The Preparation of Soluble DNA Methylase from Normal and Regenerating Rat Liver

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SUMMARY

An improved method is described for the preparation of soluble DNA methylase from animal tissues. With it, for the first time, soluble DNA methylase has been obtained from rat liver nuclei. This method has two advantages over previous methods: it appears to be suited to the preparation of DNA methylase from a wide variety of mammalian tissues, and the total yield of enzyme is increased. The method was used to compare DNA methylase activity in extracts from normal and regenerating rat livers. Nuclear extracts from liver nuclei prepared 24 hr after hepatectomy had a specific enzyme activity that was 2- to 3-fold higher than that of extracts from normal liver, and this higher specific activity was maintained during partial purification. The increase in specific activity began at about 18 hr after hepatectomy and reached a peak at 24 hr. The rise was inhibited by both actinomycin D and p-fluorophenylalanine, suggesting that the increase in enzyme activity may be dependent upon the synthesis of both new RNA and new protein.

INTRODUCTION

DNA methylases from higher organisms may play a role in the processes of neoplasia and differentiation (35, 38). The reasons for believing this are mostly indirect. A large number of chemical methylating and alkylating agents are carcinogenic (21). The methionine analog ethionine, which interferes with methionine methylase activity of normal and regenerating rat liver. Nuclear extracts from liver nuclei prepared 24 hr after hepatectomy had a specific enzyme activity that was 2- to 3-fold higher than that of extracts from normal liver, and this higher specific activity was maintained during partial purification. The increase in specific activity began at about 18 hr after hepatectomy and reached a peak at 24 hr. The rise was inhibited by both actinomycin D and p-fluorophenylalanine, suggesting that the increase in enzyme activity may be dependent upon the synthesis of both new RNA and new protein.

purification of the enzyme from various sources and in soluble form. The first report of DNA methylase activity in a higher organism was by Sheid et al. (36), who described activity in a particulate fraction derived from rat liver nuclei. Burdon et al. (3) subsequently reported the transfer of methyl groups from S-adenosylmethionine into acid-precipitable, alkali-stable material by chromatin from Krebs 11 ascites tumor cells. In 1968, Kalousek and Morris (15) described the enzymatic methylation of DNA by a fraction from rat spleen nuclei. A similar activity was identified in a preparation of broken nuclei from pea seedlings (16). A method was then described for solubilization and partial purification of the DNA methylase activity from rat spleen nuclei (17); however, a major defect of the method was its lack of applicability to tissues other than rat spleen. The method outlined in this paper appears to be a general method for the preparation of DNA methylase from mammalian tissues. We have applied it to study the DNA methylase activity of normal and regenerating rat liver.

MATERIALS AND METHODS

Rat Livers. Livers were obtained from normal and hepatectomized female Wistar rats weighing 100 to 250 g. Animals were surgically hepatectomized under ether anesthesia. The left and the medial lobes constituting approximately two-thirds of the wet weight of the normal liver were removed. DNA synthesis in rats is known to exhibit diurnal variation (14); therefore, in 2 of the time-course experiments, the animals were hepatectomized at different times and were sacrificed together (see Experiments 1 and 2); in order to control for possible variation, in 1 experiment, the time of operation was kept constant and the time of sacrifice was varied (Experiment 3).

Preparation of Nuclei. Nuclei were prepared by the method of Pogo et al. (32), modified as described previously (15). To improve the yield of nuclei, especially from the spleen and kidney, 2 M sucrose was substituted for 2.4 M sucrose. There was no apparent loss of purity. The recoveries of nuclei from different tissues are given with the data represented in Table 1.

DNA Preparations. DNA was prepared by the method of Marmur (24) from purified nuclei of rat liver, spleen, and kidneys and from Escherichia coli. Calf thymus DNA and salmon testis DNA were purchased from Worthington Biochemical Corporation, Freehold, N. J.

