Protein Synthesis by Heart Muscle Ribosomes: An Effect of Insulin Independent of Substrate Transport

Abstract. *Ribosomes from heart muscle of diabetic animals perfused for 15 minutes with insulin, but without added substrate, were more active in the catalysis of protein synthesis than were ribosomes from hearts perfused without insulin. This effect of insulin on the efficiency of protein synthesis seems not to result from transport of some critical substrate into muscle, nor is the effect on ribosomes mediated by a product of a tissue other than muscle.*

Insulin stimulates protein synthesis in muscle, but exactly how is unknown (*1*). Our analysis of the problem was materially aided by the demonstration that ribosomes from heart muscle of rats catalyze the transfer of significant amounts of radioactivity from transfer RNA (tRNA)-phenylalanine-C^{14} into protein (*2*). Using that system, we found (*3*): (i) that insulin added in vitro to ribosomes from heart muscle did not alter their ability to carry out protein synthesis; (ii) that ribosomes from animals made diabetic with alloxan incorporated less amino acid into protein than those from normal animals did; and (iii) that administration of insulin to normal and, especially, to diabetic animals 1 hour before they were killed resulted in increased ribosomal protein synthesis.

The experiments with ribosomes from animals treated with insulin have relevance to several of the theories of the hormone's action. The accelerated rate of protein synthesis is not likely to have resulted from an increase in the net rate of transport of amino acids (*4*), for synthesis in vitro is from tRNA acylated with a full complement of amino acids. Moreover, the aminoacyl-tRNA is added in equal amounts to the ribosomes from control animals and animals treated with insulin. Similarly, the increase in protein synthesis is not likely to have been the result of an increase in the transport of any other substrate. However, the experiments do not allow one to rule out with certainty the possibility that the effect of insulin on the ribosome is the secondary result of an increase in the transport of some critical substrate in the hour between the administration of insulin and the isolation of the ribosomes. That interpretation requires that the change in the ribosome, once initiated by the substrate, persist even after the ribosome is isolated and that the concentrations of all substrates and co-factors in control and experimental assay systems be made equal.

Table 1. Incorporation into protein of radioactivity from tRNA-C^{14}-phenylalanine by ribosomes from heart muscle of diabetic animals perfused with Krebs-Henseleit bicarbonate buffer with (+) and without (0) insulin.

Insulin	Incorporation (count/min)	Increase in incorporation (%)
0	387	
+	479	24
0	281	
+	366	30

Male Sprague-Dawley rats (120 to 140 g) were fasted overnight and then made diabetic by the rapid intravenous administration of alloxan monohydrate (60 mg/kg of body weight); the surviving rats were used 4 days later, at which time the average concentration of glucose in the blood (in animals that had not been fasting) was 426 mg/100 ml of blood in one experiment and 500 mg/100 ml in another. The procedure for the removal of hearts and the apparatus and technique used for their perfusion with a small volume of recirculating medium was essentially that described by Morgan, Henderson, Regen, and Park (*5*) and slightly modified by Scharff and Wool (*6*). Perfusion was for 15 minutes with Krebs-Henseleit bicarbonate buffer (*7*) with or without insulin (0.1 unit/ml). Ribosomes were prepared (*2*) from groups of perfused hearts (usually 20) and assayed for their ability to effect the transfer of radioactivity from C^{14}-phenylalanyl-tRNA to protein in a system in which the extent of protein synthesis was directly proportional to the concentration of ribosomes (*3*). The reaction was carried out in a final volume of 1 ml; incubation was for 30 minutes at 37°C. The assay mixture contained: 50 μmole of tris-(hydroxymethyl) aminomethane (Tris)-HCl buffer, *p*H 7.8; 15 μmole of $MgCl_2$; 80 μmole of KCl; 1 μmole of adenosine-triphosphate (ATP); 0.4 μmole of guanosine triphosphate; 10 μmole of β-mercaptoethanol; 10 μmole of phosphoenolpyruvate; 100 μg of pyruvate kinase; 2.5 mg of dialyzed protein from the supernatant fluid of homogenized liver centrifuged at 105,000*g* (*3*); 125 μg of *Escherichia coli* tRNA charged with C^{14}-phenylalanine (366 μc/μmole) and 19 additional C^{12}-amino acids (*8*) —the amount of radioactivity was 5.31 $\times$ 10^3 count/min; and ribosomes (60 μg of ribosomal RNA). The method for the isolation of protein and the determination of its radioactivity has been described (*9*).

In two experiments, ribosomes from heart muscle of diabetic animals perfused for 15 minutes with insulin, but without added substrate, were more active in carrying out protein synthesis than were ribosomes from heart muscle of diabetic animals perfused without insulin (Table 1).

It would appear that the effect of insulin on the efficiency of protein synthesis by ribosomes is not derived from the net transport of a critical substrate into muscle, for the effect was obtained when no substrate was added to the extracellular fluid. Our experiments do not exclude the possibility that the effect of insulin in increasing protein synthesis is secondary to an influence on the transmembrane distribution of ions. However, there is no compelling evidence in support of that possibility; indeed, there is evidence against it (*10*). The results are not inconsistent with the idea that the effect of insulin, at least with respect to protein synthesis, is on some intracellular process.

In previous experiments (*3*) the effect of insulin on ribosomal protein synthesis has required that the hormone be administered in vivo. For that reason the first site of insulin action could not be identified. It was possible that insulin acted initially on a tissue or organ other than muscle, causing the release of some substance that then affected the muscle ribosome. Our results clearly indicate that the effect of insulin is not mediated by a product of another tissue, but is directly on muscle.

