cholesterol[10] and phospholipid[11] levels did not show any significant change in a period of 18 h after the treatment with a 100 μg/kg s.c. dose. The blood total ketone bodies[12] increased from 0.20 ± 0.03 mg/100 ml pretreatment level to 1.00 ± 0.20 mg/100 ml 6 h after treatment. At this time the triglyceride contents of liver and heart muscle were elevated, as well, by 92 and 50% ($p < 0.01$), respectively, compared with controls. The cholesterol and phospholipid contents of these organs did not change significantly.

This in vivo effects of porcine β-LPH are closely similar to those of the previously isolated lipolytic fractions of porcine pituitary, the fraction H[13] and the peptid I and II[14]. These polypeptides, however, are unlike the porcine β-LPH regarding their molecular weight and other chemical characteristics. The apparent failure of β-LPH to exert a serum triglyceride enhancing effect as described for the above-mentioned peptides can be attributed to the low doses applied in our experiments[7].

Zusammenfassung. Es wird gezeigt, dass ein aus Schweinehypophysen gewonnenes lipotropes Hormon (β-LPH), in vitro und in vivo bei verschiedenen Tierarten untersucht, eine Erhöhung der Lipolyse herbeiführt. Die Wirkung war deutlich bei Kaninchen, Meerschweinchen und Schweinen, während bei Hunden, Ratten und Mäusen keine lipolytische Wirkung nachzuweisen war.

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Studies on DNA Methylase Activity in Mammalian Tissue

It has been previously demonstrated that the 5-methylcytosine in DNA of mammalian tissues is acquired by the enzymatic methylation of the preformed polynucleotide[1]. This concurs with the origin of the methylated bases (6-methyladenine and 5-methylcytosine) in the DNA of microbial origin[2,3]. The DNA methylase of mammalian tissues was shown to be localized in an insoluble nuclear subfraction in rat tissues[1] and in Krebs II ascites tumor[4]. It has also been identified as a nuclear enzyme in HeLa cells[5]. Recently, KALOUSEK and MORRIS[6] employed the exact procedure of SHEID et al.[1], and confirmed the observations of these authors with spleen. However, they found lower enzyme activity in rat liver. This may be attributed to the lability of the liver enzyme, and the greater difficulties in extracting and separating it from inhibitor(s) in liver nuclei[1].

A modification of the DNA methylase isolation and some properties of the enzyme in rat liver and Reuber minimal deviation hepatoma nuclei is reported in this communication.

Materials and methods. Salmon testis DNA was purchased from the Worthington Biochemical Corp. The active methyl donor (methyl ^{14}C)-S-adenosylmethionine, was obtained from the New England Nuclear Corporation. Holtzman and A × C male rats (120–150 g) were used in all of the experiments. The Reuber H-35 tumor was transplanted i.p. every month into the A × C rats. Radioactivity was determined in a Nuclear Chicago low background, end-window, gas-flow counter. Protein was assayed by the method of LOWRY et al.[7]. Deionized water was used throughout all of the experiments, and all manipulations of the rat tissues were performed at 4 °C.

Four grams of liver or hepatoma was homogenized by hand with a loosely fitting Ten Broeck homogenizer in 15 ml of 0.25 *M* sucrose-1×10^{-2} *M* EDTA. The nuclear fraction was isolated and washed in this media, then purified and subfractionated by a procedure described in an earlier paper[1]. The only modification in the subfractionation was the omission of magnesium from all of the extraction solutions. Enzyme assays were performed immediately after the nuclear extracts were prepared. The incubation mixture of 3–4 ml consisted of 2 ml of 0.02 *M* *Tris* (pH 8.9), 0.02 ml (10 μg) of RNase, 0.25 ml of freshly prepared glutathione (2 μmoles), 0.1–0.8 ml of enzyme extract (0.25–2 mg protein), and ammonium acetate where indicated. After 15 min of incubation at 38 °C, 0.02 ml (0.2 μC) of (methyl-^{14}C)-S-adenosylmethionine was added and the mixtures reincubated for 1 h at 38 °C. Saturation levels of ^{14}C incorporation into DNA, and chromatographic identification of the enzymatically synthesized methylated base was accomplished essentially by the procedures described by SHEID et al.[1].

Results. The Table depicts the effect of varying concentrations of EDTA used for the isolation of the nuclear fraction on DNA methylase activity, and the corresponding enzyme blank values which consists of enzyme extract plus radioactive methyl donor minus DNA. Nuclei isolated from solutions containing low amounts of EDTA have as much enzyme activity as nuclei isolated from solutions with higher quantities of EDTA. However, the enzyme blank values are markedly increased. EDTA added directly to the assay incubation mixture had no effect on the enzyme activity.

Figures 1A and 1B illustrate the effect of ammonium ions on DNA methylase activity in the normal Holtzman rat liver and Reuber hepatoma nuclear subfractions respectively. The addition of 0.15 *M* ammonium to the

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incubation mixtures resulted in approximately 95% inhibition of the liver and hepatoma enzymes. Magnesium ions have no effect on the DNA methylase activity in either tissue.

