Methylation of Newly Synthesized and Older Deoxyribonucleic Acid

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Billen (1968) demonstrated that in *Escherichia coli* methylation of DNA normally occurs only on nascent DNA. In mouse L929 cells synchronized by treatment with aminopterin there is a burst of DNA methylation that peaks about 1 h after the time of maximum DNA synthesis (Burdon & Adams, 1969). However, the rate of methylation does not fall to zero in cells in phase G1, and moreover (in the presence of 2 mM-hydroxyurea) methylation still occurs at 35% the control rate although DNA synthesis is inhibited by 95%. These results suggested that in animal cells DNA could be methylated at a time other than immediately after synthesis, a suggestion that is substantiated by the following results.

Mouse L929 cells were grown in Roux flasks in minimum essential medium (Eagle's) containing 10% (v/v) of calf serum. After incubation for 1–3 h with [^{32} P]orthophosphate (2 μ Ci/ml) and L-[Me- ^{3} H]-methionine (2 μ Ci/ml) in the presence of 5-bromodeoxyuridine (20 μ M), sodium formate (20 mM) and aminopterin (0.2 μ M), DNA was extracted by the method of Kirby (1964) and subjected to equilibrium centrifugation in alkaline caesium chloride to separate the heavy, newly synthesized, ^{32} P-labelled DNA from light DNA. Tritium radioactivity is associated with both peaks and was shown by chromatography to be present almost exclusively in 5-methylcytosine.

When the cells were prelabelled with [^{32} P]orthophosphate in the presence of 5-bromodeoxyuridine and then incubated with L-[Me- ^{3} H]methionine in the presence of hydroxyurea (2 mM) tritium is again found in both peaks, suggesting the occurrence of methylation of recently synthesized DNA as well as of older DNA.

On release from stationary phase L929 cells do not make DNA for at least 12 h (Adams, 1969). In such cells, and also in phytohaemagglutinin-stimulated horse lymphocytes, there is no methylation of DNA before the onset of DNA synthesis. Moreover, as L929 cells re-enter a stationary phase of growth and the rate of synthesis of DNA slows there is an almost parallel fall-off in the rate of methylation.

Thus although methylation of old DNA does occur it seems unlikely that the time-lag between synthesis and methylation can be more than a generation.

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Effects of Insulin and Adrenaline on Rat Epididymal-Fat-Pad Pyruvate Dehydrogenase

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When fat-pads were incubated in Krebs bicarbonate buffer insulin stimulated fatty acid synthesis from glucose or fructose to a greater extent than explicable by its action on sugar transport. For example, with fructose (2 mg/ml) insulin (0.01 unit/ml) increased the rate of fatty acid synthesis to $180 \pm 13\%$ (15) of the control rate whereas the outputs of pyruvate and lactate into the incubation medium fell to 70 ± 4 and $57 \pm 4\%$ respectively of the control. An additional action of insulin on pyruvate metabolism to fatty acids is therefore indicated, as also concluded by Halperin (1970) and Jungas (1970).

We measured maximal activities of enzymes involved in the pathway of fatty acid synthesis from pyruvate after 30 min incubation of fat-pads. No effect of insulin was observed on pyruvate carboxylase, citrate synthase, ATP citrate-lyase or acetyl-CoA carboxylase, but pyruvate dehydrogenase was stimulated by $87 \pm 12\%$ (16). The mitochondrial marker enzyme glutamate dehydrogenase was unaffected by insulin, and the effect on pyruvate dehydrogenase was observed with or without added glucose or fructose.

Adrenaline (1 μ g/ml) or dibutyryl cyclic AMP greatly diminished the effect of insulin on fat-pad pyruvate dehydrogenase when present in the incubation mixture. On collagenase-released fat-cells incubated in Krebs bicarbonate buffer with 2% bovine serum albumin adrenaline decreased pyruvate dehydrogenase activity even below the value observed in the absence of insulin. This adrenaline effect was antagonized by the β -adrenergic inhibitor propanolol (10 μ g/ml).

The total activity of pyruvate dehydrogenase and the magnitude of these effects of insulin and adrenaline may be sufficient to account for the effects of these hormones on rates of fatty acid synthesis in epididymal adipose tissue. They are substantially greater than those given by Jungas (1970). The present findings provide an explanation for earlier observations on the interactions of insulin and adrenaline on fatty acid synthesis in epididymal fat-pad (Denton & Halperin, 1968) and are in accord with the suggestion that these hormones