# III. DECREASE OF LABELED DNA IN CELLS OF THE ADRENAL MEDULLA AFTER INTERMITTENT EXPOSURE TO COLD

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## ABSTRACT

Italico rats were injected with thymidine-$^3$H 6 hr after the end of 300 hr of intermittent cold treatment. This plan of experiment ensured replacement in the adrenal medulla of lost DNA which is specifically sensitive to cold treatment and has a labeling index sufficiently high for statistical evaluation. The labeling index in the adrenal medulla decreases to one-half of the initial value within 10 days in animals subjected to further intermittent cold treatment and within 32 days in animals kept at room temperature. The very low mitotic index and the absence of doubling of the labeling index show that the observed labeling cannot be ascribed to pre-mitotic DNA synthesis. The concept of metabolic DNA adequately explains the findings.

## INTRODUCTION

It has been shown that labeled precursors are incorporated into DNA during pre-mitotic synthesis (1–3). During mitosis labeled DNA is shared out between daughter cells; therefore, the number of grains above their nuclei is half that of the parent nuclei and the number of labeled nuclei doubles. The total amount of thymidine-$^3$H incorporated in a tissue should remain constant with time; any loss of labeled DNA with time could only be justified by loss of some labeled cells.

However, a number of papers have reported more incorporation of thymidine-$^3$H than required for mitosis: for example, in the seminal vesicle of the mouse (4–6), in mouse prostate grown in organ culture (7), and in differentiating cells in bean roots (8), in nervous cells and heart muscle of adult animals (9). Criticism of these experiments (10, 11) has been discussed (9).

Recently, incorporation of thymidine-$^3$H has been found in cells of the adrenal medulla of rats which were intermittently exposed to cold for a period of 300 hr and subsequently brought back to room temperature. Previous studies had shown (12) that after 300 hr of cold treatment the adrenal medulla nuclei lose 40–45% of their DNA. This loss was compensated for a few days after returning the animals to room temperature. Concurrently, incorporation of thymidine-$^3$H started immediately after the end of the cold exposure and continued for 3 days (13). Since the adrenal medullary cells are considered either as nondividing cells (14) or as having a very low

TABLE I

*Plan of the Experiment*

33 Albino Italico Rats were exposed intermittently to cold for a total of 300 hr (20 days). All animals were injected with thymidine-$^3$H 6 hr after transfer to room temperature and were left at room temperature for 3 hr. Three animals were sacrificed at that time. The rest were divided into 2 groups and subsequently treated as set out in this table.

| No. of animals | Time after injection before sacrificing | Exposure | |
|---|---|---|---|
| | | Cold temp. | Room temp. |
| 3 | 3 hr | − | + |
| 3 | 1 day | + (15 h) | − |
| 3 | " " | − | + |
| 3 | 3 days | + (50 h) | − |
| 3 | " " | − | + |
| 3 | 7 " | + (100 h) | − |
| 3 | " " | − | + |
| 3 | 13 " | + (200 h) | − |
| 3 | " " | − | + |
| 3 | 20 " | + (300 h) | − |
| 3 | " " | − | + |

mitotic activity (10, 15, 16), these observations cannot be accounted for by pre-mitotic synthesis, and have been tentatively explained in terms of a metabolic turnover of DNA (12, 13), produced, or in some way accelerated, by cold treatment.

In order to test this hypothesis, the behaviour of the label incorporated in nuclei of cells of the adrenal medulla of rats has been studied in detail. The changes in the amount of thymidine-$^3$H incorporated into DNA in these nuclei during the recovery period following 300 hr of exposure to low temperature (4°C) have been determined for animals kept at room temperature and for rats reexposed intermittently to low temperature for different periods. It will be shown that after 20 days from the injection of thymidine-$^3$H, there is a loss of labeled DNA which is much larger in animals exposed to cold than in those kept at room temperature.

