UTILIZATION OF THYMIDINE-H^3 LABELLED DNA OF THYMOCYTES BY CELLS OF THE REGENERATING LIVER

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The phenomenon of reutilization of nucleic acids in living systems has been proved. On unicellular organisms in vitro investigators have established the possibility of the reutilization of native DNA and its metabolites and have also presented proof of the genetic transformation ensuing after this [2, 8, 14-16]. It is not known in what form the nucleic acids are reutilized in vivo in complex multicellular systems. The data in the literature on this are contradictory. Thus, some researchers [3, 4, 17, 19] consider that reutilization of DNA in vivo proceeds only at the level of free nucleotides and nucleosides. Other authors [9-12, 18, 20] allow the possibility of reutilization of high-molecular DNA. The lymphoid tissue is usually considered to be the main source of nucleic acids or their degradation products, which are repeatedly taken up into the cells of the organism [13].

The present work is devoted to a study of the role of thymocytes as the source of nucleic acids for cells of the regenerating liver.

METHOD

The experiments were carried out on male rats of the hybrid line C57BL × CBA weighing from 18 to 20 g. Two-thirds of their liver was removed by the Higgins-Anderson method. At 20 and 36 h postoperation the animals were injected intravenously with a 0.5 ml suspension of thymocytes (10^8 cells in 1 ml) labelled with thymidine-H^3. Constant infusion of unlabelled thymidine in a dose of 30 mg per day was carried out on three mice from the time of hepatectomy until they were killed. For this purpose we used a special apparatus which we described earlier [1]. Three mice of the same line which were injected with $5 \cdot 10^7$ thymocytes 52 and 36 h before sacrifice served as the control.

The thymocytes for injection, obtained from mice of the same line, were incubated with thymidine-H^3 for 1 h at 37° (specific activity of thymidine-H^3 was 8.4 Ci/mM) at a rate of 1 μCi of labelled nucleoside per 1 ml of suspension containing 10^8 cells. After incubation the cells were washed 3 times with physiological salt solution and injected simultaneously into all animals. Before injection we made smears of the cell suspension, which were fixed with methanol.

The liver of the experimental and control animals after killing was fixed in Carnoy's fluid and embedded in paraffin. The serial sections of the liver, 5 μ thick, and the smears of the cell suspensions were covered with type M nuclear emulsion (NIKFI). The radioautographs were exposed for 45 days. The preparations were stained after development with hematoxylin-eosin. In the analysis we determined the index and intensity of labelling of the thymocytes, hepatic parenchymatous cells, and other elements of the liver. In each preparation we examined at least 100,000 cells.

RESULTS

Upon incubation of the suspension of thymocytes with thymidine-H^3 only 10.2% of the cells incorporated the isotope (see table).

Labelling Index and Intensity of Various Elements of the Regenerating Liver After Injecting Mice with Thymocytes Labelled with Tymidine-H^3

Experimental setup	Lymphocytes		Cells			
			hepatic		others	
	labelling index	labelling intensity	labelling index	labelling intensity	labelling index	labelling intensity
Incubation of thymocyte suspension	10.2	24.15±7.82	–	–	–	–
Control	0.02	15.87±4.32	0	0	0	0
Injection of thymocytes in partially hepatectomized mice	0.1	15.79±3.34	0.01	3–4	0.005	3–4
Injection of thymocytes in partially hepatectomized mice and addition of unlabelled thymidine	0.3	15.33±3.06	0.01	3–5	0.004	3–4

Comment. The labelling index is the percent of labelled cells; the labelling intensity is the average number of silver grains on the labelled cell.

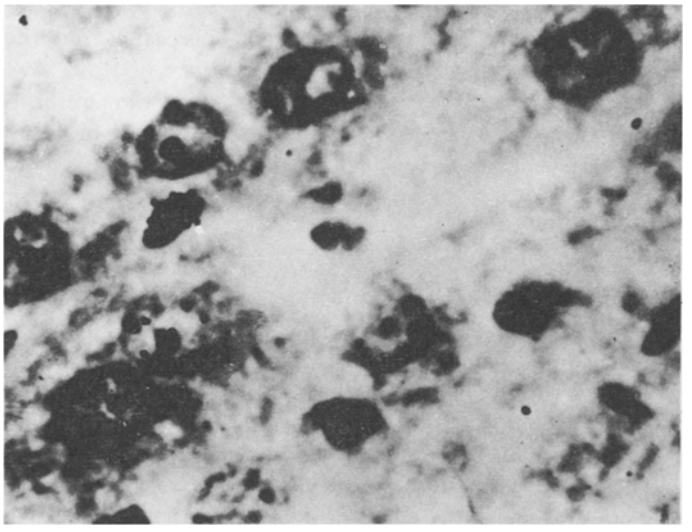

Fig. 1. Hepatic parenchymatous cell with weakly labelled nucleus.

