hibit the same spectral characteristics as those induced in vivo (*5*).

Cytochrome P-420, considered to be a degradation product of cytochrome P-450 (*13*), occurred in large amounts in the microsomes isolated from cultured cells but not in the microsomes isolated from rat liver under identical conditions. We cannot ascertain whether the cytochrome P-420 in vitro is intracellular, as appears to be the case with Morris hepatomas (*14*), or whether a factor or factors (such as lysosomal proteases) liberated from ruptured cells during homogenization produce cytochrome P-420 during the isolation procedure.

Hepatocytes cultured in the presence of phenobarbital ($2 \times 10^{-3}M$) from day 5 to day 10 were examined by electron microscopy and compared with cells cultured in the absence of phenobarbital. The hepatocytes on the floating collagen gels were fixed first with 3 percent glutaraldehyde at room temperature and then with 2 percent osmium oxide on ice. The cells were stained with a 2 percent aqueous uranyl acetate; the sections were stained with lead citrate. Subcellular organelles and lamellar rough endoplasmic reticulum were adequately preserved. Clusters of tightly aggregated vesicles of SER were seen in abundance (Fig. 3) in the cytoplasm of the phenobarbital-treated hepatocytes. The proliferation of SER was not restricted to any particular region of the cytoplasm and occurred in most of the phenobarbital-treated hepatocytes. Comparable proliferation of vesicles of SER did not occur in cells cultured in the absence of phenobarbital.

In previous studies of primary hepatic cultures and of cell lines derived from normal and neoplastic liver there were dissimilarities in the structure and biochemical function of these cells compared with liver parenchymal cells in vivo [see Bissel *et al.* (*15*)]. In this laboratory we have demonstrated (*16*) the similarity between the ultrastructure of hepatic cells maintained on floating collagen membranes and the ultrastructure of these cells in vivo. We have also shown that the enzyme ornithine oxoacid aminotransferase (E.C. 2.6.1.13) can be induced by glucagon in vitro as well as in vivo (*17*). The data reported herein may be useful in studies of drug metabolism, carcinogenesis in vitro, and the rapid assay of environmental carcinogens.

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Ischemic Areas in Perfused Rat Hearts: Measurement by NADH Fluorescence Photography

Abstract. *Fluorescence emission of reduced nicotinamide adenine dinucleotide (NADH) from the surface of perfused rat hearts was photographed to provide a two-dimensional recording of NADH levels. Sodium Amytal inhibition of NADH oxidation resulted in a homogeneous increase in NADH fluorescence, while lowering the perfusion pressure from 55 to 10 torr caused a heterogeneous increase in NADH fluorescence, reflecting the heterogeneous oxygen delivery at this low pressure. Local ischemia resulted in a well-defined region of high NADH fluorescence that corresponded to the region of ischemic insult. The sharp transition between the ischemic and normoxic areas demonstrated that the hypoxic interface separating the two areas must be quite small.*

Coronary artery occlusion, the common cause of heart attacks, results in regional derangements of normal myocardial function and metabolism that may result in irreversible tissue damage. Efforts now under way to minimize the size of the ischemic area caused by a coronary occlusion (*1*) require techniques suitable to determine the exact size of the ischemic area (*2*).

We report the direct measurement of the area of the epicardium after an ischemic insult in a perfused rat heart. The basis of the technique is the fluorescence of the reduced nicotinamide adenine dinucleotide (NADH) component of the NADH-NAD couple. This fluorescent property has been particularly useful in monitoring metabolic processes in tissues, especially processes occurring during mitochondrial metabolism (*3*). Mitochondrial NADH fluorescence is an efficient indicator of intracellular oxygen concentrations (*4*) and changes in metabolic states (*5*).

Two-dimensional scanning (*6*) and photographic recording (*7*) of NADH fluorescence in the cerebral cortices of rats have been reported; however, the methods used had insufficient time resolution to "freeze" mechanical and metabolic fluctuations in a perfused heart beating three to five times a second. Therefore, an approach was developed whereby the ultraviolet component of a xenon flash was used as an excitation source. This technique allows photographic recording of NADH fluorescence emission from the heart with good time and spatial resolution.

Fluorescence photographs were taken with a Fairchild oscilloscope camera (model 453-1) with a 40-mm focal length lens added to the front of the existing lenses to provide an image on the film twice the actual size of the heart. A filter pack containing a Corning 9788 and a Wratten 45 filter was placed over the camera lens to allow transmission of NADH fluorescence in the 430- to 510-nm region. Photographs were recorded on Kodak Royal Pan film.

Fluorescence excitation was provided by two xenon flashtubes (EG&G FX-100), one mounted on either side of the camera lens. Flashtubes were covered by Corning 5840 filters to provide 330- to 380-nm excitation. Triggering of the flash was synchronized to occur during the diastolic phase of the cardiac cycle.

