NUCLEAR RIBOSOMES, AN EARLY FACTOR IN TISSUE REPARATION [1]

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Received October 16, 1963

The role of cytoplasmic ribosomes in protein synthesis has been studied extensively in the past; more recent discoveries suggest (Noll et al, 1963) that the functional units or protein synthesis are ribosomal aggregates, "ergosomes", consisting of particles held together by m-RNA. The origin of cytoplasmic ribosomes is a matter of some speculation but a current favorite is that ribosomal RNA is synthesized in the nucleus.

The purpose of our report is to present some data which indicate that nuclear ribosomes are labeled earlier than cytoplasmic microsomes during tissue reparation, suggesting nuclear origin of the cytoplasmic microsomes. The studies were carried out on subcellular fractions of infarcted and normal cardiac muscle.

MATERIAL AND METHODS

Myocardial infarction was produced in dogs by ligation of branches of the anterior descending coronary artery, so that a homogeneous infarct was obtained.

Glycine-2-C^{14} (specific activity 15.1 mc/mMol) was injected intravenously, 30 μc per kg body weight. The animals were sacrificed

[1] This work was supported by Grant No. H-5043 from the U.S. Public Health Service, The American Heart Association, The Michigan Heart Association, Life Insurance Medical Research Fund, Tobacco Industry Research Committee, The Burroughs-Wellcome Fund, and The John A. Hartford Foundation.

at various intervals after infarction, from 4 hours up to 6 days. Four hours prior to death the fasting animals were injected with the labeled glycine.

Cell fractionation and nuclear extraction were performed according to the method of Sibatani et al (1962). The nuclear fraction (containing also the bulk of myofibrils) was able to incorporate in vitro labeled amino acids (C^{14} protein hydrolysate) into their proteins according to the method of Allfrey et al (1956).

The nuclear fractionation consisted of: (1) The extraction of nuclear ribosomes (N_I) together with other proteins of the soluble phase of the nucleus; the particles were then removed from the extract by centrifugation at high speed. (2) Extraction of DNA, RNA and nucleoproteins (N_{II}) with 1M NaCl. (3) Phenol treatment of the residue and centrifugation which resulted in the separation of an aqueous phase which overlay a considerable interphase layer, a phenol phase, and a sediment at the bottom of the tube. This phenol treatment separates the RNA into two phases: (a) an "aqueous phase" RNA fraction of relatively low activity (p-RNA), and (b) an "interphase" fraction with very high radioactivity (m-RNA).

The protein in the phenol phase and interphase was labeled N_{III} and the protein fraction from the sediment at the bottom of the tube was labeled N_{IV} and contains the bulk of contractile proteins. The nucleic acid fractions were hydrolyzed with 1 N NaOH or 1.6 N PCA and purified on charcoal columns. RNA and DNA were tested spectrophotometrically for purity with Beckman DK-2. The radioactivity of nucleic acids was determined in a liquid scintillation counter using the dioxane scintillator of Bray (1960). The protein fractions were treated with TCA at 70°C to remove possible residual mucleic acids; lipids were removed with ethanol, ethanol-ether and acetone. The protein was then washed neutral, dried and the radioactivity was

determined by suspending the powdered protein in a toluene-gel scintillator.

RESULTS AND DISCUSSION

An attempt is made in this study to follow the reparative process in cardiac muscle, following myocardial infarction, as connective tissue replaces the necrotic muscle.

Table I shows the specific activity of protein fractions from normal and infarcted tissue two days after infarction. The greatest increase in incorporation is observed in nuclear ribosomes, cytoplasmic microsomes and mitochondria, whereas there is subnormal incorporation into N_{IV} fraction, containing contractile proteins.

Table I

Specific activity of myocardial protein fractions in cpm/10 mg 2 days after infarction.

Sample	Normal*	Infarct
Cytoplasma	951	1249
Microsomes	1243	2239
Mitochondria	911	1739
Nuclear Ribosomes	890	2099
N_{II}	740	1122
N_{III}	606	837
N_{IV} **	590	464

*Normal cardiac muscle sample and the infarcted muscle sample were obtained from the same heart.

**N_{IV}-fraction contains the contractile protein. Isolated dog myosin subjected to identical treatment was collected as N_{IV}-fraction.

Table II illustrates the specific activity of some nucleic acid fractions and the increased activity in the infarcted tissue. Of particular interest is the marked difference in specific activity of "nucleolar-RNA" (p-RNA) and "messenger-RNA" (m-RNA).

