It happens often that the cold hypo solution, even highly oversaturated, does not crystallize. In this case a small grain of hypo dropped into solution produces an immediate effect—recrystallization of salt.

Certainly any other salt, differently soluble in cold and hot water, may be used for this purpose with the same result as hypo. F. e. sodium sulfate or washing soda are very good. In the case of washing soda the same solution may be used at first for boiling shale within and then, when boiling alone would be found insufficient, for crystallization through cooling.

I. TOLMACHOFF

CARNEGIE MUSEUM,
PITTSBURGH, PA.

SPECIAL ARTICLES

TOPOGRAPHIC SIMILARITIES BETWEEN MATERIALS REVEALED BY ULTRA-VIOLET LIGHT PHOTOMICROGRAPHY OF LIVING CELLS AND BY MICRO-INCINERATION[1]

LUCAS,[2] Wyckoff and Ter Louw,[3] Lucas and Stark,[4] and Wyckoff, Ebeling and Ter Louw[5] have recently published excellent ultra-violet photomicrographs of various living tissues. While examining these photographs, especially those of Wyckoff, Ebeling and Ter Louw, it occurred to me that there was a striking similarity between the shadow casting material (that which absorbed ultra-violet light of wave-length 2750 Å) and the mineral ash deposits in micro-incinerated cells of similar types. The idea is not altogether a new one, as Policard[6] had already pointed out that the calcium content of the nucleus might furnish an explanation of its capacity for absorbing ultra-violet rays as previously noted by Köhler.[7] The following observations were made in an attempt to ascertain the precise degree of similarity which exists. The method of microincineration employed was essentially that of Policard[8, 9] with some later modifications and improvements in methods of observation introduced by Scott[10, 11] and by Scott and Horning.[12]

(1) BACILLUS SUBTILIS

The ultra-violet photomicrographs of *B. subtilis* published by Wyckoff and Ter Louw serve as an excellent starting point, since the morphology of this organism is relatively simple. They show that the ultra-violet rays of wave-length 2750 Å are intensely absorbed by spores (Figs. 2, 4, 5 and 6). The authors indicate that the spores do not arise from a gradual merging together of preexisting cell bodies having similar physical properties. Indeed, at their first appearance they have already acquired their ultimate size and shape. Furthermore, the cytoplasm of spore-bearing cells is said to be of the same degree of opacity as the younger forms.

Smears of spore-bearing cultures of *B. subtilis* were dried in air and some colored with Giemsa's stain, while others were incinerated. The spore was found to be the locus of greatest mineral deposit. The residual ash was whitish grey tinged in places with a definite brownish red indicative of the presence of iron oxide. The mass of mineral was not doubly refractive when viewed with polarized light, hence, it was probably devoid of silica. The remainder of the cell was almost free of inorganic salts, with exception of its limiting membrane, which in most instances could be made out rather clearly. There were no definite concentrations of ash in the cytoplasm but only faint, finely divided deposits about the spore. Microchemical tests demonstrated that calcium was present in the ash.

From these findings it is apparent that the material which shows the greatest ultra-violet light absorption is exactly comparable to the mineral ash deposits in incinerated *B. subtilis*. Furthermore, a heavy deposition of salt in the spore lends weight to the frequently expressed opinion that spore formation is a concentration of cellular substance accompanying dehydration.

(2) RESTING NUCLEI OF TUMOR CELLS

Lucas gives photographs of cells of a mouse tumor which had previously been fixed and cut but not stained. In this particular instance the comparison of ultra-violet photographic results and those obtained by incineration is more exact, as tissues must of necessity be fixed in the course of preparation for the latter kind of study. In Lucas' Fig. 7 a number of "optical

[1] Aided by an appropriation from a grant made by the Rockefeller Foundation to Washington University for research in science.

[2] F. F. Lucas, *Proc. Nat. Acad. Sci.*, 16: 599, 1930.

[3] R. Wyckoff and A. Ter Louw, *J. Exp. Med.*, 54: 449, 1931.

[4] F. F. Lucas and M. Stark, *J. Morph. and Physiol.*, 52: 91, 1931.

[5] R. Wyckoff, A. Ebeling and A. Ter Louw, *J. Morph.*, 53: 189, 1932.

[6] A. Policard, *Bull. Hist. app.*, 5: 260, 1928.

[7] A. Köhler, *Zeitschr. f. wiss. Mikrosk.*, 21: 128 and 272, 1904.

[8] A. Policard, *Bull. Soc. Chim. Fr.*, 33: 1,551, 1923.

[9] A. Policard, *Protoplasma*, 7: 464, 1929.

[10] Gordon H. Scott, *Compt rend. Acad. Sci.*, 190: 1,323, 1930.

[11] Gordon H. Scott, *Proc. Soc. Exp. Biol. and Med.*, 29: 349, 1932.

