Myosin Isozyme Synthesis and mRNA Levels in Pressure-Overloaded Rabbit Hearts


The in vivo synthesis rates of myosin isozyme heavy chains β and α were measured in right ventricular (RV) muscle at 2 and 4 days following pulmonary artery constriction in rabbits, together with measurements of their relative mRNA levels. The synthesis rate of β-myosin heavy chains was elevated in 2-day (0.27 ± 0.06 day⁻¹ or 2.5 ± 0.7 mg/g RV/day, mean ± SD) and in 4-day (0.25 ± 0.08 day⁻¹ or 2.8 ± 1.0 mg/g RV/day) pressure overload, when compared to untreated rabbits (0.15 ± 0.04 day⁻¹ or 1.5 ± 0.4 mg/g RV/day). However, the synthesis rates of α-myosin heavy chains in the same hearts were not altered significantly. There was a differential increase in the fractional synthesis rate of β vs. α heavy chains in 2-day and 4-day pressure overload and in 2-day shams, suggesting switching toward β heavy chain synthesis had occurred at these time points. β heavy chain synthesis, as a proportion of total (α + β) heavy chain synthesis, was significantly higher in 4-day pressure overload (78 ± 9%) than in 4-day sham rabbits (63 ± 6%). This increase in relative β-synthesis was associated with a significant increase in the relative proportion of β heavy chain mRNA level (76 ± 13% vs. 56 ± 7%). Furthermore, relative β-synthesis and the β-mRNA levels correlated linearly with each other in all experimental groups. We conclude that during the early stages of pressure overload 1) the synthesis rate of β-myosin heavy chains is accelerated without a reciprocal decrease in α-myosin heavy chain synthesis, and 2) an increase in β-myosin heavy chain expression appears to be achieved mainly by modulation of pretranslational events. (Circulation Research 1987;60:692–699)

Three myosin isozymes (V₁, V₂, and V₃) have been shown to exist in ventricular tissue of rabbit and rat hearts. V₁ and V₂ isozymes are homodimers consisting of 2 α and 2 β heavy chains, respectively. V₃ isozyme appears to be a heterodimer, composed of 1 α and 1 β heavy chain. The primary structure of the α- and β-myosin heavy chains differ on the basis of the sequence of the cDNA corresponding to their respective mRNA's. The proportions of myosin isozymes have been found to change under various conditions. During pressure overload, there is a shift toward the V₃ isozyme in the rabbit and rat heart. The proportion of this isozyme also is enriched in cardiac atrophy associated with hypothyroidism while it is reduced in hypertrophy due to thyrotoxicosis and exercise. Furthermore, relative β-synthesis and the β-mRNA levels correlated linearly with each other in all experimental groups. We conclude that during the early stages of pressure overload 1) the synthesis rate of β-myosin heavy chains is accelerated without a reciprocal decrease in α-myosin heavy chain synthesis, and 2) an increase in β-myosin heavy chain expression appears to be achieved mainly by modulation of pretranslational events. (Circulation Research 1987;60:692–699)

Materials and Methods

Animals and Pressure-Overloaded Preparation

Male New Zealand white rabbits (litter mates 5–6 weeks old, 700–900 g) were maintained on food and water ad libitum in the University of Vermont Animal Care Facility for approximately 4 days prior to surgery or killing. Antibiotics were not used.

Right ventricular hypertrophy was induced by reducing the diameter of the pulmonary artery by 50% with a spiral metal constrictor for 2 or 4 days. Unjected and sham-operated rabbits were studied for comparison.

Labelling of Myocardial Proteins With ³H-Leucine

In vivo labelling of rabbit heart tissue was accomplished using the constant infusion method described
by Everett et al. Each rabbit received approximately 15 mCi of $^3$H-leucine (L-[4,5-$^3$H]-leucine, Amersham Corp., Arlington Heights, IL) over 100 minutes. Multiple blood samples (75 μl) were taken throughout the infusion period from a vein in the uncatheterized ear to determine the specific radioactivity of plasma-free leucine. After 100 minutes, the rabbit was killed by a blow to the head and the heart quickly removed. The right and left ventricles were dissected and weighed. The RV was frozen immediately in liquid nitrogen, powdered, and stored at $-85^\circ$ C.

