

lesions ranged between 8.85:1 and 13.5:1, while the cells taken from normal tissues of the control animals and the tumor animals ranged between 15.3:1 and 29.7:1. Cytological study of the malignant cells showed not only large nucleoli but also great irregularity in the shape, staining properties and number of nucleoli present in one nucleus—factors which we previously stressed as characteristic of the malignant cells from human tumors. The difference between malignant and non-malignant cells as regards nuclear-nucleolar ratio is more marked in our series of experimental tumors than it was in our series of human lesions which may partly be explained by the fact that the controls in this study were healthy tissues not the seat of inflammatory changes.

*Conclusions.* Study of experimental tumors produced by ultraviolet rays in the albino rat revealed low average values for the nuclear-nucleolar ratio of groups of 100 cells. The nucleolus from experimentally produced malignant tumor cells was found to vary greatly in size and number as it does in malignant cells from human lesions.

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#### Copper Hematoxylin, a Stain for the Acidophils of the Human Hypophysis.

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Cushing's<sup>1</sup> announcement that a pleuriglandular syndrome, designated by him as pituitary basophilism, was associated with basophilic tumors of the anterior lobe, attracted special attention to the basophils of the human hypophysis, including those that invade the posterior lobe. The latter variety, arising from the indifferent-staining cells of pars intermedia,<sup>2</sup> have been brought into prominence in connection with hypertension.<sup>3</sup>

Similarity in morphology and staining properties of the basophilic cells of the anterior and those in the posterior lobe has been been

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<sup>1</sup> Cushing, H., *Bull. Johns Hopkins Hosp.*, 1932, **50**, 137.

<sup>2</sup> Rasmussen, A. T., *Am. J. Anat.*, 1930, **46**, 461.

<sup>3</sup> Cushing, H., *Am. J. Path.*, 1934, **10**, 145.

advanced as evidence that the latter have migrated from the anterior lobe. Dayton<sup>4</sup> calls attention to the similarity in their behavior in various physiological and pathological conditions. Of those who have noted the great similarity in staining reaction, Kasche,<sup>5</sup> Biedermann<sup>6</sup> and Burgdorf<sup>7</sup> may be mentioned.

MacCallum, Fitcher, Duff and Ellsworth,<sup>8</sup> in connection with a typical case of Cushing's syndrome with a tumor in the anterior lobe, decided that copper hematoxylin stained the basophils of the anterior lobe but not those in the posterior lobe. Since the tumor cells did not stain with this technique and had morphological features somewhat similar to the cells of pars intermedia, they concluded that the tumor probably arose from pars intermedia, which, of course, arouses new interest in this part of the hypophysis. At the recent meeting of the Association for the Study of Internal Secretion at Kansas City, Lissner reported a certain amount of recovery in a patient with Cushing's disease after partial removal of a hypophyseal adenoma. That the tissue removed at operation was from a pars intermedia neoplasm, was at least partly inferred from the staining reaction with copper hematoxylin and the assumption that this technique stained the basophils of the anterior lobe, but not those that arise from pars intermedia, as announced by MacCallum, *et al.*<sup>9</sup>

The facts are that copper hematoxylin, as given in McClung's Microscopical Technique<sup>9</sup> or by substituting potassium dichromate for neutral potassium chromate, stains the acidophils rather than the basophils of either lobe. Bailey,<sup>10</sup> years ago, grouped Weigert's myelin sheath stain (a copper hematoxylin method) with the more or less specific stain for the acidophilic granules. Copper hematoxylin is also a mitochondrial stain and the difficulty in separating mitochondria from the acidophilic granules in the hypophysis is proverbial.

Sections from human hypophyses fixed in formalin, Bouin's, Helly's, Regaud's, Zenker's and Zenker-formol (half the acetic acid replaced by formalin) fluid, all give essentially the same results. We have selected specimens that happened to have groups of cells

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<sup>4</sup> Dayton, T. R., *Ztschr. f. Anat. u. Entwicklungsgesch.*, 1926, **81**, 359.

<sup>5</sup> Kasche, F., *Ztschr. f. mikr-anat. Forschung*, 1926, **6**, 191.

<sup>6</sup> Biedermann, H., *Virchow's Arch. f. path. Anat. u. Physiol.*, 1927, **264**, 217.

<sup>7</sup> Burgdorf, A. L., *Endokrinologie*, 1935, **16**, 148.

<sup>8</sup> MacCallum, W. G., Fitcher, T. B., Duff, G. L., and Ellsworth, R., *Bull. Johns Hopkins Hosp.*, 1935, **56**, 350.

<sup>9</sup> McClung, C. E., *et al.*, 1929, p. 203.

<sup>10</sup> Bailey, P., *J. Med. Research*, 1921, **42**, 349.

that could be identified without question. One section of a pair of adjacent 5 $\mu$ . sections was stained by the well-known Mallory's acid fuchsin-aniline blue-orange G method, which has been used extensively on the hypophysis, and the other one by the copper hematoxylin method. Sections have been stained in copper hematoxylin and then counter-stained with other acidophilic stains and no acidophils were seen that were not already stained by copper hematoxylin. We have drawn particular cells in colors under high power and decolorized and then stained with copper hematoxylin. In all instances it is perfectly clear that the basophils of the anterior lobe, like those in the posterior lobe, are not stained in fully differentiated copper hematoxylin slides, while the acidophils are. The basophils may have a darker grayish-brown tone than the chromophobes, but this is not sufficiently marked to serve as a method of separating basophils from the chromophobes. All acidophils do not hold the stain with equal tenacity. This is more or less true of all methods and applies to positive staining (by other methods) of basophils as well.

The basophils can usually be identified among the unstained cells in the copper hematoxylin method because of their greater vacuolation. Having sectioned serially the hypophysis of three cases of pituitary basophilism, we can corroborate the findings of Crooke,<sup>11</sup> who described hyaline areas of various sizes in the cytoplasm of the basophils of the anterior lobe and to some extent also of the posterior lobe. This modified cytoplasm also serves to identify the basophilic cell among those not stained with copper hematoxylin. While the basophils of the posterior lobe are more resistant to these changes than are those of the anterior lobe, some of them show exactly the same change, giving further proof of the great similarity between the basophils in the two locations.

It is, therefore, concluded that copper hematoxylin is not a stain that will differentiate between the basophils of the anterior lobe and those of the posterior lobe, by staining the former and not the latter, because neither retains the stain. On the contrary, as has been recognized by others, it is a good positive stain for the acidophils of the human hypophysis.

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<sup>11</sup> Crooke, A. C., *J. Path. Bac.*, 1935, 339.