Biologic Crystals and Particles Produced in Tissue Culture*

I. Introduction

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SUMMARY

A method for the cultivation of embryo chick tissues which gave rise to numerous and unusually large crystals and particles is detailed. Forms were helical, tubular, ribbon-like, hexagonal, rhomboidal, and filamentous. Rhomboids and hexagonals often had surface areas several times the size of the broad and flat tissue-cultured cells, and helices were observed up to 300 μ in length. Transformations of helical and related forms were observed. Their chemical identity could not be determined by conventional cytochemical technics. With modifications, however, helical and related forms were shown to give a positive reaction to pyronin staining which was inhibited by prior digestion in ribonuclease.

In the summer of 1959, multipurpose culture chambers (14, 15, 24) were fabricated with confronting cultures of 11-day embryo chick thyroid and lung tissues. These explants were completely separated from the nutrient vault of the chambers by interposing sheets of dialysis cellophane (19–21, 23) and thereby differed from cultures established by the formerly described cellophane strip technic (24). The strip technic permitted a flow of the nutrient around the edges of the cellophane (2, 22, 25), so that the explants were potentially affected by all the ingredients in the nutrient, whereas the sheet technic permitted only a dialysate of the nutrient into the culture environment.

After a month of cultivation the thyroid and lung tissues appeared to have reached a static state with respect to their degree of emigration and population mass. Also, cells in the original explant sites were highly differentiated. The explants still appeared to be unaltered at 3 months, although their contours were gradually changing. Smooth muscle activity in the lung was viewed as a "lazy" nonrhythmic contraction which perforce accounted for the contour change. Rhomboidal and filamentous crystalline forms also were noted around the lung explants, and the epithelial arrangements in both the lung and thyroid remained highly differentiated.

At 6 months of cultivation the explants again were relatively unchanged, except for contour, and smooth muscle activity persisted. It was then decided to retain these cultures to determine how long differentiation would persist before their demise or transformation to a neoplastic growth. Other workers (12, 26) had indicated that tissue cultures, regardless of their origin, reverted to neoplastic strains if kept sufficiently long; yet it appeared that these embryo chick cultures isolated by the cellophane molecular screen might become an exception to this usual conclusion. Smooth muscle contractions persisted for 10 months of cultivation.

Finally, the explants were retained for 25 months without histologic evidence of neoplastic growth or changes in the total cell masses. Although the final tissue organization was compact and without the well defined architecture observed in the young cultures, the emigrating cells were of a "normal" character, and mitoses were not observed.

This and subsequent experiments which utilized embryo chick tissues separated from the nutrient vault by sheets of dialysis cellophane revealed sev-
in major points of interest: (a) cultures did not show histologic evidence of neoplastic growth; (b) mitosis was markedly reduced; (c) normal function, when evident, was prolonged; (d) degenerative phenomena were minimized; (e) fibroblastic outgrowths were diminished; (f) fat droplets in the fibroblasts were significantly reduced or eliminated; and (g) cultures from many types of tissues ultimately contained crystalline and particulate arrays of varying sizes and shapes. Three general and provisional groupings of these megaparticles have been categorized according to their origin, morphology, and behavior: (a) tubules, helices, and ribbons; (b) rhomboids and hexagonals; and (c) filamentous forms.

This preliminary report details general descriptions of the form and size of the observed megaparticles by phase-contrast and bright-field (hematoxylin-stained) micrographs. Time-lapse sequences also are used to denote: (a) relationships of cells to crystals; (b) malleability of helical forms; and (c) transformations of the particles occurring over an interval of time. Responses to cytochemical, solubility, and thermal tests are presented.

MATERIALS AND METHODS

Specimens.—Embryo chicks incubated for 11–19 days were used to procure explant material for these experiments. Specimens were dissected from the cerebrum, cerebellum, spinal cord, thyroid, lung, pancreas, gonad, kidney, proventriculus, large intestine, small intestine, striated muscle, and leg bone (minus periosteum and endosteum) areas.

