ACTION OF HYDROCORTISONE ON DNA SYNTHESIS IN EPITHELIAL CELLS OF THE BILIARY TRACT

P. A. Dyban

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Autoradiographic studies of the epithelium of the biliary tract of rats and guinea pigs using thymidine-H^3 showed that hydrocortisone lengthens the time of DNA synthesis. There is a parallel delay of the cells in the G_1-period. Analysis of the numerical results showed correlation between the number of cells synthesizing DNA and the intensity of incorporation of thymidine-H^3 into them.

KEY WORDS: hydrocortisone; labeled cells; biliary tract; mitotic cycle.

Corticosteroids reduce the number of mitoses and prolong the mitotic cycle as a whole as well as its individual phases [1, 2, 4-7, 13-16]. The unequal duration of the cell cycles is connected, according to Laguchev [7], with differences in the sensitivity of the tissues to hydrocortisone.

The object of this investigation was to study the action of hydrocortisone on the proliferative activity of the epithelium of the biliary tract.

This epithelium is particularly interesting when the antimitotic effect of the corticosteroids is examined for its mitotic activity is at a low level, in sharp contrast to the rapidly proliferating intestinal epithelium, with which it shares the same origin [3].

EXPERIMENTAL

Investigations were carried out on male albino rats weighing 270-300 g and male guinea pigs weighing 230-350 g. Hydrocortisone (Richter) was injected intraperitoneally into the rats in a dose of 10 mg/100 g and into the guinea pigs in doses of 5 and 10 mg/100 g. The animals were killed 4 and 8 h after injection of the hormone. Thymidine-H^3 was injected into the albino rats 1 h before sacrifice in a dose of 0.7 μCi/g (specific activity 4.1 Ci/mmole). The gall bladder of the guinea pigs and the common bile duct of the rats were fixed with Bouin's fluid. Paraffin sections were stained by the usual histological methods and sections through the common bile duct of the rats receiving thymidine-H^3 were coated with type M emulsion. The exposure lasted 35 days. Nuclei on the autoradiographs were taken as labeled if no fewer than five grains of silver were present above them. The mitotic coefficient (MC) in the epithelium of the biliary tract was calculated per thousand cells, the distribution of mitoses in the different parts of the mucous membrane was determined, and in the epithelium of the common bile duct in addition the percentage of labeled mitoses, the number of grains per mitosis, the number of labeled cells, the radioactive index (RI) per thousand cells, and the number of grains of reduced silver per nucleus were investigated.

RESULTS

A significant decrease was found both in RI and in the number of grains per mitosis was found in the epithelium of the common bile duct of the rats 4 h after injection of hydrocortisone, while the number of mitoses remained unchanged (Table 1). The number of mitoses 8 h after injection of hydrocortisone was significantly lower than in the control, while RI and the number of grains of silver continued to decrease

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TABLE 1. Action of Hydrocortisone on Proliferative Activity of the Epithelium of the Biliary Tract (M ± m)

Object studied	Number of animals	Time of action of hydrocortisone (in h)	Dose (mg/100 g body wt.)	RI (in ‰)	Number of grains per nucleus	MC (‰)	Number labeled mitoses (in %)	Number of grains per mitosis
Epithelium of common bile duct of rats	6	Control	—	15,3±1,5	16,7±1,1	2,6±0,4	11,3±3,5	11,5±1,3
	4	4	10	9,7±1,6	12,4±0,8	2,9±0,7	10,9±3,4	13,4±1,5
		P	—	<0,05	<0,05	>0,1	>0,1	>0,1
	4	8	10	3,9±1,0	9,3±1,3	0,8±0,3	25,6±4,5	19,5±2,7
		P	—	<0,01	<0,01	<0,02	<0,05	<0,005
Gall bladder epithelium of guinea pigs	12	Control	—	—	—	1,8±0,3	—	—
	4	4	5	—	—	1,9±0,8	—	—
		P	—	—	—	>0,1	—	—
	4	4	10	—	—	1,9±0,6	—	—
		P	—	—	—	>0,1	—	—
	5	8	5	—	—	0,8±0,2	—	—
		P	—	—	—	<0,01	—	—

Note: P calculated relative to the control.