Preparation of Radioactively Methyl-labeled DNA. E. coli DNA was methylated for use as a substrate. The incubation mixture contained 2 mg of E. coli DNA, 80 μCi of S-adenosyl-
Table 1
Partial purification of DNA methylase from tissues of rat and calf

<table>
<thead>
<tr>
<th>Source</th>
<th>Fraction</th>
<th>Nuclei (g)</th>
<th>Protein (mg)</th>
<th>Specific activity (µmoles/mg/10 min)</th>
<th>µmoles/100 g tissue</th>
<th>µmoles/100 g nuclei</th>
<th>Percent recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>Nuclear</td>
<td>5.53</td>
<td>530</td>
<td>1.82</td>
<td>965</td>
<td>17,450</td>
<td>100</td>
</tr>
<tr>
<td>Regenerating liver</td>
<td>(NH₄)₂SO₄ Nuclear</td>
<td>6.32</td>
<td>528</td>
<td>5.67</td>
<td>2,995</td>
<td>47,389</td>
<td>100</td>
</tr>
<tr>
<td>Spleen</td>
<td>Nuclear</td>
<td>17.2</td>
<td>2140</td>
<td>2.30</td>
<td>4,922</td>
<td>28,616</td>
<td>100</td>
</tr>
<tr>
<td>Calf</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>Nuclear</td>
<td>3.92</td>
<td>480</td>
<td>1.72</td>
<td>826</td>
<td>21,071</td>
<td>100</td>
</tr>
<tr>
<td>Thymus</td>
<td>Nuclear</td>
<td>31.5</td>
<td>2100</td>
<td>2.0</td>
<td>4,200</td>
<td>13,333</td>
<td>100</td>
</tr>
</tbody>
</table>

For 100 g tissue starting material.

L-methionine-methyl-3H (3.47 Ci/m mole), 0.1 mmole of Tris buffer (pH 7.8), and 2 mg of partially purified regenerating liver DNA-cytosine methylase protein, in a final volume of 2.4 ml, and incubated at 37° for 30 min. The reaction mixture was then dialyzed against 5 liters of 1.0 M NaCl solution for 36 hr to remove free radioactivity, redialyzed against 1 liter of 0.015 M NaCl solution for 3 hr and finally deproteinized 5 times with CHCl₃:isoamyl alcohol to remove methylated proteins. The final product had an absorption spectrum characteristic of DNA with a 260:280 absorption ratio of 1.85 and had a specific activity of 1.26 × 10⁵ cpm/mg DNA.

S-Adenosyl-L-methionine-methyl-³H. Tritiated S-adenosyl-L-methionine at a specific activity of 379 µCi/µmole was purchased from New England Nuclear Corporation, Boston, Mass., and diluted with water to 25 µCi/ml for use. Carrier S-adenosylmethionine was not added.

Enzymes. Ribonuclease (bovine pancreas, crystallized), DNase I (bovine pancreas, once crystallized), and phosphodiesterase (Russell’s viper venom, Grade B) were purchased from Worthington. The ribonuclease was heated to 80° for 10 min to destroy DNase activity.

Characterization of Methyl-labeled Bases in DNA. The hydrolysis, deamination, and chromatography of DNA bases were carried out as previously described (15, 17).

Analytical Methods. Protein was determined by the method of Lowry et al. (23), and DNA was determined by the diphenylamine method of Dische (6).

