COMPARATIVE AUTORADIOGRAPHIC EVALUATION OF RNA SYNTHESIS IN VIVO AND IN VITRO

R. I. Kaem and T. S. Ustinova

UDC 616-018.1-092.4-07

KEY WORDS: morphology, liver, RNA synthesis, autoradiography.

Autoradiography (AR) is the most accurate method of analysis of levels of biosynthesis in cells currently available. To obtain reliable information, comparative autoradiographic experiments are necessary, in which the label is incorporated either intravitally or into biopsy material. By comparison in this way, optimal conditions for reliable assessment of processes in biopsy material can be determined and compared with the corresponding processes in vivo. Investigations of this kind have been conducted to study DNA synthesis by intestinal enterocytes [3, 6]. The intensity of biosynthesis in cells can also be analyzed after incubation with ^{3}H-uridine, a labeled RNA precursor. The rate of incorporation of the label and of formation of labeled RNA de novo provides an indication of the functional activity of the cells during renewal of cell protein structures [2].

The aim of this investigation was a comparative analysis of biosynthesis taking place in cells of the liver, kidney, and skin during RNA synthesis in vivo and in vitro, and to confirm the possibility of autoradiographic evaluation of biopsy material.

EXPERIMENTAL METHOD

The test objects were the principal cells of mouse liver tissue, labeled with ^{3}H-uridine in vivo and in vitro, and also rat kidneys and mouse skin, labeled in vitro. In the experiments in vivo, mice were given an intraperitoneal injection of ^{3}H-uridine in a dose of 100 μCi/g (specific activity 23 Ci/mmole). The animals, under superficial anesthesia, were killed after 1 h, and pieces of liver were removed, fixed, and embedded in Epon-Araldite resin, as described previously [3]. In the experiments in vitro, biopsy specimens (1-2 mm^3) were incubated at 37°C for 1 h with ^{3}H-uridine (100 μCi/ml). Semithin sections were coated simultaneously for the groups to be compared, with photographic emulsion M or R, and exposed for 4 or 2 days. The morphological characteristics of the cells, the zone of localization of labeled cells in the preparations, the index of labeled nuclei (ILN, in %) for cells in 20 fields of vision, and the maximal labeling density above the cells or nuclei were studied in the autoradiographs under the light microscope with immersion objectives. The results were subjected to statistical analysis and the significance of differences in the groups determined by Student's test.

EXPERIMENTAL RESULTS

Analysis of the two groups of autoradiographic preparations of the liver, labeled in vivo and in vitro, showed that in vivo the labeled cells were distributed throughout the preparation. Different cycles of distribution of the label, reflecting successive phases of biosynthesis, could be traced in the nuclei of hepatocytes labeled in vivo (Fig. 1a, b).

