Inhibition of 1,25-dihydroxycholecalciferol production in the kidney could not be explained by lack of the precursor substrate, 25-hydroxycholecalciferol, since this was present in higher concentration in the kidneys of the treated birds than in controls.

Direct evidence for a block in 1,25-dihydroxycholecalciferol synthesis was obtained in vitro by the use of kidney homogenates. Incubations (2h) of 25-hydroxy[26,27-3H2]-cholecalciferol were performed with chick kidney homogenates (Fraser & Kodicek, 1970). Lipid extracts of the tissues were made and chromatographed on Sephadex LH-20 with chloroform–hexane (13:7, v/v) as eluent, as described above.

Whereas kidneys from control birds produced 1,25-dihydroxycholecalciferol, no detectable amount of this compound was produced in the homogenate from chicks treated with 1-hydroxyethyl-1,1-diphosphonate and cholecalciferol: in contrast, peak Va compound was produced (Fig. 1). For this response to be elicited it was necessary for the 1-hydroxyethyl-1,1-diphosphonate-treated birds to receive cholecalciferol. When a kidney homogenate from chicks treated with 1-hydroxyethyl-1,1-diphosphonate but no cholecalciferol was incubated with 25-hydroxycholecalciferol the substrate was converted into 1,25-dihydroxycholecalciferol only.

That the stimulation of the synthesis of peak Va compound was not a direct effect of 1-hydroxyethyl-1,1-diphosphonate on the renal hydroxylation enzyme was shown adding 1-hydroxyethyl-1,1-diphosphonate in vitro to the incubation system derived from vitamin D-deficient control birds (0.1 ml of 1-hydroxyethyl-1,1-diphosphonate solution/125 ng of kidney tissue). This system also produced only 1,25-dihydroxycholecalciferol.

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Holick, M. F. & DeLuca, H. F. (1971) J. Lipid Res. 12, 460-465

Effects of Heart Work and Insulin on the Incorporation of [14C]Glucose into Hexose Phosphates and Uridine Diphosphate Glucose in Normal and Diabetic Perfused Rat Heart

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Following a previous study on the effect of insulin in the normal and diabetic working and non-working rat heart (Chain et al., 1969), we decided to determine the specific
Table 1. Effects of heart work and insulin on the incorporation of \([U-^{14}C]\)glucose into hexose phosphates and UDP-glucose in normal and streptozotocin-diabetic perfused rat heart

Experimental details are indicated in the text. Results are given as means ± s.e.m. for three observations.

<table>
<thead>
<tr>
<th>Intermediate/glucose specific-radioactivity ratio</th>
<th>Normal heart</th>
<th>Streptozotocin-diabetic heart</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Langendorff</td>
<td>Working</td>
</tr>
<tr>
<td>Intermediate</td>
<td>+Insulin</td>
<td>+Insulin</td>
</tr>
<tr>
<td>No insulin (10 munits/ml)</td>
<td>No insulin</td>
<td>No insulin</td>
</tr>
<tr>
<td>Glucose 1-phosphate</td>
<td>0.409 ± 0.016</td>
<td>0.723* ± 0.120</td>
</tr>
<tr>
<td></td>
<td>0.541* ± 0.008</td>
<td>0.891* ± 0.042</td>
</tr>
<tr>
<td>Glucose 6-phosphate</td>
<td>0.209 ± 0.024</td>
<td>0.373* ± 0.046</td>
</tr>
<tr>
<td></td>
<td>0.214 ± 0.053</td>
<td>0.492* ± 0.062</td>
</tr>
<tr>
<td>Fructose 6-phosphate</td>
<td>0.316* ± 0.039</td>
<td>0.677* ± 0.065</td>
</tr>
<tr>
<td></td>
<td>0.380 ± 0.074</td>
<td>0.702* ± 0.051</td>
</tr>
<tr>
<td>UDP-glucose</td>
<td>0.276* ± 0.025</td>
<td>0.523* ± 0.082</td>
</tr>
<tr>
<td></td>
<td>0.349 ± 0.065</td>
<td>0.615* ± 0.098</td>
</tr>
<tr>
<td>Glycogen</td>
<td>0.012* ± 0.002</td>
<td>0.083* ± 0.006</td>
</tr>
<tr>
<td></td>
<td>0.019 ± 0.005</td>
<td>0.124* ± 0.014</td>
</tr>
<tr>
<td></td>
<td>0.006 ± 0.002</td>
<td>0.036* ± 0.005</td>
</tr>
<tr>
<td></td>
<td>0.017 ± 0.002</td>
<td>0.105* ± 0.008</td>
</tr>
</tbody>
</table>