## MATERIAL AND METHODS

Albino rats of the Italico strain (33 animals aged 90–120 days, weight 150–200 g), fed with Randoin and Causeret diet, were used. The animals were kept in a cold-room (+2° to +4°C) for 15 hr a day and in the animal-room (+18° to +20°C) for the remaining 9 hr of the day, over a total period of 20 days (300 hr of total exposure to cold). At the end of this period the animals were kept at normal room temperature for 6 hr and injected intraperitoneally with thymidine-$^3$H (Radiochemical Centre, Amersham, England) at the dose of 1 $\mu$c/g body weight.

The 6 hr delay before application of thymidine-$^3$H was selected because after 6 hr from return to room temperature incorporation of label is at a higher rate than immediately after exposure to cold.[1] Three animals were sacrificed after 3 hr; the others were divided into two groups: one group was again exposed intermittently to cold, the other was kept at room temperature. The animals of both groups were sacrificed at different times after the injection of thymidine-$^3$H, as summarized in Table I.

From each animal both adrenal glands were dissected out, fixed in a mixture of ethanol, chloroform, and acetic acid (6:3:1, v/v) for 3 hr, and embedded in paraffin wax. Radioautographs of 5 $\mu$ sections were prepared by the stripping film technique (A.R. 10 Kodak) and exposed for 14, 28 or 150 days in a light-proof box at −16°C. The slides were developed for 4 min with Kodak D19 (Eastman Kodak Co., Rochester, N.Y.), rinsed in water, and fixed in Kodak Rapid Fixer for 12 min. The sections were subsequently stained with Ehrlich's haematoxylin and eosin.

The labeling index (percentage of labeled nuclei) was calculated on 4000 cells and the number of grains over each labeled nucleus was counted on each slide.

In two experiments the percentage of labeled nuclei of the adrenal cortex after 30 days of film exposure has been determined. Sections treated with DNase do not show any radioautograph.

## RESULTS

### *Animals Killed 3 hr After the Injection of Thymidine-$^3$H (Tables II, III, IV)*

In agreement with the results of Viola-Magni (13), an incorporation of thymidine-$^3$H has been found in the nuclei of adrenal medullary cells of rats intermittently exposed to cold. Five per cent of the nuclei appear labeled after 14 days of film exposure. This percentage is higher than that reported by Viola-Magni (13) after 10 days of exposure by use of the dipping technique. It has to be noted, however, that in our experiment thymidine-$^3$H was injected after 6 hr of recovery from the 300 hr of cumulative exposure to cold,

[1] G. Malvaldi and M. P. Viola-Magni. In preparation.

whereas in the experiments of Viola-Magni (13) the label was injected immediately after the end of the last 15 hr of exposure to cold. It has been shown by appropriate experiments that the number of nuclei in which synthesis of DNA takes place at the beginning of the recovery period is lower than that at 6 hr later.[1] Moreover, the exposure period used in the present experiments is slightly longer than that used by Viola-Magni (13). These two facts seem adequate to account for the discrepancy between our results and those of previous studies, although other factors, related to the different technical procedures, may play a role.

After 30 days of film exposure the percentage of labeled nuclei is 7.3%. In a previous study (13) performed with the same technique and exposure period (30 days), a labeling index of 28.86% was found after 4 injections of thymidine-$^3$H administered at intervals of 2 hr during the first 8 hr after the end of the cold exposure. The average labeling index appears to be in good agreement with our results; it should be pointed out, how-

