experiments. Fig. 2 shows the rate of entry over the first 3 min in the presence and the absence of insulin. With starved cells, unlike the previous experiments, the insulin effect was greater at 3 min than at 1 min.

We have shown that the uptake of 3-O-methylglucose by 'facilitated diffusion' into suitably cultured human lymphocytes is linear for 3 min at 4°C under the conditions described. The initial rate of entry can be enhanced by preincubation of the cells in the presence of insulin.

This effect, optimized by selection of culture conditions, media and handling procedures, could become the basis of a simple and convenient bioassay for insulin in vitro.

We thank Dr. J. Birch of Searle for helpful discussions.


Pancreatic Insulin and Glucagon Content and Secretion after Infection of Mice with EMC Virus

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There has been increasing interest in recent years in the possibility that certain viruses might induce diabetes in susceptible individuals, as a result of direct or indirect damage of the insulin-producing B cells. To investigate the nature and specificity of virus-induced islet-cell damage, we have used mice infected with EMC (encephalomyocarditis) virus (Craighead & McLane, 1968; Craighead & Steinkle, 1971) to study the insulin and glucagon content, and the regulation of hormone secretion, in pancreas removed from mice at intervals after infection.

DBA/2 mice (8–16 weeks old) were inoculated intraperitoneally with 0.2 ml of a suspension of the M strain of EMC virus (Craighead & McLane, 1968) containing between 10^2 and 10^3 suckling-mouse LD_{50} per ml. Mice were given free access to pasteurized mouse chow 4RF (Charles River Ltd.) and water. They were killed by cervical dislocation at 7, 14 or 21 days after infection, and the pancreas was removed into bicarbonate-buffered saline. The pancreases were cut into 3–6 mg pieces and preincubated for 30 min before their secretory responses were tested; the use of pieces rather than isolated islets was preferred because of the possibility of damage to peripheral A cells during islet isolation and the difficulty of isolating islets from severely diabetic mice. Single pieces were then incubated at 37°C for 60 min in 0.6 ml of medium which contained 200 kallikrein inactivation units of Trasylol/ml, 1 mg of albumin/ml and 5 mm- or 20 mm-glucose, in the presence or absence of 5 mm-theophylline. After incubation the pieces were extracted with ethanol/HCl/water (460:9:140, by vol.) and after dilution with 0.1 M-phosphate buffer the hormone content of the extracts and incubation media was determined. Insulin immunoassay was performed as described by Hales & Randle (1963) with human insulin standards. Glucagon immunoassay utilized an antibody prepared by a poly(vinylpyrrolidone) procedure, with poly(ethylene glycol) to separate antibody-bound from free glucagon (Henquin et al., 1974).

Animals infected with EMC virus developed pronounced hyperglycaemia, the blood glucose concentrations being 23, 23 and 21 mm at 7, 14 and 21 days after infection.

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Table 1. *Stimulation of insulin secretion in pancreatic fragments obtained from control and infected mice*

Stimulations are expressed as means ± S.E.M. (five observations) percentage of the basal (5 mM-glucose) release obtained in the same experiment.

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>Time after infection (days)</th>
<th>0 (control)</th>
<th>7</th>
<th>14</th>
<th>21 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 mM-Glucose</td>
<td>100 ± 11</td>
<td>100 ± 15</td>
<td>100 ± 14</td>
<td>100 ± 12</td>
<td></td>
</tr>
<tr>
<td>20 mM-Glucose</td>
<td>230 ± 26*</td>
<td>123 ± 13</td>
<td>112 ± 16</td>
<td>123 ± 15</td>
<td></td>
</tr>
<tr>
<td>5 mM-Glucose + 5 mM-theophylline</td>
<td>179 ± 19*</td>
<td>153 ± 23</td>
<td>103 ± 14</td>
<td>147 ± 28</td>
<td></td>
</tr>
<tr>
<td>20 mM-Glucose + 5 mM-theophylline</td>
<td>414 ± 56*</td>
<td>129 ± 17</td>
<td>112 ± 21</td>
<td>173 ± 47</td>
<td></td>
</tr>
</tbody>
</table>

* Significant difference from 5 mM-glucose control (P < 0.05).