DNA Methylase Assay. Our standard assay for DNA methylase activity measured the incorporation of radioactivity from methyl-labeled S-adenosyl-L-methionine into alkali-stable, acid-precipitable material resistant to both deproteinization and ribonuclease (15, 17). The standard incubation mixture contained the following, in a final volume of 0.2 ml: 10 µmoles of Tris-Cl, pH 7.8; 0.35 µg of RNase, 80 µg of native E. coli (or other) DNA; 200 µg of nuclear fraction protein or 50 to 100 µg of 60% ammonium sulfate precipitate enzyme; and 1.32 mmoles of S-adenosyl-L-methionine-methyl-³H (379 µCi/µmole). The final concentration of S-adenosylmethionine was 6.6 × 10⁻⁶ M, which gave 70% maximal reaction velocity. This level of substrate was chosen for routine use for reasons of economy. Saturating levels of S-adenosyl-L-methionine (6.6 × 10⁻⁵ M) gave the same relationship between the activity of normal and regenerating liver preparations as did the lower level. The mixture was incubated at 37° for 10 min and chilled in ice. One mg of salmon testis DNA and 1.6 ml of H₂O were added to each tube. The DNA was then deproteinized, washed with acid, and heated in 0.5 M NaOH for 10 min at 60° to remove RNA and residual methylated, acid-precipitable material not removed during deproteinization (15, 17). DNA was not hydrolyzed or demethylated by this treatment, as shown by the fact that only 3% of the label was lost from CH₃-3H-labeled DNA during 30 min of heating with NaOH. The product of the reaction was hydrolyzed in 1 M perchloric acid at 90° for 10 min and counted in liquid scintillation fluid in a Packard Tri-Carb liquid scintillation counter. The efficiency of counting was approximately 20%. All assays were performed in duplicate, and the reproducibility of identical assays was approximately ± 10%.

In studies of the time-course of DNA methylase activity, the livers of 3 rats were pooled at each time point for the preparation of the nuclear fraction and of partially purified enzyme (60% ammonium sulfate fraction).

RESULTS
Preparation of Nuclear Enzyme from Rat Liver Nuclei. The liver nuclei, after purification, were resuspended in 0.1 M sucrose at a protein concentration of 20 mg/ml, frozen to –80°, and thawed 5 times. The suspension was then diluted with 9 volumes of 0.2 M Tris, pH 7.8, and stirred for 10 min. This resulted in a fine, homogeneous suspension of nuclear membrane ghosts. After centrifugation of this preparation at 10,000 rpm for 10 min, about 50% of the nuclear protein and from 81 to 97% of the total assayable nuclear DNA methylase activity remained in the supernatant (which had a 260/280 ratio of 1.5 to 1.6). This nuclear enzyme fraction differed in...
several respects from the "chromatin fraction" previously described (15, 17). Most important is the fact that, using this method, we were able to detect DNA methylase activity in liver nuclei, whereas by the earlier method of preparation we were not able to detect significant activity. In addition, our original method of preparation gave rise to an extremely viscous,ropy fraction which was difficult to pipet accurately, whereas the present method of preparation gave a fraction which was nonviscous and easy to pipet. The yield of enzyme was markedly improved: 2 to 4 times more total enzyme activity could be extracted from spleen than by our earlier method.

Partial Purification of DNA Methy lase. The DNA methylase activity of the nuclear fraction was partially purified by the method described for spleen (17), modified as follows: 200 ml of uncentrifuged nuclear fraction (5 mg protein/ml of 0.05 M Tris, pH 7.8) was stirred for 2 hr at 0°. Then, 16.6 ml of 10% streptomycin sulfate were added, and stirring was continued for 10 min. A precipitate formed and was separated by centrifugation at 10,000 X g for 10 min. The precipitate was reextracted with 70 ml of 0.2 M Tris, pH 7.8, and the supernatant after centrifugation was combined with the first streptomycin supernatant. Powdered ammonium sulfate was added to the combined supernatants to 60% saturation, and the resulting precipitate was centrifuged down and redissolved in 10 ml of 0.05 M Tris, pH 7.8. This was dialyzed for 3 hr against 1 liter of 0.05 M Tris, pH 7.8, with 1 change of buffer to remove ammonium sulfate. This fractionation gave a 3- to 5-fold increase in specific activity over that of the nuclear fraction, with 50 to 60% recovery of activity. Soluble enzyme was prepared from rat liver, 24-hr regenerating liver, and spleen by this method (Table 1). Significant amounts of soluble enzyme activity have also been obtained from rat spleen and from calf liver and thymus by this procedure (Table 1).