Cultivation procedures.—Standard tissue culture procedures were used to open fertile eggs, remove embryos, and dissect specific organs and tissues. Explant specimens were cut into pieces approximately 1 mm. \times 1 mm. with sharp scalpels in Petri dishes and washed with Fischer's V-614 fluid nutrient minus the serum component. Four to six explants were placed on coverslips of 2 × 3-inch multipurpose culture chambers (14, 15, 24) and covered with sterile pieces of cellophane (19, 20, 23) moistened with Earle's balanced salt solution (BSS). The cellophane completely enclosed the tissue fragments, so that when the rubber gaskets and closing coverslips were placed in proper position the completed chamber was divided by the cellophane into two areas: (a) a culture vault and (b) a nutrient vault. In this way serum constituents of the fluid nutrient which were not dialyzable did not come in contact with the explants, and cellular products of large size similarly did not leave the culture environment through the cellophane pores, their diameter being approximately 24 Å. Some chambers were established without tissue fragments, and other chambers were established with tissue fragments and with four No. 25 hypodermic needle perforations in the cellophane. This latter condition produced a chamber construction in which (a) all serum constituents of the nutrient could pass to the explant environment and (b) all cellular products could pass to the fluid nutrient vault and then out of the chamber when nutrients were exchanged.

Nutrient.—The fluid nutrient utilized in these experiments was a standard type of tissue culture preparation (19–21, 23) composed of Fischer's V-614 (75 per cent); calf serum (20 per cent); and whole egg ultrafiltrate (5 per cent). Chambers were filled with this nutrient immediately after fabrication and placed in a 37° C. incubator. Fluids of the nutrient vault were completely exchanged semi-weekly by syringe techniques through the rubber gaskets of the chambers. Culture vaults were not disturbed and were affected, therefore, only by molecules small enough to pass through the dialysis cellophane screen.

Observations and photography.—Cultures were observed with Bausch and Lomb phase-contrast microscopes and recorded by both still photography, with a Hasselblad 2⅜ × 2⅜ camera, and time-lapse cinemicrography, with a 16-mm. EMDECO time-lapse unit (13, 16–18, 25).

MICROCHEMISTRY

Cytochemistry.—The nutrient of cultures with megaparticles was evacuated and replaced with BSS. After this rinsing, the BSS was replaced by formal for 20 minutes. This was followed by a 20-minute water rinsing. Staining procedures were carried out through the cellophane by admitting reagents into the nutrient vault with the syringe needle which pierced the rubber gaskets, and by extending time intervals to permit adequate penetration through the cellophane screens. Positive cellular reactions were used to certify a satisfactory procedure for the following tests: general staining with acid hematoxylin (Delafield's), Feulgen's DNA (8), Brachet's RNA-DNA (1); basic protein dyeing with toluidine blue (pH 8–9) and unmordanted hematoxylin (pH 8.2) according to Fullmer and Lillie (4); polysaccharides with the PAS method of Hotchkiss (10); neutral fats with Sudan IV in carbitol according to Gomori (5); sterols-steroids with the Schultz-Smith test for cholesterol and its esters (8); and acid phosphatase according to Gomori (6). Vital dyeing using Janus green, neutral red, and acridine orange was effected also. Stronger concentrations of vital dyes than normally used were necessary to hasten the pene-
tration of the cellophane. Here again, cellular dyeing certified a satisfactory penetration of the cellophane by the dye; hence, its availability to the megaparticles.

In addition to the conventional cytochemical technics enumerated above, nutrients were removed and replaced by a 2 per cent aqueous solution of KMnO₄ for 1 hour. This fixed and lightly stained the crystalline and particulate forms but, more importantly, permitted a separation of the cellophane from the coverslip without severely distorting the tubules and helices. Formalin-fixed tubules and helices were destroyed or floated away when the cellophane was peeled off the coverslip. Potassium permanganate fixation resulted in an attachment of the particles to either the coverslip or the cellophane. Tubules and helices attached to the coverslips were subjected to pyronin staining for RNA according to the method of Brachet (1).

**Solubility.**—Nutrients were washed out and the solvents injected into the nutrient vault. In all cases the solvents came into contact with the particles by passing through the cellophane screen. Cellular response certified presence of the solvent around the megaparticles. The following solvents were used at room temperature: methanol (100 per cent), ethanol (50 per cent and 95 per cent), chloroform vapors, distilled water, HCl (m/1), NaOH (m/1), and NaCl (m/1).

**Thermal effects.**—Fluid nutrients were replaced with BSS and a vent needle left in the nutrient vault. Chambers were then immersed in a water bath at 50° C., and then at increments of 10° C. for 20-minute periods.

