A NON-INVASIVE METHOD TO STUDY METABOLIC EVENTS WITHIN SUBLOBULAR REGIONS OF HEMOGLOBIN-FREE PERFUSED LIVER

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1. Introduction

It is well known that the liver is separated into various zones or regions often referred to as pericentral (around the central vein), mid-zonal and periportal (around the portal vein). These regions or zones have different oxygen tensions [1], different enzymatic compositions [2] and possibly different functions. For example, the enzymes of gluconeogenesis are zonated near the periportal area whereas glycolytic enzymes are more concentrated in the pericentral region [3]. In addition, one form of cytochrome P450 appears to be localized to the pericentral region of the liver [4]. Nutrients and oxygen enter the liver lobule in the periportal zone and exit via the central vein.

However, little is known regarding dynamic metabolic functions in these intralobular compartments. To study dynamic events without disturbing the liver lobular structure, preparations such as the isolated, perfused rat liver are necessary. In fact, much of the information available on metabolic regulation of dynamic processes (e.g., gluconeogenesis) has been obtained with the perfused liver.

Chance et al. have utilized the native fluorescence of pyridine nucleotides and flavoproteins to index metabolic events in perfused organs [5]. The fluorescence of NAD(P)H of hemoglobin-free organs can be employed as an indirect measure of tissue oxygenation. Moreover, NADP(H) fluorescence can be detected with single optical fibers of <100 μm in diameter [6,7]. The purpose of these studies, therefore, was to examine whether or not a modified micro-light guide could be used to monitor metabolic processes in identified sublobular regions of the liver. We describe here a morphological basis for identifying sublobular regions on the surface of hemoglobin-free perfused rat liver. Utilizing this information, we further demonstrate that redox gradients exist between the periportal to pericentral regions as assessed by pyridine nucleotide fluorometry employing a modified micro-light guide.

2. Experimental

2.1. *Animals*

Female albino rats (Sprague-Dawley, 170–290 g) received lab chow and water containing 1 gm/l sodium phenobarbital [8] for 2–3 weeks prior to perfusion experiments.

2.2. *Non-recirculating liver perfusion*

The perfusion technique has been described [9]. Krebs-Henseleit bicarbonate buffer (pH 7.6) saturated with oxygen (95%) and carbon dioxide (5%) was pumped into the liver via a cannula inserted in the portal vein. The effluent perfusate was collected with a cannula placed in the inferior vena cava and flowed past an oxygen electrode before it was discarded. The oxygen tensions in the influent and effluent perfusate were determined with Teflon-shielded platinum electrodes.

2.3. *170 μm Tip micro-light guide*

A micro-light guide for determination of NAD(P)H fluorescence with a 400 μm tip has been employed successfully in [6,7]. To construct a tip small enough to selectively detect NAD(P)H fluorescence in the periportal and pericentral regions of the liver lobule, 2 loose fibers were held together at the tip with epoxy glue (fig.1). The resulting 2 fiber light-guide possessed an excitation–collection tip of ~170 μm

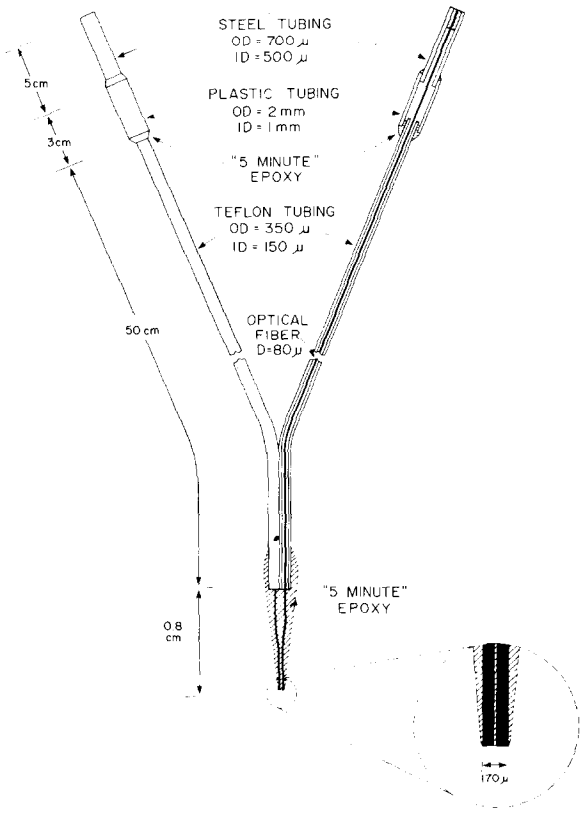

Fig.1. A schematic representation of the 170 μm tip micro-light guide. The micro-light guide was fabricated essentially as in [6,7], except that the two loose glass fibers were glued together at the tip without any steel tubing. The tip was cut with a sharp razor blade in such a way that the cut cross-sections of the fibers were smooth and parallel to each other. The light guide was used without polishing the tip. μ = μm.

