# COMPARISON OF DNA-COPYING FIDELITY DURING REPAIR AND SEMICONSERVATIVE SYNTHESIS BY RADIOACTIVE PRECURSOR DISTRIBUTION ANALYSIS

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A fundamental aspect of DNA repair is its reliability. The principal types of repair mechanisms, such as excision, postreplicative and SOS repair, are to some degree prone to mistakes [14]. They are tested, for example, with respect to mutation frequency in work with cultures and, above all, with bacterial systems.

No such tests have yet been carried out at the level of the macroorganism as a whole.

Repair was induced by cortisol, which triggers reparative DNA synthesis [2], evidently through prior activation of free-radical processes, in the same way as the action of ionizing radiation [1]. Radiation, as we know, injures the RNA molecule and, in particular, reduces the content of long pyrimidine sequences [6]. This fact is in harmony with the target theory [8]. Pyrimidines also are less resistant to the action of free radicals than purines, and accordingly, and also because of the energy migration effect [7], they draw the attacks on themselves and protect purines. Much the same picture also is observed in the course of ordinary physiological processes [3]. Since repair, insertion and excision repair, for example, takes place on a matrix of an undamaged DNA chain, the "printing press" principle can justifiably be applied to these processes [8]. In other words, the probability of appearance of a repair error is proportional to the frequency of injuries at a given locus. The intensity of repair, tested with respect to specific radioactivity of the locus, assuming adequacy of the injury and of reparative incorporation of the precursor, may be a criterion of the vulunerability of a given DNA locus.

We used this criterion in the present investigation, in the course of which we compared the fidelity of DNA copying during semiconservative and reparative synthesis under normal conditions and during cortisol-induced activation of free-radical processes, by examining the distribution of radioactivity among DNA pyrimidine isopliths.

TABLE 1. Content of Pyrimidine Isopliths (in % of their total) in Heart and Liver DNA in Normal and Experimental Rats

| Organs | Experimental conditions | I | II | III | IV | V | VI | VII | Over VII |
|---|---|---|---|---|---|---|---|---|---|
| Liver | Hydroxyurea | 12,9 | 10,7 | 8,7 | 5,4 | 4,1 | 2,9 | 1,9 | 3,4 |
| | Normal | 12,3 | 9,9 | 8,3 | 5,7 | 4,9 | 2,9 | 2,2 | 3,8 |
| | Mock operation | 12,8 | 10,8 | 8,1 | 5,6 | 4,1 | 2,6 | 2,1 | 3,9 |
| | Cortisol (6 h) | 13,1 | 11,4 | 8,7 | 5,1 | 4,2 | 2,7 | 2,2 | 2,6 |
| | Hepatectomy | 12,7 | 10,5 | 8,1 | 5,8 | 4,2 | 2,8 | 2,2 | 3,7 |
| | Cortisol (24 h) | 12,3 | 10,2 | 8,2 | 5,8 | 4,5 | 2,9 | 2,4 | 3,7 |
| Heart | Normal | 12,3 | 10,3 | 8,5 | 5,8 | 4,2 | 3,1 | 2,1 | 3,7 |
| | Hydroxyurea | 12,6 | 10,7 | 8,3 | 5,4 | 4,2 | 2,9 | 2,2 | 3,7 |
| | Cortisol (6 h) | 12,7 | 11,2 | 8,6 | 5,7 | 4,0 | 2,5 | 2,1 | 3,2 |

Legend. Standard error of arithmetic means within 15% limits (n = 5). Here and in Table 3, time from injection to analysis shown in parentheses; Roman numerals indicate number of nucleotides in isopliths.

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TABLE 2. Incorporation of $^3$H-Thymidine (in cpm/mg DNA) into DNA of Various Organs under Normal and Different Experimental Conditions (M ± m)

| Organs | Experimental conditions | SR | Experimental conditions | SR | Experimental conditions | SR |
|---|---|---|---|---|---|---|
| Liver | Normal | 7146±422 | HE | 254 863±2737 | HE + cortisol | 79 779±327 |
| | Normal + hydroxyurea | 2539±99 | HE + hydroxyurea | 9 490±1594 | HE + five injections of cortisol | 15 408±164 |
| Heart | Normal | 650±35 | — | — | — | — |
| | Normal + hydroxyurea | 457±49 | — | — | — | — |

Legend. SR) Specific radioactivity, HE) hepatectomy.

## EXPERIMENTAL METHOD

Experiments were carried out in the fall and winter on male Wistar rats weighing 120-140 g. Two-thirds of the liver was removed by the method in [12]. Cortisol in 0.9% NaCl solution was injected intraperitoneally in a dose of 2.5 mg/ml/100 g body weight. $^3$H-thymidine also was injected intraperitoneally as a single dose of 500 μCi/100 g body weight 1 h before decapitation. $^3$H-uridine, in a dose of 100 μCi/100 g, was injected 30 min before decapitation of the animals. To analyze reparative DNA synthesis, the rats were given an intraperitoneal injection of hydroxyurea in 0.9% NaCl solution 1.5 h before decapitation to inhibit semiconservative synthesis, in a dose of 50 mg/ml/100 g body weight. The injections were given and decapitation performed under superficial ether anesthesia.

DNA was isolated from various organs of the animals with chloroform [13], hydrolyzed in formic acid with diphenylamine [10], after which its isoplith composition was determined by thin-layer chromatography [4]. Radioactivity of nucleotide material in the isopliths was measured by counting in appropriate zones of the chromatograms in toluene scintillator.

