removed at varying time periods after exposure to radiation. Homogenates were prepared from these marrows and incubated at 38° C in the presence of α-C^{14}-glycine[2] as a hemoglobin precursor, as previously described (*1, 2*). Oxygen consumption of these homogenates was determined by means of the usual Warburg-Barcroft technique using modified 300-ml Warburg vessels. After incubation, hemoglobin was isolated by precipitation with 2.8 *M* phosphate buffer according to Green (*3*); from this preparation protoporphyrin dimethyl ester was isolated according to Grinstein (*4*) and globin according to Anson and Mirsky (*5*). The C^{14}-activity of the products isolated was determined as described briefly in a previous publication (*6*). The relationship between oxygen consumption and hemin and globin synthesis under conditions which result in changes in the ability of the marrow to synthetize hemin and globin is apparent from the data presented in Table 1.

TABLE 1

Time after exposure to 800 r of x-radiation*	O_2-uptake in μl/g wet wt of marrow/3 hr	mM of hemin synthesized × 10^{-6}/mM of hemin isolated	mM of globin synthesized × 10^{-3}/mM of globin isolated
No radiation	218	6.2	3.3
0 hr	640	31.2	6.7
48 "	120	2.1	9.7
72 "	82	1.4	3.6
1 week	.....	0.7	2.1
2 weeks	71	1.6	1.3
3 "	151	4.0	0.5
4 "	261	8.6	3.0

* The animals were sacrificed and bone marrows removed at the time indicated.

The number of millimoles of hemin and globin synthesized was calculated on the basis of the following considerations. The capacitance of the ionization chamber used for C^{14}-analysis was determined. The change in voltage per minute permitted calculation of the number of radioactive atoms decaying per minute by use of the number of ion pairs produced by each particle. The number of atoms decaying per minute was related to the number of radioactive atoms present in accordance with the decay function. By correcting this number by a dilution factor, the number of atoms synthesized was obtained.

The results indicate a difference in the time relation between oxygen consumption and the synthesis of hemin and globin, respectively. It can be seen from Table 1 that hemin synthesis as well as oxygen consumption increases considerably in a parallel manner in homogenates from bone marrows removed immediately after radiation. Furthermore, oxygen consumption and hemin synthesis appear to reach a minimum about 1 week after exposure to radiation at a time when marked degenerative changes prevail in the marrow. Globin synthesis also increases after radiation, but, in contrast to hemin synthesis, it reaches a maximum 48 hr after radiation at a time when oxygen consumption already approaches a minimum value. However, the decay curve of globin resembles in its shape that of oxygen consumption and hemin synthesis. Recovery of all three functions begins 2–3 weeks after radiation, which agrees well with histological findings.

A detailed investigation of the unexpected finding of increased oxygen consumption and hemin and globin synthesis in the early period following radiation is now in progress.

[2] We are indebted to the Isotopes Branch, Oak Ridge National Laboratory, for supplying the α-C^{14}-glycine (C^{14}-activity: 11.36 μc/mg glycine) used in these experiments.

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Extensibility and Minimum Number of Polypeptide Chains in the Collagen Micelle[1]

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The orderly pattern in which collagen diffracts x-rays is believed to depend on the presence of constituent ribbonlike units, or "micelles" (*1, 2*). Their accepted thickness is estimated to consist of about 4–6 parallel polypeptide chains (*3*), but neither the minimum total number of polypeptide chains forming each collagen micelle nor the number of chains lying in continuity within the micellar length is, to my knowledge, mentioned in the literature. Hence, in an attempt to estimate these figures, data on the physicochemical structure of collagen will be correlated.

From the study of the x-ray diffraction pattern of silk fibroin (*4, 5*) it is possible to infer that the polypeptide chain is probably the structural backbone in the molecule of the proteins of the fibrous class, to which both silk and collagen belong. In silk, where this chain is practically fully stretched and where the angles between bonds are almost planar, the individual amino acid residues take origin at a distance of 3.5 A from each other (*5*). The length of an amino acid residue in collagen is about 2.85 A (*6*), and this indicates that in this polypeptide chain the angles between bonds are smaller than the corresponding ones in

[1] This investigation was supported by the Institutional Grant of the American Cancer Society to the University of California School of Medicine.
[2] Damon Runyon clinical research fellow of the American Cancer Society, as recommended by the Committee on Growth, National Research Council.
[3] I am indebted to H. P. Lundgren, Western Regional Research Laboratory, Albany, Cal., for his criticism.

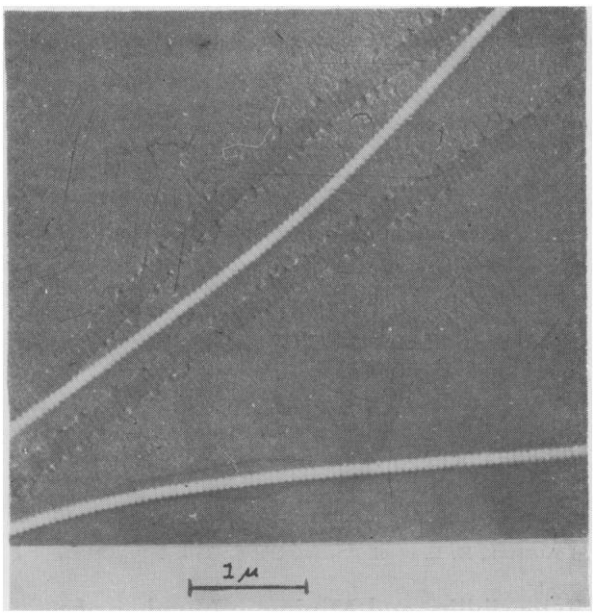

FIG. 1. Electron micrograph of individual collagen fibrils. Palladium shadowing (× 14,600).

the electron microscope (Fig. 1) and was taken as a measure of the over-all length of the "molecule" (*7, 8*). A simple calculation indicates that a certain degree of curling takes place in the collagen "molecule" if the chemically individuated 421 amino acid residues (*10*) placed at intervals of 2.85 A occupy a total length of only 640 A and not of 1,200 A as anticipated. On the other hand, it will be realized that the deformational properties of individual fibrils allow a considerable stretching of the axial periods before rupture occurs (Fig. 2).