**RESULTS**

**Descriptive Morphology**

A general orientation and comparative analysis of crystalline and cellular size may be obtained from the low-power micrographs in Figures 1 through 4. These show outgrowths from 11-day embryo chick cerebrum after 58 days of cultivation and their association with specific types of crystalline accumulations. Tubules, spirals, and ribbons are shown in Figure 1; rhomboids in Figure 2; small and refractile hexagonals in Figure 3; and filamentous forms in Figure 4. Crystals appeared as early as 8 days after cultivation had commenced, though more commonly several weeks elapsed before many became apparent. All the tissues investigated, with the exception of leg bone, produced crystalline displays to varying degrees as shown in Figures 1 through 58.

Tubules are shown at low and high powers in Figures 5 through 9. The emigrating fibroblast (fb) of a 19-day embryo chick gonad (Fig. 5) is shown at low power after 27 days of cultivation surrounded by a sea of needle-like tubules. Generally, the tubules rested on the surface of the cellophane; but, when the coverslip and cellophane were closely apposed, crystals and cells were in the same plane so that contacts were unavoidable. Tubules often became entangled in debris and appeared in clusters as those shown at high power (Fig. 6) from a 35-day culture of a 13-day embryo chick stomach. Note their uniform diameter. Macrophages and other wandering cells frequently became closely associated with tubules. The cell in Figure 7 sent probing extensions along a tube, both to the left and to the right, as it passed over it. Small granular masses sometimes were observed along tubes of old cultures (Fig. 8) and occasionally, sharp angular bends or angular attachments between tubules were noted (Figs. 9, 28).

Spirals of Figure 1 are shown again at a medium power in Figure 10 after 58 days and again in Figure 11 after 76 days of cultivation. The cellophane scratches along the right margin of these micrographs made convenient markers for the transformations which occurred. The large central spiral A became longer and its ribbon width broader; spiral B also elongated. The four finger-like processes of ribbon C similarly elongated and the ribbon to the far right with a few spirals which crossed the cellophane scratches in Figure 10 generally unfolded during this interval (Fig. 11). Other helical elements from the same cerebral culture are shown after 58 days in Figure 12. Spiral D had a broad unwinding in its central portion, and spiral E appeared to have nearly completed an unwinding. Other stages of spiraling are shown though not specifically labeled.

Helical forms were lightly stained with Delafield's hematoxylin after formal fixation and a prolonged (24 hours) staining in the chamber through the cellophane. The micrographs of hematoxylin-stained helices in Figures 15 through 16 derived from 11-day embryo chick gut after 62 days of cultivation are shown in unusual arrangements suggesting duplication processes. Pairs of spirals were noted to coincide precisely, and in Figure 16 there are three coinciding spirals. In Figure 14 the ribbon of the major spiral F appeared denser in its left extremity than its right, suggesting that the lower portion of the pair (G) peeled from the upper or major portion F.

Other suggestions of replication are shown in the phase-contrast micrographs in Figures 17 through 19. A pair of spirals or a single spiral which had split down the middle of the ribbon is shown in Figure 17. A spiral which had split at one end and...
Figure 18. The tubule in Figure 19 appeared to have unwound in its upper extremity, and a second spiral to the left coincided with it in the same spacing arrangement as those of Figures 13 through 16. Figures 21 and 23 are micrographs of two tubules at a focus through their mid-portion. It was observed that these tubules were not smooth-walled but were periodically dashed with dark areas in alternate positions on opposite sides of the tubules. In reality periodic dashes were thickenings which coincided with the pitch of the early-forming spirals observed at a high focus (Figs. 20, 22). The tubule of Figures 22 and 23, which is the particle shown in the sequence of Figures 34 through 38, had commenced to unwind at both ends.

Generally, the tubes and spirals were observed as straight forms, although occasionally they were bent or bowed by migrating cells or even by a gentle finger pressure on the coverslip. The loose spiral in Figure 24 had formed a loop and remained unchanged during a week of observation. The spiral in Figures 30 and 31 shows the straight and curved position induced by finger pressure on the coverslip. Tubules snapped back to the straight position rapidly after being bent by finger pressure, and spirals returned more slowly, if at all.

