REUTILIZATION OF LABELED DNA BY REGENERATING ADRENAL CELLS

(UDC 612.45 : 612.6.03].015.348)

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 61, No. 1,
pp. 73-76, January, 1966
Original article submitted June 8, 1964

Thymidine-H^3, which is a specific precursor of DNA, is widely used for the study of cellular kinetics. The conclusions drawn as a result of the first studies with thymidine-H^3 were based upon the fact that after a single introduction of the labeled nucleoside, it is utilized for DNA synthesis only for 45-60 min, since after this it is excreted from the organism [7, 15, 16, 18, 20].

However, the results of a number of studies give evidence of the possibility of reutilization of the isotope for DNA synthesis by various cells of the organism. This process is detected in bone marrow cells [11-14], epidermal cells of the skin [9], and cells of the liver and small intestine [5, 6]. Most authors consider the nuclear elements of the blood, primarily the lymphocytes, as the source of the isotope in its reutilization [5, 6, 8, 9].

The purpose of this work was to determine the possibility of reutilization of labeled DNA by cells of the regenerating adrenal gland.

EXPERIMENTAL METHODS

The experiments were conducted on males of the hybrid line of mice $C_{57}Bl$ and CBA, weighing 28 g. Thymidine-H^3, with specific activity 2.9 C/mM, was injected intraperitoneally. Two animals received 3 injections each of thymidine-H^3 in doses of 30 microcuries at 12 h intervals. Two mice received a summary dose of 90 microcuries of thymidine-H^3 in a single injection.

A day after the injection of thymidine-H^3, the animals were operated upon – one adrenal was removed entirely, and one third or one fourth of the other was resectioned. This method of operation induces the most pronounced regeneration of the organ [1, 3]. The operation may also be considered as a stressor. In stress reactions, the mass of the adrenals increases substantially in a short period of time. Moreover, numerous mitoses appear in the organ [19].

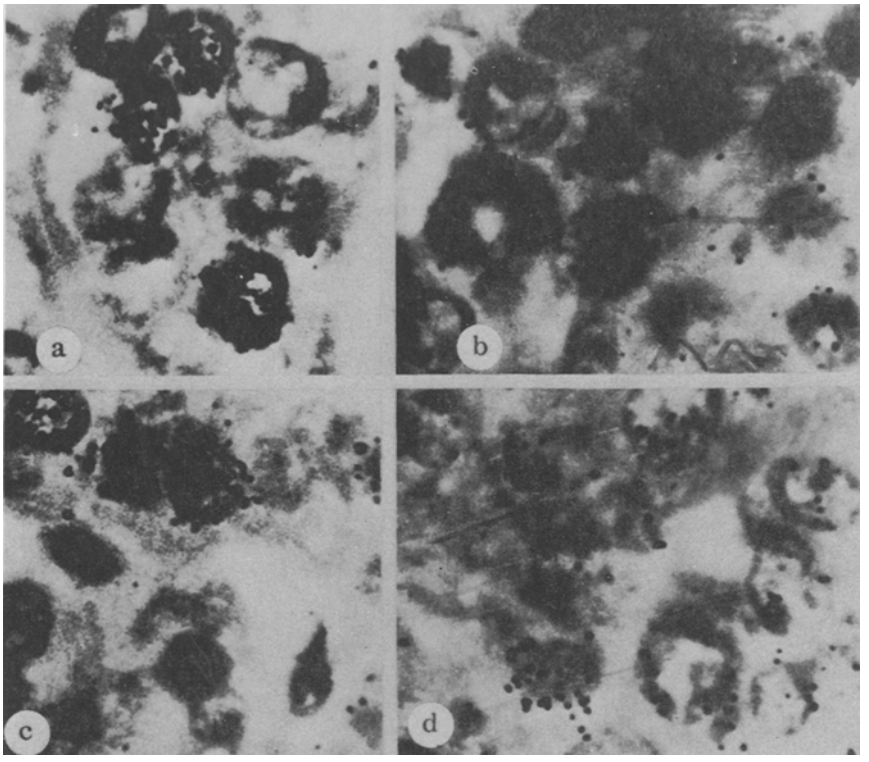
All the mice were killed on the third day after the operation and on the fourth day after the injection of thymidine-H^3.

