THE RELATION OF RNA AND PROTEIN SYNTHESIS TO THE
SEDIMENTATION OF MUSCLE RIBOSOMES: EFFECT OF
DIABETES AND INSULIN*

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Ribosomes from muscle of alloxan-diabetic rats function less efficiently in the
synthesis of protein than ribosomes from normal animals.1–4 The nature of the
change in function is unknown. The decrease in protein synthesis is not due to
a deficiency in the amount of messenger RNA, but rather the result of a defect in its
utilization or translation by ribosomes.1, 2

An important unresolved problem is the nature of the putative change in the
ribosomes that leads to an alteration in the translation of messenger RNA. It is our
hope that a study of the chemical and physical properties of the ribonucleoprotein
particles from muscle of diabetic animals will provide a solution to the problem.
We have begun the study with an examination of the sedimentation of muscle
ribosomes and of the relation of RNA and protein synthesis to the influence of
diabetes and insulin on those properties.

Materials and Methods.—The following have been described in detail before: the source of the
materials,4, 4 the nature of the animals (whose weight was 120–140 gm) and the method of making
them diabetic with alloxa,n, 4 the isolation of ribosome from skeletal muscle,4, 4 the preparation
of aminoacyl-tRNA charged with one C14-labeled amino acid and 19 C14-amino acids,1 the mak-
ing of supernatant protein ("transfer enzyme preparation"),1 the means of assaying protein syn-
thesis by ribosomes,4, 4 and the determination of the radioactivity of the protein.3

Density gradient analysis: Ribosomes were suspended in medium A (0.01 M MgCl2; 0.08 M
KCl; 0.05 M tris-HCl, pH 7.6) to a final concentration of 10 OD260 units/ml; 0.2 ml (2 OD260
units) was layered on 5.2 ml of a linear sucrose gradient (15–30%) containing medium A. (Ma-
terial absorbing at 260 mυ was first removed from the sucrose by heating a 60% solution with
7.2% Norit A (decolorizing carbon) to 80° for 1 hr and passing the mixture through Whatman no. 3 filter paper. The 60% sucrose contained less than 0.04 OD260 units/ml.) The gradients
were centrifuged for 75 min at 30,000 rpm in the SW 39 rotor of a Spincro model L ultracentrifuge.
The distribution of ribosomes in the gradient was determined by displacing the gradient from the
bottom with 50% sucrose containing potassium phthalate (3 mM) at a rate of 0.25 ml/min
with an Instrument Specialities Co., Inc. (ISCO) model D, density gradient fractionator and
analyzing the effluent at 254 mυ with an ISCO model UA-2 UV analyzer. The potassium acid
phthalate is responsible for the terminal absorption (cf. Fig. 1).

Sedimentation analysis in the analytical ultracentrifuge: Ribosomes, suspended in medium A
to a concentration of 1.5–11 mg/ml, were examined in a Spincro model E ultracentrifuge at 5–12°
using schlieren optics; the final speed of centrifugation was 30,000 rpm. The rates of sedimenta-
tion of the ribosomes were calculated from the distance that the boundary moved with time. For
that purpose the photographic plates were projected onto graph paper marked with the appropriate
reference lines and distances from the center of the rotor. Similar coefficients of sedimentation
were obtained when the method was checked with a Gaertner comparator. The percentage of the
total ribosomal particles contributed by each component was determined from photographs, en-
larged ten times, by cutting out and weighing tracings of the area bounded by the schlieren peak
and solvent line. Double-sector cells were used; one sector contained medium A and the other
sector ribosomes suspended in medium A. The values were corrected for dilution due to cen-
trifugation in a sector-shaped cell. The $s_{20, w}$ was calculated by plotting six concentrations of
the ribosome suspension (1.5–8 mg/ml) from normal and from diabetic animals against the
$s_{20, w}$ of each component and extrapolating to zero concentration.