Preparation of Samples for Measurement of $^3$H-Leucine Radioactivity

Plasma samples were precipitated with cold ethanol and centrifuged. The supernate, containing plasma-free amino acids, was dried down and stored at $-20^\circ$ C for subsequent analysis of $^3$H-leucine specific activity (see below).

A separate set of experiments was conducted to allow measurement of tRNA-bound leucine specific radioactivity. Radiolabelled leucine was infused for 100 minutes, after which the chest was opened and a blood sample rapidly withdrawn from the heart. The tissue was then frozen quickly with clamps precooled in liquid nitrogen. The heart tissue was pulverized and stored at $-85^\circ$ C for further analysis. Transfer RNA was extracted and purified as previously described prior to determination of tRNA-bound leucine specific radioactivity (see below).

Separation of Myosin Isozyme Heavy Chains

Separation of cardiac myosin isozymes was carried out using the pyrophosphate polyacrylamide gel electrophoresis methods of Hoh et al., with a slight modification. Following electrophoresis, the gels were stained for 15 minutes in 0.1% Coomassie brilliant blue R-250, 50% methanol, and 10% acetic acid and destained in cold water. The purified $V_1$ and $V_3$ bands were removed with a razor blade using only gels in which the $V_1$, $V_2$ and $V_3$ bands were separated clearly (see Figure 1, left). Generally, 100 to 120 gel slices of each isozyme were obtained for each RV.

A control experiment was performed to test for contamination between $V_1$ and $V_3$ isozymes. Infusion of $^3$H-leucine was carried out in a propyl-thiouracil-treated rabbit in which only $V_3$ isozyme exists. Tissue extracts from this heart were mixed with those of the heart from another rabbit that was not infused and that contained predominantly $V_1$. The $V_1$ and $V_3$ bands of

![Figure 1](https://example.com/figure1.png)

**Figure 1.** Left: Pyrophosphate gel electrophoresis of native cardiac myosin isozyme sample from RV of a control rabbit. Original gel and densitometric scan are shown. Similar separation was obtained for all samples used in this study. $V_1$, $V_2$, and $V_3$ represent myosin isozyme differing mobility rates, using the nomenclature of Hoh et al. Right: SDS polyacrylamide gel (10%) of purified pyrophosphate $V_3$ isozyme. Pyrophosphate gel slices (10–15) were loaded on SDS gel separating myosin isozyme into heavy chains (HC) and light chain 1 (LC1) and light chain 2 (LC2) as described in "Materials and Methods."
the mixed sample were sliced out, the heavy chains separated on SDS gels as described below, and counted. No radioactivity was recovered in the V, band, indicating no contamination from the V, band.

The pyrophosphate gel slices containing V, or V, isozyme were then subjected to SDS-gel electrophoresis employing a modified procedure of Pagani and Julian22 to separate the myosin light chains from the heavy chains (Figure 1, right) prior to determination of the specific activity of leucine in protein (see below).

Finally, to determine the relative amount of each isozyme, several pyrophosphate gel slices from each RV preparation were quantitated by densitometry.28

Analysis of Total Myocardial Protein

Approximately 35 mg of frozen powdered right ventricular tissue was homogenized in extraction solution with a mM concentration of sodium pyrophosphate 40, sodium phosphate 20, pH 7.0, and ethylenediaminetetraacetate (EDTA) 0.5. Ten percent SDS was then added at a final concentration of 1%. The mixture was heated and centrifuged at room temperature. The protein concentration was measured in the supernatant.29 An aliquot also was used to determine leucine specific radioactivity, and the remaining supernate was stored at −20°C for the dansyl chloride assay.

