of the Federation of American Societies for Experimental Biology. Since it has not yet been found possible to elaborate the technique, with protocols, in a longer article, as had been hoped, it seems best to publish these suggestions now and so to make them generally available for adaptation to individual problems in enzyme research.

The method is based on a reduction in the density of pieces of exposed photographic film by the release of silver through the progressive proteolysis of the gelatin layer. The relative densities before and after exposure to the enzyme solution are read against a suspension of the same gelatin-silver emulsion in a Duboscque type colorimeter, or by means of a photometer.

The film: Eastman Commercial, 8″ x 10″; 2 to 6 in a pile, irradiated by Roentgen ray, 50,000 v., 10 m.a., 25″ target distance, 2 min. exposure. (Small rectangles may be defined and numbered by lead strips and figures cemented to the cassette.) The exposed film is fully developed, fixed in plain hypo (no hardener), washed, dried, rewashed, dried again and cut into rectangles 2 x 2.5 cm.

The cells: No. 14 (1.5 mm) copper wire coiled around a 1.3 cm rod is snipped off in nearly complete circles (0.5 cm opening), bent flat and sealed with paraffin on 1.5 x 2 cm glass slips, with the opening on a 2 cm side. Backs are glass slips 1.5 x 2 cm. Cell, film and back are clipped together with a spring clothes-pin.

The colorimeter: A Klett, Bausch and Lomb or other Duboscque type is used, with spring clips under the tube shelves to hold the film carriers. These carriers are double leaves of thin brass, lacquered flat black, with centered 1 cm holes, between which the film is slipped for insertion under the shelf in the light path. The suspension for comparison is made of gelatin-silver emulsion, dissolved off of two films in hot water. Glycerin is added to 50 per cent. to delay sedimentation. A completely cleared film is used in the carrier under this tube. Fifty per cent. glycerin solution fills the tube above the test films. Both tubes must be at the same level when readings are made.

Method of use: Readings are made on each film before use, one film being reserved as a control. The others are each placed between a glass back and the copper ring of a cell, gelatin side to copper ring, and the whole held together with a spring clothes-pin. Enzyme solution is filled into the cell with a capillary pipette, and the cell placed upside down, gelatin film surface forming the roof, at constant temperature for a carefully timed interval. (A separate film is used for each determination desired.) Then the clothes-pin is released, the film is rinsed quickly in cold water and dried rapidly, clipped by one corner, before a fan. Units should be started at not less than fifteen-second intervals to allow for this rinsing of the successive films. When dry the density of each film is again read against the gelatin-silver suspension. Any change in the density of this suspension is revealed by a recheck on the control film, and the other control readings are corrected accordingly. Results are obtained in percentage of gelatin unaffected—the ratio of the final to the (corrected) control reading on each film. 100 minus this ratio, (*i.e.,* the percentage of gelatin affected) is proportional to the enzyme activity at the time and under the conditions of the test.

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SPECIAL ARTICLES

THE PRODUCTION OF HOMOZYGOTES THROUGH INDUCED PARTHENOGENESIS

BOTANISTS attempting to produce species hybrids have frequently obtained plants which resemble the maternal species exclusively. I have myself noted such results in various efforts to cross species of Nicotiana and of Fragaria. Obviously the most likely explanations of these phenomena (apart from certain special cases to which it is unnecessary to refer in this note) are (a) induced development of vegetative tissue, such as that of the nucellus, and (b) induced parthenogenesis.

More than a decade ago I endeavored to determine the true cause by an experiment on certain species of Nicotiana; but was unable to obtain positive results owing to the difficulty in finding satisfactory quantitative characters in the species employed. Some six years ago a similar experiment was started on the genus Fragaria. Two similar types of *F. vesca* ($2n = 14$) were crossed, in order to study the inheritance of the contrasting characters, red fruit and white fruit, and pink flower and white flower. These characters proved to be due to independent pairs of factors *R* and *r* and *P* and *p*, in which dominance of color was virtually complete. Accordingly, a first generation hybrid RrPp was pollinated with pollen from species such as *F. chiloensis* and *F. virginiana* ($2n = 56$). A number of maternals were obtained

exhibiting the gametic types RP, Rp, rP, and rp, thus indicating induced parthenogenesis. If induced nucellus division had been indicated, the resulting plants must all necessarily have been red-fruited and pink-flowered.

