# A METHOD OF SELECTIVE STAINING OF NUCLEI AT DIFFERENT STAGES OF THE CELL CYCLE WHEN STUDYING THE MYOCARDIUM

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KEY WORDS: myocardium; cell cycle.

The aim of this investigation was to discover if the method of selective straining of nuclei, developed for tissue culture [3], can be used to study paraffin sections of the myocardium. As a result of staining, chromosomes, nucleoli, and nuclei in the S-phase ought to appear orange-red, whereas nuclei in other stages of the cell cycle ought to appear various shades of green. It was necessary to discover whether in fact the red nuclei in paraffin sections of the myocardium are actually in the phase of DNA synthesis, i.e., whether they take up thymidine label.

## EXPERIMENTAL METHOD

Newborn noninbred rats were given an intraperitoneal injection of $^3$H-thymidine with specific activity of 1.3 Ci/mmole in a dose of 5 $\mu$Ci/g body weight 15 min before sacrifice. Paraffin sections 5-7 $\mu$ thick were coated with type M emulsion and developed [1] after exposure for 3 weeks. The time of staining the preparations was modified. Staining was carried out as follows: 1) dewaxed sections were immersed for 1-2 sec in a 0.5% solution of safranin in 0.025 M borax solution; 2) the preparation was rinsed in distilled water; 3) it was stained for 5-10 min in picroindigocarmine (50 ml of 1% indigocarmine and 100 ml saturated picric acid in McIlwain's buffer, pH 4.0); 4) rinsed in distilled water; 5) it was taken quickly through 96 and 99% ethyl alcohol, cleared in xylol, and mounted in Canada balsam.

The labeled red nuclei, labeled green nuclei, and unlabeled red nuclei in the sections were counted. Self-absorption of radiation by the section was taken into consideration [2] and nuclei in the upper layer of the section were counted. The results were subjected to statistical analysis.

## EXPERIMENTAL RESULTS

Red staining of the nuclei was found to coincide with labeling in most of the nuclei studied (Table 1).

Of the nuclei which were stained red, 93.4 ± 0.7% had taken up the label.

TABLE 1. Agreement between Red Staining of Nuclei and Thymidine Labeling

| Animal No. | Number of nuclei | | |
|---|---|---|---|
| | red, unlabeled | red, labeled | green, labeled |
| 1 | 38 | 320 | 40 |
| 2 | 50 | 600 | 34 |
| 3 | 57 | 1 100 | 78 |
| 4 | 137 | 2 060 | 214 |
| 5 | 33 | 200 | 9 |
| 6 | 38 | 720 | 47 |
| Total | 353 (6,1%) | 5 000 (86,5%) | 422 (7,4%) |

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On the basis of these results the method of selective staining of nuclei can be recommended for estimation of the number and topography of DNA-synthesizing nuclei during the investigation of paraffin sections.

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# IMMUNOLOGIC DETERMINATION OF EPIDERMAL $G_2$-CHALONE AS MARKER OF SQUAMOUS-CELL STRUCTURES IN RAT LUNG TUMORS


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KEY WORDS: epidermal chalone; squamous-cell metaplasia; lung tumors.


The writers previously found that an epidermal $G_2$-chalone isolated from rat skin possesses antigenic properties and showed that it is a tissue-specific antigen. Immunologic methods of its determination in tissues were developed [3]. It was found that epidermal $G_2$-chalone (its antigenic determinant) is contained in all tissues which undergo keratinization under physiological conditions (skin, tongue, esophagus, pancreas, and vagina) [6]. This is evidence that the process of squamous-cell differentiation in different tissues is always accompanied by synthesis of this particular tissue-specific antigen. It was logical to suggest that this antigen is synthesized also during squamous-cell metaplasia of tissues that do not become keratinized under normal conditions, such as is observed, for example, in neoplastic transformation. The idea thus arose that epidermal $G_2$-chalone could be used as an immunologic marker of squamous-cell tumors in different situations.

The content of epidermal $G_2$-chalone in induced rat lung tumors was studied.

## EXPERIMENTAL METHOD

Altogether 17 lung tumors were studied in 10 male rats from the "Rappolovo" nursery. Tumors were induced by subcutaneous injection of diamylnitrosamine (0.05 ml/kg body weight injected once a week for 40 weeks [2]). The neoplasms had the appearance of nodules 0.5-2 cm in diameter, located beneath the pleura and in the depth of the lung tissue. Each tumor was cut into two halves, one of which was used for standard histological investigation, the other for immunologic investigation. Lung tissue from intact male rats of the same age served as the control.

The presence of antigen in the tissues was determined by the counter-immunodiffusion test in gel or by indirect immunoautoradiography. The sensitivity of the methods was 2-3 $\mu$g and 60-80 ng antigen/ml extract, made up in the proportion of 100 mg tissue to 1 ml physiological saline buffered at pH 7.2, respectively. The method of obtaining rabbit antibodies against epidermal $G_2$-chalone, details of the immunologic methods used to detect this antigen in rat tissues, and its principal biochemical characteristics were all described previously [3, 4, 6].

## EXPERIMENTAL RESULTS

Histological investigation of the rat lung tumors showed that they were glandular neoplasms (adenomas and adenocarcinomas) and a squamous-cell carcinoma. Mixed tumors in which foci of squamous-cell carci-


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