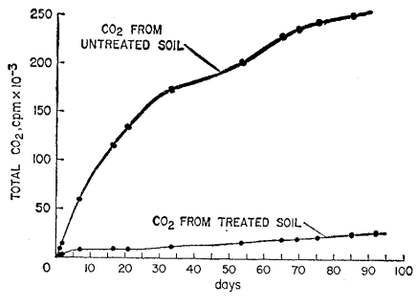

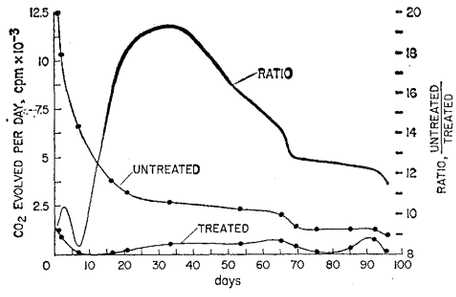

Fig. 1. (left). Total $^{14}CO_2$ evolved by LLS soil, treated and untreated. Fig. 2 (right). Specific activity per day of $^{14}CO_2$ evolved by LLS soil, treated and untreated.

(*12*). The solution was then assayed under standard conditions in a Packard Tricarb liquid-scintillation counter, model 3000; statistical error, within 5 percent.

The relative carbon-oxidatic activities of the three soils were determined by incubation for 24 hours at 22° ± 2°C, and the CO_2 was trapped and assayed by the technique described above; the results appear in Table 1. In other typical experiments, the ratio of a radioactive CO_2 has varied from 2.0 to 10.0, depending on the soil and the conditions of incubation.

LLS soil being easily available, it was selected for a long-term experiment. The contents of the KOH traps were removed at various intervals and assayed (Fig. 1); conditions were the same as those for the results in Table 1.

After 96 days, the total radioactivity trapped in KOH above the treated control was 2 percent of the total ^{14}C added as graphite. It seems reasonable to suggest that the substantial increase in the untreated : treated ratios of Fig. 2 is characteristic of soil enrichment by microorganisms capable of utilizing graphitic carbon.

It was essential to rule out nonbiologic catalysis of the observed oxidation of graphite in soils. Treatment of soils by each of the widely differing methods described in Table 2 should have had different effects on nonbiologic catalysts, yet should be equally able to destroy biologic activity at the cellular level; this proved to be so.

McLaren, Luse, and Skujins have shown that the bacterial population of a soil approaches zero when the soil is treated with a 2-Mrad dose of electron irradiation, and that 4 Mrad are required to insure complete sterility (*13*). Under such irradiation, however, phosphatase and urease activity is still manifest in the presence of suitable substrates. Table 2 shows that oxidation of graphite by soils was inhibited by all the methods of sterilization; apart from steam, all procedures yielded essentially the same results. This fact suggests that a biologic process was implicated, because a nonbiologic catalyst would not be expected to respond identically to the widely differing procedures used for sterilization.

The spontaneous formation of graphitic suboxides (*6*), especially at lattice defects, might well be carried through to complete oxidation to CO_2 by microorganisms. This process would expose additional carbon atoms to partial oxidation—again followed by complete oxidation to CO_2 by such organisms. Since this oxidative erosion of the graphitic macromolecule would selectively first remove the highly accessible lattice defects, the remaining graphite crystal should become progressively less reactive to these oxidations. An alternative possibility, based on the remarkably uniform activity ratio observed for any given soil, might be as follows: An agent, perhaps eventually of biologic origin, liberates or dislocates carbon atoms from the graphite matrix, which are then oxidized by what is terminally a nonbiologic process. Postgate has shown that such processes may occur in the reduction of sulfur by *Desulfovibrio desulfuricans* (*14*).

The expected progressive decrease in the rate of graphitic oxidation as a function of time is clearly demonstrated in Fig. 2.