Table II

Specific activity of nucleic acids in cpm/mg 2 days after infarction.

	Normal*	Infarct
Microsomal	891	4107
Mitochondrial	981	1518
Nuclear Ribosomal	323	1314
p-RNA	108	360
m-RNA	1.840	8.401

*Normal cardiac muscle sample and the infarcted muscle sample were obtained from the same heart.

Figure 1 shows the incorporation into subcellular protein fractions of infarcted tissue compared to the control sample as a function of time after infarction. Each point represents the mean of 3 to 7 experiments. The nuclear ribosomal fraction precedes all other fractions, followed by mitochondria and cytoplasmic microsomes. The N_{IV} fraction, containing contractile proteins, shows an early fall in incorporation and the incorporation remains low.

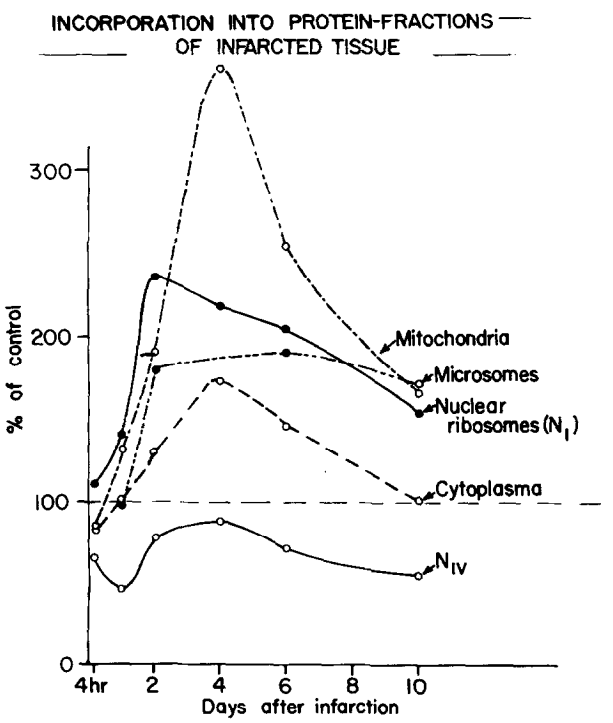

Fig. 1. Incorporation of glycine-2-C^{14} into subcellular protein fractions of infarcted heart muscle compared to normal left ventricular muscle of the same heart. N_{IV} = nuclear fraction, contains also the contractile proteins.

These data seem to indicate that immediately following infarction and destruction of myocardial tissue there is a rapid response and renewal of cell material replacing the necrotic muscle. The early renewal of cell material was further indicated by the incorporation of glycine-C^{14} into nucleic acids of the infarcted tissue (Fig. 2). The earliest and greatest increase in incorporation was observed in "nucleolar" or p-RNA, followed by nuclear ribosomal RNA. DNA shows an increase in activity 48 hours after infarction, which coincides with the appearance of new fibroblasts. The first step in the reparative process seems to be synthesis of nuclear material, followed by reconstruction of mitochondria and microsomes. The chronological order of labeling indicates the nuclear origin of cytoplasmic particles, and emphasizes the early increase in labeling of nuclear ribosomes.

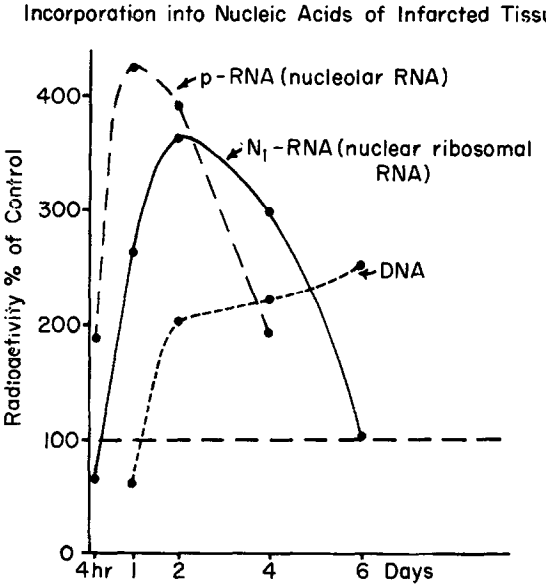

Fig. 2. Incorporation of glycine-2-C^{14} into nucleic acids of infarcted heart muscle compared to control muscle. Each point represents the mean value of 3-7 experiments.

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