The adrenals removed during the operation and those in the killed animals were fixed in Carnoy's fluid and imbedded in paraffin. Sections 5 μ thick were stained with hematoxylin-eosin and covered with liquid nuclear emulsion type M (Scientific Research Institute of the Motion Picture Film Industry) [2]. Part of the preparations was stained with methyl green-pyronine through the emulsion after development. The preparations were exposed for a month. In the analysis of the radioautograph, the following indices were determined: 1) the percent content of labeled cells, in this case from 5000 to 26,000 cells were counted in various zones of the adrenal cortex and adrenal cortex and adrenal medulla; 2) the intensity of the label (number of grains of silver over each labeled cell), on the basis of these indices, the average number of grains of silver per labeled cell and that per 1000 cells were calculated; 3) the percent content of labeled pairs of cells with respect to all the labeled cells. A labeled pair of cells was considered to be those possessing two labeled nuclei with a distance between them not exceeding the diameter of one of the nuclei (see figure, a and b).

Content of Labeled DNA in Adrenal Cells (Thymidine-H^3 Introduced One Day before Operation on Adrenals)

Animal No.	Zone of adrenals	No. of labeled cells (in %)				No. of labeled pairs of cells (in %)			Average No. of silver grains per labeled cell			No. of silver grains per 1000 cells		
		adrenal		multiplication factor (op/int)	P	adrenal		multiplication factor (op/int)	adrenal		multiplication factor (op/int)	adrenal		multiplication factor (op/int)
		intact	operated			intact	operated		intact	operated		intact	operated	
1	Glomerular	1,64	4,17	2,5	<0,0005	12,7	9,4	0,8	17,4	14,0	1,3	285,4	585,9	2,0
	Renal	0,13	0,73	5,6	<0,0005	6,7	8,2	1,2	17,7	15,3	1,2	22,3	111,6	5,0
	Medulla	0,49	0,55	1,1	>0,1	4,0	5,3	1,3	16,6	17,0	1,0	81,4	119,0	1,5
2	Glomerular	1,62	5,25	3,2	<0,0001	10,8	12,1	1,1	25,5	18,0	1,4	465,6	945,7	2,0
	Renal	0,12	1,20	10,0	<0,00005	8,3	10,9	1,3	26,8	16,4	1,6	31,2	209,2	6,7
	Medulla	0,89	1,45	1,6	<0,001	17,0	10,7	0,6	32,0	26,5	1,2	286,5	384,0	1,4
3	Glomerular	0,74	5,46	7,4	<0,00002	5,8	12,9	2,2	11,3	9,3	1,2	83,5	507,9	6,0
	Renal	0,10	1,15	11,5	<0,00005	0	5,0	—	11,7	8,6	1,4	11,2	99,0	9,0
	Medulla	0,28	1,30	5,0	<0,0002	7,0	10,3	1,5	19,5	10,1	1,9	54,0	131,3	2,6
4	Glomerular	0,76	6,25	8,2	<0,00001	3,0	14,0	4,7	23,0	8,6	2,7	173,8	533,7	3,0
	Renal	0,16	2,26	14,2	<0,00003	0	6,4	—	21,4	9,9	2,2	33,1	225,6	7,0
	Medulla	0,45	2,35	5,2	<0,0002	0	8,5	—	19,0	8,7	2,2	83,0	205,0	2,5

Notations: op – operated; int – intact.



Labeled cells in intact and operated adrenals. a) "Glomerulus" of cortical matter of adrenals. Labeled pair of cells in outer portion and cell with intense label deep layer of glomerular zone. Ob. 100 ×, oc. 12.5 ×; b) group of intensely thymidine-H^3 labeled cells in outer portions of glomerular zone of cortical substance in intact adrenal. Ob. 100 ×, oc. 11 ×; c) group of cells with weak label over nuclei in cortical layer of operated adrenal on third day after resection of part of it. Ob. 100 ×, oc. 11 ×; d) multiple label in connective tissue elements of sharply thickened subcapsular layer on third day after operation on adrenals. Ob. 100 ×, oc. 11 × and ob. 100 ×, oc. 8 ×.

The silver grains were counted at a microscope magnification of 1350 ×. The background of the emulsion comprised 0-1 grains per 100 μ^2. The nucleus was considered labeled when 3 or more grains of silver were situated above it.

EXPERIMENTAL RESULTS

The index of the label was higher in all zones of adrenal removed during the operation in mice that received three injections of thymidine-H^3, 30 μ each (numbers 1 and 2) than in the animals (3 and 4) that received single injections of thymidine-H^3 in a dose of 90 μ (see table). The number of silver grains per labeled cell in all layers of the organ was higher in mice numbers 1 and 2 than in mice numbers 3 and 4.

In the adrenals of animals killed on the third day after the operation, a substantial increase in the number of labeled cells was noted in comparison with the adrenals removed during the operation. This difference was especially pronounced in the renal zone. The number of labeled pairs of cells in this case was essentially unchanged. The intensity of the label dropped somewhat; however, this decrease was substantially less than the average number of labeled cells. The bulk of the labeled cells was detected in the glomerular zone and the outer layers of the renal zone of the adrenals. Labeled nuclei were encountered considerably less often in the deep layers of the renal zone and especially rarely at the boundary with the medullary substance. In the glomerular zone and outer layers of the renal zone, labeled cells were usually arranged in the form of nestlike accumulations. This peculiarity was also retained in the operated adrenal, only in the latter cells with a relatively weak label predominated (see figure, b and c).

