has not yet been determined; however, preliminary experiments suggest that this RNA consists of a very complex mixture of molecules present in low abundance, as would be expected from the estimates of the relative base-sequence complexities of polyribosomal and nuclear polyadenylated RNA molecules (Getz et al., 1975; Kleiman et al., 1977).

It is clearly important to determine whether any of these polyadenylated RNA sequences, which are nucleus-confined in the Friend cell, are potential mRNA molecules, which become polyribosome-associated in cells of different phenotype, or whether they are non-coding sequences, which may play some other, as yet unknown, role in the metabolism of eukaryotic cells.

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Some Factors Affecting Polyrribosomes Isolated from the Skeletal Muscle of Diabetic Rats Treated with Insulin

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The synthesis of protein by the skeletal muscle of diabetic rats is subject to translational control by insulin (Wool et al., 1972). Thus when the hormone is administered to rats made diabetic by alloxan the polyribosomes rapidly aggregate, even if the synthesis of new RNA is prevented (Stirewalt et al., 1967). As a prelude to studying the mRNA of skeletal muscle, we have been examining polyribosomes isolated by different methods. We report here that when a medium of high ionic strength is used to isolate polyribosomes from the skeletal muscle of rats made diabetic by alloxan the effect of insulin is obscured, apparently by the action of a nuclease. This does not occur if the polyribosomes are extracted in a medium of low ionic strength, or if the rats are made diabetic with streptozotocin.

In their original studies, Wool and co-workers (Stirewalt et al., 1967) isolated ribosomes from skeletal muscle using a method in which the initial homogenization of the tissue was in a medium of low ionic strength (Florini & Breuer, 1966). However the use of a higher ionic strength (250mM-, rather than 80mM-, KCl) gives a greater yield of ribosomes, and we decided to use this method (Stirewalt et al., 1971) in our own work. Figs. 1(a) and 1(b) show that there was a similar proportion of polyribosomes when ribosomes were extracted from the thigh and gastrocnemius muscle of normal 135 g male rats at either high or low ionic strength. At 2 days after intravenous injection of alloxan (8 mg) there was extensive disaggregation of the polyribosomes extracted at high ionic-strength (Fig. 1c), but this was apparently not reversed 30 min after intraperitoneal injection of 5 units of insulin (Fig. 1d). However, when the ribosomes were extracted in a medium of low ionic strength, the polyribosomes did reaggregate (Figs. 1e and 1f). Moreover, when insulin was injected into rats 3 days after induction of diabetes with streptozotocin (9 mg), re-formation of the skeletal-muscle polyribosomes was seen even if the ribosomes were extracted at high ionic strength (Figs. 1g and 1h).

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Ribosomes (150 μg) were resuspended in 0.2 ml of buffer containing 50 mM-Tris/HCl (pH 7.6), 200 mM-KCl and 5 mM-MgCl₂ and applied to a 5.2 ml linear gradient of 15–45% (w/v) sucrose in the same buffer. Centrifugation was at 4°C for 35 min at 234,000 g in a Beckman SW50.1 rotor. Analysis was by upward displacement by using the density-gradient scanner attachment of a Gilford 240 recording spectrophotometer. (a) Normal rat; low ionic strength. (b) Normal rat; high ionic strength. (c) Alloxan-diabetic rat; high ionic strength. (d) Alloxan-diabetic rat + insulin; high ionic strength. (e) Alloxan-diabetic rat; low ionic strength. (f) Alloxan-diabetic rat + insulin; low ionic strength. (g) Streptozotocin-diabetic rat; high ionic strength. (h) Streptozotocin-diabetic rat + insulin; high ionic strength.

These results suggest that alloxan causes the induction or activation of a ribonuclease which is extracted into the postmitochondrial fraction if the skeletal muscle is homogenized in a medium of high ionic strength. This causes the degradation of polyribosomes that have reassembled *in vitro* in response to insulin. The fact that streptozotocin does not have this side effect testifies further to the superiority of this drug in the study of experimental diabetes (Rerup, 1970), and our results emphasize the need for careful assessment of studies on protein synthesis in rats made diabetic with alloxan.

We do not know what is the intracellular substrate for the putative nuclease produced in response to treatment with alloxan. The enzyme is clearly not involved in the degradation of skeletal-muscle RNA during diabetes, for the extent of this degradation is similar in both types of diabetes (3 days after injection of alloxan or streptozotocin the RNA/DNA ratio was decreased to 73 and 74% of normal respectively). Moreover, we
Table 1. Ribonuclease activity of postmitochondrial supernatant from skeletal muscle of normal and diabetic rats

Postmitochondrial supernatant (containing about 5 mg of protein) was incubated for 120 min at 37°C with 32P-labelled rRNA (33000 c.p.m.) and non-radioactive yeast RNA (0.5 mg) in 0.2 M-sodium acetate (pH 7.6) in a total volume of 2 ml. Undigested RNA was precipitated and the acid-soluble radioactivity determined (Barrett, 1972).

<table>
<thead>
<tr>
<th>Conditions of homogenization</th>
<th>Normal</th>
<th>Alloxan-diabetic</th>
<th>Streptozotocin-diabetic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low ionic strength</td>
<td>453</td>
<td>330</td>
<td>226</td>
</tr>
<tr>
<td>High ionic strength</td>
<td>602</td>
<td>581</td>
<td>533</td>
</tr>
</tbody>
</table>

found no increase in ribonuclease activity in the postmitochondrial supernatant from the muscle of alloxan-diabetic rats when we followed the conversion of 32P-labelled rRNA into acid-soluble fragments (Table 1). It is likely therefore that the putative ribonuclease is a relatively specific endonuclease releasing large oligonucleotides which are insoluble in acid.

In view of the net breakdown of RNA during diabetes it might have been expected that there would be an increase in the activity of ribonuclease, similar to that seen in dystrophic muscle (Abdullah & Pennington, 1968). However, this does not appear to be the case, for we also found no increase during diabetes in the acid ribonuclease present in unfractionated homogenates of skeletal muscle (L. H. Fahmy & D. P. Leader, unpublished work). It is therefore possible that the loss of skeletal-muscle RNA during diabetes is caused solely by a decrease in the rate of its synthesis, in a manner analogous to that established for the control of the total protein of skeletal muscle (Millward et al., 1976).

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Conformational Properties of the Opiate Peptide C-Fragment, and Related Peptides from Lipotropin

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The opiate peptide, C-fragment, (residues 61–91 of lipotropin, LPH), isolated as an intact peptide present in substantial quantity in pig pituitary, has been shown to have high affinity in vitro for the opiate receptors in the brain (Bradbury et al., 1976). We have