WILLIAM S. STIREWALT
IRA G. WOOL
Departments of Physiology and *Biochemistry, University of Chicago, Chicago, Illinois*

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Rheumatic-like Cardiac Lesions in Mice

Abstract. *A single intraperitoneal injection of a sterile extract of sonically disrupted group A streptococcal cells induced an inflammatory process in the hearts of mice. The cardiac lesions, in terms of their distribution and histological features, are similar to the cardiac lesions of rheumatic fever.*

The properties of a toxic moiety found in extracts of sonically disrupted group A streptococcal cells have been described (*1*). The inflammatory reaction of rabbits that had been given a single intradermal injection of this toxic moiety was characterized by remissions and exacerbations observed over a 90-day period. The toxic material appeared to be cell wall fragments of a limited range of particle sizes, made up of C carbohydrate and mucopeptide (*2*). These observations served as a basis for the concept that fragments of group A streptococcal cell walls can act as durable toxic materials and can induce the acute and chronic inflammatory reactions associated with nonsuppurative sequelae of group A streptococcal infections, including rheumatic fever. This concept has been tested in experiments in which extracts of sonically disrupted group A streptococcal cells (type 3, strain D58) were injected intraperitoneally into mice.

Each of 54 Swiss Webster mice weighing from 20 to 22 g was injected intraperitoneally with 4 ml of sterile crude extract of sonically disrupted group A streptococcal cells suspended in 0.038*M* phosphate buffer *p*H 7.6. The extract contained 890 μg/ml of rhamnose (*3*). One group of controls consisted of 54 mice, each of which was injected with 4 ml of crude extract of sonically disrupted group D streptococcal cells suspended in the same buffer. This extract contained 899 μg/ml of rhamnose. A second group of controls consisted of 48 mice, each of which was injected with 4 ml of the buffer in which the cell extracts were suspended. Twenty-nine mice that received the group D extract died within 96 hours after injection. The mice that received buffer and the mice that received group A streptococcal extract were divided into groups and sacrificed at 1, 2, and 3 days, and at weekly intervals for 8 weeks after injection. Groups of mice that survived injection of group D streptococcal extract were sacrificed at 1, 2, and 3 days and at weekly intervals for 6 weeks after injections. Blocks of tissues were fixed in 10 percent buffered formalin, and sections were stained with hematoxylin and eosin.

The extracts of group A and D streptococcal cells stimulated a generalized acute fibrinopurulent peritonitis which evolved into a chronic focal granulomatous process and continued throughout the 8-week period of observation in animals receiving extract of group A cells. The chronic peritonitis was not observed in animals injected with the extract of group D cells and sacrificed 4 to 6 weeks later. Both extracts caused focal granulomas of the liver. Granulomas of the group D material were not observed after 4 weeks, although they continued to be present for 8 weeks in animals receiving extracts of group A cells. The spleens of both groups of animals showed an acute inflammatory reaction in animals sacrificed during the first 3 days of the experiment. No chronic focal lesions of the spleen were observed. In kidneys from animals sacrificed 2 and 3 weeks after injection with extracts of group A cells, a moderate degree of thickening of the stroma of the glomeruli was associated with an increase in glomerular cells and a decrease in the blood in the glomerular capillaries. Kidneys from the other animals showed no lesions.

With the exception of a single microscopic focus of interstitial inflammation seen in the myocardium of one mouse sacrificed for study 48 hours after injection, no lesions were seen in the hearts or other organs of the animals injected with buffer. Hearts of animals that were injected with extracts of group D cells and sacrificed for study 1, 2, 3, and 7 days later showed scattered microscopic foci of myofiber degeneration and necrosis associated with accumulation of neutrophils and mononuclear cells. This acute reaction was limited to the myocardium and did not evolve into a chronic process. Hearts of animals of this group sacrificed for study 2, 3, 4, 5, and 6 weeks after injection showed no lesions.

No lesions were observed in the hearts of mice sacrificed 1 day after inoculation with extracts of group A streptococcal cells. Sections of the hearts of animals injected with extracts of group A streptococcal cells and sacrificed 2 days later revealed foci of myofiber degeneration and necrosis associated with accumulations of mononuclear cells. Hearts collected for study 3 days to 8 weeks after injection of extracts of group A streptococcal cells revealed an inflammatory process that involved the pericardium, myocardium, and endocardium of the left atrium and left ventricle, the coronary arteries, the mitral and aortic valves, and the proximal portion of the aorta. Focal inflammatory lesions of the pericardium consisted of an accumulation of neutrophils and mononuclear cells in edematous subepicardial tissue, and adjacent myocardium and collections of similar cells on the surface of the epicardium. Lesions of the myocardium observed in animals sacrificed 3 days to 2 weeks after injection were characterized by foci of necrosis surrounded by a variety of cells, including mononuclear and giant cells with irregular basophilic cytoplasm, indistinct cell borders, and nuclei of the type observed in Anitschkow cells (*4*) (Fig. 1). The granulomatous lesions observed in the hearts of animals sacrificed later in the experiment were similar to those described; the only differences were that the areas of necrosis were less prominent and there was an increase in the fibroblasts in and around the lesions. Some of the focal granulomatous lesions of the myocardium were adjacent to or surrounded a coronary artery (Fig. 2); others were not clearly related to an artery (Fig. 1). Changes in the cusps and roots of the mitral and aortic valve were noted in animals sacrificed 3 days to 8 weeks after injection of extracts of group A streptococcal cells (Figs. 3 and 4). There was edema of the stroma of the valves associated with accumula-