TOTAL REPLACEMENT OF PARENCHYMAL LIVER CELLS INDUCED BY DIPIN AND PARTIAL HEPATECTOMY

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UDC 616.36-089.873+615.277.3:547.861.3]-07:616.36-003.9-018.15-07

KEY WORDS: regeneration of the liver; hepatocarcinogenesis; preneoplastic nodules; alkylating agents; autoradiography.

The ability of the liver to regenerate after massive injury and removal of part of the organ is well known. This paper describes restoration of the hepatic parenchyma accompanied by total elimination of pre-existing hepatocytes and their replacement by new cells. This phenomenon has been observed by the use of a new method of inducing cell damage, namely by means of a lesion of the genetic apparatus. The method consists essentially of injecting the alkylating agent dipin into an animal, and performing standard partial hepatectomy 2 h later. Dipin, which has the properties of a mutagen and clastogen*, interacts with the genetic material of the whole population of dormant hepatocytes. As the results of previous experiments showed [2], after stimulation of mitosis the latent lesions are realized as structural changes in the chromosomes. Cells with multiple chromosomal aberrations are nonviable, they degenerate, and are gradually eliminated from the tissue. In response to depression of function and loss of the cells a regenerative response develops in the form of nodular zones of hepatocellular proliferation, which gradually displace and replace the pre-existing hepatocytes in the tissue.

In this paper the phenomenon of total replacement of the parenchymal cells of the liver was demonstrated by autoradiography.

EXPERIMENTAL METHOD

CBA × C57BL/6 mice weighing 22-24 g and aged 2-2.5 months were given an intraperitoneal injection of 0.3 ml of a freshly prepared solution of dipin in 0.9% NaCl in a dose of 60 mg/kg, and 2 h later, under ether anesthesia, a standard partial hepatectomy was performed. Starting 30 h after the operation, and thereafter every 12 h for 4 days, the animals were given injections of ^{14}C-thymidine (specific activity 51 mCi/mmole) in a dose of 0.5 μCi/g body weight; the total number of injections was eight. The experiment ended immediately after saturation with labeled thymidine, i.e., 5 days (five animals), 5 months (five animals), 8 months (three animals), and 10 months (two animals) after the beginning. Paraffin sections were cut from the liver by the usual methods, covered with type M autoradiographic emulsion, exposed for 1 and 3 months, developed, and stained with hematoxylin.

EXPERIMENTAL RESULTS

In animals taken immediately after the end of the ^{14}C-thymidine injections, and 5 days after injection of dipin and partial hepatectomy, part or virtually the whole of the hepatocyte population was intensely labeled with ^{14}C (Fig. 1a). Usually at this time no morphological signs of injury or chromosomal fragments are observed, because the hepatocytes are in a state of prolonged premitotic blockade [3, 4].

After 5 months (Fig. 1b, c) multiple nodules of proliferating unlabeled cells were present in all the animals in autoradiograph sections among the labeled parenchymal tissue. Labeled hepatocytes were greatly enlarged and carried traces of mitotic aberrations: deformed

*Presumably something which induces chromosomal breaks.

Laboratory of Cytology, N. K. Kol'tsov Institute of Developmental Biology, Academy of Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 105, No. 1, pp. 77-80, January, 1988. Original article submitted September 24, 1986.

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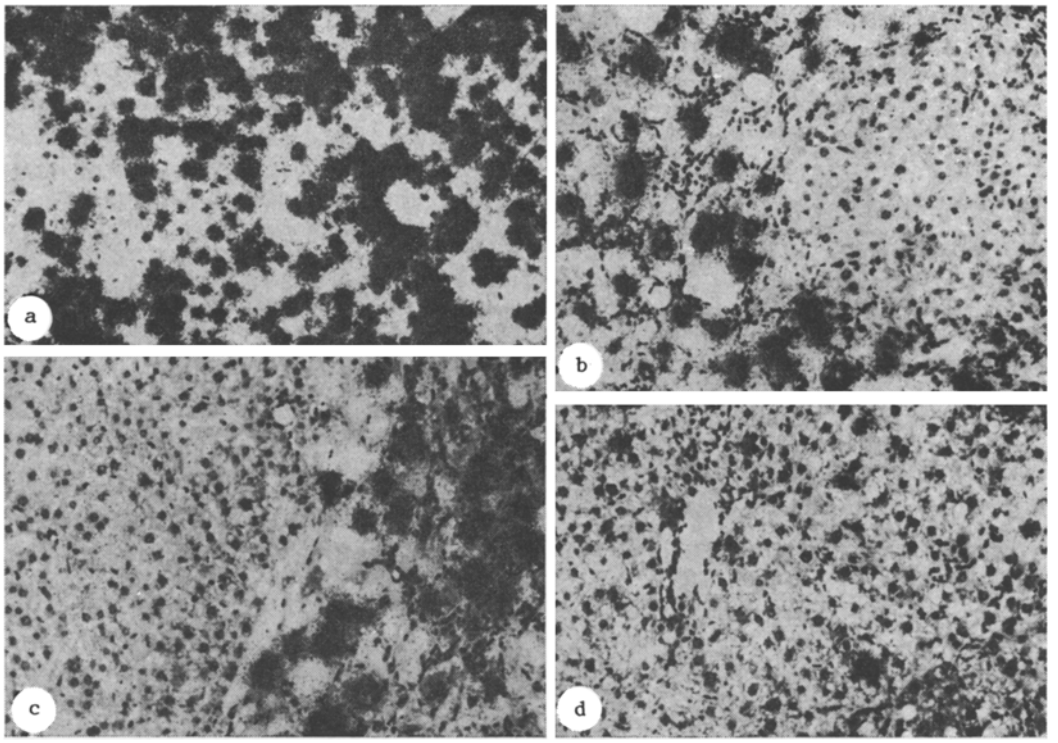