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Results.—Sedimentation of skeletal muscle ribosomes: Preparations of ribosomes from skeletal muscle were analyzed in linear sucrose gradients (Fig. 1) and in the model E analytical ultracentrifuge (Fig. 2); the sedimentation coefficients obtained by the latter procedure were used to calibrate the sucrose gradients. We have assumed that the 80S particle is the monomer and the 114S particle is a dimer stabilized by magnesium. The assumption is based on the report that the ribosome monomer in rat liver has the same sedimentation coefficient (82 ± 3S),6 and on the results of the experiments with ribonuclease (see below). We do not know the structure of the 105S particle—one possibility is that it is the product of the association of an 80S monomer and a ribosomal subunit.6 Ordinarily, we found no 60S or 40S subunits in the gradients; that may be because they are lost in the preparation of ribosomes by the method we used (the ribosomes are centrifuged through 1 M sucrose during their isolation), or because the subunits associate with other particles. We consider aggregates having sedimentation coefficients of 145 or greater to be polysomes; the 145S particle may contain three ribosomes, the 175S four, the 200S five, and so on. A portion of the ribosome population, consisting of polysomes larger than 225S, forms a pellet at the bottom of the sucrose gradient or is not clearly resolved, at least not in the circumstances we used. From analyses in the analytical ultracentrifuge the relative proportions of each of the ribosomal particles and aggregates were determined. The results are in Table 1.

Effect of ribonuclease: Preparations of normal and diabetic ribosomes were treated with ribonuclease and examined in the analytical ultracentrifuge (Table 1). As was to be expected, the enzyme produced a decrease in the relative number of polysomes and a large increase in the percentage of 80S particles as well as a substantial increase in 114S material—observations that are in accord with the assumption that the 80S particle is the monomer and the 114S particle a magnesium-stabilized dimer. The 145S aggregates were not significantly reduced in number by ribonuclease. We do not know whether that is because the digestion did not

![Fig. 1.—Sedimentation of muscle ribosomes on linear sucrose gradients: effect of diabetes and insulin. One group of diabetic rats was given (intraperitoneally) 5 units of insulin 5 min before the ribosomes were isolated. An aliquot of the ribosome preparation analyzed on each gradient was assayed for its ability to catalyze the transfer of radioactivity from trNA-C14-phenylalanine to protein. The results (in cpm/30 µg of ribosomal RNA) were: normal, 477; diabetic, 304; diabetic treated with insulin, 442. The sedimentation coefficients were calculated from schlieren patterns obtained in the analytical ultracentrifuge (cf. Fig. 2).](image-url)
proceed far enough or because some of the 145S assemblies are stabilized by magnesium rather than bound to messenger RNA. Ribonuclease produced little change in the proportion of 105S particles, but did reduce the percentage of 90S particles.

**Effect of diabetes:** The ribosomal preparations from alloxan-diabetic animals contain a far smaller proportion of larger aggregates (sedimentation coefficient of 145 or more) and a greater percentage of the smaller particles (80–114S) (cf. Fig. 1 and Table 1). A particularly striking effect of diabetes is the increase in the rela-

**TABLE 1**

Sedimentation Coefficients and Per Cent Composition of Ribosomal Particles From Skeletal Muscle

<table>
<thead>
<tr>
<th>Sedimentation Coefficient (S)</th>
<th>Normal (3)</th>
<th>Diabetic (3)</th>
<th>Diabetic + RNase (1 µg/ml for 10 min at 0°C)</th>
<th>Per Cent of Total Ribosomal Particles</th>
<th>Diabetic (2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>60</td>
<td>22</td>
<td>5</td>
<td>41</td>
<td>1.00</td>
<td>22</td>
</tr>
<tr>
<td>80</td>
<td>3</td>
<td>5</td>
<td>3</td>
<td>0.05</td>
<td>6</td>
</tr>
<tr>
<td>105</td>
<td>4</td>
<td>6</td>
<td>5</td>
<td>0.10</td>
<td>6</td>
</tr>
<tr>
<td>114</td>
<td>10</td>
<td>10</td>
<td>6</td>
<td>0.10</td>
<td>9*</td>
</tr>
<tr>
<td>145</td>
<td>10</td>
<td>10</td>
<td>9*</td>
<td>0.10</td>
<td>9*</td>
</tr>
<tr>
<td>&gt;175</td>
<td>55</td>
<td>63</td>
<td>63</td>
<td>1.00</td>
<td>45</td>
</tr>
<tr>
<td>&gt;175</td>
<td>75</td>
<td>75</td>
<td>75</td>
<td>1.00</td>
<td>45</td>
</tr>
</tbody>
</table>

The values were calculated from schlieren patterns obtained by sedimentation of ribosome preparations in the analytical ultracentrifuge; the procedure is described in Methods. The results are the mean of the analysis of the number of preparations in parentheses.