Other unusual forms of the tubes and helices are shown in Figures 25 through 29, and in Figures 32 and 33. In the series, Figures 25 through 27, a helix is shown enveloping a tubule. This form was observed with some regularity, though it was a sparse finding among the many other particles. The micrographs were made at three focal levels to show unequivocally that the helix enclosed the tubule. The high focus in Figure 25 denotes the right-handed spiraling which was characteristic of all embryo chick helices observed. The long tube H in Figure 28 had a sharp bend at the top, globular internal images in the center and at one side, and a spiral unwinding of the lower extremity. In this same figure, two other tubules (I and J) are shown in a partially unwound state. The large tubule K in Figure 29 had partially unwound to a greater diameter at its lower portion than generally observed. In the lower right, tubule L is shown with spiraling surface images as the tubules in Figures 20 and 22. One of the more unusual relationships between tubules and spirals is shown in Figures 32 and 33. In this instance, one tubule (M) penetrated another tubule (N). The upper half of N had uncoiled, producing a situation comparable to that in Figures 25 through 27. The image of a piece of a very wide helical form focused at the mid-region is labeled O, and a high-power view of the helical portion of N surrounding the tubule M is shown in Figure 33.

A relationship between particulate or crystalline formation and cellular activity was not observed. However, on many occasions, particulate and crystalline elements were observed in close proximity with cells which often disturbed their geometrical form by breaking tubes or unwinding spirals. One incidence which occurred in a 20-day culture from a 12-day embryo chick pancreas over a 96-hour interval is recorded in Figures 34 through 38. The tubule P which had commenced to unwind at both ends (P1, upper extremity; P2, lower extremity) in Figure 34 is shown after 18 hours in Figure 35. At this time it had been broken by the local cells as documented by a 16-mm time-lapse movie sequence. The two fragments remained in the same microscopic field for the first 24 hours (Figs. 36, 37) but after 96 hours they had become separated by two microscopic fields of view. The upper end (P1) which had been broken off as a spiral element had unwound further (Fig. 38a), whereas, the tube or lower portion of the complex (P2) showed considerable elongation (Fig. 38b).

On only one occasion was a crystalline form observed to be completely contained within a cell. In this instance, a malleable ribbon-like crystal (Q) was located in a multinucleated giant cell (Figs. 39, 40) of a 56-day culture derived from a 12-day embryo chick pancreas. These photographs were taken at a 2-hour interval and show the rapid change in cell contour and malleability of the enclosed crystalline form. A time-lapse cinematographic recording of this cell showed the crystal to protrude sharply at one point and then to return to a position entirely within the cell.

A series of hexagonal and rhomboidal plates are shown in Figures 41 through 44. One cell is shown near crystals in Figure 41. Careful inspection of the micrographs indicated the many layers of crystals and the stairstep pattern of growth. Angular measurements are indicated in the legend.

Cultures constructed with strips of cellophane have never produced crystalline or particulate forms. Similarly, the cultures constructed with cellophane sheets containing the four needle holes did not give rise to these elements.

Microchemical Responses

Megaparticles did not react positively to any of the conventional procedures of cytochemical analysis listed in the section "Materials and Methods."
ods.” However, fixation with the potassium permanganate solution apparently altered the molecular structure of the helical forms sufficiently to permit an interaction with the pyronin (pH 5). Helices, ribbons, hexagonals, and rhomboidal forms all stained pink but failed to do so after 1 hour of digestion in a solution of ribonuclease. The organic solvents (methanol, ethanol, and chloroform) effectively dissolved all megaparticles, and definite alterations by heat were recorded. Hexagonal and rhomboidal crystals commenced to show a rounding of angular points and a distortion of straight sides at 80°C., whereas, the tubules and helices were markedly distorted at this temperature. Water replacement immediately (less than 1 minute) lysed the cells without evident changes to the megaparticles over a 24-hour observation. HCl produced an immediate fixation of the tissues and a precipitation of the fluid in the culture environment without any obvious effect on the megaparticles. On the other hand, the response to NaOH was an immediate lysis of the cells and a slow (15 min. to 2 hr.) destruction of the megaparticles. The disturbance to the megaparticles produced by heat and NaOH was similar. The effect of NaCl was not apparent after a 24-hour exposure. Toluidine blue solutions stained helical particles which had been fragmented by short exposures to NaOH (1/M) metachromatically (red) and was further evidence for their protein quality.

**DISCUSSION**

Forty-four micrographs have been used to illustrate a variety of particulate elements produced by cultures of embryo chicks isolated under dialysis cellophane screens on coverslips of multipurpose culture chambers. The origin of these forms, their detailed biochemical identity, and usefulness to the culture environments in which they were found remain obscure though temptingly speculative at this time.