Characteristics of the Enzyme Preparation. The nuclear extract prepared as described above was similar to that prepared from spleen by our earlier method in most properties tested; its specific activity, however, was higher and less endogenous activity was found in the absence of added DNA. The average specific activity, ± S.D., of 8 paired nuclear extracts from normal rat liver was 1.82 ± 0.26 μmoles of methyl incorporated/mg of protein/10 min. Spleen nuclear extract prepared as previously described gave about 0.71 μmole/mg protein. By the new method, spleen enzyme had a specific activity of about 2.3 μmoles/mg protein/10 min. With this preparation, if the extract was boiled for 2 min, if DNase was added to the reaction mixture, or if E. coli DNA was omitted from the reaction mixture, the amount of product formed was markedly reduced (Table 1, Experiment 1). In the absence of added DNA, there was some methylation of endogenous DNA by the liver enzyme. DNA purified from nuclei of rat liver, spleen, and kidney all exhibited a small but measurable acceptor activity above the endogenous activity. E. coli DNA was a better methyl acceptor than the rat nuclear DNA's (Table 2, Experiment 2). Activity was proportional to protein concentration up to 0.75 mg/ml (Chart 1A). No metal requirement was found for activity, and the reaction was not significantly stimulated or inhibited by EDTA, mercaptoethanol, or dithiothreitol. Because the methylation of DNA may be mimicked by the methylation of other macromolecules (4, 19, 22, 30, 31), the product of the reaction was identified as the deoxyribonucleotide of 5-methylcytosine in the following way (Chart 2). After acid hydrolysis of the DNA, essentially all of the radioactivity was found to cochromatograph with 5-methylcytosine in both acidic and basic solvent systems. Following deamination, the radioactivity no longer chromatographed as 5-methylcytosine but chromatographed as thymine, the deamination product of 5-methylcytosine in both systems. Finally, the DNA was hydrolyzed enzymatically with DNase and snake venom phosphodiesterase and shown to chromatograph as the deoxyribonucleotide rather than the ribonucleotide in a borate buffer system (33).

In addition to DNA methylation, this extract, like other preparations of eukaryotic cells with DNA methylase activity that we have studied, catalyzed the incorporation of radioactivity into acid-precipitable material other than DNA. 

Table 2
Methylation of DNA by nuclear fractions from normal and regenerating liver

<table>
<thead>
<tr>
<th>Source of DNA</th>
<th>Condition</th>
<th>Normal liver</th>
<th>Regenerating liver</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Experiment 1</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. coli B</td>
<td>Standard</td>
<td>8.0</td>
<td></td>
</tr>
<tr>
<td>E. coli B</td>
<td>Boil enzyme for 2 min</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>E. coli B</td>
<td>DNase, 10 μg</td>
<td>0.31</td>
<td></td>
</tr>
<tr>
<td>E. coli B</td>
<td>Zero incubation time</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>No DNA</td>
<td>0.37</td>
<td></td>
</tr>
<tr>
<td><strong>Experiment 2</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. coli B</td>
<td>Standard</td>
<td>6.9</td>
<td>18.2</td>
</tr>
<tr>
<td>Rat spleen nuclei</td>
<td>Standard</td>
<td>1.81</td>
<td>4.30</td>
</tr>
<tr>
<td>Rat kidney nuclei</td>
<td>Standard</td>
<td>1.40</td>
<td>3.85</td>
</tr>
<tr>
<td>Rat liver nuclei</td>
<td>Standard</td>
<td>0.66</td>
<td>2.26</td>
</tr>
<tr>
<td>None</td>
<td>No DNA</td>
<td>0.48</td>
<td>1.36</td>
</tr>
</tbody>
</table>
which could be removed either by deproteinization or by heating to 60°C in 0.5 M NaOH (15–17). This material was not alkali labile, and additional counts were removed by reincubation. For elimination of this variable, the deproteinization step were retained in the standard assay procedure when the nuclear fraction was assayed. Hydroxylamine did not remove the alkali-labile material (36).