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8. By D. L. Peck, Hawaii Volcano Observatory.
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Polyribosome Disaggregation during Metaphase

Abstract. *Polyribosomes as examined by both sucrose-gradient analysis and electron microscopy disaggregate during metaphase in both normal HeLa cells and those arrested in metaphase by treatment with colchicine.*

Autoradiographic studies of cultured animal cells show that during mitosis there is a rapid decrease in the rate of protein synthesis (*1, 2*). In the case of HeLa cells, this depression is most pronounced during metaphase and anaphase (*1*) which together last 20 to 30 minutes (*3*). Since aggregates of ribosomes (polyribosomes) associated with messenger-RNA molecules are the site of nascent protein synthesis in many cell systems (*4, 5*), ribosomal organization, as determined by sucrose-gradient analysis and electron microscopy, has been investigated in metaphase cells.

Large populations of normal HeLa (strain S_3) mitotic cells were selectively detached by mild agitation of monolayer cultures grown in low-calcium medium (*6*). In experiments requiring larger numbers of mitotic cells (as for sucrose-gradient analysis), the yield was increased tenfold by combining thymidine synchronization (*7*) with selective detachment. Cells obtained in this manner complete division, show no morphological abnormalities, and clone with a normal plating efficiency (*8*). When the cells were harvested quickly, approximately 60 percent were in metaphase, and 40 percent were in anaphase or telophase. For more quantitative comparison of cells in metaphase and interphase, populations—98 percent of which were arrested in metaphase—were collected by treating monolayers with colchicine (0.1 μg/ml) for 2 to 4 hours before detachment. Control of the effect of the drug in itself was provided by exposing a suspension culture of interphase cells to colchicine for 2 to 4 hours, at the end of which time less than 15 percent were arrested in metaphase.

Polyribosomes were prepared from cells that had been "pulse" labeled with C^{14}-amino acids (algal hydrolyzate, New England Nuclear Corporation), washed three times in Earle's salt solution, and then suspended in hypotonic buffer ($10^{-2}M$ KCl, $10^{-2}M$ tris-HCl buffer, *p*H 7.2, and 1.5×10^{-3} $MgCl_2$) (*5*). Cells were disrupted either by treatment of the whole cell with 0.5 percent deoxycholate (DOC) or by Dounce homogenization. The cell lysate was subsequently separated from the residual nuclear pellet in the case of interphase cells and from the sedimented chromatin in the case of metaphase cells. There was no significant difference in the results obtained by either method.

Cell lysates were layered on the top of linear sucrose gradients (15 to 30 percent) (*9*), and after centrifugation (SW 25.1 Spinco swinging bucket rotor) the gradients were analyzed for ultraviolet-absorbing material at 260 nm by means of a recording spectrophotometer (Gilford), and for labeled, acid-precipitable material by treatment of samples with an equal volume of 10 percent trichloroacetic acid and collection of the precipitate on Millipore filters (*10*).

For electron microscopy cell monolayers were grown on carbon-coated coverslips, and mitotic cells were located prior to fixation and embedding (*3*). After fixation in 5.5 percent glutaraldehyde followed by 2 percent osmium tetroxide, they were dehydrated in an ascending series of alcohol concentrations and embedded in Epon. Sections were cut with a diamond knife on the Porter-Blum ultramicrotome, mounted on Formvar-coated grids, stained with lead citrate and uranyl acetate, and examined in the Siemens Elmskop 1A electron microscope (accelerating voltage of 80 kv and a 50-μm aperture).

