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# CHANGES IN THYMIDINE-$^3$H CONTENT IN DNA OF LIVER AND SKIN CELLS AFTER ADMINISTRATION OF SYNGENEIC TISSUE EXTRACTS

B. P. Shadrin and G. V. Bulava

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The literature on reparative regeneration of organs in fully grown mammals is extensive. One of the most probable mechanisms by which the body recognizes the location of injury and stimulation of cell division in the residual part of an organ is considered to be immunologic reactions [1]. The most demonstrative data in support of this view have been obtained by the use of regenerating rodent liver as the model. Blood, plasma, serum, and spleen cells of animals after removal of about two-thirds of the volume of the liver have been shown to acquire for a certain length of time the ability to stimulate mitotic activity of liver cells in syngeneic recipients [1, 4]. If this effect is assumed to be connected with the development of an immunologic reaction, induced by factors arriving from the injured parenchyma of the liver, their presence in normal liver likewise cannot be ruled out.

The object of the present investigation was to study the effect of parenteral injection of liver and skin extracts on proliferative activity of liver and skin cells in syngeneic mice on the basis of incorporation of thymidine-$^3$H.

## EXPERIMENTAL METHOD

Six male CC57 white mice weighing 16 g were given an intravenous injection of 0.2 ml of liver extract from syngeneic donors containing 180 µg protein. The intravenous injection of extract was repeated 40 days later in a dose of 90 µg (as protein) and an intraperitoneal injection of thymidine-$^3$H (5-methyl derivative, specific activity 0.5 mCi/mole) in a dose of 1 µCi/g body weight was given at the same time.

The liver extract was prepared from a native tissue homogenate in distilled water by freezing to —12°C and thawing at 37°C five times, followed by centrifugation at 12,500g.

An extract of skin or physiological saline and thymidine-$^3$H were injected by a similar scheme into two other groups of mice, differing in number.

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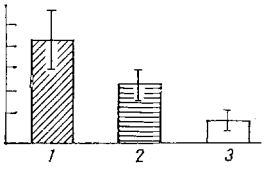


Fig. 1

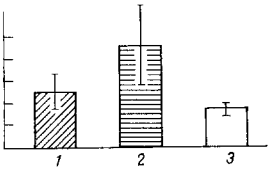


Fig. 2

Fig. 1. Number of labeled cells (in %) in liver of mice receiving liver extract (1), skin extract (2), and physiological saline (3).

Fig. 2. Number of labeled epidermal cells (in %) in mice receiving liver extracts (1), skin extracts (2), and physiological saline (3).

Before the first injection of extracts or physiological saline, a skin incision 1 cm long, passing through all its layers, was made in all experimental animals in the interscapular region, under aseptic conditions.

The mice were killed 72 h after injection of the thymidine-$^3$H and second injection of the extracts, after which the liver and a piece of skin where the incision was made were fixed in Bouin's fluid and embedded in paraffin wax. Sections 4 μ thick were coated with type M liquid nuclear emulsion (Photographic Chemical Research Institute), exposed, and developed. During analysis of the microautoradiographs, the number of labeled cells per 4000 cells was counted in specimens from the liver and skin of each animal. A cell was taken to be labeled if at least seven grains of metallic silver were present above its nucleus. The numerical results were subjected to statistical analysis by the Fisher–Student method, after which the confidence interval was calculated for each index at a 95% level of significance.

## EXPERIMENTAL RESULTS

Two intravenous injections of liver extract led to an increase in the number of liver cells incorporating thymidine-$^3$H in the syngeneic recipients 72 h after a single injection of labeled nucleoside (Fig. 1). The skin extract had a weaker action. Intravenous injection of skin extract had a stronger stimulating action than injection of liver extract on incorporation of the radioisotope into epidermal cells (Fig. 2).

The results indicate a tendency for liver and skin extracts to predominantly stimulate proliferation of cells from the liver and skin respectively. This suggests that pathologically unchanged tissues contain factors which, in an autologous or syngeneic system, can induce a response which affects proliferation of the corresponding cells and the connected processes of DNA synthesis. The increase in number of labeled cells can be explained not only by stimulation of incorporation of free thymidine-$^3$H, but also by reutilization of the radioactive label of the DNA, for the free nucleoside does not remain in the blood plasma longer than 15 min [2]. Circulating lymphocytes could be the source of the reutilized label [3, 5-8].

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