INCORPORATION OF LABELED THYMIDINE INTO MOUSE LIVER CELLS AFTER PRELIMINARY ADMINISTRATION OF CORTISONE

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Administration of cortisone acetate to rats caused a decrease in the number of polyploid and binuclear cells in the liver. Maximal incorporation of labeled thymidine was observed 40 h after discontinuing cortisone injections. Label was incorporated mainly into tetraploid nuclei. The number of labeled diploid nuclei 56 h after injection of thymidine was increased more than three-fold. It is suggested that this was due to direct division of polyploid nuclei.

The suggestion has often been made that physiological regeneration in the liver takes place not only by mitotic cell division, but also by direct division of polyploid nuclei [1-5, 7].

The method which demonstrates most clearly the fate of different classes of liver cells is autoradiography, and it has shown that labeled thymidine is incorporated into both mononuclear and binuclear cells of different ploidy [8, 13].

The present investigation was carried out to study the dynamics of incorporation of thymidine-H^3 into mouse liver cells after preliminary administration of cortisone to the animals as an agent causing degenerative changes in the liver [6].

EXPERIMENTAL METHOD

Experiments were carried out on C_{57} mice weighing 20 g. Every day the mice received 4 injections of cortisone acetate (2.5 mg). Labeled thymidine was injected intraperitoneally in a dose of 1 μCi/g, 16, 24, and 40 h after the last injection of cortisone. The animals were sacrificed 1 h after injection of thymidine-H^3. In addition, three mice receiving thymidine 40 h after injection of cortisone were sacrificed 56 h after injection of thymidine. The liver was fixed in Carnoy's fluid. Type M (Motion Picture Research Institute) emulsion was applied to sections 5 μ in thickness and exposure took place for 22 days at 4°. The number of labeled nuclei was counted in 5000 cells, counting the label separately in diploid, tetraploid, and polyploid cells, and also the unlabeled mitoses.

EXPERIMENTAL RESULTS

The number of polyploid and binuclear cells in the liver 16 h after the last injection of cortisone was small, but diploid cells were numerous. In the liver of adult mice under normal conditions few diploid cells are present [4, 5, 9]. The number of diploid cells fell sharply 40 h after injection of cortisone (Table 1), whereas the number of binuclear and polyploid cells increased. A sharp increase in the number of binuclear cells was noted toward the end of the experiments.

TABLE 1. Changes in Number of Binuclear Diploid and Polyploid Cells (in ‰) after Injection of Cortisone

Type of cells	Time after injection of cortisone (in h)			
	16	24	40	56
Diploid	220	151	26,9	46,7
Polyploid	7,1	25,6	43,2	37,0
Binuclear	7,1	16,0	14,2	50,6

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TABLE 2. Incorporation of Thymidine-H^3 into Liver Cell Nuclei (number of labeled nuclei in ‰)

Labeled nuclei and cells	Time after injection of cortisone (in h)			
	16	24	40	56
Polyploid nuclei	—	0,15	10,1	0,07
Tetraploid "	0,15	1,02	18,7	8,06
Diploid "	—	0,07	2,51	7,62
Binuclear cells	—	—	1,17	0,07
Mitoses	—	—	—	—
Total number of mitoses (in ‰)	0,15	1,24	32,4	15,82
	—	—	0,8	0,7

During the first hours after ending the cortisone injections, incorporation of label was very slight (Table 2). Incorporation of thymidine-H^3 did not reach its maximum until 40 h after the last injection of cortisone.

At all times of the investigation label was incorporated mainly into tetraploid nuclei. Post and Hoffman [12, 13] consider that labeled tetraploid nuclei are in fact diploid nuclei in the phase of DNA synthesis which subsequently divides by mitosis. In our experiment the number of mitoses was very small and no labeled mitoses whatsoever were found.

Polyploid nuclei incorporated label particularly intensively at the 40-h stage. The number of labeled diploid nuclei increased with an increase in time elapsing after injection of cortisone. The number of binuclear labeled cells was very small. The total number of labeled nuclei decreased over the period of 56 h after injection of labeled thymidine by almost 50%. During this period there was a marked decrease in the number of labeled polyploid and tetraploid nuclei with a simultaneous increase in the number of labeled diploid nuclei. Labeled diploid nuclei evidently appeared because of division of polyploid and tetraploid cells whose nuclei had incorporated label 40 h after injection of cortisone.

Probably many of the polyploid nuclei in our experiment underwent simple division, as indicated, first, by the very small number of mitoses in general and, second, by the fact that the liver cell replication time (in mice, in particular) is very great [10, 11, 13]. It is evidently by direct division of tetraploid and polyploid nuclei that the population of labeled diploid nuclei increases. The cell itself does not divide immediately after direct division of the nucleus, as shown by the increase in number of labeled binuclear cells 40 h after injection of cortisone.

LITERATURE CITED

1. V. Ya. Brodskii and A. A. Kushch, Dokl. Akad. Nauk SSSR, 147, No. 3, 713 (1962).
2. V. Ya. Brodskii, Uspekhi Sovr. Biol., 58, No. 3, 367 (1964).
3. L. N. Zhinkin, Arkh. Anat., Gistol. i Émbriol., No. 1, 3 (1962).
4. T. A. Zaletaeva, Byul. Éksperim. Biol. i Med., No. 8, 112 (1963).
5. T. A. Zaletaeva, Byul. Éksperim. Biol. i Med., No. 10, 93 (1963).
6. T. A. Zaletaeva, Dokl. Akad. Nauk SSSR, 165, No. 2, 421 (1965).
7. S. S. Laguchev, Tsitologiya, No. 5, 598 (1964).
8. R. Basserga and W. E. Kiselski, J. Nat. Cancer Inst., 28, 331 (1962).
9. F. Doljanski, Internat. Rev. Cytol., 10, 137 (1960).
10. R. A. McDonald, Arch. Intern. Med., 107, 335 (1961).
11. C. Pilgrim and W. Maurer, Exp. Cell. Res., 37, 183 (1965).
12. J. Post and J. Hoffman, Exp. Cell Res., 36, 111 (1964).
13. J. Post and J. Hoffman, Exp. Cell Res., 40, 333 (1965).