Needle-like crystals were observed as early as 8 days after cultivation commenced, but generally 2–3 weeks elapsed before defined elements could be perceived easily by 10X objective microscopic scanning. Particulate growth was slow yet persistent. Rhomboids followed other rhomboids in specific areas, but so slowly that time-lapse cinematography at one frame per minute failed to detect a growth rate. The highly refractile hexagonal crystals, as shown in Figure 3, were usually a late element and appeared to be built upon other tubes or ribbons. Larger hexagonals (Figs. 41 through 42) were also late-appearing and occasionally appeared to be built upon ribbons and filamentous forms. An abundance of filamentous forms were more frequently observed in cultures of the central nervous system.

The most prominent of all particles were the tubules, which proceeded through a transformation to tightly wound spirals, to loosely coiled spirals, to gently folded ribbons, and frequently to straight ribbons. The spirals were always dextro-helical or produced a right-handed threading. The malleability of the spirals (strength), snap-back of the tubules (elasticity) after having been bowed, uncoiling procedures, and occasional double helices were unique characteristics.

Observations of the developing forms denote two basic points: (a) the dialysis compartment with living cells was required to produce the illustrated particles and crystals and (b) living cells were not observed to give rise to the crystals. The second part appears to exclude the first; however, cells have been observed in the living form by phase contrast (7, 27, 28) and in the fixed form by electron microscopy (3, 9, 27, 28) to contain crystals, presumably to give rise to them (27). Particles in this report were observed to enlarge extracellularly in the microscopic range of size. It is not, therefore, unreasonable to consider that their origin in the submicroscopic realm was first intracellular and that growth continued extracellularly owing to the barricading effect of the cellophane which prevented their passage to the nutrient compartment; when their size reached 0.25–0.3 μ or better they were observed optically. Further, this culture environment was highly desirable for the maintenance of differentiating embryonic tissue, and the particulate and crystalline formation apparently did not in any way adversely affect this cellular growth. As a matter of fact, this differentiated growth was, in all probability, responsible for their production, which, because they could not leave the environment, simply interlocked and grew in physical dimensions from submicroscopic to microscopic sizes.

Further, it appeared that those cultures with the most differentiated growth produced the largest number of megaparticles and crystalline forms, whereas cultures composed exclusively of fibroblasts were totally devoid of them. Since these megaparticles and crystals gave positive responses to RNA and protein staining, it seems probable that they are protein products of the cultivated cells and visible evidence that these cells are carrying out their differentiated functioning capacity of secretion. The elongating growth of tubules and spirals in uniform dimensions, their malleability, and distortion by heat and alkali suggested a poly-
meric construction, whereas the layered growth of rhomboids and hexagonalons, their rigid forms, and melting response to heat and alkali suggested a true crystalline structure. Further, the resistance of all forms to the conventional cytochemical techniques was indicative of their homogeneous solid state. Furthermore, formalin fixation is not considered a good fixative for nucleic acids, since it blocks a large number of the reactive groups to both basic and acid dyes.

As indicated by the title, this report is simply an introduction into a new field of study and admittedly is far from complete. For instance, it is not certain that all the particulate and crystalline forms have been identified morphologically and admitted is far from complete. For instance, it is not certain that all the particulate and crystalline forms have been identified morphologically and the sequences of transformations have only partially been followed. Their precise solubilities, responses to enzymes, chemistry, and molecular patterns as revealed by electron microscopy and X-ray diffraction are some of the essential analyses required to determine specific biostructural identity. Obviously, the tubular forms were not biochemically the same as the ribbons, nor were the rhomboids the same as the hexagonals; yet there were interrelationships between these variations which suggested biosynthetic processes at a magnified level.

Human embryo and neoplastic tissue cultures similarly are being evaluated for their megaparticulate productivity. Recent experiments have displayed their capacity to give rise to similar forms.

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REFERENCES

PLATES 1–10.—Abbreviations.

sp, spiral.
ri, ribbon.
tu, tubule.
rh, rhomboid.
he, hexagonal.
fi, filamentous particles.
ex, explant.
fb, fibroblast.
ma, macrophage.
Nu, nucleus.
gi, globular images within and exterior to tubes.
A, B, C, etc.—specific particles and crystals mentioned in text.
1, 2, 3, etc.—specific areas of spirals at two levels of focus (Figs. 20, 21).
a, b, c, etc.—specific angles in rhomboids and hexagonals.
Hi, high microscopic focus.
Mi, mid-microscopic focus.
Lo, low microscopic focus.
Oh, 18h, etc.—initial photograph (zero time), 18 hours after Oh, etc.
Od, 16d, etc—initial photograph (zero time), 16 days after Od, etc.

