lyophilized.[3] The procedure resulted in various degrees of elongation of the axial periods. In a series of 75 individual fibrils so treated, the greatest elongation found in an axial period was of about 7,000 A, as close as could be measured (Fig. 3). This measured value seems to parallel the figures of the obtained estimates.

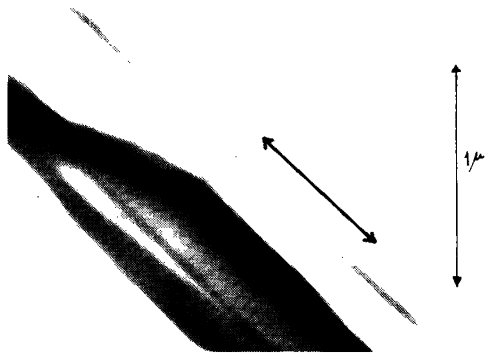

FIG. 3. Electron micrograph of a collagen fibril stretched and lyophilized. The period parallel to the arrow measures approximately 7,000 A (× 35,000).

The stretching undergone by the fibrils may be thought of as the summational effect of the uncoiling of the constituent molecules. This uncoiling is believed to be conditioned and limited by the rotational possibilities about the involved bond. In the established concept (*9*) that the collagen micelle contains parallel chains of the general form

$$\text{—CH(R}_1\text{)—NH · CO—CH(R}_2\text{)—}$$

proline and hydroxyproline are built into chains forming imino links

$$\text{=N—CO—}$$

believed to restrict rotation about these atoms. Because of the large proportion of such radicals, approximately 1 in 4 (*10*), full extension of the polypeptide chain in collagen cannot be achieved and complete flattening of the bond angles cannot be obtained.

If 7,000 A is accepted as the correct value, it then seems logical to infer that no less than 6 such chains are linked in series within one axial period of 640 A in order to account for the observed magnitude of extensibility in the fibril. It is interesting to compare this figure with the number of intraperiodic bands or elevations demonstrable with special techniques in collagen fibrils.

The six intraperiodic bands that can be resolved in fibrils "stained" with phosphotungstic acid (*7*) indicate a preferential affinity for the acid at six distinct levels of the axial period and suggest the presence of six repeating elements within its over-all length. The six intraperiodic elevations visible in electron micrographs of unstained but shadowed collagen concur with this suggestion.

From the considerations made above, the minimum total number of polypeptide chains in the collagen micelle will be:

$n = 6$ (polypeptide chains linked in series) × 4–6 (polypeptide chains linked in parallel) = 24–36.

[3] Details will be published elsewhere.

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The Use of Oxalate to Produce Free-living Cells from Carrot Tissue Cultures

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At the turn of the century Haberlandt (*1*) formulated the concept of plant tissue culture but was unsuccessful in obtaining proliferating cultures from single isolated cells. Various workers (*2*) since that time have tried in vain to obtain tissue cultures derived from different kinds of single isolated plant cells. The sole exception to these negative results is an unconfirmed report by Schmucker (*3*), who obtained division of the spongy mesophyll cells of *Bocconia*. White (*4*) has suggested that the cause of failure is the use of mature, and hence unsuitable, cells. The purpose of the present report is to describe a chemical method whereby single viable bacteria-free cells of varying age and size may readily be obtained from plant tissue cultures.

The method was suggested by the researches of Herbst (*5*), who demonstrated that fertilized eggs of *Echinus*, when grown in artificial sea water deficient in calcium, divide to form cells that soon separate. Since the cementing material of plant cells is commonly calcium pectate (*6*), it was assumed that the omission of calcium might cause separation of the cells. Heller (*7*) recently grew carrot tissue on filter paper in the absence of calcium and noted a decreased growth rate, but made no mention of the friability of the tissues. In the present experiments the oxalate method of Pringsheim (*8*) was used to bind free calcium. The basal growth medium was that of White (*9*), with sodium nitrate substituted for calcium nitrate. The medium contained 10–7 *M* naphthalene acetic acid, 10–5 *M* cysteine hydrochloride, 10–6 *M* thiamine hydrochloride, and 10% coconut juice. The pH was adjusted to 5.7. To this medium increments of ammonium oxalate were added in the amounts of 0,

100, 200, 300, 400, 500, 600, 700, 800, 900, and 1,000 μg/ml (ppm), respectively.