diam., much smaller than the 500–1000 μm av. diam. of the liver lobule. The measured fluorescence arises from that portion of the tissue which is both illuminated by the excitation light and is in the field of view of the collection fiber [6,7]. This 'overlap volume' forms a prolate spheroid with axes of 320 μm and 125 μm and with 3×10^6 μm^3 vol. This corresponds to the space occupied by ~600–700 hepatocytes.

2.4. *NAD(P)H fluorescence measurement with the 170 μm tip micro-light guide*

After the 2-strand micro-light guide (fig.1) was placed on a periportal or pericentral region of the liver (see below), one strand was connected to a near-ultraviolet light source (100 W Hg arc lamp, Illumination Indust., Sunnyvale, CA), and the other strand to a photomultiplier (EMI type 9824B). The liver was illuminated with the 366 nm mercury arc line isolated with a Corning glass filter no. 5840, and the NAD(P)H fluorescence (450 nm) of the tissue was passed through Kodak gelatin filters (2E and 47) before it was detected by the photomultiplier. The signal from the photomultiplier was filtered, amplified and recorded essentially as in [10].

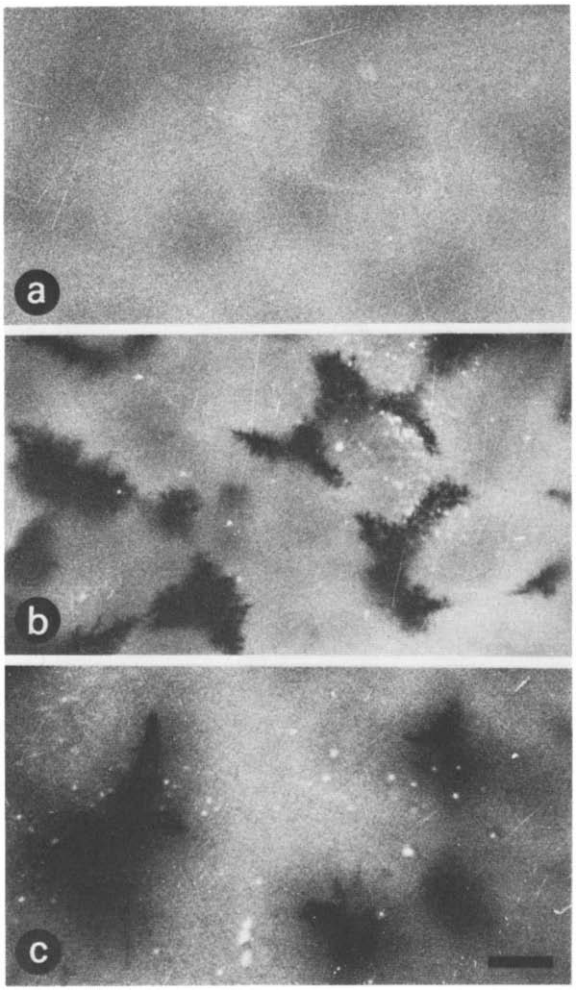

Fig.2. Photomicrographs of perfused liver surface before and after perfusion with india ink. In (a) note pigmented dark spots and light areas present before india ink infusion. In (b) india ink was infused via the portal vein, and flow was stopped as soon as ink appeared at the liver surface. Notice that the ink has entered the light areas surrounding the pigmented dark spots. In (c) india ink was infused via the vena cava. India ink staining appears first within the dark pigmented spots. Bar is 250 μm.