Periods elongated up to 5,800 A have been reported (*7*). However, the total obtainable increment compatible with the integrity of a fibril being stretched as a result of the physical changes occurring in the supporting metallic grid cannot be measured directly on electron micrographs. In fact, the maximally stretched period remains at its newly acquired length only for a fraction of the time needed for a photographic exposure. Nevertheless, this maximal elongation may be estimated fairly accurately by photographing a stretched axial period, by then evaluating the extent of the residual elongation that will culminate in its rupture, and finally by correcting accordingly the measurements taken on the picture. Estimates obtained with this method indicate that an axial period may elongate up to 6,700 A–6,900 A before breaking. In another set of experiments, collagen fibers were forcibly stretched in water, quick frozen and

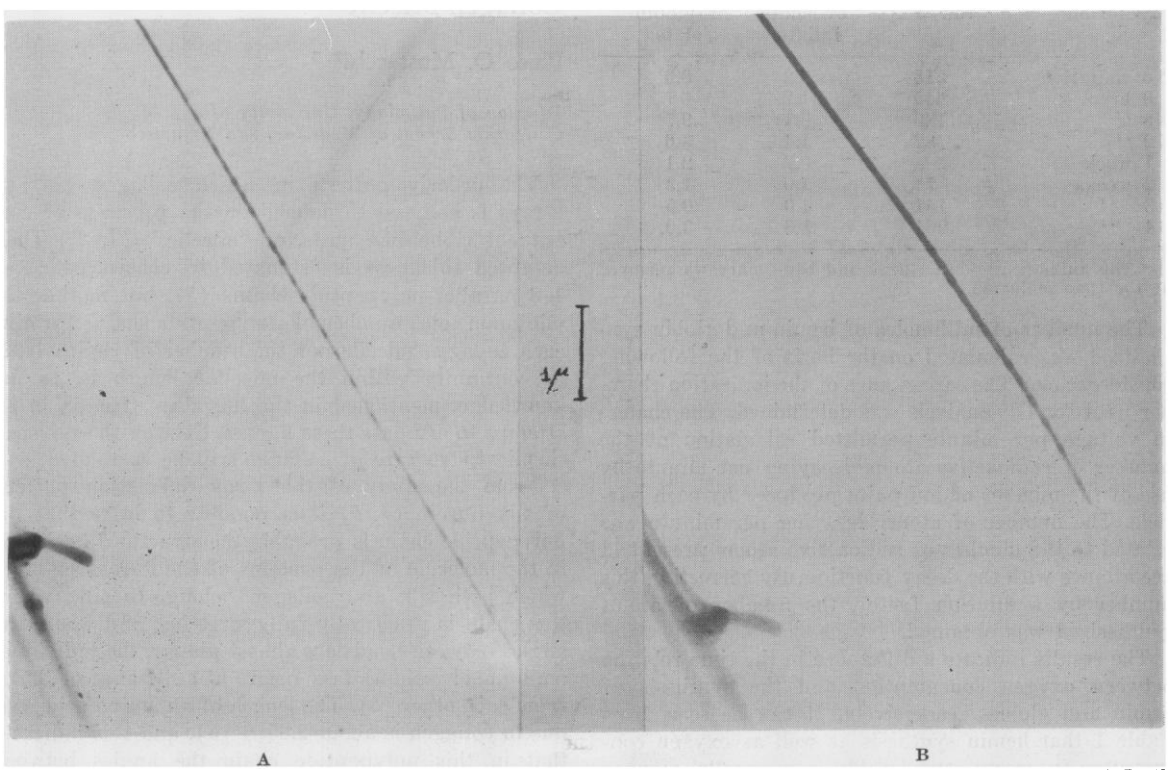

FIG. 2. Electron micrographs of single collagen fibril: *A*, the elongated axial periods of a stretched fibril, and *B*, the same fibril after rupture (× 12,700).

fibroin and that, therefore, the polypeptide chain in collagen is, as a whole, slightly contracted.

The presence of fundamental periods averaging 640 A along the collagen fibril, first deducted from x-ray diffraction studies (*4, 5*), is clearly demonstrable with

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.

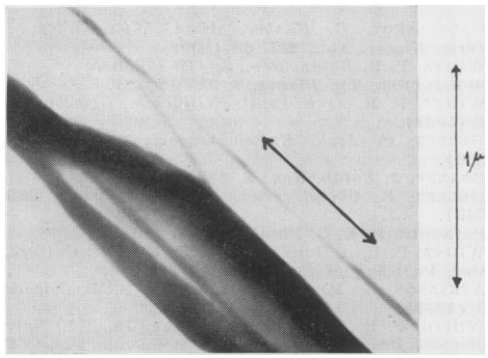

FIG. 3. Electron micrograph of a collagen fibril stretched and lyophilized. The period parallel to the arrow measures approximately 7,000 A (× 35,000).

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

$$-\mathrm{CH}(R_1)-\mathrm{NH}\cdot\mathrm{CO}-\mathrm{CH}(R_2)-$$

proline and hydroxyproline are built into chains forming imino links

$$=\mathrm{N}-\mathrm{CO}-$$

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,