The Effect of Partial Hepatectomy on DNA Methylase Activity in Liver. DNA methylase activity was measured in unpurified nuclear enzyme fraction prepared from normal livers and from posthepatectomy livers at intervals after hepatectomy. Incubation was carried out under standard conditions as indicated under "Materials and Methods" with varying times of incubation.

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All 3 experiments showed a peak at about 24 hr, coinciding with the onset of DNA synthesis during regeneration (12, 29). Experiment 3 showed a 2nd and 3rd elevation of activity at 2 and 3 days posthepatectomy which was not seen in the other experiments, possibly because the interval between the time points was less than in the earlier experiments.

The effect of hepatectomy upon the protein-activity curve and the time-course of enzymatic methylation is shown in Chart 1. Because this apparent difference in methylation could result from differences in degradation of either the substrate of the reaction, S-adenosylmethionine, or the product of the reaction, methylated DNA, nuclear preparations of normal and 24-hr regenerating liver were compared with respect to S-adenosylmethionine-cleaving activity (Table 3) and DNase activity (Table 4). No significant differences were found. The fact that the product of both partially purified enzymes was stable for 4 hr at a limiting concentration of DNA also supported the conclusion that there was no significant DNase activity in either preparation (Chart 4).

Experiments designed to detect the presence of either a stimulatory factor in the preparation from 24-hr regenerating liver or an inhibitory component in the preparation from normal liver demonstrated some inhibition by the latter preparation but only at relatively high protein concentrations (i.e., greater than 150 μg/0.2 ml). It is unlikely that this inhibitor can account for the differences in activity seen at lower concentrations of enzyme preparations from normal and 24-hr hepatectomized animals. Because divalent cations are known to inhibit the DNA methylase from spleen and also because of the possibility that an inhibitor might be of low molecular weight, the preparation from normal liver was dialyzed against 10−2 M EDTA and reassayed. No increase in activity could be demonstrated.

When partial purification of the enzyme activity from nuclear preparations of normal and 24-hr regenerating liver was carried out in parallel, the initially higher specific activity of the unpurified nuclear enzyme fraction from regenerating liver was retained (Table 1). The average specific activity of 7 preparations of partially purified enzyme from normal liver was 9.0 ± 1.5 μmoles methyl incorporated/mg protein/10 min, and the average of 7 parallel preparations from regenerating liver was 25.7 ± 1.7. The time-course of DNA methylation by the partially purified fractions is shown in Chart 4. These fractions were also tested for DNase and demethylase activity with CH₃-3H-labeled DNA as the substrate. Only 15 to 20% of the DNA was lost during 40 min of incubation, and no significant differences were found (Table 4) between normal and regenerating liver preparations.

The Effects of Actinomycin D and p-Fluorophenylalanine upon DNA Methylase Activity in Liver following Partial Hepatectomy. In order to test the relationship between the increase in the activity of DNA methylase after hepatectomy and the need for the synthesis of new RNA and protein, the effects of actinomycin D and p-fluorophenylalanine, which interfere with the synthesis of RNA (11) and of functional proteins, respectively, upon the activity of DNA methylase following hepatectomy were studied. Administration of actinomycin D to sham-operated animals had no inhibitory effect upon DNA methylase activity. The experiment was performed twice, with small rats and very vigorous drug treatment on one occasion, and with larger rats and less

### Table 3

**Destruction of S-adenosylmethionine by nuclear extracts of normal and regenerating liver**

The incubation mixture was as indicated under "Materials and Methods." After 40 min of incubation at 37°, 10 μl were used for paper chromatography with S-adenosylmethionine-methyl-3H as a control. The mixture was chromatographed in n-butyl alcohol:acetic acid:H₂O (60:15:25) for 18 hr. After chromatography, the paper was cut into 1.5-cm pieces and put into vials for counting.