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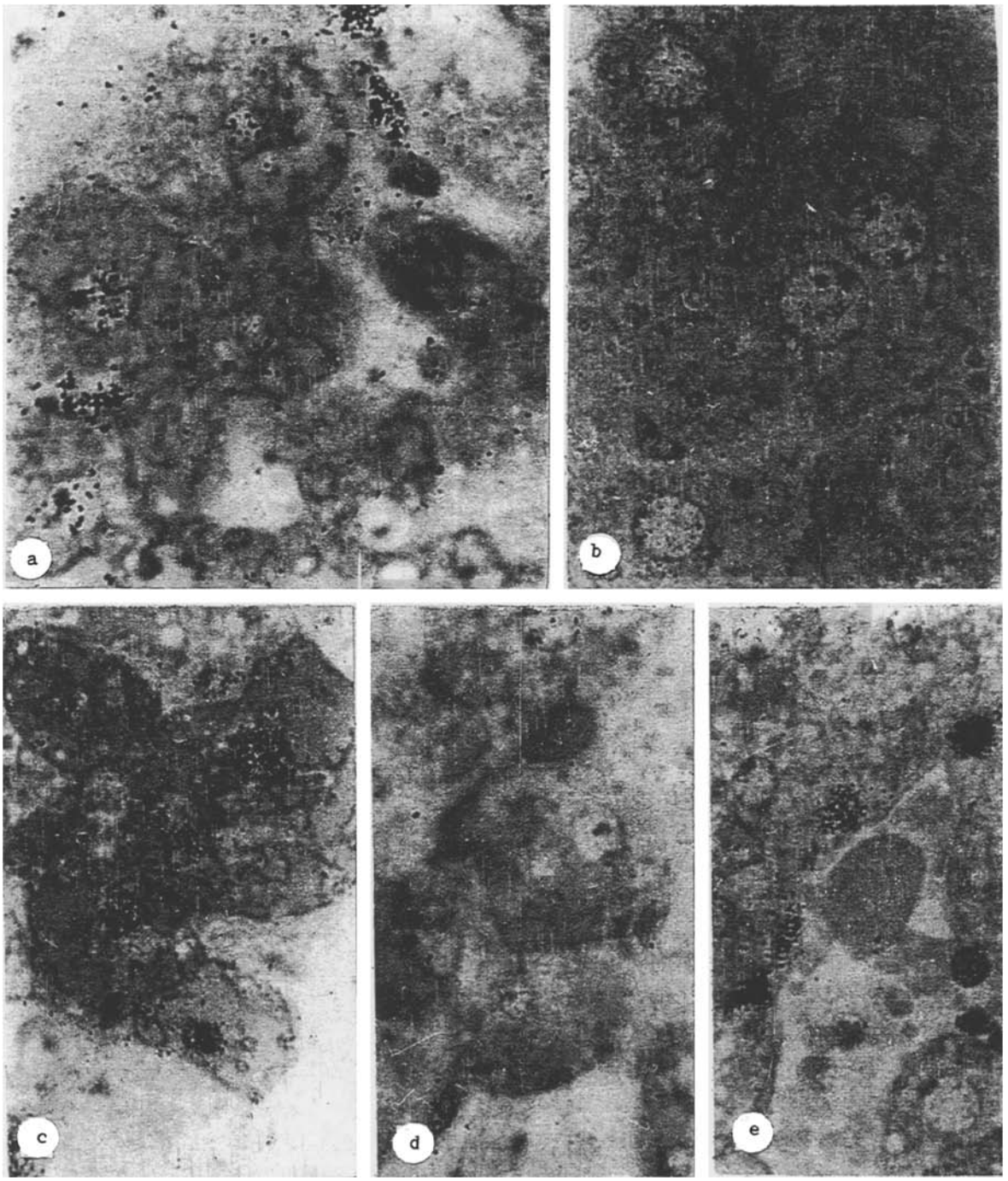

Fig. 1. Light-microscope autoradiographs of mouse liver tissue. ^{3}H-Uridine In vivo: a) uniform distribution of label in hepatocytes, endothelial cells; b) localization of label mainly above nucleoli of hepatocytes. In vitro: c) dense labeling in hepatocytes of peripheral zone; d) labeled endotheliocytes and single labeled hepatocytes in transition zone; e) in depth of preparation. 900×.

In preparations of biopsy material from the liver, the label was localized mainly in cells of the peripheral zone, extending over a distance of 80-100 μ from the edge into the depth of the preparations. Uniform intensive labeling was observed here in the hepatocytes ("dark" and "pale") and in the endotheliocytes of the capillaries (Fig. 1c). The value of ILN of the hepatocytes in vitro (55 ± 5%) was high, but lower than in vivo (79 ± 2%, $p < 0.001$). The labeling density above the hepatocyte nuclei in the peripheral zone of the biopsy material was high, and for most cells it was comparable with the labeling density obtained in vivo (Fig. 1a, b). Its value fell considerably closer to the

