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NEW HYBRIDS FROM INCOMPATIBLE CROSSES IN DATURA THROUGH CULTURE OF EXCISED EMBRYOS ON MALT MEDIA[1]

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INCREASINGLY of recent years it is becoming possible to control the activities of the living plant by chemical treatment. The success in doubling chromosomal numbers with colchicine and other stimuli naturally led to an attempt to halve the chromosomal number by some similarly simple treatment. The fact that over two hundred haploids (1*n*) had turned up spontaneously in our cultures of Daturas since 1921 showed that the plant is capable of producing individuals with half the normal 2*n* chromosomal complement. Preliminary attempts to induce the production of 1*n* offspring by treating the unfertilized egg cells with a wide series of stimuli were entirely unsuccessful. In the summer of 1940 the cooperation of Drs. J. van Overbeek and Marie Conklin was secured in a more intensive attack on the problem. Something was learned about the processes involved in embryo development,[2] but none of the stimuli tested induced the production of 1*n* embryos. In the summer of 1941 they attacked the problem from a different standpoint in order to learn more about the factors involved in embryo development and attempted to dig out the young embryos and cultivate them on artificial media. The older embryos could be thus readily cultivated, but the smaller ones (under 0.5 mm in *D.*

[1] Read before the American Philosophical Society, November 19, 1943. Contributions from the Department of Botany, Smith College, New Series, No. 12. This investigation was supported in part by the Carnegie Institution of Washington and by a grant from the American Philosophical Society. The authors are indebted to Margaret Conlin, Jean Cummings, Susanne McLean and Mary Sanders, who as graduate assistants have made a large proportion of the dissections.

[2] J. van Overbeek, M. E. Conklin and A. F. Blakeslee, *Am. Jour. Bot.*, 28: 647–656, 1941.

sent crude extracts. Considerable work will be required to determine fully the range of biological activity and the chemical nature of the active principle. In view of the low and variable yields thus far obtained it is apparent that further work is necessary to determine the optimum conditions for the accumulation and extraction of chlorellin. The cell mass may prove to be a valuable source of the active principle. These investigations are progressing.

Chlorellin is unique in the constantly growing list of antibiotics which have been reported in the literature, because, since it is derived from an autotrophic organism, its production does not entail the use of expensive and troublesome organic culture media, only inorganic salts, carbon dioxide and light being required.

These investigations have been carried out as a cooperative project by staff members of the College of Pharmacy of the University of California and of the Division of Plant Biology of the Carnegie Institution of Washington.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

NEW INDICATORS TO REPLACE LITMUS IN MILK

THE use of litmus milk has gained wide popularity in bacteriological procedures because of the large number of biochemical reactions that can be determined with one inoculation. It has been our experience to find it very difficult to duplicate the density of the color. This may be due to the fact that litmus is not a single substance but a mixture which varies with the lichen and preparation used.

A search for a single compound which would serve both as a pH indicator and an E_h (oxidation-reduction) indicator in the proper ranges proved disappointing. A combination of indicators was decided upon, and those embodying the most desirable properties both alone and in combination were Chlor Phenol Red (or Brom Phenol Red) for the pH indicator and Methylene Blue for the E_h indicator.

Chlor Phenol Red is a member of the sulfonphthalein dyes which are widely used in bacteriological media. The pH range is from 5.2 to 6.8. Its yellow color on the acid side and red color on the basic side does not mask the reactions of the Methylene Blue. A stock solution is prepared by dissolving 0.1 gram in 10 cc of absolute alcohol.

The Methylene Blue stock solution is prepared by dissolving 0.625 grams (total dye concentration 84 per cent.) in 250 ml of distilled water. The stock solutions are kept in well-stoppered light-tight bottles.

The formula for the preparation of the milk is as follows:

Dry milk powder	90 grams
Chlor Phenol Red (stock solution)	1 ml
Methylene Blue (stock solution)	2 ml
Water	make up to 1 liter

The milk is then sterilized by the fractional method or autoclaved at 15 pounds for 15 minutes. The color of the milk changes to a light pink when strongly heated, but the original color returns as the milk cools.

Autoclaved milk requires a somewhat longer time for acid coagulation, but this is foretold by the very rapid reduction of Methylene Blue in this reaction.

The color changes in the milk as compared with Ridgeway's Color Standards (1912) are summarized as follows:

Uninoculated	Pale Glaucous Green
Slightly acid	Yellowish Glaucous
Acid with reduction	Ivory Yellow
Alkaline	Pale Russian Blue
Alkaline with reduction	Pale Pinkish Cinnamon
Alkaline peptonization	Clear Transparent Red

Inoculation of organisms of known biochemical character readily overcomes confusion in transposing the same reactions found on litmus milk.

This medium has been successfully used in a number of beginning and advanced classes in bacteriology. Students using milk for the first time have no difficulty in determining the reactions which have taken place.

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BOOKS RECEIVED

ABRAMS, LEROY. *Illustrated Flora of the Pacific States.* Volume II. *Buckwheats to Kramerias.* Illustrated. Pp. viii + 635. Stanford University Press. $7.50.

WEIDENREICH, FRANZ. *The Skull of Sinanthropus Pekinensis; A Comparative Study on a Primitive Hominid Skull.* Illustrated. Pp. xxi + 484. Published by the Geological Survey of China. For sale at the office of G. E. Stechert and Company.