# ACTION OF HYDROCORTISONE HEMISUCCINATE ON THE INCORPORATION OF $^3$H-THYMIDINE INTO DEOXYRIBONUCLEIC ACID OF SOME A2G MICE ORGANS

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The process of DNA biosynthesis and retention of $^3$H-thymidine-labelled DNA in some A2G mice organs has been studied after administration of hydrocortisone hemisuccinate (HCHS). The results indicated the stimulation of biosynthesis and retention of DNA in thymus after administration of HCHS, in contrast with the spleen, where inhibitory effects were observed. HCHS did not influence these processes at the level of liver and small intestine.

The effect of stress-agents is mainly exerted by ACTH stimulation of glucocorticoid hormones secretion [15], [16], which induces metabolic changes at the level of some organs, especially in liver and lymphatic organs [2], [6], [11]. By their interaction with intracellular receptors, especially nuclear receptors, these hormones regulate nucleic acid and protein metabolism [3] [4] [14].

The reason of hormones penetration into cell nuclei from extracelullar space may be to modify the activity of some genes, which influences cell metabolism and proliferation [7] [9].

In the present work the action of hydrocortisone hemisuccinate (HCHS) on the incorporation of $^3$H-thymidine into DNA of thymus, spleen, liver and small intestine and the retention of labelled DNA were studied.

## MATERIAL AND METHODS

Two hundred A2G mice weighing 25 — 35 g were used. The animals were kept under standard conditions and 18 hours before killing they received no food. The animals were injected i.p. with 40μCi methyl-$^3$H-thymidine (per 100 g of body weight) dissolved in 0.9 per cent NaCl solution. The specific activity of radioactive labelled substance was 5 Ci per mmol (purchased from R. C. Amersham). Fifty mice received i.m. injections containing 1 mg and another 50 animals — 5 mg HCHS dissolved in a mixture of methylglycine and natrium-hydrocarbonate solution (purchased from Uzina de Medicamente București).

The determination of DNA was made using a technique described previously [1]. The specific radioactivity of labelled DNA was determined after the neutralization of DNA extracts by 1 N KOH. The samples were mixed with 0.5 ml hyamine hydroxide (Nuclear Enterprise, Chicago) and 10 ml of Bray's solution. The radioactivity of samples was measured at 10°C by means of a Betaszint BF-5003 scintillation spectrometer.

## RESULTS

$^3$H-thymidine is an excellent precursor of DNA biosynthesis, which incorporates only into *de novo* synthesized DNA of living cells. As reference

value the specific radioactivity of DNA was used, obtained one hour after injection of $^3$H-thymidine.

Our results indicated that the administration of 1 mg or 5 mg HCHS significantly increased the specific radioactivity of thymus DNA after 24 and 48 hours (Table 1), but 5 mg HCHS after 72 hours deter-

*Table I*

**Incorporation of $^3$H-thymidine into thymus DNA and retention of labelled DNA after administration of HCHS. (The results represent the mean values of specific radioactivity of DNA in DPM per mg DNA).**

| Time (h) | 1 | 3 | 10 | 24 | 48 | 72 |
|---|---|---|---|---|---|---|
| **Control groups** | | | | | | |
| X | 2 118 | 1 931 | 1 916 | 1 837 | 1 265 | 1 020 |
| S.E. | ±89 | ±145 | ±127 | ±157 | ±120 | ±146 |
| n | 8 | 6 | 6 | 6 | 6 | 6 |
| $PC_1$ | 100 | 91.2 | 90.5 | 86.7 | 59.7 | 48.2 |
| $P_1$ | — | >0.05 | >0.05 | >0.05 | <0.001 | <0.001 |
| **1 mg HCHS** | | | | | | |
| X | | 1 953 | 2 068 | 2 793 | 2 649 | 1 852 |
| S.E. | | ±126 | ±91 | ±159 | ±235 | ±174 |
| n | | 5 | 6 | 5 | 5 | 5 |
| $PC_1$ | | 92.2 | 97.6 | 131.9 | 125.1 | 87.4 |
| $P_1$ | | >0.05 | >0.05 | <0.01 | <0.05 | >0.05 |
| $PC_2$ | | 101.1 | 107.9 | 132.2 | 209.4 | 181.6 |
| $P_2$ | | >0.05 | >0.05 | <0.001 | <0.001 | <0.01 |
| **5 mg HCHS** | | | | | | |
| X | | 2 414 | 2 388 | 2 939 | 2 651 | 1 105 |
| S.E. | | ±229 | ±152 | ±235 | ±193 | ±138 |
| n | | 5 | 5 | 5 | 5 | 5 |
| $PC_1$ | | 114.0 | 112.8 | 138.8 | 125.2 | 52.2 |
| $P_1$ | | >0.05 | >0.05 | <0.01 | <0.05 | <0.001 |
| $PC_2$ | | 125.0 | 124.6 | 160.2 | 209.6 | 108.3 |
| $P_2$ | | >0.05 | >0.05 | <0.01 | <0.01 | >0.05 |

X = mean value, S.E. = standard error, n = number of animals.