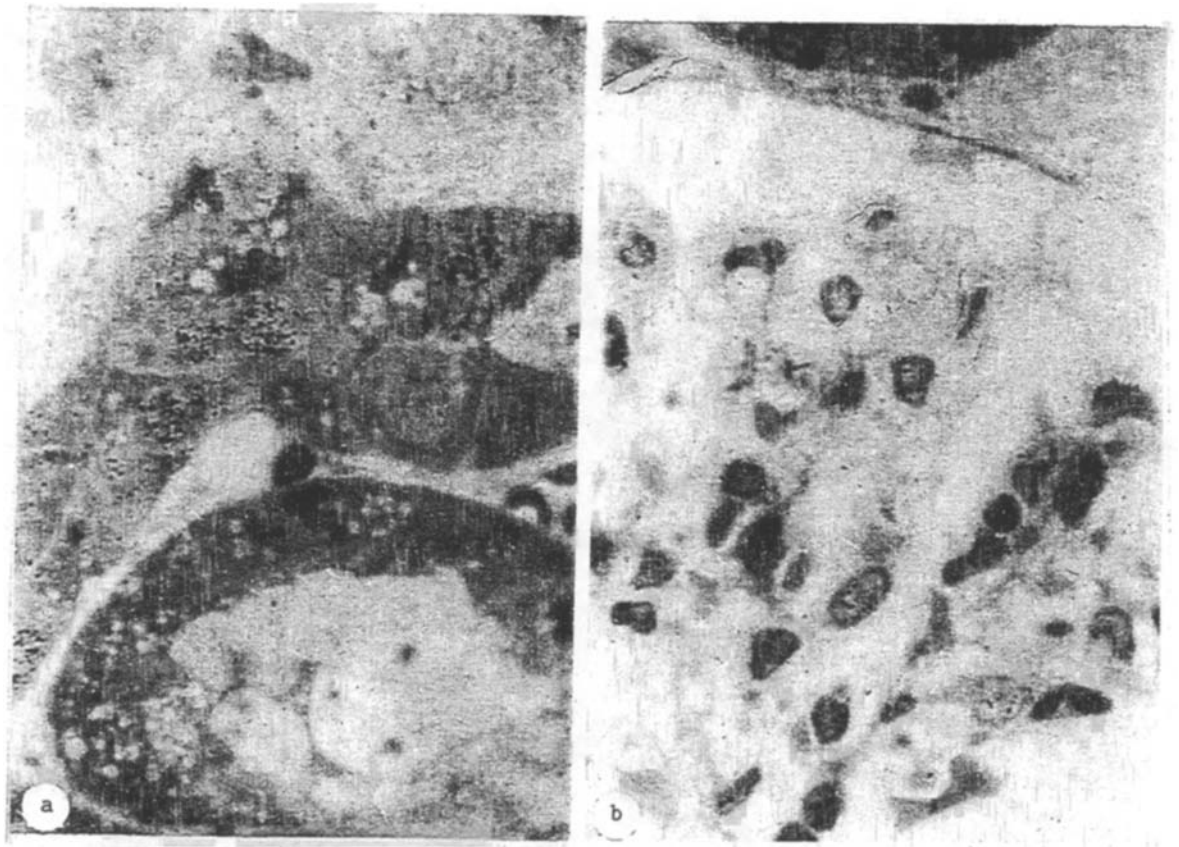

Fig. 2 Light-microscopic autoradiographs of rat kidney biopsy material. ^{3}H-Uridine. a) Labeled nephrocytes of convoluted tubule, weak labeling in degeneratively changed nephrocytes, absence of label and marked degenerative changes in nephrocytes of a tubule located deeper in preparation; b) moderately dense labeling in endotheliocytes and podocytes of a glomerulus. 90×.

center of the preparations, as also did the value of ILN, for the hepatocytes, although it was high for the endotheliocytes (Fig. 1d, e). The degree of preservation of the structures determined the labeling intensity, and in the biopsy material it was high in the peripheral zone. Morphological changes in the depth of the fragments increased in severity, and took the form of swelling of the hepatocytes and homogenization of the cell cytoplasm. In preparations labeled in vivo, no such dystrophic changes could be seen as a rule.