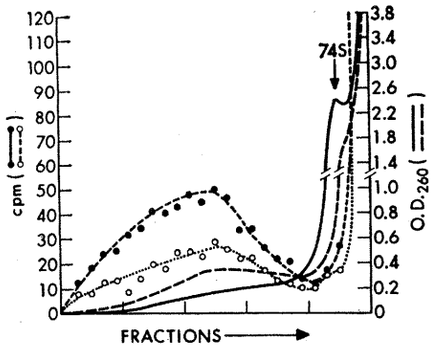

Fig. 1. Polyribosomes in mitotic and early interphase cells: HeLa S_3 cells, grown in monolayer culture (30 Blake bottles), were synchronized after they were treated with 2.0 mM thymidine for 12 hours and then with fresh medium that did not contain thymidine for 10 hours. The mitotic cells (4×10^7) were collected, and half of the 60-ml sample was immediately exposed to 20 μc of C^{14}-labeled amino acids for 3 minutes. The other half of the harvested population was incubated at 37°C in suspension for 45 minutes and then exposed similarly to C^{14}-labeled amino acids. The bottom (heavy) part of the sucrose gradients are on the left. Absorbancy at 260 nm from metaphase cells——; absorbancy at 260 nm from interphase cells - - - -; acid-precipitable radioactive material from metaphase cells o · · · · · · o; acid-precipitable radioactive material from interphase cells • - - - - •; cpm, count/min.

In Fig. 1 polyribosomes and polyribosome-associated protein synthesis are compared in synchronized cells immediately after collection and 45 minutes later. Initially, 60 percent of the cells were in metaphase, and 40 percent were in anaphase or telophase. Forty-five minutes later 90 percent had entered interphase. In the initial, predominately metaphase sample there were significantly fewer large polyribosomes (ultraviolet-absorbing material in the "heavy," 150 to 350*S*, area of the sucrose gradient) and more single ribosomes (74*S*). Similarly, there was approximately half as much polyribosome-associated protein synthesis initially as 45 minutes later.

We confirmed this disaggregation of polyribosomes by examining the ultrastructure of ribosomal organization in serial sections of mitotic cells from unsynchronized monolayer cultures. In interphase cells (Fig. 2) there are numerous helical and amorphous aggregates of ribosomes scattered throughout the cytoplasm, while in metaphase cells (Fig. 3) such aggregates are difficult to find. Instead, the ribosomal pattern is predominately one of single units scattered relatively homogeneously throughout the cell with occasional polyribosomes.

In order to provide a more accurate estimate of the number and size of the polyribosomes remaining in metaphase, populations containing 98 percent metaphase cells (arrested by colchicine) were analyzed on sucrose gradients for polyribosomes and polyribosome-associated protein synthesis (Fig. 4, left panel), as well as for the relative amounts of 60*S* and 40*S* ribosomal subunits and single ribosomes (74*S*) (Fig. 4, right panel). As compared with interphase cells treated with colchicine for the same amount of time, the metaphase cells showed an absolute loss of ultraviolet-absorbing material from the polysome area of the gradient, a shift toward smaller aggregates, and a compensatory increase in single ribosomes. There was no difference between interphase and metaphase cells with respect to ribosomal subunits (right panel). Associated with the decrease in polyribosomes in metaphase cells is a significant decrease in protein synthesis associated with polyribosomes. As with interphase cells (*5*), almost all of the absorbing material in the polyribosome area of the sucrose gradient of metaphase cells is converted to single ribosomes by small amounts of ribonuclease. Colchicine-treated metaphase and interphase cells showed the same differences with respect to ultrastructural ribosomal organization as shown in Figs. 2 and 3.

Our sucrose-gradient analyses and morphological studies show that polyribosomes partially disaggregate during metaphase. Love has proposed such a change in ribosomal structure based on the absence of "granular RNA" as stained with toluidine-molybdate in