Analysis of Total Myocardial Protein

Approximately 35 mg of frozen powdered right ventricular tissue was homogenized in extraction solution with a mM concentration of sodium pyrophosphate 40, sodium phosphate 20, pH 7.0, and ethylenediaminetetraacetate (EDTA) 0.5. Ten percent SDS was then added at a final concentration of 1%. The mixture was heated and centrifuged at room temperature. The protein concentration was measured in the supernatant.29 An aliquot also was used to determine leucine specific radioactivity, and the remaining supernate was stored at 10% glycerol and 0.1% mercaptoethanol at −85°C for determination of total myosin content (see below).

Analysis of 3H-Leucine Radioactivity

The specific radioactivity of leucine in α- and β-myosin heavy chains, myocardial RNA, tRNA, and plasma was determined using a ultramicro dansyl chloride assay.27,30 The 3H and 14C counts of the dansyl-leucine spot from the polyamide layer plate were generally at least four times background. An equation was derived to determine the amount of time needed to count both 14C and 3H to obtain an error of ≤5% (see Appendix).

Calculation of Synthesis Rates

An accurate measurement of protein synthesis using isotopic leucine requires information on the specific radioactivity of tRNA-bound leucine, the obligatory precursor for protein synthesis.31,32 To facilitate this measurement, the relation between the specific radioactivity of plasma-free leucine and tRNA leucine was determined. A plateau value of the specific radioactivity of plasma-free leucine was reached within 5 to 10 minutes (see Figure 2). The tRNA-leucine/plasma-free leucine specific activity ratio was 0.82 ± 0.05 (mean ± SD, n = 7) for control animals. This ratio was unaltered in pressure-overloaded rabbits. Thus, the value of 0.82 was used as a correction factor for measurements of synthesis rates based on specific activity of plasma-free leucine.

The fractional synthesis rate of protein synthesis was calculated as follows:

\[
K_s = \frac{SR \text{ leucine protein}}{\text{mean SR plasma free leucine} \times \text{time of infusion} \times 0.82}
\]

where Ks is rate constant expressed as fractional synthesis rate (day⁻¹), SR is specific radioactivity (dpm/pmol), and 0.82 is the tRNA-leucine/plasma-free leucine specific activity ratio. Synthesis rates were calculated as mg protein/g RV/day by multiplying the fractional synthesis rate by the protein concentration in right ventricular free wall.

Quantitation of Myosin Heavy Chains: Isotope Dilution Assay

Total myosin content was determined by the isotope dilution assay.33 A rabbit heart was labelled by constant infusion procedure using 30 mCi of 3H-phenylalanine (ICN Biochemicals, Cleveland, Ohio). Myosin was isolated from the labelled heart.10 By using a 3H-phenylalanine labelled-myosin standard, we were able simultaneously to measure content and synthesis of the myosin isozymes from the same heart, since dansyl-phenylalanine (content) and dansyl-leucine (synthesis) are completely separated in our chromatographic system.30

Measurement of the Relative Amounts of α- and β-Heavy Chain mRNA (α-mRNA and β-mRNA)

Total right ventricular RNA was extracted from 80–100 mg of powdered tissue according to the method of Towle et al.34 The relative amounts of α- and β-mRNA were measured employing nuclease S1 mapping technique.35 The cDNA probe (pMHCα 81/2) used in this study encodes the carboxy terminus plus

![Figure 2. Specific radioactivity of plasma-free leucine during infusion of [3H] leucine into an untreated control rabbit. Similar curves were obtained for all untreated and experimental rabbits. Experimental protocols are described in “Materials and Methods.”](image-url)
The 3'-untranslated region of α-myosin heavy chains. Thirty to sixty micrograms of total RNA were hybridized with 25,000-50,000 cpm of cDNA probe, which was 3' end-labelled with 32P (32P-αATP, specific activity 3,000 Ci/mmol, Amersham). After reacting with 1,000, 2,000, and 4,000 units of S1 nuclease, the digested products were separated on a 5% polyacrylamide-urea sequencing gel. Two bands with 126 and 42–46 nucleotides, representing α- and β-mRNA, respectively, were localized by autoradiography, excised, and counted. The ratio of both bands was the same for each S1 nuclease concentration.

