A Turbidimetric Method for Determining the Effect of 2,4-D Upon the Growth of Yeast

FRED R. WEST, JR., and JAMES H. M. HENDERSON

The G. W. Carver Foundation, Tuskegee Institute, Alabama

A considerable amount of work has been reported concerning the effect of 2,4-dichlorophenoxyacetic acid (2,4-D) upon the development or growth of plant tissues. The subject has been reviewed recently by Mitchell and Marth (*1*). In view of the widespread use of this substance as a herbicidal or herbistatic agent, a study of the mechanism of its action on plant cells seemed desirable.

TABLE 1

TURBIDITY MEASUREMENTS OF YEAST CULTURES AT VARIOUS CONCENTRATIONS OF 2,4-D

Date	Concentrations of 2,4-D/ flask (ppm)	Per cent transmission			
		Flasks			Average
		1	2	3	
10–10–47	Control*	19	19	18	19
10–25–47	Control	18	20	22	20
"	0.001	20	23	21	21
"	0.01	22	22	21	22
"	0.1	22	21	21	21
"	1.0	22	21	22	22
10–10–47	1.0	18	18	20	19
"	10	22	23	23	23
"	100	46	46	47	46
"	1,000	82	81	82	82

* Control contains no 2,4-D.

Since growth and respiration are generally closely allied, it seemed plausible to use respiration as a means of studying the underlying mechanism of 2,4-D. In making such a study, it was thought desirable to run preliminary experiments on living yeast cells. This study could then serve as groundwork toward establishing the most effective concentration of 2,4-D to be used in experiments on mechanism. Yeast cells were selected (1) because of their ease of growth and manipulation and (2) because it is assumed that cell reactions of plants and animals, regardless of phylogenetic rank, generally are the same. In this particular phase of the work to be reported, the effect of 2,4-D on the growth of yeast is measured, by determining the turbidity of the medium, using a Cenco Photelometer.[1]

Cultures of *S. cerevisae* were grown on fort agar (*2*) slants at a pH of 4.8 and incubated for 48 hrs at 30° C. These were stored at 4° C. To 500 ml of a Czapek's solution were added 25 gm of glucose and 0.12 γ of biotin. Twenty-five ml of this solution was put into 15 125-ml Erlenmeyer flasks which were autoclaved at 15 lbs pressure for 15 min.

[1] Cenco-Sheard-Sanford "Photelometer," Type C-5, Central Scientific Company, Chicago, Illinois.

A stock solution of 2,4-D was prepared at a concentration of 1,000 ppm, and from this, concentrations of 100, 10, and 1 ppm were prepared. All four concentrations were then autoclaved at 15 lbs pressure for 15 min.

The 15 sterile flasks containing the Czapek's solution were divided into 5 groups of 3 each. The first group served as the control (no 2,4-D). Each flask was then inoculated with yeast cultures and incubated at 30° C. At the end of 7 days, growth was determined by the turbidimetric method. A red filter was used throughout.

The results, as shown in Table 1, indicate that concentrations of 2,4-D from 10 to 1,000 ppm have the most effect upon the growth of yeast. Concentrations of 1 ppm have practically no effect. This finding was further substantiated when another batch of yeast was subjected to 2,4-D in which the concentration ranged from 0.001 to 1.0 ppm. The same procedure was used as described above. With the exception of one aberrant value, the results were similar, *i.e.* the same as the control.

The test described above, which is based on the assumption that growth is directly proportional to turbidity, is relatively simple and can be run in a comparatively short time.

References

1. MITCHELL, J. W., and MARTH, P. C. *Growth regulators.* Chicago: Univ. Chicago Press, 1947.
2. PARFITT, E. H. *J. dairy Sci.*, 1933, **16**, 141.

A Simple Adaptation of the Mercury Calibration of Warburg Manometer Sets to Insure Interchangeability

SHANNON C. ALLEN

Pharmacology Division, Bureau of Agricultural and Industrial Chemistry, U. S. Department of Agriculture, Albany, California

Almost anyone who has worked with the Warburg technique has experienced the irritation and delay which follows the breaking of a flask. Even with reasonable care, vessels do get broken, and unless one has extra sets, runs may be delayed until the flask has been repaired and recalibrated. This can be avoided by the use of flasks and manometers with interchangeable joints and an extra set of flasks. In this laboratory the most commonly used flask is the simple double-side-arm type, and extra flasks were procured to use either with a set of four Summerson manometers or as replacements on the other manometers. In planning the calibration of the glassware it was realized that, if the usual calibration methods were used, it would be necessary to calibrate each of these vessels both with its Summerson manometer and with its intended replacement manometer and that without a great deal of extra calibration interchangeability was strictly limited. To increase the interchangeability of flasks and manometers the following scheme was worked out.

Any flask in the replacement set is weighed empty and then filled, according to the method of Burris (*1*), with