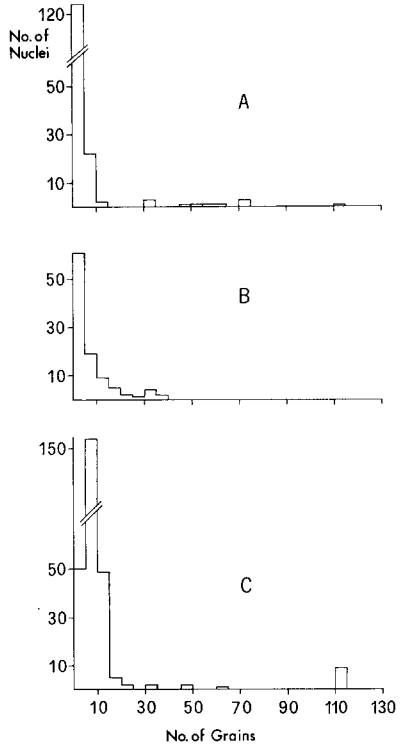


FIGURE 1 Histograms of the distribution of the label in the nuclei of adrenal medulla cells of rats exposed intermittently to cold for 300 hr, injected with thymidine-$^3$H after 6 hr at room temperature, and sacrificed: *A*) 3 hr after the injection of thymidine-$^3$H; *B*) after 300 hr (20 days) of intermittent exposure to cold following injection of thymidine-$^3$H; *C*) after 20 days at room temperature following injection of thymidine-$^3$H. Exposure time of the slides, 150 days.

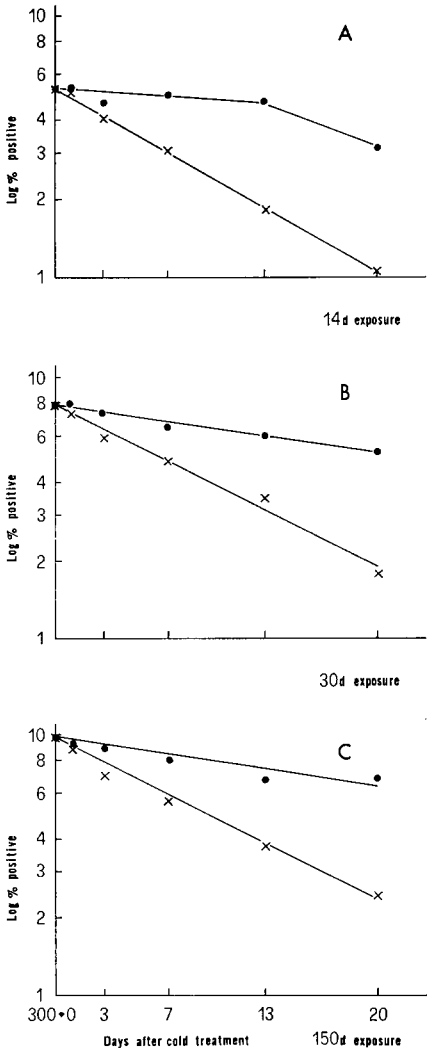


FIGURE 2 Plots of log labeling index versus time after injection. *X*, animals reexposed intermittently to cold; ●, animals left at room temperature; *A*, 14 days of film exposure; *B*, 30 days of film exposure; *C*, 150 days of film exposure.

TABLE II

*Changes in the Percentage of the Labeled Nuclei in the Adrenal Medulla Cells of Rats Exposed Intermittently to Cold for 300 hr and Injected with Thymidine-$^3H$ After 6 hr at Room Temperature*

Some of the rats were subsequently re-exposed to cold; others were kept at room temperature as controls.