Figs. 1–4.—Low-power phase-contrast micrographs of outgrowths from a 12-day embryo chick cerebrum after 58 days under a full sheet of cellophane in a multipurpose culture chamber. A magnification line for Figs. 1–4 is in Fig. 4. X180.

Fig. 1.—Spirals (sp) and ribbons (ri) peripheral to the cerebral explant (ex).

Fig. 5.—Numerous overlapping rhomboid (rh) crystalline forms next to the cerebral explant (ex).

Fig. 3.—A cluster of highly refractile hexagonal (he) crystalline forms accumulated in a nestlike space of cerebral cells. Hexagonal crystals of this smaller and denser variety were not always a perfect six-sided form, but usually one end was perfectly pointed, sometimes both ends. (See also Figs. 41 and 45).

Fig. 4.—Filamentous forms (fi) with gentle sweeping and circular contours adjacent to the cerebral explant (ex).

Figs. 5–9.—Low- and high-power phase-contrast micrographs of tubular (tu) elements. A magnification line is in Fig. 6 for Figs. 6–9.

Fig. 5.—Many tubular (tu) elements at the periphery of a 19-day embryo chick gonad explant (ex) after 20 days of cultivation. A large fibroblastoid (fb) cell serves as a good index of the particulate size and population density in some areas. X200.

Fig. 6.—High-power phase-contrast micrograph of a cluster of tubular (tu) elements observed in a 35-day culture of 12-day embryo chick proventriculus. X1800.

Fig. 7.—High-power phase-contrast micrograph of a macrophage passing over a tubule (tu) in a 28-day culture of 12-day embryo chick pancreas. Arrows indicate extension processes formed by the macrophage along the tubule as it passed over it. X1800.

Fig. 8.—High-power phase-contrast micrograph of a tubule (tu) observed in a 42-day culture of 12-day embryo chick pancreas showing granular elements aligned along the surface of the tube. This was a common observation on old particles in areas in which the cellophane and glass coverslips were closely apposed. X1800.

Fig. 9.—High-power phase-contrast micrograph of a tubule (tu) from a 42-day culture of 12-day embryo chick pancreas showing a sharp angular bend. X1800.
FIGS. 10–12.—Medium-power phase-contrast micrographs of helical and ribbonlike spirals from the cerebral culture shown in Figs. 1–4. A magnification line is in Fig. 10 for Figs. 10, 11. X360.

FIGS. 10 and 11.—The specific area shown in Fig. 1 is reproduced at higher power in Fig. 10. Particulate elements A, B, and C are indicated. In Fig. 11 the same area is again shown after 76 days of cultivation or after an 18-day lapse of time between the two micrographs. The helical forms A and B were enlarged and elongated, and the division of the partitions of ribbon C was considerably elongated. Spiral A appeared to be composed of two adjacent ribbons.

FIG. 12.—A rather long helix (D) was in a semi-unwound form through its mid-section. Another helical form (E) was loosely coiled. Other unlabeled helices and ribbons are shown. X535.
Fig. 13.—Low-power bright-field micrograph of helical forms derived from a 64-day culture of 11-day embryo chick intestine. These helices were weakly stained in the chamber with hematoxylin after formol fixation. X733.

Figs. 14–16.—High-power bright-field micrographs of hematoxylin-stained helical spirals from the culture shown in Fig. 13. In Fig. 14 the helix F is shown with a secondary helix G on its right-hand portion. The helix F was observed to be denser in its left extremity, suggesting that helix G split from the right extremity of F. A similar record is shown of the helical form in Fig. 15, and in Fig. 16 there is a suggestion of a tripling of helical forms. X1500.

Fig. 17.—High-power phase-contrast micrograph of twin helices which appeared to have resulted from a longitudinal divisioning. This was derived from a 64-day culture of 11-day embryo chick cerebrum. X1555.

Fig. 18.—A low-power phase-contrast micrograph of a helical form which had divided at its right-hand extremity to form two antennalike components. This helix was derived from a 60-day culture of 11-day embryo chick cerebrum. A lesser-sized helix is shown crossing the antennalike ends. X360.

