fication, involving condensation and resublimation, as well as thermal "cracking" and alkali scrubbing.

It is possible, however, that the effect may be a physical one, affecting the exchange of CO_2 between blood and lung space. In this event it may be a temporary imbalance that might be corrected by adjustments in the tidal volume and rate of breathing in the course of a longer experiment than was carried out in this instance. It has been our experience that a temporary ventilation imbalance is also caused by breathing helium-oxygen mixtures, although without such overt symptoms. When feasible, this aspect of the administration of SF_6-oxygen mixtures will be explored further.

It is our hope that these observations will guide others about to consider the use of SF_6 in respiratory experiments. We would appreciate any comments or observations that may be helpful to our projected experiments on breath velocity patterns.

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Fungistatic Potencies of Some Fluorinated *p*-Benzoquinones

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Halogenation has been shown to increase fungicidal effectiveness in the benzoquinone series (*1*) and has resulted in the recognition and commercial development of important, practical, and highly effective fungicides (*2, 3*). Interest, however, has centered mainly in halogenation with bromine and chlorine, for iodine substitution products are highly unstable, and interest in the production of fluorinated benzoquinones is recent (*4*).

In March 1949, there became available from the Division of Fluorine Chemistry, Illinois State Geological Survey, several fluorinated *p*-benzoquinones having structural formulas analogous to those of certain chlorinated *p*-benzoquinones known to possess high fungicidal potencies. Preliminary tests showed that these compounds possessed fungicidal powers, and this fact was mentioned by Finger *et al.* (*4*) in 1949. With Arthur F. Kunes, Jr., as assistant, these compounds were given bioassays to determine their fungistatic potencies in relation to spores of *Macrosporium sarcinaeforme*.

Five compounds were subjected to examination: 2-fluoro-1,4-benzoquinone, 2,5-difluoro-1,4-benzoquinone, 2-fluoro-5-chloro-1,4-benzoquinone, 2-fluoro-5-bromo-1,4-benzoquinone, and 2-fluoro-5-methyl-1,4-benzoquinone. These compounds were prepared under the direction of G. C. Finger, chemist in charge of fluorine research, Illinois State Geological Survey. Their preparation and their physical characteristics were described by Finger *et al.* (*4*) in 1949.

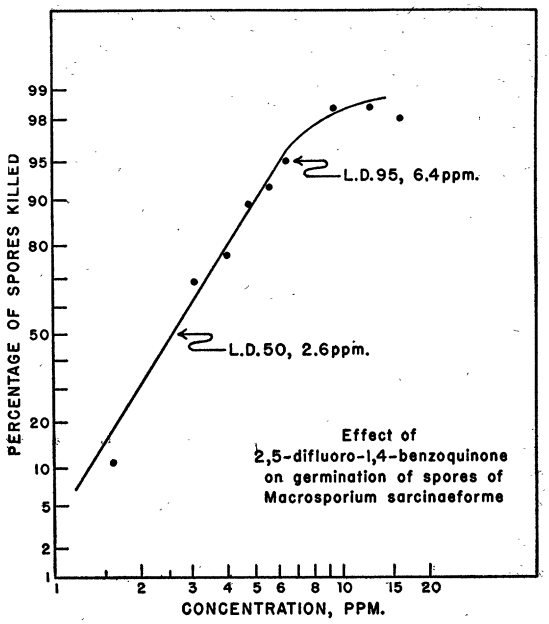

FIG. 1. Dosage-response curve obtained with 2,5-difluoro-1,4-benzoquinone and spores of *Macrosporium sarcinaeforme*, using the Peterson method of bioassay.

Bioassays were made by the Peterson (*5*) method, with minor modifications. The test fungus was obtained from Boyce Thompson Institute and presumably was a subtransplant of the strain of *M. sarcinaeforme* used by McCallan (*6*). For production of spores, the fungus was grown on thin oat agar slants for 20 or 21 days at 25° C. Mixtures consisting of a standard 50,000/ml spore suspension and of graded concentrations of the candidate compound first dissolved in 95% ethyl alcohol and then dispersed in distilled water were pipetted upon chemically clean, sterile 12-mm glass circles previously impressed in a thin coat of petrolatum on 75 × 25-mm glass microscope slides. Three circles were used per slide, and slides were arranged for incubation in groups of 4 in water-sealed, inverted 7 × 20-cm culture dishes provided with wet paper bottom pads and glass U's to support the slides above the pads, as directed by McCallan (*6*). Suitable checks were employed. Both circles and slides were so arranged as to give non-duplicating random arrangements of all concentrations in each of 4 culture dishes, thus replicating all concentrations 4 times.