Fig. 1. Formation of new parenchymal tissue and replacement of injured hepatocytes, demonstrated by autoradiography with ^{14}C-thymidine. a) 5 days after injection of dipin combined with partial hepatectomy and repeated injections of ^{14}C-thymidine: virtually total labeling of hepatocytes; b, c) 5 months after beginning of experiment, individual hepatocellular nodules (b) and large adenomas (c) containing unlabeled cells can be seen among the labeled degenerating hepatocytes; d) 8 months after beginning of experiment: disappearance of labeled pre-existing cells, one labeled hepatocyte in the field of vision. Stained with hematoxylin. 200×. Autoradiographs exposed for 1 month.

nuclei, edges, and multiple micronuclei. Many cells had vacuolated or pycnotic nuclei, with dystrophic cytoplasm. In all cases there were signs of activation of the stromal part of the organ, an inflammatory reaction, and also oval-cell proliferation, characteristic of pretumor changes in the liver [5]. The hyperplastic nodules contained only unlabeled relatively small, outwardly mature hepatocytes with no evidence of injury or degeneration. In some animals the nodules of proliferating cells appeared as solitary small speckles against the background of labeled tissue, whereas in others they were well developed, so that the liver tissue in some places consisted of confluent nodules, separated only by narrow bands of degenerating labeled cells.

After 8 and 10 months complete disappearance of the degenerated hepatocytes and their replacement by new, unlabeled tissue, took place in all the animals. Only solitary labeled cells could be found in the completely renewed organ (Fig. 1d). The tissue organization of the regenerating liver was disturbed, the course of the trabeculae was often chaotic, although in the same section alternation of portal vessels and central veins could be observed in the section. In no case did fibrosis develop. The liver with completely replaced parenchymal cells also showed macroscopic changes. The liver was enlarged a little and appeared, not homogeneous but consisting of a large number of small, whitish round formations, although it preserved its softness and elasticity.

The results are evidence that total replacement of injured mature hepatocytes is possible in the liver of adult animals, and they indicate that the way is wide open for studying sources of new tissue and mechanisms of their activation on the basis of the model suggested above.

The phenomenon of restoration of the hepatocyte population after total involvement has not been described previously. During regeneration of the liver, when studied on standard

models, the weight of tissue is usually restored through proliferative activity of residual cells. If a certain threshold level of damage is exceeded, however, regeneration will not develop [1]. The method of action on the genetic apparatus of the cells, which we suggest, enables irreversible injuries to develop, leading to disturbances of function and ultimately to death of the entire cell population. This effect develops gradually, so that it enables activation of reserve sources of new cells, giving rise to multiple centers of growth. The nature of these reserve sources is unknown. They may arise from hypothetical undifferentiated precursor cells and also from mature hepatocytes that are resistant, or have undergone repair of their injuries.

New tissue is formed in this model from nodules of proliferating cells, and it is the product of fusion of foci of growth, known in the literature as hyperplastic, neoplastic, hepatocellular nodules or adenomas [7]. Nodular proliferation is an essential stage in the development of pretumor changes it is observed in all models of hepatocarcinogenesis, and it is nowadays regarded as a manifestation of adaptive reactions of the tissue to carcinogens and to tumor growth promoters [6, 7]. It is considered that in the course of this adaptation, growth of cells resistance to the action of toxic agents, inhibiting function and proliferation of normal hepatocytes, is selectivley stimulated.

The fate of preneoplastic nodules differs. Some of them give rise to large adenomas and malignant hepatomas, whereas the majority usually undergo reduction and redifferentiation and are absorbed by the normal surrounding parenchymal tissue [8]. In the model which we suggest, damage to the hepatocytes is irreversible and it affects the whole or the greater part of the population. This encourages the complete realization of the growth potential of cells of preneoplastic nodules. New parenchymal tissue is formed by fusion of the initially sufficiently autonomous foci of growth, and it therefore has features of an atypical structure.

The fact that new parenchymal tissue is formed in response to complete disappearance of pre-existing hepatocytes proves that cells possessing high clonogenic ability and normal histogenetic powers in adult animals can be activated.

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