One set of experiments was executed in 50-ml Erlenmeyer flasks containing 4 ml of carrot medium solidified with 0.8% agar. A second set of experiments was conducted in pyrex side-arm test tubes containing 4 ml of liquid medium. Cultures in test tubes were aerated with air forced through a solution of 10% potassium dichromate in sulfuric acid, sterile water, and numerous sterile cotton plugs placed at intervals in the rubber tubing connecting the test tubes. The cultures did not become contaminated. Each concentration of oxalate, solid and liquid medium, was run in duplicate. Inoculation was made with sterile 5-mg pieces of carrot removed by a cannula from the cambial region of a carrot root. After inoculation the cultures were placed in the dark at room temperature.

At the end of 10 days the cultures were examined. Aerated cultures were especially friable and crumbled readily. Indeed, some cultures had spontaneously broken into large or small pieces while growing. Certain tubes and flasks contained cultures with free single cells. In both tubes and flasks lower increments of oxalate (100 μg/ml) allowed both proliferation and production of loose cells. These were easily dislodged from the flask cultures by a glass needle or could be readily sucked into capillary pipettes when the tissue mass was placed in aqueous medium. Higher oxalate increments retarded the growth of the cultures. Aerated cultures had shed many cells into the surrounding medium. The cells were of many shapes and sizes, some ranging up to several hundred μg in length. Most cells were bean- or banana-shaped, but a few had single emergences resembling embryonic root hairs. Many cells were disorganized and obviously dead, but many other cells were visibly living, with nucleus, vacuole, plastids, granules, etc., plainly evident upon microscopic examination.

Viability of the cells was tested in two ways. One method was by direct microscopic observation of normal cells. This was done by sucking cells aseptically into sterile micropipettes, whose ends were then sealed with sterile paraffin. This permitted repeated observation of individual cells. Cytoplasmic streaming could be seen because of the orderly movement of material suspended in the cytoplasm, and was especially evident in the strands of cytoplasm traversing the vacuole. Some cells showed cyclosis at the end of 10 days even under these relatively anaerobic conditions. The other method of testing for viability was by means of the colorless dye 2,3,5-triphenyltetrazolium chloride. This dye turns pink or red when reduced, and has recently been widely used to distinguish living from dead cells (*10*). Cytoplasmic granules of many single carrot cells subjected to 500 μg/ml of the dye for 12 hr were pink, thus demonstrating their reducing ability, a common property of healthy protoplasm. Dead cells did not change the color of the dye.

In a recent publication, White (*11*) has commented that not enough is known about plant hormones at the cell level. It is hoped that the facility of the method here reported and the present widespread use of plant tissue culture techniques will stimulate the study of single cells and eventually lead to a better understanding of the interrelationships of plant cell and hormone. The effects of various chemicals on single carrot cells is being investigated.

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The Effect of Heated Linseed Oil on Reproduction and Lactation in the Rat[1, 2]

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A series of experiments carried out in this laboratory during the past 4 years has shown that diets containing 10% by weight, or more, of heated linseed oil are less nutritious than diets containing the same amounts of unheated linseed oil, the effects having been gauged by live-weight gains, efficiency of feed consumption, and time of survival (*1, 2*). In this connection "heated oil" signifies oil heated in the absence of oxygen at 275° C. Impairment of nutritive value has been observed with oils heated for as short a period as 4 hr. It has been shown in the test herein reported that reproduction and lactation are impaired in the female rat by diets containing 10% of the heated oil.

The experiment used 72 female albino rats distributed among 4 diet groups of 18 rats each. The rats received the experimental diets from the time of mating until the end of the subsequent lactation. Groups 1 and 3 received the heated oil diet, and Groups 2 and 4 the unheated oil diet. Rats of Groups 3 and 4 received in addition a supplement of 20 mg α-tocopherol per week.

Immediately before parturition, the females were put into individual wire-mesh cages. The cages were

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[2] This test is a part of a larger project undertaken for the Committee on Edible Fats and Oils of the National Research Council of Canada, whose financial assistance is acknowledged. Paper No. 259 of the Canadian Committee on Food Preservation.