* The values for the "145S region" were obtained by sedimentation of ribosome preparations in the analytical ultracentrifuge; the procedure is described in Methods. The results are the mean of the analysis of the number of preparations in parentheses.

† There was no distinct peak with a sedimentation coefficient greater than 145.

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tive number of 105S particles and the presence of a particle having a sedimentation coefficient of 90; the latter particle is clearly seen in the schlieren pattern obtained in the analytical ultracentrifuge (Fig. 2).

**Effect of insulin:** The administration of insulin completely reversed the effect of diabetes on the sedimentation of muscle ribosomes (Table 1). The hormone caused a reaggregation of the smaller particles to form polysomes, a marked decrease in the number of 105S particles, and the disappearance of the 90S particle; what is more, insulin did so as quickly as it increases protein synthesis, that is, in 5 minutes (Fig. 1). Obviously, the hormone acts rapidly to recruit ribosomes for the formation of large assemblies. There was a close correlation between the effect of insulin on the sedimentation of ribosomes and on their capacity to synthesize protein (Fig. 1).

**Effect of actinomycin:** An experiment was carried out to determine if the insulin-mediated recruitment of ribosomes to form polysomes required the synthesis of new RNA, and more specifically whether it required the synthesis of messenger RNA. Animals were treated with an amount of actinomycin (1 mg) shown before to effectively inhibit RNA synthesis in muscle. Treatment of animals with the antibiotic 75 minutes before they were killed did not significantly alter the sedimentation of ribosomes (Fig. 3), a finding consistent with the surmise that messenger RNA in muscle is relatively stable. After administration of actinomycin, in the all but complete absence of DNA-dependent RNA synthesis, insulin still brought about a reversal of the sedimentation pattern characteristic of ribosomes from diabetic animals.

If insulin can cause the formation of polysomes in the absence of RNA synthesis, then there must be a reservoir of messenger RNA in the muscle of diabetic animals, RNA which is not utilized. Insulin appears to condition, in some manner, the binding and translation of that preformed messenger RNA, accounting in that way

![Graph](image-url)
for the greater percentage of polysomes and the increase in ribosomal protein synthesis.

**Effect of cycloheximide:** In a parallel experiment, animals were treated with cycloheximide (5 mg) 75 minutes before they were killed and the ribosomes were isolated; 5 mg of cycloheximide is sufficient to inhibit protein synthesis in muscle by 96 per cent. The inhibition of protein synthesis by cycloheximide altered the ribosomes so that their sedimentation profile exactly resembled the pattern obtained with diabetic ribosomes (Fig. 4). A “diabetic-like profile” was also observed when ribosomes from puromycin-treated or starved (96 hr) animals were analyzed. Obviously, the “diabetic changes” in muscle ribosomes—the appearance of the 90S particle, the increase in the proportion of 105 and 114S particles, and the decrease in polysomes—is not unique to diabetes but rather is a typical change that occurs whenever protein synthesis is decreased. (Insulin is absent or present only at a reduced concentration in the plasma of diabetic and starved animals. We do not know the circulating titer of insulin after treatment with cycloheximide or puromycin; if it were reduced, then the “diabetic-like profile” might still be due to insulin deficiency.)

Cycloheximide inhibition of protein synthesis produced no further alteration in

![Figure 4](image-url)
the sedimentation of ribosomes from diabetic animals (Fig. 4), but did prevent the reaggregation of ribosomes that ordinarily occurs when insulin is administered to diabetic animals (Fig. 4). Recruitment of ribosomes by insulin to form polysomes requires either the synthesis of a specific protein(s) or protein synthesis per se.

The maintenance of a normal complement of polysomes requires nearly constant protein synthesis, despite the presence in muscle of a normal amount of messenger RNA. A reduction in the number of polysomes by cycloheximide was detected 15 minutes after administration of the antibiotic and was maximal in 30 minutes. If there is a specific protein(s) that is essential for the formation and the maintenance of ribosomal assemblies, the protein(s) is relatively unstable. We presume that cycloheximide has no effect on the sedimentation of ribosomes from diabetic animals because the specific protein(s) is not made in the diabetic, so further inhibition of protein synthesis is of no moment.

