green supravital technique. We have found that this latter technique is very reliable in predicting viability of cells and correlates closely with the microscopic growth after replantation. In contrast, the eosin exclusion technique proved to be unreliable.

It is possible that this simple technique can be applied for preservation of a broad variety of primary explants or lines of tissue culture cells, and for the preservation of other tissues and organs.

Summary. A simple and reliable technique for preservation of HeLa and Fl-amnion cells at −79°C with DMSO has been developed in our laboratory. The optimum concentration of DMSO is from 5% to 10%; using this concentration we have been able to preserve HeLa and Fl-amnion cells at −79°C for as long as 15 months. Large concentrations of cells as high as 10,000,000 per ml can be stored per vial. Studies of the toxicity of DMSO on tissue culture cells demonstrated that either prolonged exposure to low concentrations or shorter exposure to high concentrations of DMSO, at 37°C or 4°C, are toxic to the cells.

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Myosin Synthesis in Chronic Heart Failure.* (30349)

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Studies on the mechanism of chronic congestive heart failure have centered primarily on the identification of abnormalities in the energy production of the failing heart and on changes in the physicochemical properties of the contractile proteins(10). Recent work has failed to confirm alterations in molecular weight of myosin of failing hearts(9), but evidence has been presented suggesting alterations in energy metabolism of the diseased heart(6). The involvement of protein metabolism in congestive heart failure was suggested from experiments on animals with experimental left ventricular failure(8,5).

Presently an abnormality of the contractile proteins is still considered a possible factor in the pathogenesis of myocardial insufficiency, and an impairment in myocardial energy production could lead to defective protein synthesis and thus affect myosin. For these reasons investigation of myosin synthesis in the failing heart appeared to be of interest.

Material and methods. Left heart failure was produced in male rabbits of approximately the same age by constriction of the ascending aorta above the sinus of Valsalva

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according to the method of Gertler(4). The cross sectional area of the aorta was reduced approximately 50%. The main steps of the surgical procedure have been described(5). About 30% of the rabbits developed congestive heart failure within 45 days. Indications for heart failure were fluoroscopic evidence of increase of heart size, pulmonary congestion, and ascites as demonstrated by paracentesis.

Four hours before the animals were killed, one hundred μc/kg of DL Cystine-S^{35} were injected into an ear vein. Left ventricular pressures were obtained under light anesthesia by direct transthoracic puncture. An elevation of the end-diastolic left ventricular pressure above 10 mm of mercury was considered as further indication of left heart failure. The rabbits were killed by intravenous injection of Nembutal after a blood sample had been withdrawn from the left ventricle by direct puncture and placed in a test tube containing Heparin. The blood was centrifuged in the cold and the plasma was precipitated with 1.6 N perchloric acid (PCA). The heart was then excised, washed with ice cold saline and kept on ice. Skeletal muscle (5 to 10 g) was removed from the gluteal region and was immediately placed in liquid nitrogen. The extraction of myosin was based essentially on the method of Davis *et al*(3). The myosin extraction was performed on 10 to 14 g of left ventricular tissue obtained from pooled rabbit hearts. Before the myosin extraction, the ground cardiac tissue was extracted for 20 minutes with 40 ml of glass bidistilled water and the extract was then precipitated with 1.6 N PCA. This fraction will be referred to as "water extractable protein" (WEP).

The purity of the myosin obtained according to this method was tested in an analytical ultracentrifuge. The sedimentation pattern indicated a pure preparation. The myosin was precipitated with 5% trichloracetic solution. The precipitate was then washed with 95% ethanol, ethanol-chloroform (3:1), and ether and acetone in order to remove lipids. The protein was dried and stored in a desiccator before being weighed.