## EXPERIMENTAL RESULTS

As Table 1 shows, 6 h after injection of cortisol the distribution of DNA pyrimidine isopliths was shifted a little, although not significantly, toward a decrease in the number of nucleotides. Incidentally, in all processes due to free-radical action, including aging [3] and irradiation [6], a decrease in the number of nucleotides in DNA pyrimidine isopliths is observed.

Since cortisol inhibits semiconservative DNA synthesis to a degree commensurate with the action of hydroxyurea (Table 2) [11], and considering that in both cases free-radical processes are activated [9], we postulate that the mechanism of this inhibition may be similar in both cases. Hydroxyurea is known to inhibit reduction of ribonucleotides to deoxyribonucleotides [15]. Possibly free-radical peroxidation processes, activated by hydroxyurea [9], participate directly or indirectly in this inhibition, which would explain the mutagenic effect of this agent. It can therefore be tentatively suggested that an increase in activity of free-radical oxidative processes is the immediate cause of the phenomenon observed. The possibility cannot be ruled out that this mechanism is also fundamental to the inhibition of DNA synthesis as a result of the action of radiation. Hormonal inhibition of replicative DNA synthesis can be partly explained by the appearance of breaks resulting from demethylation [2]. However, we know that despite general inhibition of DNA synthesis, cortisol also induces selective amplification of particular regions of the genome [5]. This can be explained on the grounds that local inhibition of free-radical processes may take place at the replication (amplification) site, even against the background of their general activation. Association of DNA with the membrane at the replication site may be facilitated by the hydrophobic $CH_3$-group of 5-methylcytosine. In accordance with this view, the demethylation established previously [2] must lead to dissociation of the DNA–membrane complex, and incision of DNA under these circumstances activates its transcription. In fact, 2 h after injection of cortisol, incorporation of $^3$H-uridine into the acid-insoluble fraction increased from 2544 ± 243 cpm/mg DNA under normal conditions to 4406 ± 299 cpm/mg DNA, and was held at almost the same level (4338 ± 253 cpm/mg DNA) for 4 h after injection of the hormone. This parameter then fell to 3389 ± 240 cpm/mg DNA after 8 h, rose appreciably until 12 h (6457 ± 713 cpm/mg DNA), and then fell again until 24 h (4539 ± 147 cpm/mg DNA).

On turning to the analysis of reparative DNA synthesis, we note that cortisol and hydroxyurea inhibits incorporation of radioactive label into DNA by not more than half in the

TABLE 3. Relative Radioactivity of Pyrimidine Isopliths of Liver DNA under Normal Conditions and during Induction of Replicative and Reparative Synthesis

| Experimental conditions | I | II | III | IV | V | VI | VII | Over VII |
|---|---|---|---|---|---|---|---|---|
| Hepatectomy | 1,00 | 1,05 | 1,07 | 0,95 | 0,99 | 1,05 | 0,91 | 0,98 |
| Normal | 1,00 | 0,99 | 1,02 | 0,90 | 1,11 | 1,07 | 1,13 | 1,16 |
| Cortisol (6 h) | 1,00 | 0,98 | 1,01 | 0,95 | 1,14 | 1,14 | 1,24 | 1,28 |
| Cortisol (6 h) + hydroxyurea | 1,00 | 0,94 | 0,99 | 1,04 | 1,14 | 1,25 | 1,31 | 1,46 |
| Cortisol (48 h) | 1,00 | 1,02 | 0,95 | 1,03 | 1,10 | 1,10 | 1,16 | 1,21 |

Legend. Standard error of arithmetic mean within 9% limits (n = 5). For convenience of comparison, SR of isoplith I taken as 1.

liver of control rats and rats undergoing a mock operation. This is evidence that normally, almost half of total DNA-synthetic activity is accounted for by reparative synthesis in this organ (Table 2). In the heart, reparative synthesis accounts for about 90% of total incorporation of label. Cortisol inhibits semiconservative synthesis and, at the same time, activates reparative synthesis, as is shown by intensification of incorporation of the label after injection of hydroxyurea alone. This is evidently because of hormonal activation of free-radical processes [1]. The less intensive inhibition of DNA synthesis by cortisol than by hydroxyurea can also be explained by hormonal activation of amplification of particular regions of the genome [5]. In other words, unlike hydroxyurea, cortisol inhibits DNA synthesis selectively. Although the hormone increases total incorporation, it does not change the character of distribution of the label in DNA normally. Investigation of the specificity of reparative DNA synthesis, tested after injection of hydroxyurea, showed that reparative incorporation of $^3$H-thymidine into long pyrimidine isopliths takes place more intensively than into short, by contrast with semiconservative synthesis, when the label is distributed uniformly among the isopliths (Table 3). Together with the explanations given at the beginning of this paper, this fact is evidence that mistakes are more likely to arise during repair of long pyrimidine sequences than short (fewer than four pyrimidines in one isoplith).

It can thus be concluded from this investigation that injury to DNA of different organs, both directly and as a result of faulty repair, leads to shortening of the pyrimidine isopliths, i.e., to changes in the primary structure of DNA.

These data go some way toward explaining the simultaneously cytostatic, carcinostatic, and mutagenic action of irradiation, cortisol, and hydroxyurea.

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