* Significant (P < 0.05).
radioactivities of some of the phosphorylated hexose intermediates under these same conditions. These were determined by using a new automatic chromatographic separation method (A. J. Thomas, I. Das & K. C. Blanshard, unpublished work). Time curves of incorporation of \([\text{U-}^{14}\text{C}]\text{glucose}\) into the intermediates tested (glucose 1-phosphate, glucose 6-phosphate, fructose 6-phosphate and UDP-glucose) showed a steady increase up to 10 min, then reached a plateau. Determination of specific radioactivities at the plateau level were made after 30 min perfusion. The perfusion conditions for the Langendorff and working rat heart were as described by Chain et al. (1969). The results are reported in Table 1.

In all experiments the specific radioactivity of glucose 1-phosphate was considerably higher than that of glucose 6-phosphate. This indicates either compartmentation or a pathway of glucose 1-phosphate formation that does not go through glucose 6-phosphate. Higher specific radioactivities of glucose 1-phosphate than of glucose 6-phosphate were observed previously in rat liver \textit{in vivo} after intravenous injection of \([\text{U-}^{14}\text{C}]\text{glucose}\) (Das et al., 1971) and in pigeon liver homogenates after incubation with \([\text{U-}^{14}\text{C}]\text{glucose}\) (Das & Sie, 1972). The specific radioactivity of UDP-glucose is always lower than that of glucose 1-phosphate. As both compounds were present in rat heart muscle in approximately equal amounts (about 0.12 \(\mu\text{mol/g dry wt.}\)), this can be explained either by a dilution of this compound by some unknown pathway of UDP-glucose biosynthesis involving a non-radioactive precursor or by some unknown metabolite superimposed on the UDP-glucose fraction in the chromatographic separation. This latter possibility is not likely, as the UDP-glucose peak disappears completely when the compound is destroyed by mild acid hydrolysis. In the diabetic Langendorff heart the specific radioactivity of UDP-glucose was very low, and was increased fivefold by insulin, whereas that of glucose 1-phosphate was increased only twofold. Work compensated for the diabetic effect and brought up the specific radioactivity of UDP-glucose to normal values. Insulin increased the specific radioactivity of this compound still further. Similar results were observed previously with regard to glycogen synthesis (Chain et al., 1969). As could be expected, the specific radioactivities of glucose 6-phosphate and fructose 6-phosphate are similar in all cases.

We thank Dr. Anne Beloff-Chain for her encouragement and useful discussions.


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\textbf{Phosphorylation of Histones from Pregnant and Lactating Guinea-Pig Mammary Gland}

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The biological function of histone phosphorylation has been studied in a number of systems, and there have been reports of an involvement with both DNA replication and RNA transcription. In regenerating liver, for instance, phosphorylation of lysine-rich histone (F1) occurs at the time of DNA synthesis and reaches a peak at mitosis (Balhorn et al., 1971), but the phosphorylation of a non-lysine-rich fraction increases with the start of RNA synthesis (Ord & Stocken, 1969). On the other hand it has been shown that the lysine-rich histone is phosphorylated on treatment of rat liver with glucagon (Langan, 1969), a hormone that causes RNA synthesis and enzyme induction.

Mouse mammary gland maintained in organ culture can proliferate in the presence of insulin and cortisol. Addition of prolactin to the medium not only induces the specific milk protein \(\alpha\)-lactalbumin but also increases the incorporation of \(^{32}\text{P}\) from \([^{32}\text{P}]\text{P}\) into histone fractions F2b and F2a2 (Turkington & Riddle, 1969). Since we have been

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