Fig. 19.—A tubular form which had unwound to form a helix and possibly to give rise to a secondary helix. This particle was observed in a 78-day culture of an 11-day embryo chick lung. X1900.
FIGS. 50–53.—High-power phase-contrast micrographs of two tubules observed in a 30-day culture of a 12-day embryo chick pancreas. The magnification line is shown in Fig. 52 for Figs. 50–53. X3400.

FIGS. 29 and 52.—High-focal level micrographs (Hi) showing the striping which occurred on the surface of the tubes. In Fig. 52 a helical unwinding occurred at both extremities. Specific spirals in Fig. 29 are numbered.

FIGS. 51 and 53.—Mid-focus micrographs (Mi) of the spirals of Figs. 29 and 52 showing the prominent dashing produced by the forming spirals. This was particularly evident in the tube in Fig. 51, delineated by the numbered arrows which matched the numbered spirals in Fig. 29.
Fig. 54.—Low-power phase-contrast micrograph of a helix which had been distorted so that a single, though symmetrical, loop was formed. This was observed in a 60-day culture of a 12-day embryo chick pancreas. At the top of the loop there is a nonrefractile hexagonal-forming crystal. X560.

Figs. 55–57.—High-power phase-contrast micrographs of a helix which surrounded a tubule taken at three focal levels (high, Hi; mid, Mi; low, Lo). X8700.

Fig. 58.—Three tubular forms (H, I, J) are shown with partially unwound spiraling (sp) extremities. The tubule H also had a sharp elbow bend and globular (gl) internal and external images. X1650.
FIG. 59.—High-power phase-contrast micrograph of a broad unspiraling portion of tube $K$. A lesser-sized tubule ($L$) with encircling helical images and several other tubes were in this field. $\times 1600$.

FIGS. 30, 31.—Two medium-power phase-contrast micrographs of a tube which was bowed after the coverslip of the culture chamber was gently wiped with lens tissue for cleansing purposes. Within a few minutes this bowed helix had returned to the straight form. $\times 600$.

Fig. 32.—High-power phase-contrast micrograph of two tubes, $M$ and $N$. Tube $N$ apparently surrounded tube $M$. The unspiraling which occurred in the upper extremity of $N$ produced the encircling helical form similar to that of Figs. 25–27. A portion of a large spiral $O$ is also shown. $\times 1700$.

Fig. 33.—High-power phase-contrast micrograph of the tubule $M$ encircled by the helical extremity of $N$. $\times 5750$. 
FIGS. 34–38.—Medium-power phase-contrast micrographs of a tubule-spiral complex (P₁, P₂) observed in a 20-day culture of a 13-day embryo chick pancreas. Numbers in the lower left portion of each illustration indicated the hours between micrographs. A magnification line for Figs. 34–38 is shown in Fig. 34. X750.

Fig. 34.—Complex (P₁, P₂) as it was positioned between two cellular elements.

Figs. 35 and 36.—During the first 18 hours the tubule-spiral complex was separated into a spiral component P₁ and a tubular component P₂ by the cellular activity.

Fig. 37.—After 24 hours the cellular elements separated the components P₁ and P₂.

Fig. 38.—By 96 hours the two components were so widely separated that two separate micrographs were made (Figs. 38a and 38b). P₁ is shown in Fig. 38a to have uncoiled and elongated 54 times its original length as shown in Fig. 37. Crystalline element P₂ is shown in Fig. 38b to have elongated to a greater dimension than the original complex P₁, P₂ shown in Fig. 34.
FIGS. 39, 40.—High-power phase-contrast micrographs of a macrophagic multinucleated cell containing an elongated particle (Q) of uncertain type. This was observed in a 56-day culture of a 12-day embryo chick pancreas. The change in contour of the particle during the 2 hours separating the micrographs denoted its malleable quality. The magnification line is in Fig. 39 for Figs. 39–40. X4700.
FIGS. 41–44.—High-power phase-contrast micrographs of hexagonal (he) and rhomboid (rh) crystalline elements. A magnification line is in Fig. 44 for Figs. 41 through 44. ×1735.

FIGS. 41, 42.—Hexagonal forms from a 66-day culture of a 13-day embryo chick pancreas. Angles a (107°) and b (146.5°) are labeled. All hexagonal crystals were formed of these angular measurements.

FIGS. 43, 44.—Rhomboids formed by angles c (80°) and d (100°) from a 322-day culture of an 11-day embryo chick lung. Note the many forming layers.
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