[12] Gordon H. Scott and E. S. Horning, *J. Morph.*, 53: 1932, in press.

sections" of a fixed section of mouse tumor are pictured. One is struck almost immediately by the hyperchromatic condition of the nuclei of the tumor cells, which supports Cowdry's[13] observation that in tissue cultures they exhibit a more pronounced Feulgen reaction than do non-malignant cells. The formed chromatic material is densely packed at the margin of the nucleus, with one or more heavy flakes, which may perhaps be nucleoli, near its center. The cytoplasm of the tumor cells shows a rather greater absorbing power for the ultra-violet rays than one expects after examining photographs of other types of normal cells. Definite granules are quite prominent in the cytoplasm.

The micro-incineration method has already been used in the study of neoplastic tissues by Policard and Doubrow[14] and by Scott and Horning,[15, 16] and photographs and drawings are available in the literature. When incinerated sections of a neoplasm are examined the observer is at once aware of the large amount of mineral ash in the cells. The area of invading growth is clearly delimited from the surrounding stromal tissue by its increased salt content (Fig. 1, Scott and Horning). In the cells shown in Fig. 2 (Scott and Horning) the increased ash deposit at the periphery of the nucleus is brought out clearly. The arrangement and distribution of the mineral deposit corresponds in every way to the formed chromatic material of the stained nucleus. A comparison of Lucas, Figs. 7 and 9 with Scott and Horning's Figs. 1 and 2 leaves but little doubt of the similarity of the nuclear materials shown by ultra-violet photomicrography and those remaining after micro-incineration.

The relatively heavy density of the cytoplasm—its absorptive power for ultra-violet light—is in keeping with the results obtained by incineration. Neoplastic cells of the types examined showed a greater concentration of salt in the cytosome than did normal cells of similar types.

(3) Tissue cultures of fibroblasts

Wyckoff, Ebeling and Ter Louw have discussed the similarity in appearance of ultra-violet photomicrographs and Feulgen staining of cells in tissue cultures. Their comparison, to be sure, is more or less fortunate as regards the formed chromatic material but not so of the cytoplasmic constituents common to protoplasm. Their photographs show that in the nuclei of resting fibroblasts the only material strongly absorbing ultra-violet rays is that of the nucleolus but that the nuclear membrane is distinct (especially Figs. 3 and 4). The nucleoplasm has practically the same translucency as the cytoplasm. Flakes of chromatin are frequently seen in the nucleus. In Fig. 2 (Wyckoff, Ebeling and Ter Louw) a fibroblast is illustrated which has many small highly absorptive granules scattered throughout its cytoplasm. Amongst these granules are seen several vacuolar structures which possess rims not transparent to ultra-violet light. The fibroblasts of their Figs. 1, 3 and 4 also show the same structures.

Micro-incinerated specimens of fibroblasts and fibroblast-like cells have been described by Horning and Scott.[17] The nucleolus leaves a residue of very dense, whitish ash simulating in appearance the structure revealed in Wyckoff, Ebeling and Ter Louw's Figs. 1, 2, 3 and 4. Occasionally the rim of the nucleus can be made out as a distinct ash deposit arranged in a very fine line. In some instances tiny bead-like deposits are visible at the margin of the nucleus quite like those shown in Fig. 3 (Wyckoff, Ebeling and Ter Louw).

The cytoplasmic residue of incinerated fibroblasts gives an appearance almost exactly like that seen in the ultra-violet photographs. There are in some instances diffuse deposits with larger clumps of ash of an entirely different appearance scattered about in an irregular fashion. In other cells large, empty, ring-like spaces with heavy borders of mineral material are found. The appearance of a similar type of cell after incineration is shown in Fig. 1 (Horning and Scott). The boundaries of the incinerated tissue culture cell are usually sharply outlined by a definite ash deposit. A comparison of Fig. 2 (Wyckoff, Ebeling and Ter Louw) with Fig. 1 (Horning and Scott) shows that the minute granules in the ultra-violet photographs are of the same order of magnitude as the individual ash deposits in the incinerated cell. This point has been checked by measurements at the same magnification of the granules in the photograph and in the incinerated cells. The average diameter of the light-absorbing masses in the photograph is calculated to be equal to 0.83 microns in the living cell, whereas the mean diameter of the ash deposits is 0.79 microns.

(4) Tissue Cultures of Macrophages

Wyckoff, Ebeling and Ter Louw's Fig. 12 shows the nucleus and the surrounding cytoplasm of macrophages to have about the same opacity to ultra-violet light; but there are present in the cytoplasm certain intensely absorbent granules.

[13] E. V. Cowdry, SCIENCE, 68: 40, 1928.

[14] A. Policard and S. Doubrow, *Ann. d'Anat. path.*, 1: 163, 1924.

