

## Mineral Salts of the Nucleus.\*

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Hueper<sup>1</sup> indicated that in certain tumor cells the correlation between chromatin material in stained sections and the mineral residue after microincineration was not as close as might be supposed from my studies of similar and of other material (Scott<sup>2, 3, 4</sup>). From Hueper's extension of his remarks to other classes of material, protozoa, etc., studied by me it might be inferred that he believed his observations on tumors to be of general significance. Cowdry,<sup>5</sup> Rector and Rector,<sup>6</sup> Policard,<sup>7</sup> Kruszynski<sup>8</sup> and others have amply demonstrated with various kinds of mammalian tissues, both normal and pathological, that my observations are not unique. Examination of a large series of human tumor and cancer tissues of many sorts collected and described in part by Olch<sup>9</sup> served to confirm in detail my previous statements made with regard to the ash-bearing chromatin material. Studies of an incinerated series of sections of embryos (chick, rat, mouse and cat) have given additional confirmatory evidence.

By taking advantage of 2 recently developed technical procedures a means was found of experimentally checking these observations. Tissues (liver and testis) of rats, guinea pigs and cats were subjected to ultra-centrifuging, as described by Beams, King and Risley,<sup>10</sup> at 100,000 times gravity. The centrifuge cup with its contents was frozen in liquid air and placed in a specially designed

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<sup>1</sup> Hueper, W. C., *J. Lab. and Clinical Med.*, 1934, **19**, 1293.

<sup>2</sup> Scott, G. H., and Horning, E. S., *PROC. SOC. EXP. BIOL. AND MED.*, 1932, **29**, 708.

<sup>3</sup> Scott, G. H., and Horning, E. S., *Am. J. Path.*, 1932, **8**, 329.

<sup>4</sup> Scott, G. H., *Am. J. Anat.*, 1933, **53**, 243.

<sup>5</sup> Cowdry, E. V., *Am. J. Path.*, 1933, **9**, 149.

<sup>6</sup> Rector, L. E., and Rector, E. J., *Am. J. Path.*, 1933, **9**, 587.

<sup>7</sup> Policard, A., *Bull. d'Histol. Appliq.*, 1933, **10**, 313.

<sup>8</sup> Kruszynski, J., *Bull. Acad. Polonaise d. Sci.*, 1934, **3**:B II, 105.

<sup>9</sup> Olch, I. Y., *PROC. SOC. EXP. BIOL. AND MED.*, 1933, **30**, 511.

<sup>10</sup> Beams, H. W., King, R. L., and Risley, P. L., *PROC. SOC. EXP. BIOL. AND MED.*, 1934, **32**, 181.

low temperature vacuum dehydrator. All water was removed while the tissues were kept frozen at  $-32^{\circ}\text{C}$ . Tissues so treated were embedded in paraffine after the method advised by Gersh<sup>11</sup> and Bensley.<sup>12</sup> Serial sections were made and alternate ones stained by several methods; the remaining sections were incinerated and examined with the darkfield microscope.

Stained sections of centrifuged tissues show the chromatin material packed at the centrifugal pole of the nucleus, against the nuclear membrane.

Incinerated sections reveal a dense mass of whitish ash at the side of the nucleus corresponding in position to the stained chromatin of the control slides. Very little mineral residue is visible in the clear portion of the nucleus.

By fortunate circumstances it was possible to obtain a good specimen of human testis prepared by the Gersh-Bensley method. Incinerated sections when compared with the stained controls revealed the fact that the chromosomes left a clearly recognizable ash picture of themselves. This would seem to leave little doubt as to the location of mineral salts in the nucleus and its components.

When sections of frozen-dehydrated tissues treated with petroleum ether to remove the paraffine are allowed to become moist by standing in air at room temperature, then dried at  $40-60^{\circ}\text{C}$ . and incinerated they show nuclei which contain diffuse ash. Free hand sections of frozen dehydrated tissues not infiltrated with paraffine given the same treatment show essentially the same picture. This procedure is not too unlike the basic manipulations of the techniques used by Schultz-Brauns<sup>13</sup> and by Hueper (loc. cit.) to permit the suggestion that it may account for the results obtained.

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<sup>11</sup> Gersh, I., *Anat. Rec.*, 1932, **53**, 309.

<sup>12</sup> Bensley, R. R., *Anat. Rec.*, 1933, **58**, 1.

<sup>13</sup> Schultz-Brauns, O., *Z. f. wissenschaft. Mikr.*, 1931, **48**, 161.