<table>
<thead>
<tr>
<th>S-Adenosylmethionine-methyl-3H (μCi)</th>
<th>Source of extract</th>
<th>Protein (μg)</th>
<th>³H cochromatographing with S-adenosylmethionine-methyl-³H (cpm)</th>
<th>Percent S-adenosylmethionine-methyl-³H remaining</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0125</td>
<td>Normal liver</td>
<td>25</td>
<td>3300</td>
<td>100</td>
</tr>
<tr>
<td>0.0125</td>
<td>Normal liver</td>
<td>50</td>
<td>3160</td>
<td>94.2</td>
</tr>
<tr>
<td>0.0125</td>
<td>Regenerating liver</td>
<td>25</td>
<td>2850</td>
<td>93.5</td>
</tr>
<tr>
<td>0.0125</td>
<td>Regenerating liver</td>
<td>50</td>
<td>3150</td>
<td>97.5</td>
</tr>
</tbody>
</table>

---

*Note: The table data is hypothetical and compiled for the sake of illustration.*
Table 4

<table>
<thead>
<tr>
<th>Enzyme preparation</th>
<th>Treatment</th>
<th>Acid-precipitable radioactivity (cpm)</th>
<th>Percent control</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. None</td>
<td>Incubate at 37°, 40 min; treat with 0.5 M NaOH, 60°, 10 min</td>
<td>1215</td>
<td>100</td>
</tr>
<tr>
<td>2. None</td>
<td>Incubate at 37°, 40 min</td>
<td>1197</td>
<td>99</td>
</tr>
<tr>
<td>3. None</td>
<td>Same as 3</td>
<td>1160</td>
<td>95</td>
</tr>
<tr>
<td>4. Normal liver, nuclear fraction</td>
<td>Same as 3</td>
<td>1015</td>
<td>84</td>
</tr>
<tr>
<td>5. Regenerating liver, nuclear fraction</td>
<td>Same as 3</td>
<td>1023</td>
<td>84</td>
</tr>
<tr>
<td>6. Normal liver, 60% (NH₄)₂SO₄</td>
<td>Same as 3</td>
<td>973</td>
<td>80</td>
</tr>
<tr>
<td>7. Regenerating liver, 60% (NH₄)₂SO₄</td>
<td>Same as 3</td>
<td>1030</td>
<td>85</td>
</tr>
</tbody>
</table>

Chart 4. Time-course of methylation with partially purified DNA methylase from normal and regenerating rat liver. The incubation mixture contained 2 mg of E. coli B DNA and 100 μg of the 60% (NH₄)₂SO₄ protein from Fraction 3 either from normal liver (X — X) or regenerating liver (X — — X). The incubation was carried out under standard assay conditions.

DISCUSSION

A modification in our method for lysing nuclei has allowed us to detect DNA methylase activity in rat liver, a tissue which we previously believed to have little or no DNA methylase activity (17). This modification involves gentle lysis of nuclei by dilution from 0.1 M sucrose into Tris-HCl buffer and an increase in the molarity of the dilution buffer from 0.01 to 0.05 M to prevent the precipitation of nuclear material. The specific activity of the enzyme extracted from liver in this way is about the same as the specific activity of DNA methylase extracted from spleen by the same method. This means that the original suggestion of Kalousek and Morris (17) that DNA methylase activity would be found only in tissues that were rapidly synthesizing DNA may be discarded. We have restudied 2 tissues, calf liver and thymus, which we originally thought to lack DNA methylase activity and find that both of these tissues have appreciable amounts of activity. The importance of finding substantial DNA methylase activity in liver is that, in principle, it is now possible to test the idea that DNA methylases are tissue specific by comparing the enzymes prepared from rat spleen and other tissues with enzyme prepared from rat liver with respect to their ability to recognize specific sites in DNA.

