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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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 μg 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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noma adjoined foci of adenocarcinoma were frequently found. This structure of the tumors can be explained by early squamous-cell metaplasia of the glandular epithelium and its malignant transformation.

Since epidermal G_2-chalone was used to detect squamous-cell structures in the tumors, the available material was divided into three groups: 1) glandular neoplasms with no sign of squamous-cell metaplasia (five cases); 2) glandular neoplasms with regions of squamous-cell metaplasia and small foci of squamous-cell carcinoma (seven cases); 3) squamous-cell tumors (five cases).

No antigen was found in the unchanged lung tissue of intact rats (control) by either the direct or the immunoautoradiographic method.

By the use of direct immunodiffusion, epidermal chalone was found only in all five tumors of group 3. By the immunoautoradiographic investigation antigen was found in the tumors of group 3 and also in most (in five of seven) tumors with squamous-cell metaplasia, as well as in one tumor belonging to group 1.

The absence of antigen in two tumors of group 2 and its discovery in one case in a "purely" glandular tumor are evidence, in the writers' view, of the irregular distribution of foci of squamous-cell metaplasia in the tumors, as a result of which single foci of this type could occur either in the sample investigated immunologically, or only in the region taken for histological study.

On the whole, the results of the investigation are evidence that epidermal G_2-chalone is a sufficiently reliable marker of squamous-cell structures in the rat lung. Such structures can be seen when squamous-cell tumors or squamous-cell metaplasia are present. The latter is regarded by some workers as a factor attended by an increased risk of development of cancer [1].

Squamous-cell tumors of the human lungs, unlike adenocarcinomas, can acquire sensitivity to the action of epidermal G_2-chalone [5]. Comparison of this fact with the results of the present investigation indicate that a similar tissue-specific substrate is very probably synthesized by such tumors and that epidermal G_2-chalone can be used as an immunologic marker of such neoplasms in man.

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