| | | | 14 Days of film exposure | | | | |
|---|---|---|---|---|---|---|---|
| Period of cold exposure | No. of nuclei counted | No. of labeled nuclei | Per centage | Period of room temperature exposure | No. of nuclei counted | No. of labeled nuclei | Per centage |
| 0 | 4000 | 210 | 5.2 | | | | |
| 0 | 4000 | 206 | 5.1 | | | | |
| 0 | 4000 | 187 | 4.7 | | | | |
| | | | 5.0 | | | | |
| 15 hr (1 day) | 4000 | 212 | 5.3 | 1 day | 2000 | 103 | 5.1 |
| 15 hr (1 day) | 4000 | 204 | 5.1 | 1 " | 4000 | 205 | 5.1 |
| 15 hr (1 day) | 3500 | 194 | 4.8 | 1 " | 4000 | 204 | 5.1 |
| | | | 5.1 | | | | 5.1 |
| 50 hr (3 days) | 4000 | 151 | 3.8 | 3 days | 4000 | 169 | 4.2 |
| 50 hr (3 days) | 4000 | 186 | 4.6 | 3 " | 4000 | 205 | 5.1 |
| 50 hr (3 days) | 4000 | 157 | 3.9 | 3 " | 4000 | 185 | 4.6 |
| | | | 4.1 | | | | 4.6 |
| 100 hr (7 days) | 4000 | 99 | 2.5 | 7 " | 4300 | 174 | 4.1 |
| 100 hr (7 days) | 4000 | 142 | 3.5 | 7 " | 4000 | 231 | 5.8 |
| 100 hr (7 days) | 4000 | 129 | 3.2 | 7 " | 4000 | 210 | 5.2 |
| | | | 3.1 | | | | 5.0 |
| 200 hr (13 days) | 4000 | 72 | 1.8 | 13 " | 4000 | 199 | 5.0 |
| 200 hr (13 days) | 4000 | 72 | 1.8 | 13 " | 4000 | 193 | 4.8 |
| 200 hr (13 days) | 4000 | 69 | 1.7 | 13 " | 4000 | 179 | 4.5 |
| | | | 1.8 | | | | 4.8 |
| 300 hr (20 days) | 4000 | 39 | 1.0 | 20 " | 4000 | 130 | 3.2 |
| 300 hr (20 days) | 4000 | 39 | 1.0 | 20 " | 4000 | 123 | 3.1 |
| 300 hr (20 days) | 4000 | 46 | 1.1 | 20 " | 4000 | 122 | 3.0 |
| | | | 1.0 | | | | 3.1 |

ever, that the value reported by Viola-Magni (13) represents the sum of different contributions to the total labeling index, the relative magnitudes of which have not been worked out in sufficient detail.

The labeling index rises to 9.8% after 150 days of film exposure. Most of the labeled nuclei show 2 – 10 grains, and only a few nuclei are heavily labeled (100 grains per nucleus, Fig. 1 *A*). These findings are in agreement with previous results, which showed that the nuclei appear weakly labeled also after multiple injections (13).

### *Animals Kept Intermittently in the Cold-Room After the Injection of Thymidine-$^3H$*

The labeling indexes obtained after different exposure times (14, 30, 150 days) decrease progressively with the time of intermittent cold-treatment after the injection of thymidine-$^3$H (Fig. 2; Tables II–IV). After 20 days (300 hr of cold exposure) the labeling index is reduced to 20–24% of that found in animals sacrificed 3 hr after the injection of thymidine-$^3$H. The average number of grains per nucleus remains approxi-

TABLE III

*Changes in the Percentage of Labeled Nuclei in the Adrenal Medulla Cells of Rats Exposed Intermittently to Cold for 300 hr and Injected with Thymidine-$^3H$ After 6 hr at Room Temperature*

Some of the rats were subsequently re-exposed to cold; others were kept at room temperature as controls.