Statistics
Statistical comparisons of untreated group with 2- and 4-day pressure overload and 2- and 4-day sham groups were carried out using the Bonferroni method (p < 0.05) that of the untreated rabbits in 2-day and 4-day pressure overload groups, respectively, again with no change in the sham groups (Table 2).

Changes in Synthesis of β- and α-Myosin Heavy Chains
The fractional synthesis rates of the β- and α-myosin heavy chains in hearts from untreated rabbits were 0.15 ± 0.4 and 0.12 ± 0.10 day⁻¹, respectively, as shown in Figure 3. The fractional synthesis rate of the β heavy chains was significantly higher in the 2-day (0.27 ± 0.06 day⁻¹, p < 0.01) and 4-day pressure overloaded RV (0.25 ± 0.08 day⁻¹, p < 0.05) than untreated animals. In contrast, the fractional synthesis rate of the α heavy chains was not significantly altered. Values for shams were not significantly different from the untreated group. The fractional synthesis rates of β heavy chains were significantly faster than α heavy chains in 2-day and 4-day pressure overload and in 2-day shams when both values were compared within individual tissue samples (paired t test, p < 0.05, Figure 3).

The synthesis rate (mg/g RV/day) of the β heavy chains was 1.7 times (p < 0.01) and 1.9 times (p < 0.05) in the 2-day pressure overload and 1.6 times (p < 0.05) in the 4-day pressure overload when compared to values of the untreated group. The fractional synthesis rate of the sham groups was not significantly different from the untreated rabbits.

*Results*

Body and Heart Weight
Body weights were not significantly different among groups (data not shown). RV weights normalized to the body weight or to total ventricular weight were significantly higher in the 2-day and 4-day pressure overload than untreated rabbits (Table 1). There also was an increase in normalized RV weight for the 2-day sham. This suggests that there may be effects of surgical stress on the right ventricle (see below).

Synthesis of Total Myocardial Protein
Commensurate with growth of tissue, the fractional synthesis rate of total RV protein was measured 1.9 times (p < 0.01) in the 2-day pressure overload and 1.6 times (p < 0.05) in the 4-day pressure overload when compared to values of the untreated group. The fractional synthesis rate of the RV groups was not significantly different from the untreated groups.

The synthesis rate of RV protein (mg protein/g RV/day) was 1.8 times (p < 0.01) and 1.6 times (p < 0.05) that of the untreated rabbits in 2-day and 4-day pressure overload groups, respectively, again with no change in the sham groups (Table 2).

<table>
<thead>
<tr>
<th>Group</th>
<th>RV (gm)</th>
<th>RV Body weight (gm/kg)</th>
<th>RV RV + LV × 100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated (n = 8)</td>
<td>0.37 ± 0.07</td>
<td>0.48 ± 0.07</td>
<td>25 ± 2</td>
</tr>
<tr>
<td>2-Day PO (n = 5)</td>
<td>0.56 ± 0.12*</td>
<td>0.74 ± 0.11†</td>
<td>32 ± 3†</td>
</tr>
<tr>
<td>4-Day PO (n = 4)</td>
<td>0.66 ± 0.15†</td>
<td>0.78 ± 0.19‡</td>
<td>33 ± 4‡</td>
</tr>
<tr>
<td>2-Day sham (n = 4)</td>
<td>0.51 ± 0.17</td>
<td>0.68 ± 0.06*</td>
<td>29 ± 3</td>
</tr>
<tr>
<td>4-Day sham (n = 4)</td>
<td>0.45 ± 0.05</td>
<td>0.53 ± 0.05</td>
<td>27 ± 2</td>
</tr>
</tbody>
</table>

Values are mean ± SD; PO, pressure overload; RV, right ventricle; LV, left ventricle.