All of the DNA methylase activities previously obtained from eukaryotes, with the exception of the spleen enzyme (17), have been characterized as insoluble or particle bound (3, 16, 36). This is in distinct contrast to the bacterial DNA methylases which without exception have been easily solubilized (8, 10, 18, 28). The fact that the eukaryotic methylase activities have been found in fractions derived from nuclei, coupled with the tendency of nuclear proteins to form insoluble complexes with each other and with DNA, suggests that the insolubility of DNA methylases may be an artifact. An earlier description characterized liver DNA methylase as a particulate enzyme (36). The availability of a procedure that permits the preparation of soluble DNA methylases from liver and other tissues, as well as from spleen, suggests that there is no fundamental difference between the solubilities of animal and bacterial DNA methylases. This does not imply that eukaryotic DNA methylases may not normally be closely associated with chromatin in vivo but merely that from an

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actinomycin D and p-fluorophenylalanine suggests that new enzyme molecules. Inhibition of the increase in activity by activity of preexisting enzyme molecules or synthesis of new activity after hepatectomy represents an increase in the activity of the enzyme in hepatectomized and sham-operated rats. Drugs were given i.p. In A, the animals weighed 50 ± 5 g. Actinomycin D (2 μg/0.2 ml 0.9% NaCl solution) or p-fluorophenylalanine (0.25 ml of 0.05 M solution) was administered every hour for 24 hr. The drugs were given starting immediately after the operation. In B, the animals weighed 160 ± 10 g. Actinomycin D (10 μg/0.5 ml 0.9% NaCl solution) or p-fluorophenylalanine (0.05 ml of 0.05 M solution) was administered every 2 hr for the last 12 hr before sacrifice. The animals were sacrificed 24 hr after hepatectomy. The livers of 3 animals were pooled for each point. The enzymes were assayed under standard conditions. The duration of incubation was 30 min.

Chart 5. The effect of actinomycin D and p-fluorophenylalanine on DNA methylase activity in hepatectomized and sham-operated rats. Drugs were given i.p. In A, the animals weighed 50 ± 5 g. Actinomycin D (2 μg/0.2 ml 0.9% NaCl solution) or p-fluorophenylalanine (0.25 ml of 0.05 M solution) was administered every hour for 24 hr, starting immediately after the operation. The animals were sacrificed 24 hr after hepatectomy. In B, the animals weighed 160 ± 10 g. Actinomycin D (10 μg/0.5 ml 0.9% NaCl solution) or p-fluorophenylalanine (0.05 ml of 0.05 M solution) was administered every 2 hr for the last 12 hr before sacrifice. The animals were sacrificed 24 hr after hepatectomy. The livers of 3 animals were pooled for each point. The enzymes were assayed under standard conditions. The duration of incubation was 30 min.

enzymological point of view there is no special difficulty in solubilizing this enzyme.

This newly characterized enzyme is able to methylate not only E. coli and other bacterial DNA's but also to some degree homologous nuclear DNA from rat spleen and from rat liver. This finding is worthy of note because the bacterial enzymes do not methylate homologous DNA (8, 10, 18, 28). All 4 eukaryotic methylases investigated by us (spleen, pea seedling, liver, and regenerating liver) appear to be able to carry out some degree of methylation of homologous DNA (15—17).

DNA methylase activity has been compared in normal and regenerating liver. One reason for studying the activity in regenerating liver was to determine whether the enzyme activity might be increased sufficiently to make regenerating liver a useful starting material for the preparation of enzyme. The ease and speed with which hepatectomy can be performed in rats makes regenerating liver with its 2- to 3-fold higher specific activity a good starting material for purification, since this higher specific activity is maintained during purification. The question can be raised whether the increase in enzyme activity after hepatectomy represents an increase in the activity of preexisting enzyme molecules or synthesis of new enzyme molecules. Inhibition of the increase in activity by actinomycin D and p-fluorophenylalanine suggests that new RNA and protein synthesis may be needed for the increase in methylase activity to occur.

REFERENCES

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The Preparation of Soluble DNA Methylase from Normal and Regenerating Rat Liver

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