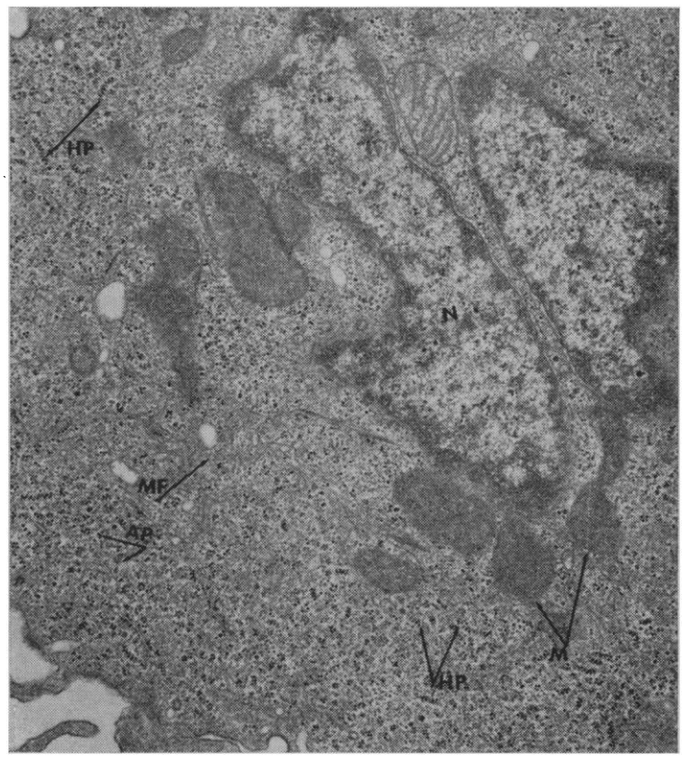

Fig. 2. Electron micrograph of normal interphase cell showing the striking tendency of the ribosomes to accumulate in helices and amorphous aggregates. *N*, nucleus; *M*, mitochondria; *HP*, helical polyribosomal aggregates; *AP*, amorphous polyribosomal agggregates; *MT*, microtubules (× 7000).

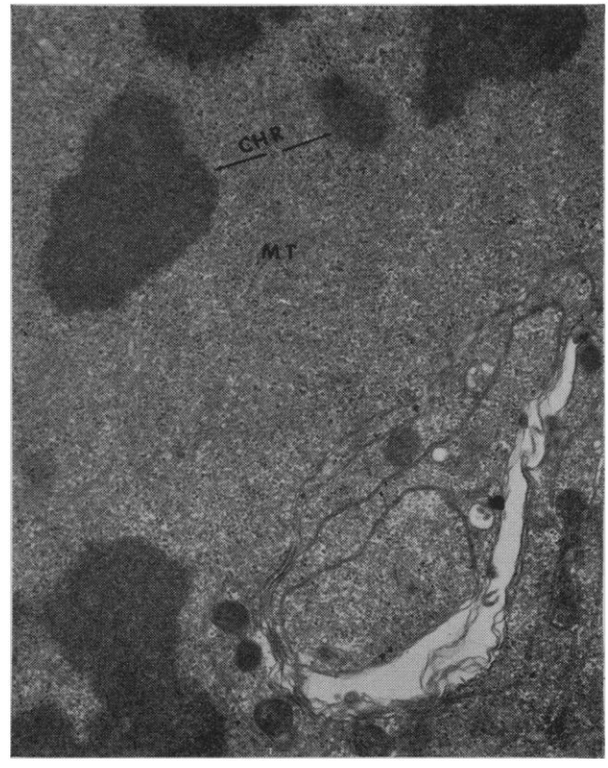

Fig. 3. Normal metaphase cell showing the fairly homogeneous distribution of single ribosomes scattered throughout the cytoplasm freely intermixed with chromosomes and spindle tubules. *CHR*, Chromosomes; *MT*, microtubules (spindle tubules) (× 7000).