3. Results

3.1. *Identification of periportal and pericentral zones of the liver*

It was noted that light areas and dark spots of 300–500 μm diam. could be detected on the surface of the hemoglobin-free perfused liver with the naked eye or with a low power dissection microscope (fig.2a). When india ink was perfused into the liver via the portal vein, the light areas surrounding the dark spots turned black prior to any staining of the dark spots (fig.2b). This identifies the light areas as periportal regions (zone 1 in [1]). In contrast, retrograde perfusion via the vena cava stained the dark spots first and hence identifies each dark spot as a pericentral region (fig.2c). Thus, the natural distribution of liver pigments can be used to identify periportal and pericentral areas of the liver lobule. Since the intensity of the dark spots was enhanced following treatment with phenobarbital, a phenobarbital-inducible protein such as cytochrome P450 may be responsible for this pigmentation. The experiments reported here were, therefore, carried out with phenobarbital-treated rats.

3.2. *Differential response of periportal and pericentral pyridine nucleotide systems*

When the light guide was placed on a light (periportal) area, the basal fluorescence intensity was found to be ~50% higher than the basal fluorescence of the dark (pericentral) zone, presumably due to a higher absorption coefficient of pigments in the pericentral cells at either one or both of the two wavelengths involved (366 nm and 450 nm) (table 1; fig.3). Fig.3 provides a typical example of the differential metab-

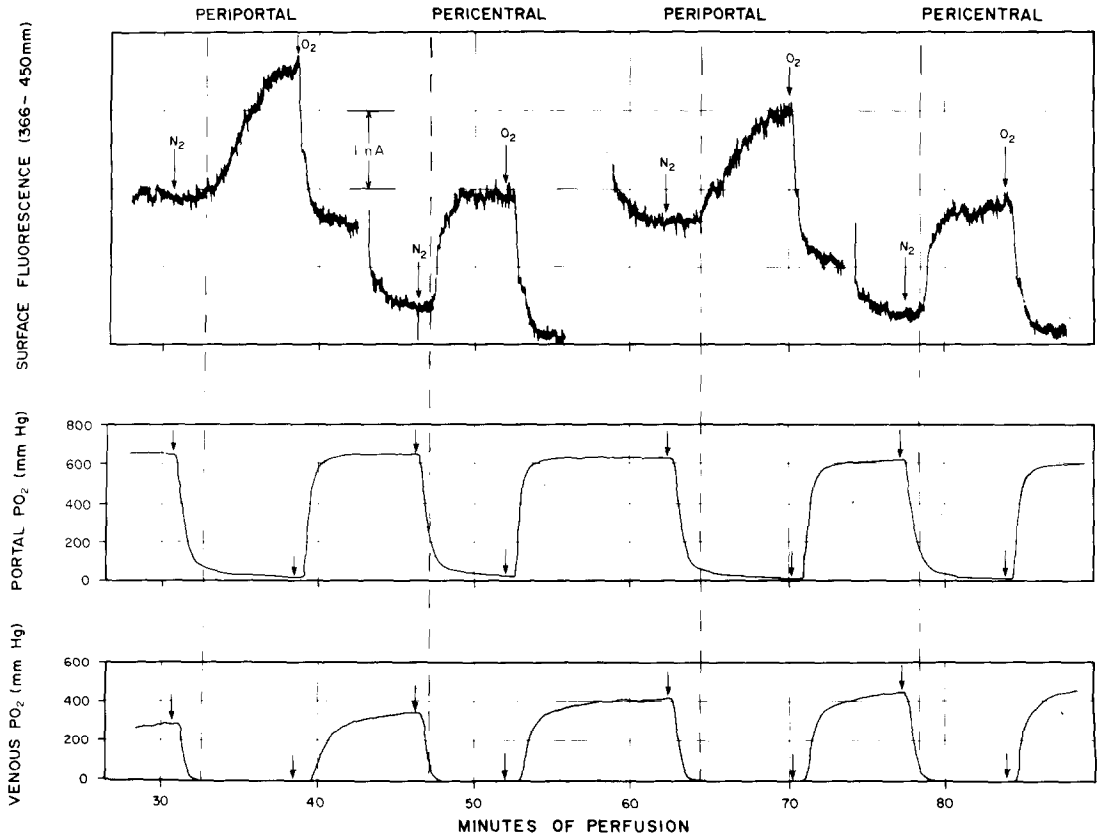

Fig.3. Dynamic fluorescence responses of the periportal and pericentral areas to anoxic perfusion. The 170 μm tip micro-light guide (fig.1) was placed on dark spot (pericentral) or light areas (periportal) on the surface of the perfused liver via a flexible mechanical arm. The micro-light guide was glued near the tip to one end of an L-shaped flexible hypodermic steel tubing (o.d. = 200 μm, i.d. = 140 μm) which was firmly attached on the other end to the mechanical arm. The pressure exerted by the tip of the light guide on the liver surface was minimized by varying the position of the mechanical arm. Arrows indicate that the equilibrating gas mixture was changed either to 95% N_2 and 5% CO_2 (N_2) or 95% O_2 and 5% CO_2 (O_2).