The summary histograms of the labeled cells of the glomerular and renal zones in the operated and nonoperated adrenals represent single vertex curves with a peak in the region of cells containing 4 to 6 silver grains over the nucleus. However, in the operated adrenals, this peak is higher in the region of weakly labeled nuclei.

Noteworthy is the content of the label in the connective tissue elements of the capsule on the third day after the operation. Usually the adrenal capsule consists of two to three rows of elongated dark-colored nuclei, among which one to two labeled nuclei are detected on the entire section. The intensity of the label of these nuclei, as a rule, is equal to 18-20 grains of silver. In the operated adrenal, the capsule is substantially thickened, chiefly on account of connective tissue elements of the subcapsular layer. The latter forms a wedge, turned with its broad side toward the site of the operation trauma, and consists of cells with very pale cytoplasm; their nuclei are large, circular or oval, weakly colored; almost every one of them contains a label in 3 to 5 silver grains or more (see figure, d). At the base of the wedge-shaped expansion of the capsule, six to seven rows of the cells described are counted; they are most likely fibroblasts and similar cells.

At the level of the wedgelike expansion of the capsule, the glomerular zone of the cortex of the organ is displaced inward. The structure of the zone close to the operated section is disturbed. In the parenchyma of the organ, along the line of the cut there is an outgrowth of strands of connective tissue. Outside them lies a structureless mass of protein secretion, a fibrin clump, which is stained a pale rose color by eosin and methyl green-pyronine. On the inside of this clump grow elements of connective tissue, while on the outside it is adjoined by a mass of formless detritus, stained by nuclear dyes. In this mass of nuclear detritus, a diffuse label is detected, which is 20-25 times as great as the background activity.

As was indicated above, most researchers believe thymidine-H^3 is rapidly utilized for DNA synthesis under the conditions of the organism. Thus, all the processes in the operated adrenal took place against a background of the absence of free thymidine-H^3 in the blood plasma.

Under these conditions, we detected a substantial increase in the number of labeled cells in various layers of the adrenal. This fact cannot be explained by division of the labeled cells, since the increase in the number of labeled cells was paralleled by an increase in the intensity. There is some basis for believing that there is still some dilution of the label as a result of division of the cells; however, it cannot explain the increase in the number of labeled cells. The latter is due chiefly to secondary utilization of the isotope liberated in other tissues of the organism for DNA synthesis in the adrenal. It cannot be stated on the basis of the results of our experiments what tissues liberate the labeled DNA in this case. It is believed [5, 6] that the donors of the label for reutilization are the mononuclear blood cells. The degree of polymerization of the labeled products of DNA that participate in the process of reutilization is unknown. It may be assumed that the bulk of them are comprised of low-molecular compounds — nucleosides and nucleotides [6, 7]. However, in this case the possibility of reutilization of high-polymer compounds cannot be entirely excluded.

The operation may be considered as a strong stress factor. It is known that in the case of stress influences the processes of proliferation are most actively expressed in the renal zone [4, 19]. Our data indicating that the increase in the number of labeled cells after the operation on the adrenals is especially pronounced in the renal zone agree with this.

Of special interest are the changes in the balance of renal activity in the capsule of the operated adrenal. There is no doubt that the high content of labeled elements in the thickened capsule cannot be due to division of single labeled cells, encountered in the capsule of the intact organ. The labeled elements may also be "immigrants" with respect to the focus of inflammation.

In the zone of operative intervention, there is nuclear detritus with an especially high content of the label. This label is not washed out by histological treatment, which may be evidence of a high molecular state of the labeled material. Whether the adrenal can directly utilize this labeled material for the synthesis of DNA of the proliferating elements is unknown.

SUMMARY

Mice ($C_{57}Bl$ and CBA) weighing 28 g were given intraabdominal injections of thymidine-H^3 (90 mc) 24 h before an operation on the adrenals (excision of one gland and part of the other). On the third day after the operation a considerable increase in the index was noted, as well as of the total grain count in all the layers of the adrenal

operated upon and in the medullar layer. This increase was particularly notable in the fascicular zone of the cortex and in the connective-tissue elements of the subcapsular layer, which was markedly thickened after the operation. The accumulation of label in the organ operated upon took place in the absence of free H^3-thymidine in the blood plasma. This fact may point to the process of reutilization of DNA metabolic products occurring in the mouse body.

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