Fig. 1. *Changes in insulin and glucagon secretion from mouse pancreatic fragments 7, 14 and 21 days after EMC-virus infection*

Results are shown as the mean ± S.E.M. percentage of the rates of secretion obtained in uninfected animals (day 0). ---, Glucagon; ----, insulin.

by comparison with normal values of 7 mM. The insulin content of the pancreas fell by 75% by 7 days after inoculation and remained at low values at 14 and 21 days. Insulin secretion by surviving islets within the fragments showed an almost complete failure to respond to 20 mM-glucose or to 5 mM- or 20 mM-glucose plus theophylline at 7 or 14 days. By 21 days, however, there was some indication of recovery in responsiveness of the B cells to 20 mM-glucose in the presence of theophylline (Table 1),
although this was not statistically significant. Stimulation of secretion was expressed in Table 1 as the percentage of the basal secretory rate on each day; the overall decrease in insulin secretion is illustrated in Fig. 1, in which secretion rates in normal mice are compared with those on days 7, 14 and 21 after infection. Glucagon secretion was more variable after EMC-virus infection, although a stimulation of glucagon secretion by 5mM-theophylline in the presence of 5mM-glucose was consistently observed at 7, 14 and 21 days as well as in control pancreas. The alterations of basal rates of glucagon secretion are shown in Fig. 1, from which it is clear that secretion was significantly increased on day 7, but returned to normal values or less by days 14 and 21. This pattern is not reflected in comparable alterations of glucagon content of the pancreas at similar times after infection.

There is thus a clear distinction between the responses of A and B cells of the islets after inoculation of mice with EMC virus. For the B cells the pattern is clear: there is within 7 days a failure of the cells to secrete insulin in response to glucose and/or theophylline, and a significant fall in the insulin content of the cells. Ultrastructural evidence (Wellman et al., 1975), which we have confirmed, suggests that this results from damage to the structure of the B cells after viral attack. In the A cells the pattern is much less clear: the cells respond to theophylline stimulation at 7, 14 and 21 days after infection, and there is hypersecretion at day 7, but normal or decreased overall secretion at days 14 and 21. The reason for this pattern is uncertain; it is clear, however, that under these conditions EMC virus does show some degree of specificity for the B cells of the islets of Langerhans.

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Craighead, J. E. & McLane, M. F. (1968) Science 162, 913–914
Hales, C. N. & Randle, P. J. (1963) Biochem. J. 88, 137–146

**High-Molecular-Weight Forms of Diuretic Hormone from *Rhodnius prolixus***

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The hormonal control of diuresis in *Rhodnius prolixus* is well established (Maddrell, 1971). An earlier attempt to purify and characterize the hormone demonstrated that approx. 80% of the total hormone activity could be sedimented at 100000g (Aston & White, 1974). As the activity present in the supernatant was particularly labile, the present work was directed towards that activity associated with high-molecular-weight material. Mesothoracic ganglionic masses were dissected from fifth-stage larvae, taken 8–11 days after feeding, and placed in a solution of 158mM-NaCl/40mM-KCl/10mM-EDTA, pH 7.0. Batches of 100 ganglionic masses in 0.5 ml of solution were frozen in liquid N₂ and stored at −25°C before use. A total of 1000 ganglionic masses were thawed to 4°C, sonicated for 2 min and subjected to differential centrifugation. In each case sedimented material was resuspended in the same solution that the ganglionic masses had been stored in. The protein content of the various fractions was measured by the method of Lowry et al. (1951) and hormone activity was determined by a method modified from that described by Maddrell (1969). Malpighian tubules for use in the assay were dissected from the fifth-stage larvae taken 7–10 days after feeding,