Protein Synthesis by Ribosomes from Heart Muscle: Effect of Insulin and Diabetes

Abstract. *Incorporation of phenylalanine into protein by ribosomes from the heart muscle of rats is decreased when the ribosomes are from alloxan-diabetic animals. Protein synthesis is increased when the ribosomes are obtained from nondiabetic animals 1 hour after treatment with insulin. The change due to insulin appears to be an alteration in the function of the ribosome.*

Insulin stimulates protein biosynthesis in muscle, but the means whereby the hormone does so is not known (*1*). An analysis of the mechanism would be materially aided by the preparation of a cell-free ribosomal system, from muscle, capable of catalyzing the incorporation of significant amounts of amino acid into protein. We have prepared ribosomes from heart muscle and now report on the influence of insulin and diabetes on the incorporation of radioactivity from soluble RNA (sRNA) charged with C^{14}-phenylalanine into protein.

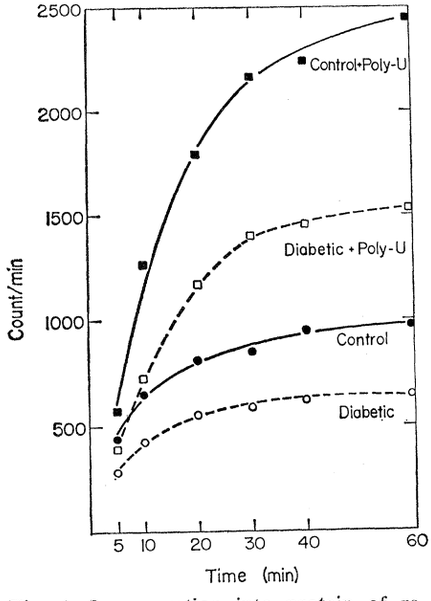

Fig. 1. Incorporation into protein of radioactivity from sRNA–C^{14}-phenylalanine by ribosomes from heart muscle of diabetic rats. Diabetes was induced by the rapid intravenous injection of alloxan monohydrate (60 mg/kg), and the surviving rats were used 7 days later, at which time the average blood glucose concentration (in animals that had not been fasting) was 736 mg per 100 ml of blood (range 680 to 990). The reaction was carried out in a final volume of 1 ml at 37°C. The assay mixture contained: 50 μmole of tris-HCl buffer, *p*H 7.8; 15 μmole of $MgCl_2$; 80 μmole of KCl; 1 μmole of ATP; 0.2 μmole of guanosine triphosphate; 10 μmole of β-mercaptoethanol; 10 μmole of phosphoenolpyruvate; 100 μg of pyruvate kinase; 2.5 mg of dialyzed 105,000*g* supernatant protein from liver; 150 μg of *Escherichia coli* sRNA charged with C^{14}-phenylalanine (9.52×10^3 count/min); and 200 μg of ribosomal RNA. The amount of polyuridylic acid, when present, was 125 μg. The ribosomes from control animals had an absorbancy ratio (260:280 mμ) of 1.75; those from diabetic animals, of 1.68.

Ribonucleoprotein particles were prepared from heart muscle of Sprague-Dawley rats (130 to 160 g). The whole-tissue homogenate was centrifuged at 105,000*g*, the sediment was treated with deoxycholate, and ribosomes were prepared by differential centrifugation of the deoxycholate-treated material. The procedure yields particles of reasonable purity and with characteristics similar to those of ribosomes from other mammalian tissues (*2*). Soluble RNA from *Escherichia coli* was charged with C^{14}-phenylalanine (297 mc/mmole) and 19 additional C^{12}-amino acids (*3*). The "transfer enzyme" was prepared from the soluble fraction of homogenate of rat liver; the homogenates were centrifuged at 105,000*g*, and the supernatant was dialyzed against buffer (0.01*M* tris-HCl, *p*H 7.8; 0.01*M* $MgCl_2$; 0.01*M* KCl; 0.005*M* β-mercaptoethanol) for 4 hours. In the assay, the number of aminoacyl transfers from sRNA–phenylalanine-C^{14} to protein was proportional to the concentration of ribosomes. The exact conditions of the assays are in the legends of Figs. 1 and 2.