olic responses of the periportal and pericentral areas of the liver lobule toward changes in oxygen tensions in the perfusate. Summary data are presented in the accompanying table. When oxygen is replaced with N_2 in the perfusate (anoxia), the pericentral region became anoxic as indicated by an increase in surface fluorescence at 1.0 ± 0.1 min after initiation of anoxia whereas the periportal region took a longer time (1.6 ± 0.2 min) to become reduced (table 1). Also, the pericentral area became reduced at a higher portal PO_2 than the periportal region. Furthermore, the initial rate of anoxia-induced fluorescence increase was about twice as fast in the pericentral region as in the periportal (table 1). Upon restoring oxygen to the liver, tissue fluorescence also decreased about twice as rapidly in the periportal area than the pericentral (table 1).

4. Discussion

The native fluorescence of intracellular pigments (e.g., NAD(P)H) has been studied non-invasively from living tissues such as the hemoglobin-free perfused rat liver and has yielded considerable insight into the regulation of metabolic processes of whole organs [11,12]. Micro-light guides consisting of single glass fibers have been employed to study NAD(P)H fluorescence in localized tissue areas of liver and heart [6,7]. Studies employing fluorescence of small frozen cubes of liver [13] or multiple oxygen electrodes placed on the liver surface [14] have shown that microheterogenous distribution of the oxygen tensions exists in the liver. However, these studies did not correlate the observed difference with specific zones within the liver lobule by histology or other means. Here we have modified the light guide (see fig.1) so that the exposed tip is <200 μm diam. considerably smaller than the average diameter of the liver lobule [1]. Further, it was observed that dark and light spots exist on the liver surface which correspond to pericentral and periportal areas, respectively, as proven with the infusion of india ink (fig.2). Thus, it is now possible to study dynamic metabolic events from the surface of specific regions (e.g., periportal and pericentral) of the liver lobule noninvasively using the 170 μm tip micro-light guide.

Two observations suggest that the dark spot may be due to absorption of light by cytochrome P450:

(1) The basal fluorescence (450 nm) of the dark was ~60% that of the light spot (table 1, fig.3), suggesting absorption by the P450 heme of excitation or emission light, or both;
(2) The dark spot was intensified following pretreatment of the rat with phenobarbital, an agent known to induce cytochrome P450 [8].

The observation that, following anoxia, the lag time preceding an increase in fluorescence was much longer in the periportal than in the pericentral region (table 1, fig.3) indicates that a substantial oxygen gradient exists across the liver lobule. This conclusion is supported by the observations that, for periportal (light) regions as compared to the pericentral (dark) regions:

(1) The initial rate of fluorescence increase due to N_2 was slower;
(2) The lag time for return of fluorescence to the base line was shorter;
(3) The influent (portal) PO_2 at which fluorescence began to increase was lower (table 1).

Thus, these experiments establish a correlation between local redox gradients and liver lobular structures.

5. Conclusion

Two developments were necessary before the measurement of intralobular redox gradients reported here could be carried out:

(1) The morphological identification of the dark spots of the liver surface as the pericentral regions and the light areas as periportal zones (fig.2) represents an important advance.
(2) The construction of the 170 μm tip micro-light guide that can record the anoxia-induced alterations in tissue pyridine nucleotide fluorescence non-invasively is essential to measure fluorescence in areas of liver surface 300–500 μm in diameter.

These two developments enabled us to experimentally demonstrate for the first time a correlation between local redox gradients and the sublobular structure of the liver. This technique provides a novel and simple method to compare dynamic metabolic events in different zones of the liver lobule non-invasively and can readily be applied to studies of micro-circulation and metabolic regulation at the sublobular level employing a variety of fluorescent species.

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