verstärkt und durch Rhodanid abgeschwächt [7, 12]. Weiter wird bei regenerierenden Tieren von *Spirostomum ambiguum* der Abstand des Cytostoms vom Hinterende der Zelle bei Lithiumbehandlung vergrößert und bei Rhodanidbehandlung verkleinert [11]. Wir meinen, daß die antagonistische Beeinflussung von morphogenetischen und molekularbiologischen Vorgängen bei den Ciliaten durch eine einheitliche Wirkung der entsprechenden Ionen erklärbar ist.

Säugerzellen zeigen im Bereich der DNS-Synthese keine antagonistische Reaktion auf die Behandlung mit Lithium- und Rhodanidionen. Nach unserem Wissen fehlen Untersuchungen über den Einfluß der beiden Ionen auf das morphologische Differenzierungsgeschehen bei Säugerkeimen. Damit muß die Frage offen bleiben, ob eine Kopplung von morphogenetischen und molekularbiologischen Vorgängen fehlt, oder ob Säuger allgemein auf die beiden Ionen nicht antagonistisch reagieren.

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Methylation of Deoxyribonucleic Acid in Regenerating Rat Liver

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(Z. Naturforsch. **28 c**, 460–462 [1973]; received February 14, 1973)

Hepatectomy, DNA-methylation, DNA-synthesis, 5-Methylcytosine, Endoxan

In double label pulse experiments DNA methylation was compared to DNA synthesis after partial hepatectomy. 24 hours after the operation the highest ^{14}C-thymidine incorporation rates were found as well as the highest 5-methylcytosine labelling derived from (^{3}H-methyl)-methionine. However, synthesis was much more elevated than DNA methylation. Applying Endoxan DNA methylation is reduced to a significantly higher extent than DNA synthesis. Our results indicate that DNA methylation occurs not only combined with DNA synthesis.

Introduction

Since it has been known that DNA methylation occurs at the polymeric level several experiments on bacteria and tissue cultures have demonstrated the dependence of DNA methylation on DNA synthesis [1–10]. Recently we reported analogous results with mammalians *in vivo*. We found that organs having a high DNA synthesis also showed a high DNA methylation [11]. The present paper deals with the question whether the same could be found for regenerating rat liver too. Regenerating liver is used as more or less synchronized system, in which molecular mechanisms within the cell cycle can be separated from each other.

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Abbreviations: 5 MC, 5-methylcytosine; DNA, deoxyribonucleic; dUMP, deoxyuridinmonophosphat; TMP, thymidinmonophosphat; T, thymine.

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Methods and Materials

Partial hepatectomy (about two thirds) was performed by the method of Higgins and Anderson [12] on female Wistar rats, weighing 80 – 100 g. DNA was isolated by a modified Colter procedure [13]. DNA determination and DNA base separation by thinlayer chromatography were accomplished as reported earlier [11]. 5-Methylcytosine was the only methylated base detected in DNA of normal and regenerating liver. This confirms the results reported by Craddock [14]. The protein content of the isolated DNA was less than 1.5% as measured by the method of Lowry *et al.* [15]. RNA contamination in the DNA preparation could not be detected by the orcinol reaction [16].

L-methionine (methyl-T) with a specific radioactivity of 2.9 Ci/mmole (Batch 23) and thymidine-2-C^{14} with a specific radioactivity of 60 mCi/mmole were purchased from the Radiochemical Centre, Amersham, England. Endoxan (cyclophosphamide) is a gift from ASTA-Werke, Brackwede, Germany.

Results

a. Synthesis and methylation of DNA

^{14}C-thymidine and ^{3}H-methionine are used as precursors in double label puls experiments for measuring DNA synthesis and DNA methylation. As shown in Fig. 1 we observed an extremely depressed incorporation of both labels into DNA 6 – 12 hours after hepatectomy. In the period of DNA synthesis (20 – 24 hours after the operation) we found the highest DNA methylation rate. Thereafter, both synthesis and methylation are reduced consecutively.

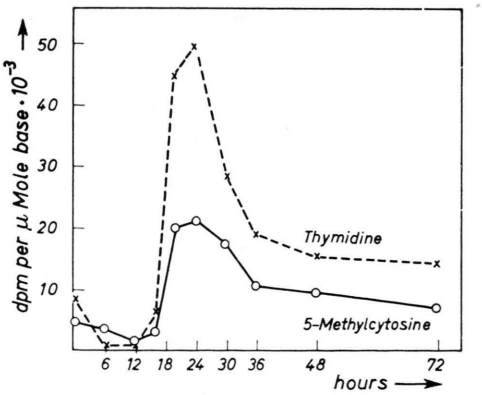

Fig. 1. DNA synthesis and DNA methylation in regenerating rat liver. At the times indicated after hepatectomy the rats were killed. 2 μCi ^{3}H-methionine/g body weight or 0.1 μCi ^{14}C-thymidine/g body weight were injected 1 hour or 20 min, respectively, before decapitation. Mean values of 4 – 10 rats.