The average labelling index of the thymocytes was 24.15±7.82 grains of silver on the labelled nucleus. When labelled thymocytes were injected we observed their dilution by unlabelled lymphocyte recipients, which is indicated by the appreciable drop of the labelling index of the lymphocytes in the hepatic tissue in all series of the experiment. The fewest labelled lymphocytes were found in the liver of the control animals. After hepatectomy their number increased fivefold in comparison with the control and with the simultaneous infusion of unlabelled thymidine, 15-fold. These data confirm the conclusions about the selective aggregation of lymphocytes at places of high cellular proliferation [6].

The labelling intensity of the thymocytes in all variants of our experiments was the same. The lower labelling intensity of the lymphocytes in the hepatic tissue in comparison with that of the thymocytes in the smears can be explained by the partial dilution of the label by division of these cells and by differences in the conditions of obtaining the radioautographs of the smears and sections. The relationship between the hepatic parenchymatous cells and the lymphocytes in the control and experiment was virtually unchanged.

Labelled hepatic cells are encountered with equal rarity in both experimental variants and have a weak labelling intensity (see table and Fig. 1). A few liver cells with labelled cytoplasm were found in the hepatectomized

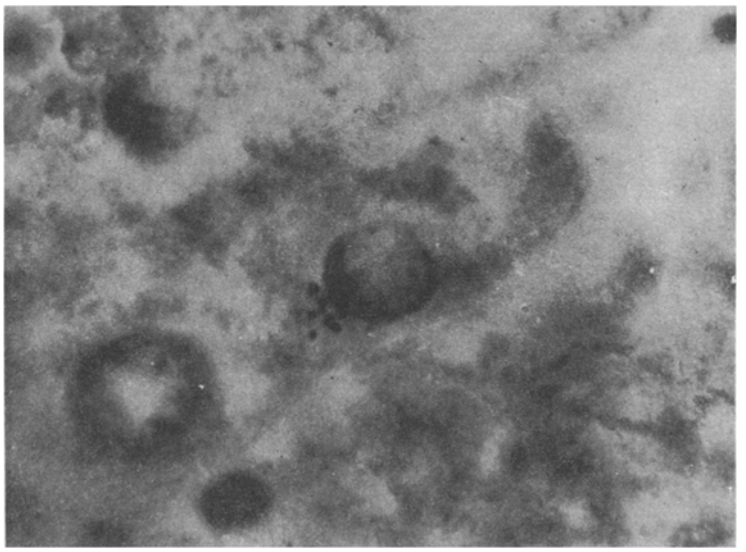

Fig. 2. Hepatic parenchymatous cell with labelled cytoplasm.

mice that did and did not receive unlabelled thymidine. The label usually occupies a limited portion of the cytoplasm in the form of a segment (Fig. 2).

A few weakly labelled cells of the reticuloendothelial system, endothelium of the vessels and bile ducts were noted in both experimental variants.

Three-fold washing of the thymocyte suspension to remove the thymidine-H^3 incorporated into the DNA during incubation permits us to consider that the label was injected into the mice in the composition of the high-polymer nucleic acid. The total quantity of isotope injected in this case into the mice was a small fraction of 1 μCi. In the case of disintegration of the thymocytes in the recipient mouse and the subsequent splitting of DNA to low-molecular fractions, a small amount of the isotope should be uniformly distributed throughout all tissues.

Here it is impossible to detect the label by the method of microradioautography, since distinct radioautographs of the cells when thymidine-H^3 is injected can be obtained only at doses exceeding 0.1 μCi per 1 g of animal weight. Consequently, the demonstration in our experiments of labelled liver cells can attest to some kind of local transfer of the isotope, in which case it is found in the composition of relatively high-molecular compounds. If the free thymidine-H^3 were to be transferred, the infusion of large quantities of unlabelled thymidine should lead to a substantial dilution of the label, as was the case in the experiments of other authors [19]. No effect of infusion of large amounts of unlabelled thymidine on the process of isotope transfer from thymocytes to liver cells was elicited.

The liver cells with labelled cytoplasm are of interest. Since such a label is found in both cases, this attests to high-molecular DNA being in the cytoplasm. This can be explained by the incorporation of a large fragment of DNA from the labelled thymocyte.

In the regenerating liver we did not find nuclei whose labelling intensity could be compared with that of the liver lymphocytes. This fact does not agree with the conclusions that the DNA of one cell can be completely transferred to another [9-12].

The increase in the number of labelled lymphocytes can indicate that the injected labeled lymphocytes primarily accumulate at places of enhanced cell proliferation. Possibly this is caused by lower vitality of the injected cells.

In the experiment with the infusion of unlabelled thymidine, the number of labelled lymphocytes in the regenerating liver was triple that in the experiment without the injection of unlabelled nucleoside. This can indicate that the excess of unlabelled thymidine prevents to some extent the destruction of the injected thymocytes.

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