Insulin added in vitro to ribosomes from heart muscle did not affect the ability of the particles to catalyze the incorporation of radioactivity from sRNA–phenylalanine-C^{14} into protein, whether or not polyuridylic acid was present. (The lack of effect of the hormone when it is added in vitro to the cell-free system is a disappointment, but no alteration of the experimental design or circumstances has yet changed the outcome.) However, ribosomes isolated from the heart muscle of animals made diabetic with alloxan were less effective in catalyzing the transfer of C^{14}-phenylalanine from sRNA to protein. The difference was generally small; incorporation into protein was decreased by diabetes 10 to 20 percent on the average, but with greater duration of the diabetes (7 days) the difference was considerably increased (Fig. 1). The defect in protein synthesis attributable to the diabetes was still manifest in the presence of artificial template RNA (in this case, polyuridylic acid); what is more, the defect persisted at several concentrations of polyuridylic acid (50 to 200 μg/ml) and at each concentration of ribosomes (50 to 250 μg/ml of ribosomal RNA) tested.

It is never certain that a particular biochemical alteration occurring in diabetes is attributable primarily to a deficiency of insulin. The decrease in the transfer of C^{14}-phenylalanine from sRNA to protein by ribosomes from animals made diabetic with alloxan is no exception, and it, too, may be the secondary consequence of some other change. However, that does not seem the most likely possibility, for the decrease in protein synthesis was detected as early as 2 days after the administration of alloxan and persisted for several weeks. Moreover, the defect attributable to the diabetes was rapidly reversed by the administration of insulin. Ribosomes from diabetic animals treated with insulin 1 hour before the animals were killed showed a 33 percent increase in the number of aminoacyl transfers from sRNA to protein [untreated diabetic, 757 ± 35; insulin-treated diabetic, 1008 ± 37—the results, in count/min per 100 μg of ribosomal RNA, are the mean ± the standard error of the mean (SEM) of three observations; the difference is significant, $P < 0.01$].

While insulin added in vitro did not influence incorporation, the ribosomes isolated from animals given insulin 1 hour before they were killed showed an increase in the catalysis of incorporation of C^{14}-phenylalanine into protein (Fig. 2). The stimulation due to the administration of insulin was not great, being on the average about 15 percent; nonetheless, the difference was reproducible and, in two separate experiments, statistically significant (untreated control, 730 ± 27; insulin-treated, 807 ± 16—the results, count/min per 100 μg of ribosomal RNA, are the mean ± SEM of three observations, and the difference is significant, $P < 0.02$). We do not know why insulin did not produce a more substantial stimulation of protein synthesis. Perhaps "back-

ground" synthesis was high owing to the secretion of endogenous insulin, or the effect of insulin was in part counterbalanced by the secretion of adrenal medullary and adrenal cortical hormones in response to hypoglycemia; epinephrine (*4*) and adrenal cortical steroids (*5*) are inhibitory of protein synthesis in muscle.

The assay was carried out in circumstances in which incorporation was proportional to the concentration of ribosomes; that is, where all other factors necessary for protein synthesis were added in optimal or excess amounts. In those circumstances, the insulin effect was present even when polyuridylic acid was added in concentrations from 25 to 150 μg/ml; moreover, the stimulation due to insulin treatment was observed at each of several concentrations of ribosomes (50 to 250 μg/ml of ribosomal RNA).

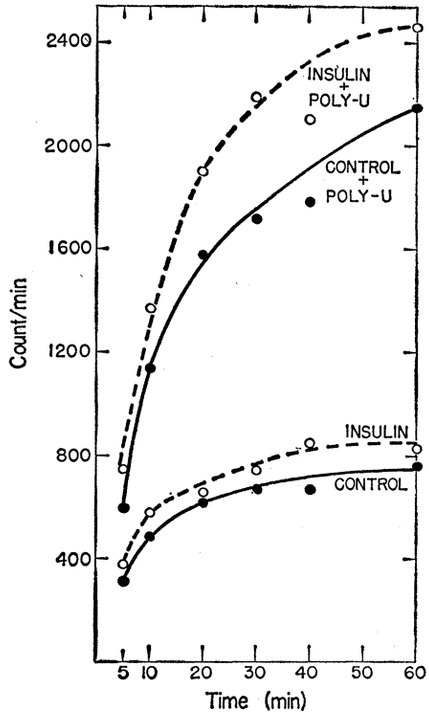

Fig. 2. Incorporation into protein of radioactivity from sRNA charged with C^{14}-phenylalanine by ribosomes from heart muscle of insulin-treated animals. The treated animals received 1 unit of insulin intraperitoneally 1 hour before they were killed; at that time the average blood glucose concentration was 43 mg per 100 ml of blood (range 31 to 77). The assay was carried out as described in Fig. 1, except that 150 μg of sRNA from *Escherichia coli* charged with C^{14}-phenylalanine (8.47×10^3 count/min) and 100 μg of ribosomal RNA were added. The amount of polyuridylic acid, when present, was 100 μg. The ribosomes from control animals had an absorbancy ratio (260:280 mμ) of 1.73; those from insulin-treated animals, an absorbancy ratio of 1.74.