But even 72 hours after hepatectomy the values of unoperated animals are not reached again.

Investigating the liver of rats having received tritiated methionine we found a remarkable amount of ^{3}H-counts in the thymine of the DNA. Since this radioactivity is achieved by labelling a precursor for DNA synthesis (dUMP → TMP) it is possible to correlate DNA methylation with DNA synthesis by only one labelled compound. The correlation of DNA methylation to DNA synthesis is indicated as ratio of the ^{3}H-radioactivity in DNA 5 MC to DNA thymine (Table I). It is obvious that DNA

Table I. Correlation of DNA methylation (dpm per μmole 5 MC) to DNA synthesis (dpm per μmole T) after labelling with ^{3}H-methionine.

Hours after Hepatectomy	dpm per μmole Base [5 MC] [T]	% of Control
Control	15.40	100.00
18	14.30	92.90
20	10.20	66.20
24	9.55	62.00
30	12.45	80.90
36	16.60	84.50
48	13.00	107.80

synthesis is more pronounced than DNA methylation. 24 hours after hepatectomy there is a maximum deviation between these two processes. 48 hours after the operation the ratio reaches the value of control animals.

b. Influence of Endoxan

Several hours after hepatectomy Endoxan inhibits DNA synthesis as well as DNA methylation (Table II). However, methylation is more reduced than DNA synthesis. The ^{14}C-thymidine incorporation values are well corresponding to the ^{3}H-labelling of DNA thymine derived from the tritiated methionine. Both thymine labels indicate that Endoxan affects DNA synthesis less than DNA methylation.

Discussion

In agreement with previously reported results [11] on different organs we could demonstrate in the regenerating liver that an increased DNA synthesis rate implies an increased DNA methylation rate. However, DNA synthesis is much more elevated than DNA methylation. Applying Endoxan we found DNA synthesis being less suppressed than DNA

Table II. Influence of Endoxan on DNA synthesis and DNA methylation in regenerating rat liver. Endoxan (100 μg/g body weight), ^{3}H-methionine (5 μCi/g body weight) and ^{14}C-thymidine (0.1 μCi/g body weight) were injected 2 hours, 60 or 20 min, respectively, before decapitation at the times indicated after hepatectomy. Mean values of 2–6 rats.

Hours after Hepatectomy	DNA-synthesis		DNA-methylation
	Thymine [^{14}C]	Thymine [^{3}H]	5-Methyl-cytosine [^{3}H]
	dpm per μmole Base		
Control	8 367	292	4 495
21	44 700	1 968	20 171
21+Endoxan	19 350	800	5 402
24	49 533	2 225	21 225
24+Endoxan	19 600	805	6 750
36	18 930	817	10 607
36+Endoxan	8 450	361	3 680

methylation. This effect does certainly not depend on different pool utilization as observed in the case of other drugs [17]. We could demonstrate that Endoxan equally affects the incorporation of ^{14}C-thymidine and $^3H-CH_3$ of $_3$H-methionine into DNA thymine.

It is well known that eucaryotes show an obligatory dependence of DNA methylation on DNA synthesis. However, it es uncertain whether the process of DNA methylation is completed immediately after DNA synthesis. Investigating this question Adams [18] could demonstrate that older non replicating DNA can well be methylated. In mouse fibroblast cultures Adams [8] found 17% of DNA methylation being not joined to DNA synthesis. This methylation occurs obviously later than within the DNA synthesis phase. To explain this Adams supposed two distinct processes of DNA methylation in eucaryotes [8].

Regenerating liver is known as a synchronized system in which biochemical reactions within one cell cycle are separable. In the DNA synthesis phase (20–24 hours after hepatectomy) DNA methylation is less elevated than DNA synthesis. This could mean that only that much methylation has been measured which is immediately following synthesis. Interruption of the cell cycle by Endoxan reduces DNA methylation to a significantly higher extent than DNA synthesis. Considering our results we suppose in agreement with Adams [8–18] that DNA methylation within one cell cycle occurs not only combined with DNA synthesis.

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