[15] Gordon H. Scott and E. S. Horning, *Proc. Soc. Exp. Biol. and Med.*, 29: 708, 1932.

[16] Gordon H. Scott and E. S. Horning, *Amer. J. Path.*, 8: 329, 1932.

[17] E. S. Horning and Gordon H. Scott, *Proc. Soc. Exp. Biol. and Med.*, 29: 704, 1932.

Examination of macrophages which have been incinerated shows very little ash in the nucleus; the nucleolus leaves a definite residue though the nuclear membrane is very feebly marked. The cytoplasm is filled with heavy, highly refractive masses of ash which are birefringent in polarized light, indicating the presence of silica. These ash deposits are obviously the remains of granules similar to those showing so clearly in the ultra-violet photographs.

(5) Nucleated Erythrocytes

When adult chicken erythrocytes are photographed with ultra-violet light the nucleus shows as a highly absorbing mass revealing but little structure (Fig. 8, Wyckoff, Ebeling and Ter Louw). The cytoplasm is moderately dense, indeed rather more so than that of the fibroblasts and macrophages.

Adult chicken blood was prepared in a thin, even smear and incinerated. The nuclei appeared as dense deposits of greyish white ash quite sharply separated from the surrounding cytoplasm. The cytoplasmic residue was finely granulated and evenly distributed throughout the cell. The limiting membrane of the erythrocyte was not at all sharply defined, but since the blood plasma leaves little or no residue, difficulty was not experienced in determining cell boundaries.

(6) Sperm Cells

Lucas and Stark have published excellent ultra-violet photomicrographs of living sperm cells of the grasshopper. As their study is primarily one of certain differences in chromosome formation, they have pictured but few resting cells. In Fig. 1 of their paper there are three large cells near the center which may well be true examples of the resting nucleus. A close examination of these cells shows the nucleus to be quite distinctly outlined. There are definite though faint flakes of chromatic material present. In Figs. 3 and 4 of Lucas and Stark's article the true resting nucleus is admirably shown in the sustentacular cells. In these nuclei the nucleolus absorbs the ultra-violet light and hence appears as a marked shadow. The nuclear membrane is sharp and patches of chromatin material are visible.

Preparations were made of testes fixed in absolute alcohol and formalin and of rapidly dried fresh smears. The resting spermatocytes showed but slight ash in the nucleus. The nucleolus was always represented by a highly refractile deposit of minerals and occasional ash clumps were scattered throughout the nucleus.

(7) Cells during Mitosis

The actual process of cell division with the coincident formation of chromosomes in living sperm cells, as observed with the ultra-violet microscope, has been described by Lucas and Stark. Their findings are well illustrated. In general it can be said that the chromosomes absorb ultra-violet light and consequently appear to be very dark in the photograph. This holds true for all stages of division, from the earliest prophase to the daughter cells. The cytoplasm of cells in most of the earlier stages of mitosis seems to be quite transparent and cell membranes are rather indistinct (Figs. 6 and 7, Lucas and Stark). It is significant that there are no marked granulations in the cytoplasm in the later stages.

Incinerated sperm cells of grasshoppers were examined and the arrangement of the salts noted during division. The findings in this form differed in no essential respect from those in the skin of the frog and in the testicle of the white rat previously recorded by Scott.[18] In each instance chromosome form was perfectly preserved by the ash residue and but little mineral matter could be seen in other portions of the cell. Even the early changes in the division figures could be followed with considerable accuracy.

Sperm cells of different orders do not appear to vary in the inorganic constituents revealed by micro-incineration. Dividing cells of the cat and rat testicle, frog skin, intestinal epithelium of rat and of cat, epidermis of *Triturus viridescens* and developing eggs of the frog, all show a concentration of mineral in the formed chromatin—especially in the chromosomes. Very little, if any, ash residue can be seen in other parts of the nucleus either of resting cells or of cells undergoing mitosis.

Evidently in these several kinds of material available for comparison, there is a definite resemblance between the topographic distribution of the substances of the living cell which are opaque to ultra-violet light of certain wave-lengths, and the mineral ash deposits in incinerated cells of similar types. With the present evidence it is impossible to decide whether the material absorbing ultra-violet light in living cells acts as a surface or focus of crystallization for the mineral materials in the process of fixation and dehydration, or represents an area of protoplasm composed largely of an inorganic colloidal complex. The conclusion is warranted that these two techniques (ultra-violet light photography and micro-incineration) are mutually supportive insofar that they reveal the presence of similar substances. Together they constitute a new and valuable method for the investigation of the chemical constitution of living matter.

Gordon H. Scott

Washington University
School of Medicine

[18] Gordon H. Scott, *Bull. Hist. app.*, 7: 251, 1930.