

by changing the concentrations of KCl and NaCl in opposite directions, keeping the osmotic pressure constant.

It is evident that the low limit of temperature for spontaneous beats varies according to the potassium content of the solution in which the heart has been kept. Martin² found this to be true of the beats of the turtle ventricle, but since he stated that the auricles were relatively resistant to cooling, he was presumably dealing with an atrio-ventricular block rather than a complete stoppage.

The relatively high susceptibility of summer preparations, indicated in the table, is apparently due to an actual seasonal variation in the condition of the tissue, and not to the higher initial temperature from which the cooling was started. Preliminary immersion at 20° instead of 28° does not render such a preparation appreciably more resistant to cooling. Regardless of seasonal variations and initial temperature, however, any preparation can be cooled to 0° without stopping, if it has been kept for a time in a sufficiently low concentration of potassium.

The effect of the higher concentrations of KCl is reversible. A preparation kept in Ringer solution containing 0.160% KCl shows, on repeated cooling, a progressively higher temperature of stoppage until beats finally cease at room temperature. On transferring the preparation to normal frog or turtle Ringer the process is reversed, beats eventually continuing at 0°. Balancing the original solution, by adding a corresponding excess of calcium, is not effective in causing such recovery.

8784 P

Cytological Studies on Experimental Rat Tumors.

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The present investigation is the first attempt at an experimental approach to the problem of nucleolus size in cancer cells. In a previous publication, von Haam and Alexander¹ reported studies on nuclear and nucleolar measurements in fixed and unfixed cells of malignant tumors in man. After examining 10,000 cells, 5,000 of

² Martin, E. G., *Am. J. Physiol.*, 1904, **11**, 370.

¹ von Haam, E., and Alexander, H. G., *Am. J. Clin. Path.*, 1936, (in press).

which were taken from non-malignant tissues and served as controls, the authors came to the conclusion that the number of excessively large nucleoli encountered in malignant tissues was sufficient to produce a distinct difference in the nuclear-nucleolar ratio between benign and malignant lesions based on average values of at least 100 cells to a specimen.

The material for the present investigation was experimental tumors produced in the albino rat by means of radiation of the animals with ultraviolet rays over a long period. With this method, which has been described in detail by Roffo; Beard, Boggess and von Haam² obtained in 40% of their animals malignant tumors of various histological types which proved fatal to the animals. The technic of measurements of the nuclei and the nucleoli in the experimental tumors was identical with that described by von Haam and Alexander with the exception that only fixed tissue cells were used for the determinations, since it had been shown that proper fixation has no appreciable influence on the nuclear-nucleolar ratio.

Satisfactory selective staining of the nucleoli was obtained with Victoria blue or with Regaud's iron hematoxylin. Toluidine blue which had been used extensively in measurements on unfixed cells, did not give satisfactory results with fixed tissues. Twelve tumors in albino rats were examined—there were eight carcinomas of the ear, one carcinoma of the nose and three sarcomas of the eye. Skin from healthy animals, taken from regions corresponding to the location of the malignant tumors, and liver and kidney epithelium of some of the tumor animals served as controls. The results obtained are summarized in Table I.

The nuclear-nucleolar ratio of the cells examined in the malignant

TABLE I.

Prot. No.	Type of tissue examined	Nuclear Nucleolar Ratio (100 cells)	Prot. No.	Type of tissue examined	Nuclear Nucleolar Ratio (100 cells)
1T	Carcinoma of ear	12.4:1	6T	Epithelium, kidney	16.5:1
2T	" " "	11.7:1	5C	" skin	23.3:1
3T	" " "	13.5:1	4T	" liver	18.6:1
4T	" " "	13.4:1	15C	" skin	19.0:1
5T	" " "	8.85:1	1T	" kidney	16.5:1
6T	" " "	10.8:1	6T	" liver	20.8:1
7T	Sarcoma " eye	10.3:1	12C	" skin	27.4:1
8T	Carcinoma " ear	9.1:1	19C	" "	25.3:1
9T	" " nose	10.1:1	6T	" "	15.3:1
10T	" " ear	10.9:1	17C	" "	29.7:1
11T	Sarcoma " eye	11.3:1	21C	" "	27.5:1
12T	" " "	12.9:1	6C	" "	25.5:1

² Beard, H., Boggess, T. S., and von Haam, E., *Am. J. Cancer*, 1936 (in press).

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's¹ 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,² have been brought into prominence in connection with hypertension.³

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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¹ Cushing, H., *Bull. Johns Hopkins Hosp.*, 1932, **50**, 137.

² Rasmussen, A. T., *Am. J. Anat.*, 1930, **46**, 461.

³ Cushing, H., *Am. J. Path.*, 1934, **10**, 145.