The results are evidence that during incubation of liver biopsy specimens with ^{3}H-uridine, which could be preceded by mild hypothermia, factors complicating incorporation of the label could arise, such as hypoxia and slowing of diffusion of the precursor, which were not present during labeling in vivo. Sensitivity of hepatocytes to damaging factors under the incubation conditions which we used, namely at 37°C and for 1 h, is known to be particularly high for hepatocytes [5]. High labeling density in endotheliocytes located nearer the center of the preparations could be explained by the presence of preexisting pathways (capillary lumen) for ^{3}H-uridine diffusion, or complete trapping of the blood cells or collapse of the capillary lumen did not take place in the biopsy material. Consequently, the labeled endotheliocyte can be used as an in tissue marker of the depth of ^{3}H-uridine diffusion both in vivo and in vitro.

The same principles of distribution of labeled cells in the preparations were found on the whole for biopsy material from the kidneys as for liver biopsy material. Dense labeling was observed mainly in the nephrocytes of the convoluted tubules, podocytes, and endothelial cells of the renal glomeruli located in the peripheral zone of the biopsy material (Fig. 2). Accumulation or absence of the label above the structures of similar type, located side by side, could be due to differences in their functional state. The high sensitivity of nephrocytes to damaging factors, and to hypoxia in particular, may lead to heterogeneity of ^{3}H-uridine incorporation not only in different tubules, but also in the same tubule (Fig. 2a). The high functional and morphological lability that is characteristic of nephrocytes of

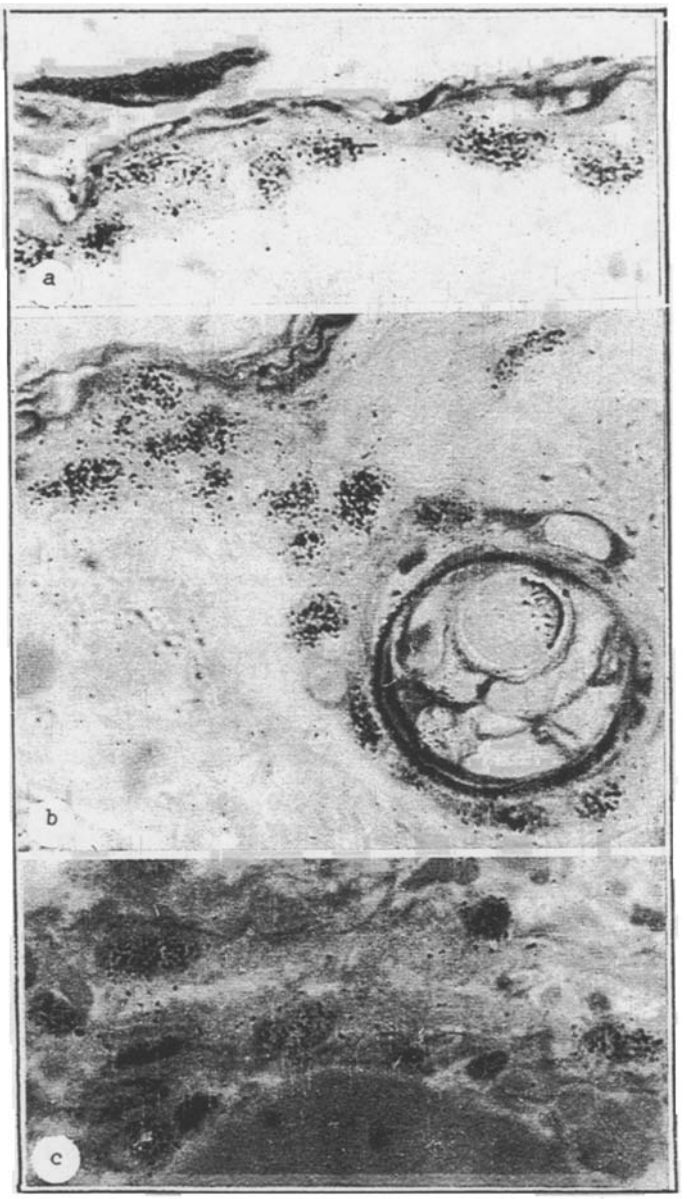

Fig. 3. Light-microscopic autoradiographs of mouse skin biopsy material. ^{3}H-Uridine. High labeling density: a) epidermocytes, b) epithelium of hair follicle, c) endothiocytes of capillary and stromal cells of dermis. 900×.