| | | | 30 Days of film exposure | | | | |
|---|---|---|---|---|---|---|---|
| Period of cold exposure | No. of nuclei counted | No. of labeled nuclei | Percentage | Period of room temperature exposure | No. of nuclei counted | No. of labeled nuclei | Percentage |
| 0 | 4000 | 303 | 7.6 | | | | |
| 0 | 4000 | 324 | 8.1 | | | | |
| 0 | 4000 | 309 | 7.7 | | | | |
| | | | 7.8 | | | | |
| 15 hr (1 day) | 4000 | 332 | 8.3 | 1 day | 5000 | 351 | 7.0 |
| 15 hr (1 day) | 4000 | 298 | 7.4 | 1 " | 5000 | 376 | 7.5 |
| 15 hr (1 day) | 4000 | 313 | 7.8 | | | | 7.3 |
| | | | 7.8 | | | | |
| 50 hr (3 days) | 4000 | 225 | 5.6 | 3 days | 4000 | 272 | 6.8 |
| 50 hr (3 days) | 4000 | 252 | 6.3 | 3 " | 4000 | 288 | 7.2 |
| 50 hr (3 days) | 4000 | 226 | 5.6 | 3 " | 4000 | 305 | 7.5 |
| | | | 5.8 | | | | 7.2 |
| 100 hr (7 days) | 4000 | 216 | 5.4 | 7 " | 4000 | 256 | 6.4 |
| 100 hr (7 days) | 4000 | 187 | 4.7 | 7 " | 4000 | 240 | 6.0 |
| 100 hr (7 days) | 4000 | 174 | 4.3 | 7 " | 4000 | 273 | 6.8 |
| | | | 4.8 | | | | 6.4 |
| 200 hr (13 days) | 4000 | 133 | 3.3 | 13 " | 4000 | 248 | 6.2 |
| 200 hr (13 days) | 4000 | 143 | 3.6 | 13 " | 4000 | 241 | 6.0 |
| 200 hr (13 days) | 4000 | 140 | 3.5 | 13 " | 4000 | 213 | 5.3 |
| | | | 3.5 | | | | 5.8 |
| 300 hr (20 days) | 4000 | 70 | 1.7 | 20 " | 4000 | 188 | 4.7 |
| 300 hr (20 days) | 4000 | 72 | 1.8 | 20 " | 4000 | 220 | 5.5 |
| 300 hr (20 days) | 4000 | 70 | 1.7 | 20 " | 4000 | 202 | 5.0 |
| | | | 1.7 | | | | 5.1 |

mately constant (Table VI). The plots of log labeling index versus time after injection show a straight-line relationship, indicating exponential decline (Fig. 2). The biological half-life, i.e. the time during which the labeling index declines to one half, varies from 9.2 to 10 days for the various exposure times of the radioautographs (Table V).

The labeled nuclei appear frequently in groups of two, three and sometimes five. However, no increase in the occurrence of groups of two labeled nuclei has been found in animals sacrificed at different periods after the injection.

For comparison, the behavior of labeled nuclei was also studied in the adrenal cortex of two animals sacrificed, respectively, 3 hr and 7 days after the injection of thymidine-$^3H$ and exposed to cold (Table VI). The percentage of labeled nuclei increases three-fold after 7 days from the in-

TABLE IV

*Changes in the Percentage of Labeled Nuclei in the Adrenal Medulla Cells of Rats Exposed Intermittently to Cold for 300 hr and Injected with Thymidine-$^3H$ After 6 hr at Room Temperature*

Some of the rats were subsequently re-exposed to cold; others were kept at room temperature as controls.

| | | | 150 Days of film exposure | | | | |
|---|---|---|---|---|---|---|---|
| Period of cold exposure | No. of nuclei counted | No. of labeled nuclei | Percentage | Period of room temperature exposure | No. of nuclei counted | No. of labeled nuclei | Percentage |
| 0 | 4000 | 420 | 10.5 | | | | |
| 0 | 4000 | 371 | 9.3 | | | | |
| 0 | 4000 | 387 | 9.7 | | | | |
| | | | 9.8 | | | | |
| 15 hr (1 day) | 4000 | 379 | 9.5 | 1 day | 2200 | 213 | 9.7 |
| 15 hr (1 day) | 3700 | 314 | 8.5 | 1 " | 2100 | 182 | 8.7 |
| 15 hr (1 day) | 4000 | 338 | 8.4 | 1 " | 4000 | 369 | 9.2 |
| | | | 8.8 | | | | 9.2 |
| 50 hr (3 days) | 4000 | 283 | 7.1 | 3 days | 4000 | 341 | 8.5 |
| 50 hr (3 days) | 4000 | 297 | 7.4 | 3 " | 4000 | 352 | 8.8 |
| 50 hr (3 days) | 4000 | 255 | 6.4 | 3 " | 4000 | 373 | 9.3 |
| | | | 7.0 | | | | 8.9 |
| 100 hr (7 days) | 4000 | 181 | 4.5 | 7 " | 4000 | 326 | 8.1 |
| 100 hr (7 days) | 4000 | 241 | 6.2 | 7 " | 4000 | 328 | 8.2 |
| 100 hr (7 days) | 4000 | 252 | 6.3 | 7 " | 4000 | 316 | 7.9 |
| | | | 5.7 | | | | 8.1 |
| 200 hr (13 days) | 4000 | 164 | 4.1 | 13 " | 4000 | 240 | 6.0 |
| 200 hr (13 days) | 4000 | 164 | 4.1 | 13 " | 4000 | 283 | 7.1 |
| 200 hr (13 days) | 4000 | 121 | 3.0 | 13 " | 4000 | 286 | 7.1 |
| | | | 3.7 | | | | 6.7 |
| 300 hr (20 days) | 4000 | 99 | 2.5 | 20 " | 3800 | 274 | 7.2 |
| 300 hr (20 days) | 4000 | 93 | 2.3 | 20 " | 4000 | 276 | 6.9 |
| 300 hr (20 days) | 4000 | 91 | 2.3 | 20 " | 4000 | 265 | 6.6 |
| | | | 2.4 | | | | 6.9 |