*p < 0.05 vs. untreated rabbits; †p < 0.01 vs. untreated rabbits; ‡p < 0.001 vs. untreated rabbits.

<table>
<thead>
<tr>
<th>Group</th>
<th>Ks (day⁻¹)</th>
<th>Total right ventricular protein (mg/g RV/day)</th>
<th>Myosin heavy chains (mg/g RV/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated (n = 7)</td>
<td>0.16 ± 0.02</td>
<td>17.2 ± 4.0</td>
<td>1.46 ± 0.41</td>
</tr>
<tr>
<td>2-Day PO (n = 5)</td>
<td>0.31 ± 0.06†</td>
<td>30.2 ± 5.4†</td>
<td>2.53 ± 0.71†</td>
</tr>
<tr>
<td>4-Day PO (n = 4)</td>
<td>0.25 ± 0.05*</td>
<td>27.5 ± 8.8*</td>
<td>2.75 ± 0.98†</td>
</tr>
<tr>
<td>2-Day sham (n = 4)</td>
<td>0.18 ± 0.04</td>
<td>18.1 ± 2.9</td>
<td>1.48 ± 0.31</td>
</tr>
<tr>
<td>4-Day sham (n = 4)</td>
<td>0.19 ± 0.03</td>
<td>19.6 ± 4.0</td>
<td>1.70 ± 0.33</td>
</tr>
</tbody>
</table>

Values are mean ± SD; PO, pressure overload; Ks, fractional synthesis rate; RV, right ventricle.

*p < 0.05 vs. untreated rabbits; †p < 0.01 vs. untreated rabbits; ‡p < 0.001 vs. untreated rabbits.
FIGURE 3. The fractional synthesis rates of \( \beta \) and \( \alpha \) heavy chains for untreated, 2-day and 4-day pressure overload, and 2-day and 4-day shams. *\( p < 0.05 \) for \( \beta \) vs. \( \alpha \) heavy chain in same animal (paired t test); ?\( p < 0.05 \) for \( \beta \) heavy chain, treated vs. untreated rabbits.

Discussion

Synthesis of Total Protein and Myosin Heavy Chain Isozymes

We found that the fractional synthesis rate of total myocardial protein and myosin isoyme heavy chains were approximately 15%/day in the untreated rabbits after corrections for the specific radioactivity of tRNA-leucine. This value is slightly lower than that reported for in vivo synthesis of total protein in the rat heart\(^{38} \) and the same as those of \( \beta \)- and \( \alpha \)-myosin heavy chains in normal rabbit hearts.\(^{26} \)

There was an approximately twofold increase in the synthesis rate of both myocardial total protein and \( \beta \) heavy chains 48 hours after pressure overload was induced. A rapid increase at the onset of hypertrophic stress has been observed by others.\(^{39-41} \) Clearly, the increase in workload can very quickly be translated into the biochemical signals responsible for hypertrophy.

Everett et al\(^{36} \) showed that thyrotoxicosis leads in rabbit heart to a threefold increase in the fractional synthesis rate of \( \alpha \) heavy chain (which becomes predominant) while there is a reciprocal decrease in the synthesis of \( \beta \) heavy chains. We have confirmed the results of Everett et al in our own laboratory (data not shown). The results indicate reciprocal changes in isoyme synthesis do not occur during the early stage of pressure-overload hypertrophy. These data indicate a different control mechanism is brought into play in the two hypertrophy models.