Figures 2A and 2B are saturation curves for DNA methylase activity in the isolated liver and hepatoma nuclear subfractions. In both instances, approximately 1.25 mg of protein from the enzyme extract is necessary for the incorporation of the maximum amount of methyl groups into the substrate DNA. The livers derived from either the Holtzman or A × C rats, and the Reuber H-35 hepatoma all had equivalent amounts of enzyme activity. In some cases, the DNA substrate after the in vitro methylations was subjected to hydrolysis and two dimensional chromatography. By this technique, 5-methylcytosine was identified as the only methylated base in DNA.

We were unable to demonstrate DNA methylase activity in rat sperm. However, there is trace enzyme activity (too low to identify the nature of the methylated base) in rat testis.

Discussion. Ammonium inhibition of DNA methylase activity is in direct contrast to the stimulatory effect of ammonium ions on the mammalian supernatant sRNA methylase activity[8–10]. The enhancement by ammonium of enzyme activity has been confirmed by our laboratory[11] for both supernatant and mitochondrial sRNA methylase activity in mammalian tissues. It is difficult to evaluate fully the inhibitory effect of ammonium ions on DNA methylase activity, since desalting or dialysis of the enzyme extract is not applicable, due to the rapid inactivation of the enzyme once it has been solubilized[1].

EDTA and magnesium free solutions used in isolating the nuclear fraction eliminates extraneous interfering protein and nuclear ribosomes which bind and sediment with the nuclear subfraction used as the source for DNA methylase[1]. The low enzyme control values achieved by employing this procedure affords greater statistical reproducibility in determining DNA methylase activity.

In sperm cells where there is no active DNA synthesis there is no demonstrable DNA methylase activity. The trace enzyme activity in rat testis may account for the methylated guanine derivatives which have been isolated from sperm[12]. sRNA methylase activity also is apparently absent in sperm. This is in agreement with the findings of BOREK and SRINIVASAN[13,14].

The effect on DNA methylase activity of variations in amounts of EDTA used in isolating rat liver nuclear fractions

Concentration of EDTA[a]	cpm[b] incorporation into total precipitate		cpm incorporation into precipitate minus DNA (enzyme blank)		cpm incorporation into DNA	
	Liver[c]	Hepatoma	Liver	Hepatoma	Liver	Hepatoma
0	1659	1579	865	792	794	787
$1 \times 10^{-4} M$	1505	1324	630	528	875	796
$1 \times 10^{-3} M$	1242	1122	400	352	842	770
$1 \times 10^{-2} M$	1066	947	241	190	825	757
$1 \times 10^{-1} M$	1039	907	239	195	800	712

[a] EDTA is used as the tetrasodium salt. [b] cpm represents ^{14}C methyl incorporation. [c] Livers excised from the Holtzman or A × C rats had equivalent amounts of enzyme activity.

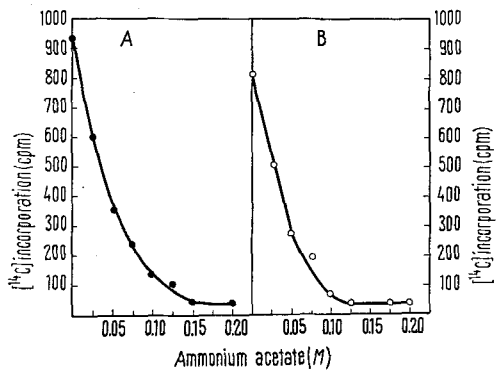

Fig. 1. (A) The effect of increasing ammonium ion concentrations on DNA methylase activity in rat liver. (B) The effect of increasing ammonium ion concentrations on DNA methylase activity in Reuber H-35 hepatoma. The assay conditions are described in the text.

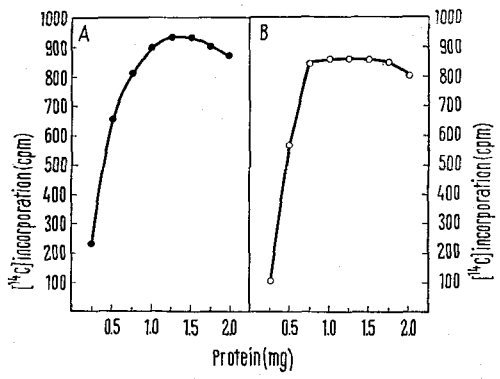

Fig. 2. (A) The activity of liver DNA methylase with increasing concentrations of protein. (B) The activity of Reuber H-35 hepatoma DNA methylase with increasing concentrations of protein. The assays were preformed without the presence of ammonium and magnesium ions.

Resumen Para aislar nucleo de mamíferos que contienen DNA metilasa actividad se utilizó como medio homogenizante $0.25 M$ sucrosa-$1 \times 10^{-2} M$ EDTA. Hígado de rata y el hepatoma de Reuber demostraron cantidad equivalente de actividad enzimática. Se observó aproximadamente 100% de inhibición con amoníaco $0.15 M$, los iones de Mg no mostraron efecto.

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[14] Acknowledgments. This study was supported by grants from the American Cancer Society (No. P-492) and the National Institutes of Health (No. CA-06576-06). We wish to thank Dr. M. D. REUBER of the National Institutes of Health, U.S. for generously supplying us with the hepatoma bearing rats.