Readings of spore germination were made after 20 hr of incubation, 200 spores being counted on each circle, and the readings obtained from the 4 replications were averaged.

Most effective in preventing germination of *M. sarcinaeforme* spores was 2,5-difluoro-1,4-benzoqui-

none. Fig. 1 shows results obtained with this compound. The dosage-response curve has a slope of about 58°. An LD_{50} value of 2.6 ppm and an LD_{95} value of 6.4 ppm may be interpolated on it.

Table 1 gives for all 5 compounds LD_{50} and LD_{95} values indicated by interpolation on their dosage-response curves and, for further comparison of the compounds, the slopes of their curves.

TABLE 1

LD_{50}, LD_{95}, AND DOSAGE-RESPONSE CURVE SLOPES OF FIVE FLUORINATED *p*-BENZOQUINONES IN RELATION TO SPORES OF *Macrosporium sarcinaeforme*

p-Benzoquinone compound	Parts per million		Approximate slope of curve
	LD_{50}	LD_{95}	
2-fluoro-	4.4	15.0	47°
2,5-difluoro-	2.6	6.4	58°
2-fluoro-5-bromo-	8.4	20.2	57°
2-fluoro-5-chloro-	13.0	23.5	67°
2-fluoro-5-methyl-	36.0	70.0	64°

The difluoro-*p*-benzoquinone was tested also against spores of *Alternaria solani,* using a strain of the fungus obtained from Boyce Thompson Institute. Spores used in testing were produced on potato dextrose agar and harvested on the ninth day. Readings consisted of counts of 100 spores on each circle after 20 hr of incubation. An LD_{50} of 7.1 ppm and an LD_{95} of 17 ppm were indicated by interpolation on the dosage-response curve. Although these values are nearly three times those obtained with *M. sarcinaeforme,* the slopes of the dosage-response curves for both fungi were the same, about 58°. For the chlorine analog an LD_{50} of 11 ppm and for chloranil an LD_{50} of 8 ppm were reported by Schoene *et al.* (*3*) for the same fungus.

According to McNew (*7*), toxicity increases as a result of halogenation in the order iodine, bromine, chlorine. In our results, the substitution of a bromine atom gives a more effective compound than the substitution of a chlorine atom, when there is also present a substituted fluorine atom. The slope of the dosage-response curve of the fluorobromo compound is, however, not so steep as that of the fluorochloro compound. Methylation appears, as would have been expected (*2,* 146; *3,* 26), to have diminished toxicity appreciably.

Although more extended bioassays should be made, using other methods of assay and other fungi as indicators, the results presented here show that fluorinated *p*-benzoquinones have high fungistatic potencies. The effective dosages are remarkably low, and the slopes of the dosage-response curves are steep. The differences shown in Table 1 between monofluoro-*p*-benzoquinone and difluoro-*p*-benzoquinone suggest that, if it becomes possible to make trifluoro or tetrafluoro compounds, those compounds would prove effective at still smaller dosages, and, if the gradation from the monofluoro-monochloro through the monofluoro to the difluoro compound illustrated in our results continues to hold true, might well be considerably more potent than chloranil.

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A Centrifugal Device for the Preparation of Embryo Extract and Tissue Minces

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Existing methods for the production of chick embryo extract used in tissue culture are cumbersome and time-consuming. As described by Cameron (*1*) in her recent manual, the small-scale procedure involves aseptic chopping with curved scissors of 8–10-day-chick embryos in a watch crystal until they are reduced to a fine pulp, addition of an equal volume of Tyrode solution, mixing in a wide-mouth pipette, transfer to centrifuge tubes, 10 min of centrifuging at 2,000 rpm, withdrawal of supernatant, and recentrifuging. The time element and the several transfers involved in this technique increase the danger of contamination.

The Pyrex homogenizer, based on the double test-tube model of Hagan (*2*), and perfected (*3*) to include a motor-driven toothed glass pestle fitting tightly into a Pyrex tube, has been adapted (*4*) for mincing 6–8 chick embryos in one operation.

The apparatus described here (Fig. 1) combines

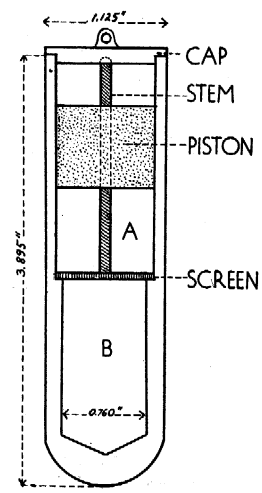

FIG. 1. Centrifugal tissue mincer.