$PC_1$ = per cent difference between the mean value of control group sacrificed after 1 h and the mean values of other control groups or treated groups.

$PC_2$ = per cent difference between the mean values of control groups sacrificed at different times and treated groups sacrificed at the same time.

$P_1$ and $P_2$ = significance of differences.

mined significant decrease of DNA retention. Comparing the specific radioactivity of DNAs extracted from thymi of animals killed after different periods against controls killed after the same periods, an increase of the specific radioactivity of DNA (especially after 24 and 48 hours) was observed.

In contrast with the thymus, in the case of spleen the incorporation of $^3$H-thymidine into DNA and the retention of labelled DNA decreased already after 10 hours (Table 2). Our experiments concerning the liver and small intestine showed that the administration of 1 mg or 5 mg HCHS did not influence the biosynthesis of DNA or the retention of labelled DNA.

*Table 2*

**Incorporation of $^3$H-thymidine into spleen DNA and retention of labelled DNA after administration of HCHS. (The results represent the mean values of specific radioactivity of DNA in DPM per mg DNA)**

| Time (h) | 1 | 3 | 10 | 24 | 48 | 72 |
|---|---|---|---|---|---|---|
| | | | Control groups | | | |
| X | 2 327 | 1 970 | 2 297 | 2 137 | 1 443 | 1 223 |
| S.E. | ±158 | ±147 | ±259 | ±175 | ±215 | ±141 |
| n | 7 | 5 | 5 | 5 | 5 | 5 |
| $PC_1$ | 100 | 84.7 | 98.7 | 94.8 | 62.1 | 52.6 |
| $P_1$ | | >0.05 | >0.05 | >0.05 | <0.01 | <0.001 |
| | | | 1 mg HCHS | | | |
| X | | 1 972 | 1 803 | 1 542 | 1 676 | 1 358 |
| S.E. | | ±147 | ±129 | ±130 | ±133 | ±41 |
| n | | 5 | 5 | 5 | 5 | 6 |
| $PC_1$ | | 84.7 | 77.5 | 66.3 | 72.0 | 58.4 |
| $P_1$ | | >0.05 | <0.05 | <0.02 | <0.02 | <0,001 |
| $PC_2$ | | 100.1 | 78.5 | 72.2 | 116.0 | 111.0 |
| $P_2$ | | >0.05 | >0.05 | <0.02 | >0.05 | >0.05 |
| | | | 5 mg HCHS | | | |
| X | | 2 024 | 1 967 | 1 828 | 1 193 | 1 206 |
| S.E. | | ±200 | ±170 | ±194 | ±130 | ±235 |
| n | | 5 | 5 | 5 | 5 | 5 |
| $PC_1$ | | 87.0 | 84.3 | 78.6 | 51.3 | 51.8 |
| $P_1$ | | >0.05 | >0.05 | <0.05 | <0.001 | <0.001 |
| $PC_2$ | | 102.7 | 83.6 | 85.5 | 82.6 | 98.6 |
| $P_2$ | | >0.05 | >0.05 | >0.05 | >0.05 | >0.05 |

The explanation is the same as for table 1.

## DISCUSSION

There are different opinions concerning the action of glucocorticoid hormones on the biosynthesis and degradation of DNA in lymphatic organs. Some authors consider that these hormones are antimitotic, inhibiting DNA biosynthesis in the thymus and lymph nodes [12] [17]. Lang and co-workers [10] suppose that hydrocortisone acts at the level of DNA biosynthesis only in the case of lymphocytes which show pycnotic or cytoplasmolytic aspects. Other data show that glucocorticoid hormones influence cell division in prophase or in the final part of interphase, increasing the activity of adenylate cyclase and the concentration of cyclic AMP [19].

Autoradiographic experiments showed that only medium and large thymic lymphocytes incorporate $^3$H-thymidine into their DNA, while small lymphocytes have no or little capacity to use this precursor in DNA biosynthesis [5] [8] [13].

Our results indicate an increase of $^3$H-thymidine incorporation into thymic DNA 24 hours after administration of HCHS, which suggests either an increased DNA synthesis at the level of medium and large lymphocytes, or an increase of the number of labelled cells in the thymus

from extrathymic tissues. In contrast with the results obtained in the case of thymus the action of HCHS at the level of spleen manifests itself by inhibition of DNA biosynthesis and retention of labelled DNA. These observations are in agreement with the well-known lymphocytolytic effect of glucocorticoids [5] [18].

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