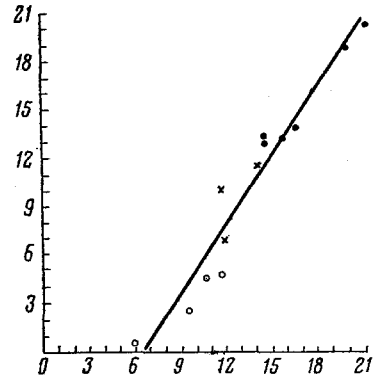

Fig. 1. Correlation between number of cells synthesizing DNA and intensity of incorporation of thymidine-H^3 into them. Filled circles – control; crosses – action of hydrocortisone for 4 h; empty circles – for 8 h. Abscissa, number of grains per nucleus; ordinate, RI (in ‰).

progressively. Hydrocortisone also had a similar action on the gall bladder epithelium of the guinea pig, an animal that produces endogenous hydrocortisone [10, 12]. In doses of 5 and 10 mg, hydrocortisone did not change the number of mitoses in the gall bladder epithelium after 4 h, and it began to exhibit its effect only after 8 h (Table 1).

A previous investigation [3] showed that epithelial cells in the gall bladder migrate from the base of the folds where the great majority of proliferating cells are situated toward the apex. The rate of migration of the cells was not observed to be slower in the experimental animals. This was probably because of the long period (normally 32 days) of migration of the epithelial layer compared with the short duration (8 h) of the experiment.

The number of labeled mitoses in the epithelium of the common bile duct of the rats and the number of grains of reduced silver above the karyokinetic figures were significantly increased 8 h after the injection of hydrocortisone (1 h after administration of the isotope) (Table 1). This could be due to lengthening of the S-period and shortening of the G_2-period. The second explanation is unlikely because of the observed [1, 6, 7, 16] lengthening of the G_2-period under the influence of hydrocortisone. The decrease in the number of grains of silver above the interkinetic nuclei, accompanied by an increase in the number of labeled mitoses and in the number of grains above the mitotic figures, confirms the data of other workers [1, 2, 4-7, 15, 16] who observed an increase in the duration of DNA synthesis; according to the present experiments this was probably connected with an increase in the number of late-replicating regions of the chromosomes.

The intensity of incorporation of thymidine-H^3 into the cell nuclei cannot always be taken as a sufficiently strict indicator of the stimulation or inhibition of DNA synthesis [8, 18]. Additional biochemical investigations were therefore carried out with the use of C^{14}-labeled sodium formate as the DNA precursor.* The number of pulses in DNA isolated from the organs of the intact and experimental rats was counted by means of a "Protok" gas-flow counter. Preliminary biochemical findings showed that hydrocortisone reduces incorporation of the isotope into DNA. The decrease in the number of cells incorporating thymidine-H^3 discovered autoradiographically and the decrease in the intensity of this label above the nuclei thus reflect

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not only the inhibitory action of hydrocortisone on thymidine- and thymidylate-kinase [9], i.e., on the reverse pathway of thymidylate synthesis, but also on other stages of DNA synthesis [9, 17].

Cells in which DNA synthesis is initiated, i.e., cells at the end of the G_1-period, are the decisive point of application of hydrocortisone [16]. Analysis of the results of the present experiments confirms this conclusion and shows that the decrease in RI took place chiefly because of delay in the departure of the cells from the G_1-period. However, it must not be forgotten that hydrocortisone, by prolonging the S-period, thereby reduces the intensity of DNA synthesis, i.e., reduces the quantity of label above the nuclei. If under these circumstances the number of grains is lower than a certain limit (in this case, less than five grains), this fraction of the cells cannot be regarded as labeled. A correction must therefore be introduced into the conclusions on the number of cells delayed in the G_1-period based on the determination of RI.

The correlation between the number of labeled cells (Y) and the number of grains above them (X) in both the control and the experimental animals will be noted; under the conditions used to prepare the autoradiographs and with the criterion used to assess labeling (five grains above the nucleus) this correlation has the following form: $y = -9.26 \pm 1.45x$ (Fig. 1).

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