The total proteins of the other fractions, that is of plasma proteins, the "water extractable proteins" (WEP) and of skeletal muscle, were isolated by a method modified from Schmidt and Thannhauser(11). The acid soluble substances were extracted with 0.3 N perchloric acid (PCA) at room temperature. The nucleic acids were extracted with 1.6 N PCA at 70° and both extracts were discarded. The proteins were washed with Tris buffer (pH 7.2). Lipids were extracted with ethanol, ethanol-chloroform, ethanol-ether and acetone. The proteins were dried at 100° and stored in a desiccator before being weighed. The radioactivity of the protein was determined in a toluene gel scintillator. This scintillator consisted of 4 g PPO (2,5-diphenyloxazole) and 100 mg POPOP (1,4-bis-2(5-phenyloxazole)-benzene) in one liter toluene. To this scintillator was added 3.5% thixotropic gel (Packard). The dried protein was powdered in a mortar, and 10 mg were weighed and suspended in the scintillator. The efficiency as determined with an internal standard was 67%.

Chronic congestive heart failure leads to accumulation of ascites and interstitial fluid, which can cause variation in the dilution and distribution of the amino acid tracer. To follow the effect of dilution due to edema and ascites and to obtain comparable results of incorporations into left ventricular myosin, the specific activity was also determined for plasma protein, skeletal muscle protein and water extractable protein of the left ventricle (WEP).

Results and discussion. Table I shows the specific activity of the protein samples studied. There were no significant differences between the specific activities of left ventricular myosin and other protein fractions obtained under conditions of heart failure, as compared to the same protein fractions in the normal animal.

TABLE I. Specific Activity of Protein.
cpm/10 mg

Protein	Control N = 24	Heart failure N = 20
Myosin	607	581
Water extractable	1,841	1,799
Plasma	5,688	5,393
Skeletal muscle	403	349

The contraction of muscle is basically an interaction between myosin, actin, ATP and ions. These basic reactions are based on the integrity of SH groups of the contractile elements and several investigators(2,12) have demonstrated that the reactions which are involved in muscular contraction are interfered with by SH group inhibitors. It was considered possible that in chronic heart failure the cardiac myosin could be affected by a decrease in the incorporation of specific amino acids, particularly cysteine, which carries the sulfhydryl group. In our experiments cystine was used and served as a source for labeled cysteine.

Labeled cystine is not an ideal amino acid for the study of protein synthesis, because this amino acid is not only incorporated into the polypeptide chain *via* the peptide bonds, but cystine can also attach to protein by formation of covalent disulfide bonds, which can be reduced with mercaptoethanol(7). Cystine was, however, chosen because of the functional importance of the SH group in the contractile protein. The injected amino acid is not incorporated as such, but after reduction *in vivo,* the cysteine is attached to transfer-RNA and incorporated into the protein. A lack or decrease of incorporation of -SH amino acids might interfere with the basic functions of myosin, as well as ATPase activity and the ability to bind actin. The present study suggests that the rate of synthesis of myosin is not an important factor in experimental myocardial failure produced by aortic constriction.

The observations of Meerson(8) and by Gudbjarnason *et al*(5) that the specific and the relative specific activity of total proteins was diminished in heart failure, does not necessarily indicate a primary involvement of myosin. They do not, however, exclude the possibility that the myosin molecule may be affected in chronic heart failure, since the configuration of myosin may be altered, independent of its synthesis. Another contributory factor in chronic heart failure might be the ATPase activity of myosin. Although some investigators(9,10) have been able to show an increase in ATPase activity of isolated myosin of failing hearts, Alpert and Gordon(1) have recently demonstrated a decrease of activity in the myofibrils of the failing heart. However, in view of the discrepancy of these findings, it is not possible to evaluate the significance of changes in ATPase activity in the development of heart failure.

Summary. Left heart failure was produced in rabbits by constriction of the ascending aorta. Incorporation of Cystine-S^{35} into cardiac myosin, water soluble enzymes, plasma protein and skeletal muscle protein was studied. There were no significant differences between the rate of synthesis of left ventricular myosin and the other protein fractions obtained under conditions of heart failure, as compared to the same protein fractions in the normal animal.

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