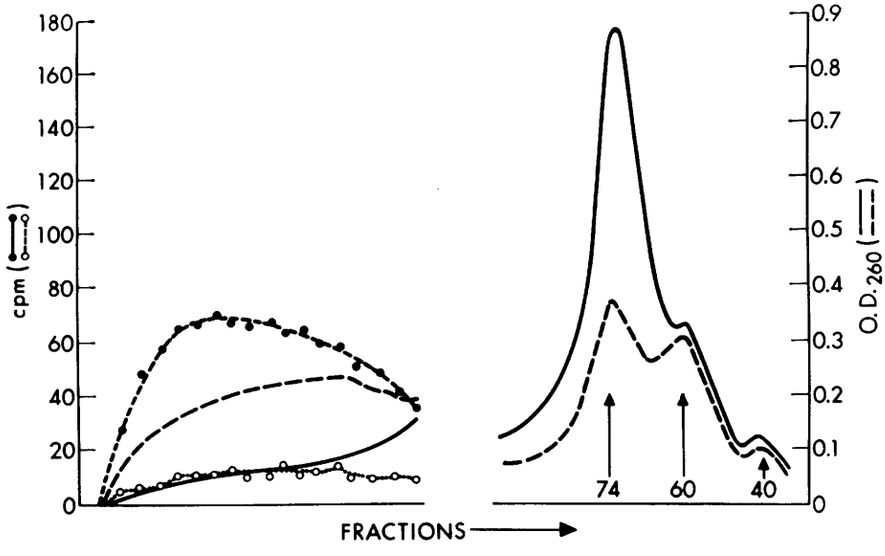

Fig. 4. Polyribosomes and polyribosome-associated protein synthesis in colchicine metaphase and colchicine interphase cells: Monolayers of HeLa S_3 cells were treated with 0.1 μg of colchicine per milliliter of medium for 3 hours, and then metaphase cells were harvested and adjusted to the same cell mass per milliliter as a random population that had been treated with colchicine for the same amount of time. Both cultures were treated with 10 μc of C^{14}-labeled amino acids for 1½ minutes, and polyribosomes were prepared by Dounce homogenization. Half of each cell lysate was centrifuged for 130 minutes at 24,000 rev/min (left panel) and half for 8 hours at 25,000 rev/min (right panel) on linear sucrose gradients 15 to 30 percent. Absorbancy at 260 nm from metaphase cells ——; absorbancy at 260 nm from interphase cells - - -; acid-precipitable radioactive materials from metaphase cells o · · · o; acid-precipitable radioactive material from interphase cells • - - - - •; cpm, count/min.

metaphase (*11*). Consistent with earlier autoradiographic studies (*1*, *2*), during metaphase there is a decrease in amino acid incorporation associated with polyribosomes. That this reflects a real decrease in protein synthesis is suggested by the finding that the size of the amino acid pool and its rate of equilibration with radioactive amino acids in the medium are the same in metaphase and in interphase cells, both of which were treated with colchicine (*12*).

The sudden and transient decrease in protein synthesis and associated breakdown of polyribosomes during metaphase suggests the existence of regulatory mechanisms that have not been detected during interphase. While there is no RNA synthesis during metaphase (*1*, *2*, *13*), this alone cannot explain the rapid disappearance of polyribosomes since messenger RNA from HeLa cells has an average half-life of about 3 to 4 hours (*5*), as compared with the 15 minutes of metaphase. Although a rapid breakdown of polyribosomes could result from the enzymatic destruction of mes-

senger RNA, sensitive techniques (*14*) have failed to demonstrate increased ribonuclease activity in metaphase cells.

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Blood Flow in the Microvasculature of the Conjunctiva of Man

Abstract. *Cinephotomicrography of capillary blood flow in conjunctival vessels of man were carried out at 116 pictures per second. Red cells did not move in streamlines but rather in a heterogenous mixing type of flow. Flow in vessels of patients whose red cells were grossly aggregated was very slow and often stagnant.*

Ever since the observations of blood flow in the capillary vessels of small animals by Poiseuille and Krogh, attempts have been made to examine these same phenomena in man. An external, transparent vascular membrane is not available in man without surgical procedure, hence the microscopy of capillary blood flow must be studied by reflected light from normally exposed surface vessels such as those in the conjunctiva of the eye, or through translucent tissues such as finger webs or nail beds. In addition to problems of resolution in these areas, observations are also difficult because of the serious artifacts of heat stress or injury when the necessarily intense light is focused on the vascular tissue under examination. Cinephotomicrography is also complicated by the fact that filming rates must be increased by a factor equal to the image magnification by the lens system in order to retain time resolution.