The results have relevance for several of the theories of insulin action. The accelerated rate of protein synthesis due to insulin (or the decreased rate in its absence) is not likely to have resulted from an alteration of the rate of transport of amino acids (*6*), because synthesis was from sRNA "charged" with a full complement of amino acids, and the sRNA was added in excess to ribosomes from both normal and insulin-treated animals; for the same reason, the increase in protein synthesis is not likely to have resulted from an increase in the transport of any other substrate. The possibility that insulin affected the ribosomes as the result of an increase in the transport of some critical substrate during the hour before the ribosomes were isolated cannot, of course, be excluded. However, that alternative requires that the change, once initiated, persists even after the ribosomes are isolated and the concentration of substrates in the assay system are made equal for the control and experimental groups. The results also make it less likely that the stimulation of protein synthesis by insulin is the secondary result of a stimulation of the generation of adenosine triphosphate (ATP), of the distribution of ATP in the cell (*7*), or of the turnover of high-energy phosphate compounds (*8*), for all were, once again, added to the assay system in optimal or excess amounts. Nor do the results support the idea that the crucial action of insulin is on the cell's cytostructure (*9*). Finally, the defect that occurs in the absence of insulin would not appear to be due to a deficiency of template RNA (*10*), for the defect persists in the presence of even large amounts of polyuridylic acid just as does the increased incorporation of amino acid into protein by ribosomes from insulin-treated animals. The results do support the suggestion that the locus of action of insulin in stimulating protein synthesis is the ribosome. Conceivably, the hormone produces an alteration in the ribosome of a type that leads to a modification in the translation of messenger RNA.

OLIVER R. RAMPERSAD
IRA G. WOOL
Departments of Physiology and Biochemistry, University of Chicago, Chicago, Illinois

References and Notes

1. I. G. Wool, in *Action of Hormones on Molecular Processes*, G. Litwack and D. Kritchevsky, Eds. (Wiley, New York, 1964), p. 422.
2. O. Rampersad, R. Zak, M. Rabinowitz, I. G. Wool, L. DeSalle, *Biochim. Biophys. Acta*, in press.
3. G. von Ehrenstein and F. Lipmann, *Proc. Nat. Acad. Sci. U.S.* **47**, 941 (1961).
4. I. G. Wool, *Amer. J. Physiol.* **198**, 54 (1960).
5. ——— and E. I. Weinshelbaum, *ibid.* **197**, 1089 (1959); E. I. Weinshelbaum and I. G. Wool, *Nature* **191**, 1401 (1961).
6. D. M. Kipnis and M. W. Noall, *Biochim. Biophys. Acta* **28**, 226 (1958).
7. J. Tepperman and H. Tepperman, *Pharmacol. Rev.* **12**, 301 (1960).
8. H. Clauser, P. Volfin, D. Eboué-Bonis, *Gen. Comp. Endocrinol.* **2**, 369 (1962).
9. M. E. Krahl, *Perspectives Biol. Med.* **1**, 69 (1957).
10. I. G. Wool and A. J. Munro, *Proc. Nat. Acad. Sci. U.S.* **50**, 918 (1963).

11. Supported by Life Insurance Medical Research Fund, the John A. Hartford Foundation, and NIH (AM-04842). One of us (I.G.W.) is the recipient of a PHS research career development award.

17 June 1965

Ultraviolet Damage to Bacteria and Bacteriophage at Low Temperatures

Abstract. *The survival of* Escherichia coli *B/r WP2 (tryptophan-requiring) from ultraviolet irradiation when suspended in 0.067*M *phosphate buffer (pH 7) has been studied over the temperature range 22° to −269°C. In unfrozen suspensions there was no appreciable change in sensitivity between 22° and −10°C. The sensitivity in the presence of ice progressively increased by a factor of 7 when the temperature was lowered to −79°C. Between −79° and −196°C the sensitivity decreased to less than four times the sensitivity at 22°C and was not appreciably different at −269°C. Evidence from experiments with bacteriophage T1 and* E. coli *WP2 HCR− (a strain unable to excise thymine dimers) indicates that a new, qualitatively different lesion, less amenable to repair, may replace the thymine dimer in* E. coli *irradiated at −79°C.*

Many physicochemical properties of liquids and solutions alter abruptly in the process of cooling to the frozen state. From present knowledge of the interaction of ultraviolet light with organic molecules in solution (*1*) one would anticipate that the efficiency of the resulting photochemical changes would also change abruptly with change of state (*2*). One example of such a change is the enhancement of the dimerization of nucleotide bases when