Effects of Surgical Stress on Sham Animals

Heart weight normalized to body weight was significantly higher in 2-day sham rabbits than that in untreated.
Regulation of Myosin Isozyme Switching

Considering the multitude of known control steps in protein biosynthesis, it is likely that regulation of gene expression differs depending on the growth of stimuli. In our model of growth, pressure-overload-induced rapid enlargement of right ventricle, we addressed the question of whether switching toward β heavy chains is controlled at translational or pretranslational steps. This was attempted by comparing the relative synthesis of the two heavy chains with the level of their respective mRNAs in the same heart.

The effects of pressure overload on myosin isozyme switching were more obvious at 4 days than at 2 days post-treatment. Thus, in the 4-day pressure overload, both the relative β-synthesis and the relative β-mRNA level were significantly higher than those in 4-day shams. In the 2-day pressure overload group, neither the relative β-synthesis nor the β-mRNA level showed a difference from the 2-day sham animals, perhaps because of the aforementioned pressure overload-like effects of surgical stress. However, in this group as well, not only did β-mRNA levels correspond closely to the relative β-synthesis but also both were linearly correlated. Furthermore, the ratio of β-synthesis/β-mRNA to α-synthesis/α-mRNA in individual hearts in all cases was approximately 1.0 (1.1 ± 0.3), indicating that preferential translation of β-mRNA over α-mRNA was not the major reason for accelerated β heavy chain synthesis following pressure overload. Based on these results, we suggest that switching toward β heavy chains during development of pressure-overload hypertrophy is mainly due to modulation of pretranslational mechanisms and that any translational regulation is of secondary, if any, importance. Previous studies of thyrotropic hypertrophy in the rabbit and the rat have led to the same conclusion.

Finally, Medford et al studied a turnover rate of myosin heavy chain mRNA in mouse L6E9 cells in vitro and showed a relatively long half-life of heavy chain mRNA (50 hours). Should cardiac myosin heavy chain mRNAs in rabbit heart have such a long half-life, the observed level of mRNA is a sum of newly synthesized mRNAs and mRNA present prior to the experimental intervention. Studies of in vitro nuclear transcription would allow measurement of the synthesis rates of new mRNA and thus provide further insights into the pretranslational regulatory mechanism of myosin isozyme synthesis in pressure-overloaded hearts.

In summary, we have found that during the early stage of pressure overload 1) the synthesis of β-myosin heavy chains increases in parallel with synthesis of total protein while that of α heavy chain remains unchanged, and 2) the increase in β heavy chain expression appears to be achieved by changes at the pretranslational level since both synthesis and mRNA level change in parallel and relative synthesis, and mRNA levels correspond in all circumstances. It also appears that the control of the two myosin heavy chain genes does not necessarily have to be reciprocal as was found to be the case in thyroid hormone-induced isomyosin shifts. The fact that myosin heavy chain expression differs in various models of cardiac growth should be useful for future studies directed toward the elucidation of mechanisms that control myosin gene expression in the heart.

Acknowledgment

We thank Barbara Jill Martin and the following University of Vermont students for their ideas, enthusiasm, and excellent technical assistance in this study: Michael Stephanides, Deborah Filler, and Jonah Houston. We also thank Dr. John Mitchell for his expertise in the isolation of RNA. We are grateful for the assistance of Dr. Elizabeth Low in our statistical analyses. Finally, we appreciate the secretarial assistance of Cathy Ward and Julie Charron.

Appendix

Accurate Counting of Samples

We derived an equation to determine the amount of time necessary for counting both 14C and 3H with 5%
error. Counts of the sample and the background during certain amount of time (t min) have potentially some error, which follows Poisson’s distribution. Therefore,

\[ N'_1 = N_1 \pm \sqrt{N_1}, \]
\[ N'_2 = N_2 \pm \sqrt{N_2}, \]

where \( N'_1 \) = counts of the sample, \( N_1 \) = mean value of \( N'_1 \), \( \sqrt{N_1} \) = standard deviation (SD) of \( N'_1 \), \( N'_2 \) = counts of the background, \( N_2 \) = mean value of \( N'_2 \), and \( \sqrt{N_2} \) = SD of \( N'_2 \). The net counts of the sample are equal to the difference between the sample counts and the background counts. The variance of the difference between two variables is calculated as follows:

\[ \sigma^2(X - Y) = \sigma^2 X + \sigma^2 Y \]

where \( \sigma^2 (X - Y) \) = variance of the difference between variable X and variable Y, \( \sigma^2 X \) = variance of X, \( \sigma^2 Y \) = variance of Y. Therefore,

\[ \frac{N'_1 - N'_2}{t} = \frac{N_1 - N_2}{t} \pm \sqrt{\frac{N_1 + N_2}{t^2}} \]
\[ n'_1 - n'_2 = (n_1 - n_2) \pm \sqrt{n_1 + n_2} \]

where \( n'_1 \) = cpm of the sample, \( n'_2 \) = cpm of the background, \( n_1 \) = mean value of \( n'_1 \), \( n_2 \) = mean value of \( n'_2 \),

\[ \frac{\sqrt{n_1 + n_2}}{t} = \text{SD of} \ (n'_1 - n'_2). \]

In the case of the dansyl assay, \( ^3H \) and \( ^14C \) counts are measured simultaneously, and the ratio of \( ^3H \) cpm to \( ^14C \) cpm is calculated. The variance of the ratio of two variables (X and Y) is also known statistically:

\[ \sigma^2(X/Y) = \frac{\sigma^2 X}{X^2} + \frac{\sigma^2 Y}{Y^2} \]

where \( \bar{X} \) = mean value of X, and \( \bar{Y} \) = mean value of Y. Therefore, the coefficient of variation (CV) is given by:

\[ CV(X/Y) = \sqrt{\frac{\sigma^2 X}{X^2} + \frac{\sigma^2 Y}{Y^2}} = \sqrt{CVX^2 + CVY^2}. \]

The CV of the ratio of \( ^3H \) cpm to \( ^14C \) cpm is given by:

\[ CV \left( ^3H/^{14}C \right) = \sqrt{CV \left( ^3H \right)^2 + CV \left( ^{14}C \right)^2} \]
\[ = \frac{\sqrt{n_1 + n_3} + n_1 + n_4}{t(n_1 - n_2)^2} \]

\[ t \geq \frac{1}{0.05^2} \left( \frac{n_1 + n_3}{(n_1 - n_2)^2} + \frac{n_1 + n_4}{(n_1 - n_2)^2} \right) \]

By substituting \( n_1 \) with 12.0 and \( n_2 \) with 6.0, which are backgrounds of our liquid scintillation counter, a diagram was obtained for getting appropriate time (Figure 6). However, when cpm of the sample is very low, a slight change in cpm of the background can induce a significant amount of error in calculating net cpm of the sample. Therefore, cpm of the sample should be at least 3 times higher than the background.

References

Myosin Isozyme Synthesis in Pressure-Overloaded Heart


34. Towle HC, Marisini CN, Oppenheimer JH: Changes in the hepatic levels of messenger ribonucleic acid for malic enzyme during induction by thyroid hormone or diet. *Biochemistry* 1980;19:579–585

35. Everett AW, Sinha AM, Umaeda PK, Jakovic S, Rabinowitz M, Zak R: Regulation of myosin synthesis by thyroid hormone: Relative change in the α- and β-myosin heavy chain mRNA levels in rabbit heart. *Biochemistry* 1984;23:1596–1599


**Key Words** • myosin isozymes • protein synthesis • mRNA • pressure overload • cardiac hypertrophy
Myosin isozyme synthesis and mRNA levels in pressure-overloaded rabbit hearts.
R Nagai, N Pritzl, R B Low, W S Stirewalt, R Zak, N R Alpert and R Z Litten

Circ Res. 1987;60:692-699
doi: 10.1161/01.RES.60.5.692

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1987 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/60/5/692

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/