Owing to the extreme lightness of the pollen of all the strawberry species, the earlier results were questioned, and the experiment was repeated under the most careful conditions. Of twenty-four plants which have now fruited, twelve are red-fruited and pink-flowered, three are white-fruited and pink-flowered, seven are red-fruited and white-flowered and two are white-fruited and white-flowered. These plants are all diploids ($2n = 14$).

Naturally the possibility is not excluded that a portion of these plants arose through the division of vegetative cells. On the other hand, at least a portion—and perhaps all—of the plants must have arisen through induced parthenogenesis. That is to say, the beginning of development must have been haploid. This being true, the ensuing diploidy is most likely to have come about through ordinary mitotic division. The plants are therefore complete homozygotes.

As yet I can not say whether the breeding results corroborate these conclusions. It will take three years more to grow selfed progeny of each maternal plant. But in looking back over data on Nicotiana species recorded before the war, I find that as high as 100 seeds per capsule yielded maternals in certain attempted crosses where the full complement of seed per capsule is about 400. Notes, photographs and measurements show that the populations resulting from these seeds were extremely uniform.

Since the production of maternals is common in many species when an attempt is made to cross distant species, since some of these cases are certainly instances of induced parthenogenesis with ensuing diploidy, I am publishing this note with the hope that geneticists working with each of the important agricultural and horticultural crops will undertake similar experiments. If the technique can be improved so that a reasonable percentage of parthenogenetic embryos can be forced to develop, and if the majority of these prove to be homozygous diploids, the importance of the procedure to agriculture can hardly be over-emphasized.

I can give very few suggestions regarding technique; but perhaps the following points may be helpful. The indications are that (a) pollen which will produce tubes that will enter the micropyle are likely to induce parthenogenesis whether or not hybrids are occasionally formed; (b) parthenogenesis is not induced when the two species are so similar that hybrids are usually formed; (c) diploidy is probably the result of mitotic division of the chromosomes without nuclear division, division then taking place normally. Possibly X-ray or radium treatment or other stimuli will produce similar results. The main point to be guarded in experiments is to use mother plants having a genetic constitution that will permit easy detection of parthenogenetic diploids.

If this means can be used for the production of complete homozygotes, the labor of producing homozygotes through long periods of self-fertilization will be eliminated. First generation hybrids of homozygous stocks of many perennials such as grapes, blackberries, strawberries, apples, plums, etc., can then be tested out. Moreover, it would be possible to use homozygotes of annuals produced in this way for plot-testing experiments, for physiological researches and for determining residual variability due to external conditions.

Professor R. A. Emerson writes to me stating that for several years he has been endeavoring to secure haploids parthenogenetically with the hope that some diploid seeds can be obtained by selfing them. Professor L. J. Stadler also has been working for and obtaining haploids by means of treatment with X-rays. In this connection it might be pointed out that possible non-disjunctions, translocations, inactivations of small portions of chromosomes, etc., in haploids might reduce the probability of obtaining complete homozygotes in this way; whereas mitotic division of each chromosome at the first nuclear division of the stimulated egg-cell (if that is what occurs) would practically insure homozygosis. Second, certain experiments can be set up which would insure the detection of parthenogenetic haploids which would not insure the detection of parthenogenetic diploids. Experiments should be planned to detect both types.

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THE PRODUCTION OF BACTERIA-FREE AMOEBIC ABSCESSES IN THE LIVER OF CATS AND OBSERVATIONS ON THE AMOEBAE IN VARIOUS MEDIA WITH AND WITHOUT BACTERIA[1]

SINCE the cultivation of *Entamoeba histolytica* by Boeck and Drbohlav five years ago, a number of investigators have attempted to obtain this amoeba free of bacteria, but without success. Various dyes have been placed in the cultures with the hope of inhibiting bacterial growth without inhibiting the growth of the amoebae or without killing them. Also the cysts have

[1] Aided by a grant from the DeLamar Mobile Research Fund.