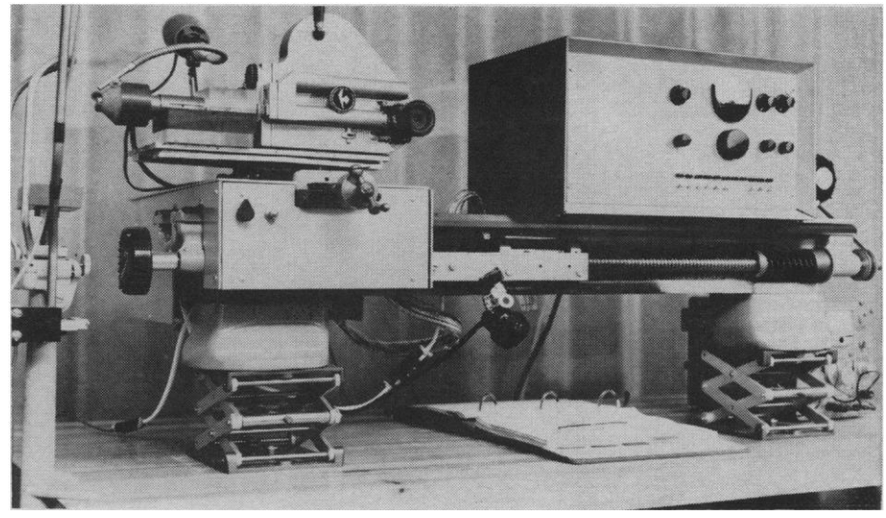

Fig. 1. Camera, lens system, and power source mounted on lathe bed. Headrest at left.

Improved techniques of pulsed high-intensity (stroboscopic) lighting, light manipulation by fiber optic bundles, and increased film speed have been utilized to overcome some of these difficulties in order that observations of blood flow in the microvasculature of man might be recorded without alteration or influence of the subject. With a modified 16-mm camera and a long-working-distance (14 mm) objective lens, mounted 180 mm from the film plane, a magnification of 20 times the true image size was obtained on film with resolution of 2 μ. Stroboscopic light synchronized with the camera was conducted from a xenon flash tube to the tissue by way of fiber optic bundles (diameter, 9.5 cm; length, 0.6 m) attached to the lens mounting. The entire unit was constructed on a heavy, 1.5-m lathe bed (Fig. 1). A precision loaded lead screw was used as the focusing mechanism. The subject was comfortably seated in front of the apparatus with his head resting in a head-and-chin rest and his gaze fixed on a small point of light off to the side at 45° angle. The mass of the instrument and the table precluded its use in the prostrate patient, but the machine could be brought to the bedside for photography of a patient in the sitting position. Filming runs lasted 4 to 5 seconds at rates of 116 pictures per second. Film type was high-speed fine-grain reversal, ASA 160 daylight. The procedure was innocuous and no untoward reactions to the brief stimulus of light were noted.

Film studies of blood flow in vessels of diameters from 10 μ (capillaries) up to 200 μ (arterioles and venules) in normal man (20 studies of seven subjects) revealed a continuous heterogenous mixing type flow rather than streamline flow (Figs. 2 and 3). Erythrocyte aggregation was commonly observed in vessels of 50-μ diameter and less, while flow within capillaries often

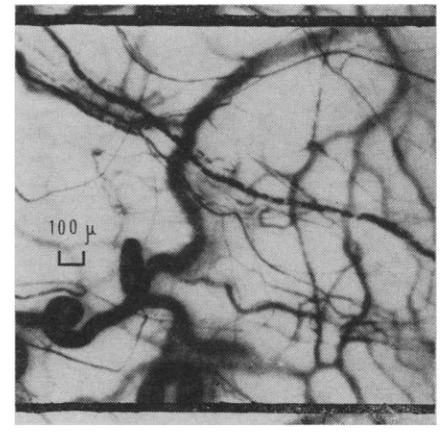

Fig. 2. Bulbar conjunctival vessels of man. Magnification on original 16-mm film was × 4.