TABLE V

*Biological Half-Life (in days) of Labeled Thymidine Incorporated into DNA of the Nuclei of Adrenal Medulla of Italico Rats*

The animals were injected with thymidine-$^3H$ after 300 hr of intermittent exposure to cold.

| Days of film exposure | Rats exposed to cold after the injection | Rats left at room temperature after the injection |
|---|---|---|
| 14 | 9.2 | — |
| 30 | 10.0 | 32.6 |
| 150 | 10.0 | 32.7 |

jection, whereas the average number of grains is reduced to about one-fourth as would be expected in a population of dividing cells.

## *Animals Kept at Room Temperature After the Injection of Thymidine-$^3H$*

A group of rats was kept continuously at room temperature after the injection of thymidine-$^3H$. The animals belonging to this group were sacrificed at the same intervals as those exposed intermittently to cold (see above).

In this experiment the number of labeled nuclei

TABLE VI

*Amount and Distribution of Label in the Adrenal Medullary Cells and in Adrenal Cortical Cells in Animals Sacrificed at Different Intervals from the Injection of Thymidine-$^3H$ and Exposed to Cold*

30 days of film exposure.

| Interval from the injection of thymidine -$^3H$ | Adrenal medulla | | | | Adrenal cortex | | | |
|---|---|---|---|---|---|---|---|---|
| | No. of nuclei counted | No. labeled nuclei | Per centage | Average number of grains/nucleus | No. of nuclei counted | No. of labeled nuclei | Per centage | Average number of grains/nucleus |
| 7 hr | 4000 | 303 | 7.57 | 6.23 | 8500 | 66 | 0.78 | 34.83 |
| 7 days | 4000 | 216 | 5.40 | 6.82 | 8500 | 203 | 2.15 | 9.24 |

shows a slight decrease with time (Tables II–IV; Fig. 2). After 20 days the labeling index is 70% of that found in the animals sacrificed 3 hr after injection. For the 30 day and 150 day exposures, biological half-lives of 32.6 and 32.7 days, respectively, can be calculated (Table V). As shown in Fig. 1 *C*, the number of grains per nucleus remains fairly constant. It is worth noting that 20 days after the injection heavily labeled nuclei (100 grains per nucleus) can still be found (Fig. 1 *C*).

## DISCUSSION

It has been observed that incorporation of thymidine-$^3H$ in adrenal medullary cells of rats kept at normal temperature is in the order of 1%.[1] This situation is particularly unsuitable for obtaining measurements of possible changes in labeling index with any degree of accuracy. Therefore, the initial conditions of the present experiment were chosen (injection of thymidine-$^3H$ after 300 hr of cold exposure + 6 hr at room temperature) to give a high labeling index of the adrenal medullary nuclei (9.8%). This produced a starting point for the subsequent study, ensuring a greater accuracy of the determinations.

The results of the present experiments show that the amount of thymidine-$^3H$ incorporated in the nuclei of the adrenal medulla of rats exposed to cold decreases with time. This loss is particularly evident when the animals are exposed intermittently to +4°C for a period of 20 days after the injection of the label.

