaternaries on wool (*2*). This report describes an attempt to relate bactericidal activities of quaternaries to the property of these agents of causing macroscopic flocculation of the test organisms. Clumping of bacteria as a result of the action of the quaternaries has been observed microscopically (*3*), but we are aware of no previous attempts to develop this observation into a macroscopic method for studying antibacterial action.

The addition of graded levels of quaternary germi-

[1] The opinions expressed herein are those of the authors and are not necessarily similar to the views of the Department of the Navy.

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.

References

1. D. N. Eggenberger, *et al., Ann. N.Y. Acad. Sci.* **53**, 105 (1950).
2. R. Fisher and S. Seidenberg, *Science* **114**, 265 (1951).
3. E. W. Kivella, *et al., J. Bacteriol.* **55**, 565 (1948).
4. M. T. Dyar and E. J. Ordal, *J. Bacteriol.* **51**, 148 (1946).
5. S. Ross, *et al., J. Colloid Sci.* **8**, 385 (1953).

Received January 25, 1954.

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

Joseph C. Muhler and William G. Shafer

Department of Chemistry and School of Dentistry, Indiana University, Bloomington and Indianapolis

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

[1] This investigation was supported in part by the Medical Research and Development Board, Office of the Surgeon General, Department of the Army, under Contract No. DA-49-007-MD-332. Paper I in this series appeared in *J. Dental Research* **31**, 798 (1952); papers II and III are in press in *J. Dental Research*.