In contrast to our results with muscle ribosomes, cycloheximide has been reported not to alter the polysome pattern of ribosomes from reticulocytes even after complete inhibition of protein synthesis; the antibiotic did prevent the reassembly of polysomes in sodium fluoride-treated reticulocytes. The interpretation was that cycloheximide inhibited some process in protein synthesis necessary for the formation of polysomes.

Discussion.—There is a close correlation between the extent of aggregation of ribosomes and their ability to catalyze protein synthesis. Diabetes reduces the number of large ribosomal assemblies that can be isolated from muscle and decreases the efficiency with which the ribosomes synthesize protein. The sedimentation characteristics of the ribosomes from diabetic animals and their function are restored to normal by insulin administration in only five minutes.

At first we focused our attention on the 90S particle and the other changes that seemed unique to the diabetic preparation. We now know, however, that the 90S particle and the other alterations occur in a number of circumstances, all characterized by a decrease in the synthesis of protein by muscle.

The effect of insulin in increasing protein synthesis in muscle has the following characteristics: it is independent of the action of the hormone to increase the transport of glucose or amino acids. The stimulation of protein synthesis does not require the simultaneous synthesis of RNA. When equal numbers of ribosomes from normal and from diabetic animals are compared in an assay system (where protein synthesis is directly proportional to the concentration of ribosomes), the ribosomes from normal animals are more efficient than those of diabetic animals. The observations that the diabetic ribosomes appear deficient in messenger RNA and that the efficiency of ribosomal protein synthesis is increased by insulin in the absence of RNA synthesis (the hormone's effect is not inhibited by actinomycin) may at first appear inconsistent. But that is not necessarily so. There may be in muscle of diabetic animals sufficient messenger RNA which is not properly used in the absence of insulin. There is another important characteristic of the action of insulin; the effect on the ribosomes requires protein synthesis.

There are two interpretations that accord with all the observations: protein synthesis per se may be required for the formation of polysomes and for the action of insulin, or, alternatively, the hormone may be necessary for the production of a specific protein(s) that is required for the binding and translation of natural mes-
senger RNA by the ribosome. The experiments with cycloheximide do not distinguish between the two possibilities. There are, however, observations that give circumstantial support to the suggestion that a specific protein is needed. Stanley and colleagues,17 and others,18–20 have found that there are protein factors that regulate translation of natural messenger RNA by ribosomes from Escherichia coli. (The factors are not necessary for translation of polyuridylic acid and certain other synthetic polynucleotides.17, 20) The protein factors appear to function by facilitating the attachment of ribosomes to messenger RNA20 and the initiation of protein synthesis.17 It is possible that similar proteins are present in mammalian cells and that their synthesis, and hence their availability, is controlled by insulin and by other hormones. Kaji and Kaji,21–23 Zak et al.,24 and Nair et al.25 have demonstrated that mild trypsic treatment (very little soluble material is released) renders ribosomes deficient in their ability to carry out protein synthesis. The deficiency is the result of an impaired ability to bind transfer and messenger RNA, and hence to form polysomes. The protein sensitive to trypsin may be the same or similar to the one we suggest is necessary for translation of messenger RNA by muscle ribosomes.

If insulin causes the synthesis of a specific protein, we assume the protein is not required for the binding and translation of polyuridylic acid, as distinct from natural messenger RNA, for the stimulation of protein synthesis by the homopolymer is as great with ribosomes from muscle of diabetic animals as with ribosomes from normal animals.4 There are known to be differences in the utilization of polyuridylic acid and natural messenger RNA by ribosomes,26 differences to be attributed in part to the relative amounts of secondary structure.27 Moreover, ribosomes bind very efficiently to polyuridylic acid and form polysomes in circumstances where their affinity for natural messenger RNA is much smaller,28–30 presumably because the latter have specific initiation sites.

A very pertinent observation is that ribosomes from L cells exposed to interferon lose their ability to bind viral RNA, but preserve their capacity to associate with the cell’s messenger RNA;21 it would appear that the ribosomes are altered selectively by a protein (interferon) so only a particular class of messages are bound and translated. The importance of the finding—its relevance for ideas concerning the means of the regulation of protein synthesis in animal cells and the analogy to our explanation of the action of insulin on protein synthesis in muscle—has not escaped our notice.

Little is known of the function of ribosomal proteins. It is possible that one or more of them serve in the binding of transfer and messenger RNA to the ribosome and that the process is susceptible to hormonal regulation.

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