The slopes of the straight lines shown in the plots of log labeling index versus time after injection (Fig. 2) are independent of exposure time (Table V). The increase in the labeling index, from 5.0% after 14 days of exposure, to 9.8% after 150 days, shows that some of the very weakly labeled nuclei are missed after short exposure times.

After division, nuclei which have incorporated thymidine-$^3H$ during pre-mitotic synthesis give rise to two labeled nuclei, each containing one-half of the original amount of labeled DNA. This situation is verified in the adrenal cortex, where our results can be taken to indicate that after 7 days the nuclei have undergone two divisions. In the adrenal medulla, however, the percentage of the labeled nuclei decreases with time, both in rats exposed to normal temperature and, more markedly, in rats exposed to cold. No increase in the labeling index is observed, even after short times.

If labeled DNA were gradually lost from a given population of nuclei one should expect an increase in the proportion of weakly labeled nuclei at short times after incorporations and a decrease in the number of more heavily labeled nuclei. This, however, is not the case, since the average grain count (Table VI) does not change significantly during the first 7 days of cold treatment, whereas the labeling index decreases. This suggests a loss of all or most of the labeled DNA from a given population of nuclei during a short period of time; however, in view of the complex nature of the histograms (Fig. 1), this interpretation can be regarded as only tentative. It is worth noting in this context that resynthesis of DNA during recovery from cold is confined to a short period of time in a given nucleus, since: a) synthesis starts at different times during recovery, and b) at all times only a proportion of the nuclei is labeled.[1]

It has been established that the total amount of labeled DNA in a tissue is proportional to the

product of the labeling index times grain count (9). Since in our experiment the average grain count remains constant whereas the labeling index decreases, the halving time of the labeling index is a good indication of the biological half-life of the labeled DNA. The biological half-life of labeled DNA is 10 days if the animals are subjected to intermittent cold-treatment after injection. Obviously, if cell-division were responsible for the replacement of lost labeled cells by unlabeled ones, similar to the process of renewal of cells encountered in the small intestine, a mitotic index of 0.2% should be observed, assuming the duration of mitosis to be 1 hr (16). In fact, 12 – 16 mitoses were found, amongst a total of 250,000–280,000 cells, in whole medullas of animals kept at room temperature (16, footnote 1), which gives a mitotic index of approximately 0.006%. For animals subjected to cold-treatment, the mitotic index is 0.004%.[1] The number of mitoses found is, therefore, clearly inadequate to account for the observed number of labeled nuclei. Altogether, none of the results of the tests we have used are compatible with cell proliferation as an explanation for the labeling in the adrenal medulla.

In view of these considerations, it appears extremely unlikely that the groups of 2–4 adjacent labeled nuclei, frequently observed in the adrenal medulla, may be due to clustering of daughter cells after cell division. The significance of this finding is not understood at present; possibly, it may be correlated in some way with a particular level of functional activity of small groups of cells.

As reported in a previous paper (12), a progressive decrease of the amount of DNA per nucleus can be observed in the adrenal medulla of animals intermittently exposed to cold. The decrease takes place during the 15 hr of exposure to +4°C and is partially compensated for by synthesis that takes place during the 9 hr at room temperature (17). The marked loss of label (80%) observed in the animals which were exposed again for 300 hr to low temperature after the injection of the precursor, as compared with the less marked loss of label (30%) observed in rats kept at room temperature after injection, can be attributed to the influence of the ambient temperature on the turnover of DNA. It must be noted, however, that all animals had been exposed intermittently to +4°C before the injection of thymidine-$^3$H. This procedure, while ensuring that DNA especially sensitive to cold-treatment is labeled, may in some way influence the subsequent response of the adrenal medulla to temperature; a comparison with the results of other experiments (12, 13, and unpublished results) suggests that this difference is a matter of degree.

