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.

References

1. CUNNINGHAM, H. S., and SHARVELLE, E. G. *Phytopathology,* **30,** 4 (1940).
2. HORSFALL, J. G. *Fungicides and their Action.* Waltham, Mass.: Chronica Botanica, **7,** 145, 146 (1945).
3. SCHOENE, D. L., TATE, H. D., and BRASFIELD, T. W. *Agr. Chemicals,* **4,** (11), 24 (1949).
4. FINGER, G. C., FINNERTY, J. L., and SCHNEIDER, H. G. *Am. Chem. Soc., Abstr. of papers, 116th meeting,* 17K (1949).
5. PETERSON, P. D. *Phytopathology,* **31,** 1108 (1941).
6. MCCALLAN, S. E. A. *N. Y. (Cornell) Agr. Exp. Sta. Memoir,* 128 (1930).
7. MCNEW, G. L. *Bull. Torrey Botan. Club,* **77,** 294 (1950).

Manuscript received July 23, 1951.

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

Theodore S. Hauschka and Edmund Poppe

Institute for Cancer Research* and *Lankenau Hospital Research Institute, Philadelphia, Pennsylvania

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

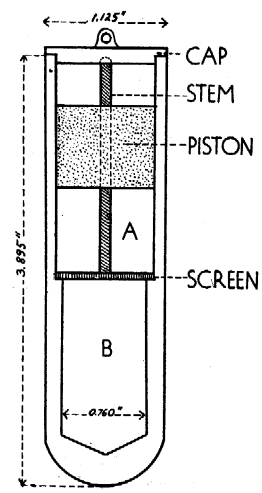

FIG. 1. Centrifugal tissue mincer.

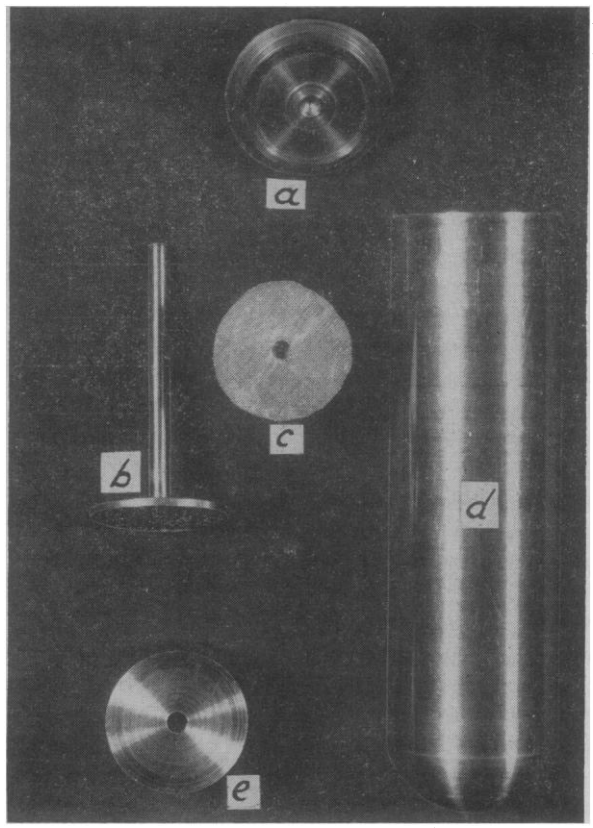

FIG. 2. Centrifugal tissue mincer disassembled.

mincing, screening, and centrifuging in a single step requiring less than 10 min, instead of more than 1 hr as under the older method. It fits the rotor of a Sorvall SS-1 superspeed angle centrifuge and is operated at 13,000 rpm at an R.C.F. of about 20,000 × G. The stainless steel construction allows dry heat sterilization and is not objectionable on chemical grounds. Since the rotor carries 8 tubes, 4 properly balanced mincing units may be run at the same time.

In practice, the tube (Fig. 2, *d*) containing only the stemmed screen (Fig. 2, *b*), is dry-sterilized in a cotton-plugged Pyrex tube; cap and piston (Fig. 2, *a* and *e*) are sterilized in a deep Petri dish. The device may be chilled before loading 4 chick embryos (or other tissues to be minced) into space *A*. The piston is picked up with sterile forceps at its upper end and slipped over the stem into the tube, which is then capped. After critical counterbalancing with a steel tube containing finest birdshot in the opposite tube-depression of the previously chilled rotor, the centrifuge is gradually brought to top speed, and shut off after 3 to 4 min at 13,000 rpm. The mincing tube is then removed, opened under sterile precautions, and with a pair of flamed pliers the screen-piston assembly is grasped by the stem and pulled out slowly to avoid a vacuum.[1] Space *B* now contains a steep bank of tightly packed dissociated tissue and about 4 ml of clear, concentrated embryo juice. This amount is more than double the yield per embryo that may be expected after mincing with curved scissors.

The dimensions in inches of the unit shown in Figs. 1 and 2 are: length without cap, 3.895; OD, 1.125; ID, space *A*, 0.939; ID, space *B*, 0.760; distance from top to shelf, 2.000; the piston diam, 0.938; piston height, 0.750; screen thickness, 0.060; screen diam, 0.938; screen stem diam, 0.125; hole through piston to accommodate stem, 0.126; cap thickness, 0.160; thickness of cap lip, 0.042; diam of cap recess, 0.937. Holes in screen are drilled with a No. 60 to 69 drill and closely spaced in concentric circles.

A disk of stainless steel mesh (Fig. 2, *c*), 80–90 wires/inch (gauge #37 B & S), with openings measuring 150–165 μ square, may be inserted above the screen for very fine mincing. This is not recommended for making embryo extract, but is useful in preparing uniform tumor suspensions.

[1] An improvement in design, since this description has gone to press, eliminates formation of a vacuum by replacing the stem (Fig. 1) with stainless tubing which opens into space *B* below the screen disk.

References

1. CAMERON, G. *Tissue Culture Technique.* New York: Academic Press (1950).
2. HAGAN, W. A. *J. Exptl. Med.*, **36**, 711 (1922).
3. POTTER, V. R., and ELVEHJEM, C. A. *J. Biol. Chem.*, **114**, 495 (1936).
4. PARKER, R. C. *Methods of Tissue Culture.* New York: Hoeber (1950).

Manuscript received July 18, 1951.

A Simple Procedure for Determination of the Approximate Lymph Space[1]

R. H. Storey, J. Moshman, and J. Furth[2]

Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee

In the course of studies of permeability changes caused by irradiation it was discovered that whatever macromolecular or corpuscular substance was injected intravenously, it disappeared faster from the circulation of x-rayed animals than from that of normals (*1*). Subsequent studies led to the observation that the substance which disappeared from the blood entered the tissue space (*2*). In order to determine the magnitude of this change, it became essential to measure the space.[3, 4]

Data on the tissue space are few and discrepant (*3*). The main difficulty in the estimation of the magnitude of this space is caused by differences in composition of the lymph in different parts of the

[1] Work performed under Contract No. W-7405-eng-26 for the Atomic Energy Commission.

[2] The authors gratefully acknowledge the constructive criticism of this manuscript by W. S. Wilde, C. W. Sheppard, A. W. Kimball, and A. S. Householder and the help of J. Z. Hearon and W. S. Wilde in the formulation of equations (*a*) and (*b*).

[3] The term "lymph" is used in the broad sense, including the fluid in the interstitial spaces and lymphatics. These two spaces combined are conventionally called tissue space.

[4] The term "space" is used instead of volume for theoretical reasons, but it is certain that the values for the two are close if not identical.