the convoluted tubules led to the development of marked dystrophic changes of the vacuolar degeneration type, and as this investigation showed, under these conditions there is a sharp fall in the rate of incorporation of the RNA precursor. The high labeling intensity of the endothelial cells of the glomeruli (Fig. 2b) was evidently associated, just as in the liver, with the presence of preexisting channels of diffusion.

The study of ^{3}H-uridine incorporation into skin biopsy material revealed dense labeling in all cells of the epidermis and dermis. The densest labeling was observed in the stratum basale of the epidermis and in the epithelium of the sheath and bulb of the hair follicles (Fig. 3a, b). Active incorporation of ^{3}H-uridine took place in the depth of the dermis, and for that reason dense labeling was observed in most endotheliocytes of the capillaries and connective-tissue cells (Fig. 3c). This particular feature of the skin biopsy material was evidently attributable, on the one hand,to the high resistance of its cells to hypoxia and, on the other hand, to the optimal conditions for diffusion of the low-molecular-weight precursor. This was shown by the absence of any appreciable degenerative changes of the

various skin elements and the distribution of cells with intensive labeling throughout the bulk of the biopsy specimens.

The ability of ^{3}H-uridine to penetrate into biopsy material is evidently determined by competition between two opposite processes taking place at different speeds during incubation of fragments. Chemical diffusion of a substance dissolved in a liquid medium into a gellike body, taking place along interphase boundaries, is known to be a slow stage and characterizes the rate of the process as a whole. Consequently, the diffusion kinetics delays passage of the precursor molecules into the bulk of the biopsy material, whereas the spread of hypoxia, when the blood flow has ceased in isolated fragments, takes place sufficiently rapidly and, under these circumstances, damages the cells [1]. Hypoxic damage may be shifted to some degree away from the edges of the zone of the biopsy specimen during incubation with additional aeration with oxygen, although this allows only some degree of widening of the marginal labeling zone [2, 4]. In the depth of the biopsy specimens, in cells of tissues highly sensitive to hypoxia (liver, kidney, etc.), dystrophic and destructive changes developed, accompanied by a sharp decline or complete loss of biosynthetic activity. Meanwhile, conditions for labeling were optimal for biopsy specimens from the skin, the components of which are quite resistant to hypoxia.

Comparison of autoradiographic preparations of the liver, kidney, and skin thus shows that, in the biopsy material studied there are cells with high activity of de novo RNA formation. Maximal labeling intensity in biopsy material from the liver and in vivo was comparable, evidence of the absence of any significant inhibition of genetically determined transcription of different forms of hepatocyte RNA of biopsy material in DNA molecules. A characteristic feature of liver and kidney biopsy material is that intensive labeling was present in cells of a peripheral zone up to 1000 μ wide, but labeling was absent in the center of the preparations due to hypoxic damage and low rates of diffusion in vitro. For tissue resistant to hypoxia and with high rates of diffusion (skin), total labeling in vitro was observed. Endothelial cells of liver, kidney, and skin can be used as an indicator of the depth of diffusion of ^{3}H-uridine within the tissues, for because of the high intensity of their labeling, the potential powers of the surrounding cells can be assessed. For light-microscopic autoradiography of biopsy material it is preferable to use coarse-grain photographic emulsions. The size of the fragments to be tested must be minimal, considering differences in the rate of diffusion of ^{3}H-uridine in concrete tissues.

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