Since the loss of label can be described by an exponential curve, the daily loss of labeled metabolic DNA in the adrenal medulla can be calculated to be 6.9% per day during intermittent cold-treatment and to be 2.1% per day at room temperature. Using Feulgen photometry, biochemical techniques, and interference-microscopy, Viola-Magni (12) found a loss of 40% to 45% after 20 days of intermittent cold-treatment, about 2% per day. The observations by radioautography show that synthesis of DNA starts almost immediately after transfer of a rat from a temperature of 4°C to one of 22°C (17), and the net daily loss of 2% during intermittent cold treatment can be regarded as the result of a loss of 6.9% during the 15 hr of cold-treatment followed by a resynthesis of 4.9% during 9 hr at room temperature. The daily loss of 2% of the DNA during intermittent cold-treatment can thus be regarded as due to a negative balance of losses and gains (17).

It is not surprising that this balance may be different in animals belonging to different strains. Tongiani and Viola-Magni (18) have shown that the cells of the adrenal medulla of Wistar rats fed with Zoofarm standard diet lose only 8% of their DNA under conditions that cause a 45% loss of DNA in Italico rats. This difference can be accounted for by the fact that synthesis of DNA (as shown by thymidine-$^3$H incorporation) takes place during the cold-exposure periods in Wistar rats, whereas it is confined only to the periods at room temperature in Italico rats.[1] If the intermittent cold treatment is prolonged for more than 300 hr, synthesis of DNA takes place during the cold also in Italico rats, thus leading to an almost null balance, over a period of a few days (17).

The two processes of loss of DNA and recovery can be separated successfully by radioautography, which is not the case when use is made of Feulgen-photometry, which only shows the net balance (19, 20).

The events during the whole of the experiment described here can therefore be summarized in the following manner. In Italico rats during the 15 hr of cold-treatment a certain amount (about

7%) of the DNA is lost from the nuclei of the adrenal medulla and a smaller amount (about 4.5–5%) is resynthesized during 9 hr at room temperature. After 20 days of cold-treatment the deficit of 2–2.5% per day has accumulated to a total loss of 45%. If thymidine-$^3$H is injected 6 hr after transferring the animals to room temperature, DNA which is being resynthesized will be labeled; the subsequent observations with radioautography refer to this proportion of the DNA. During subsequent intermittent cold-treatment, as well as at room temperature, labeled DNA is lost, though at different rates. Since 1–1.3% of the nuclei are labeled in animals kept permanently at room temperature and labeled DNA is lost at normal temperatures, it has to be assumed that loss and resynthesis of DNA are processes which take place under normal conditions and that these processes are exaggerated when the function of the cells of the adrenal medulla, namely production of catecholamines, is enhanced by exposure to cold.[1]

The nuclei of the adrenal medulla show a weak incorporation of thymidine-$^3$H (6 grains per nucleus, on the average, after 30 days of exposure, Table VI), whereas those of the adrenal cortex show a marked uptake of the label, which is characteristic of pre-mitotic synthesis (35 grains per nucleus, on the average). The significance of the weakly labeled nuclei found in many organs has been evaluated and discussed by Pelc (9), who suggests that this type of incorporation is not due to pre-mitotic DNA synthesis, but rather to a metabolic turnover of DNA (9). The existence of a DNA fraction amounting to approximately 40% of the total DNA, which is characterized by a high turnover, has recently been demonstrated in the intestine, heart, and skeletal muscle of adult mice (21). It has been suggested (22, 23) that "metabolic" DNA consists of additional copies of those genes which are active in the differentiated cells of an organ and that these copies perform the actual function of DNA, e.g., transcription to mRNA.

Our present observations and those previously reported (12, 13, 17) have shown that DNA can be lost and resynthesized; unless some of the basic concepts of molecular genetics are entirely wrong, loss of genetic DNA should lead to loss of function and to an inability of the cells to replace DNA since the templates would have been lost. Our findings can be adequately explained on the assumption that metabolic DNA is involved and that it functions as previously suggested (22, 23). The fact that a stimulus which affects the specific function of an organ affects the turnover of "metabolic" DNA of its cells strengthens the view that metabolic DNA contains extra copies of those genes which are concerned with the function of differentiated cells.

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