Hearts were rapidly excised from male Sprague-Dawley rats (weighing 200 to 220 g and anesthetized with sodium pentobarbital) and immediately perfused at 30°C with Krebs-Ringer bicarbonate buffer containing 5 m*M* glucose and 2.5 m*M*

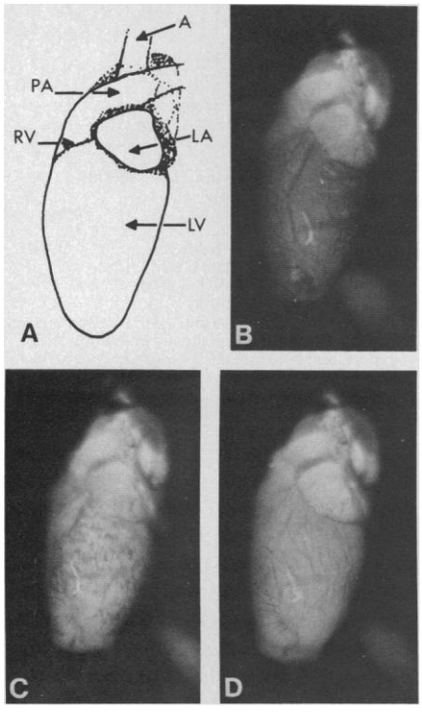

Fig. 1. (A) Diagram of the perfused rat heart; *A*, aorta; *LA*, left atrium; *LV*, left ventricle; *RV*, right ventricle; *PA*, pulmonary artery. Photographs of NADH fluorescence from the perfused rat heart. (B) Normoxic (55 mm-Hg perfusion pressure). (C) Coronary insufficiency (10 mm-Hg perfusion pressure). (D) Inhibition of mitochondrial NADH oxidation by 1 m*M* sodium Amytal in a normoxic heart (55 mm-Hg perfusion pressure).

Ca^{2+} on a Langendorff perfusion procedure (*8*). Left ventricular pressure was continuously monitored through a 25-gauge needle in the left ventricle connected to a Millar pressure transducer (model 350).

The orientation of the hearts as photographed is shown in Fig. 1A. The fluorescence from the surface of the normoxic heart is very low, with different parts of the heart showing fluorescence signals of different intensity (Fig. 1B). Near the top of the figure are brighter areas where tissue components are present which contain fluorochromes other than NADH (particularly a fluorescent, cross-linking substance in collagen) and thus fluoresce significantly under normoxic conditions. These are identified particularly with the aorta and the pulmonary artery. The ear of the left atrium is also visible as a region of relatively high fluorescence. This may be due to a combination of factors, including high concentrations of fat and connective tissue and poor atrial perfusion in the Langendorff procedure. The focus of interest here is the ventricles, which are well perfused and where the mitochondria have an adequate supply of oxygen. The ventricles appear dark because of the highly oxidized state of the NADH-NAD couple (state 3). The main fluorochrome of the heart ventricles is NADH; the NADPH (reduced nicotinamide adenine dinucleotide phosphate) component shows only a small signal that does not change significantly with the redox state of the tissue (*9*).

Coronary insufficiency caused by lowering the perfusion pressure from 55 to 10 torr resulted in an increase in fluorescence of most of the myocardium (Fig. 1C). The heterogeneous distribution of the increased fluorescence describes the distribution of NADH across the myocardium. The brighter regions represent anoxic areas, while the darker regions represent those areas that remain adequately oxygenated even at this low perfusion pressure.

Sodium Amytal (1.0 m*M*) was infused into the normoxic rat heart to inhibit mitochondrial NADH oxidation (Fig. 1D). Since Amytal is not metabolized, it established uniform concentrations throughout the tissue, and a uniform increase of fluorescence was obtained.

To simulate a heart attack, we ligated a coronary artery of a normoxic heart (Fig. 2). The ischemic area formed by the coronary occlusion is visible as a zone of increased NADH fluorescence. This area represents that portion of the epicardium where insufficient oxygen remains to support normal myocardial function. Of particular interest is the sharp line of transition of pyridine nucleotide oxidation state from ischemic to normoxic areas. Studies with isolated cardiac mitochondria (*5*) demonstrated that the oxygen concentration available to the mitochondria is approximately 0.06 torr where the NADH fluorescence is midway between normoxic and fully anoxic levels. Since the heart is perfused with oxygen at more than 600 torr, the normoxic tissue oxygen tension may be a significant fraction of this. It is estimated that the tissue oxygen concentration gradient between the light and dark areas may be in excess of 100 : 1. The sharp dividing line between the anoxic and normoxic region relates to the controversial question of whether there is a border zone of partially anoxic tissue surrounding the region of infarcts (*10*). The results from the fluorescence photographs of the perfused heart support the view that the tissue oxygen gradient between anoxic and normoxic regions is so steep that the portion of tissue that may be considered to be a hypoxic border zone is extremely small.

Our method may be useful in detecting anoxic regions of heart tissue in other models as well as under surgical conditions that might be suspected of causing local anoxia in cardiac surgery. However, our experiments were carried out under favorable conditions in which a blood-free perfusate was used. Surface fluorometry with photoelectric devices on blood perfused hearts in situ appears to be feasible (*11*); thus, more selective procedures for fluorometry may afford appropriate results on the heart in situ.

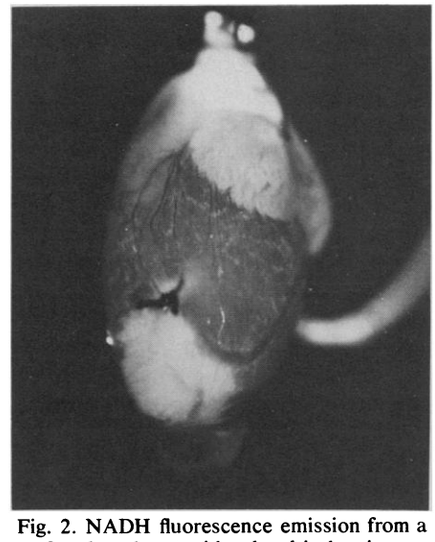

Fig. 2. NADH fluorescence emission from a perfused rat heart with a local ischemic area near the apex, caused by ligation of a coronary artery with the black silk suture that is visible at the top of the highly fluorescent ischemic area. The photograph was obtained by superimposing four flashes on a single negative over a 2-minute period.

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