The data indicate that the posterior pituitary contains a substance capable of inhibiting prolactin secretion both in vivo and in vitro. Our previous study with isolated anterior pituitary cells showed that this PIH activity of the posterior lobe had a dose-dependent characteristic and that vasopressin and oxytocin were ineffective in modifying prolactin secretion (*11*). On the basis of the reversal of the posterior lobectomy-induced prolactin increase by dopamine, the abolishment of this PIH activity in vitro by butaclamol, and the endogenous concentration of dopamine, we propose that the active PIH in the posterior pituitary is dopamine.

We have recently reported (*12*) that 85 percent of estrous female rats subjected to long-term posterior lobectomy had increases in circulating prolactin sufficient to induce pseudopregnancy for 11 days. Water consumption increased four- to fivefold immediately after posterior lobectomy, indicating a severe vasopressin deficiency, but was only twice above control water consumption after 12 to 14 days. This and the fact that the increase in prolactin was not sustained, because all posterior lobectomized females resumed normal cyclicity, could be explained by the regeneration of the posterior lobe taking place during this time (*13*).

Neither the in vivo nor the in vitro methods reported here are sufficiently refined to determine the relative role of the median eminence (long portal vessels route) compared with the posterior pituitary (short portal vessels) in the dynamic regulation of prolactin secretion. Furthermore, it is questioned whether dopamine is synthesized exclusively in hypothalamic nuclei and is only stored in the posterior lobe as is the case with vasopressin and oxytocin (*14*). Some degree of independence of hypothalamic contribution is suggested by our results showing that posterior pituitaries incubated in vitro are capable of synthesis of dopamine de novo from tritiated tyrosine. Indeed, the presence of tyrosine hydroxylase and the absence of dopamine β-hydroxylase in the posterior lobe has been reported (*5*). Thus, to gain a better insight into the participation of the posterior lobe in the regulation of prolactin secretion, a correlation should be made between possible changes in dopamine concentration or turnover in the posterior lobe and alterations in prolactin secretion under various endocrine conditions.

The present study demonstrates that there is a functional dependency between the two lobes of the pituitary, at least with respect to one of the anterior pituitary hormones. In view of the high concentration of other releasing or inhibiting hormones such as thyrotropin-releasing hormone (*15*), somatostatin (*16*), corticotropin-releasing hormone activity (*17*), and the enkephalins (*18*) in the posterior lobe, other types of interactions might also occur in the pituitary.

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Centrilobular Injury Following Hypoxia in Isolated, Perfused Rat Liver

Abstract. *Hypoxia was produced in isolated, hemoglobin-free, perfused rat liver by reducing the flow rate of oxygen-carrying fluid entering the organ. The procedure caused anoxia in centrilobular regions. In these anoxic areas, structural derangements developed rapidly, characterized by bleb-like protrusions of hepatocyte plasma membrane through fenestrations in the sinusoidal endothelium. Periportal tissue remained normoxic and was completely spared. Cellular injury resulting from localized anoxia may play an important role in the pathogenesis of centrilobular liver disease.*

Centrilobular liver injury occurs as the consequence of circulatory disorders and the toxicity of various drugs (*1*). This type of injury has been postulated to reflect the fact that the central region of the liver lobule is the last to receive blood-borne oxygen and metabolites (*2*). Long-term exposure to alcohol, in particular, causes centrilobular injury in experimental animals and man (*3*). In addition, both long- and short-term exposure to alcohol causes an increase in oxygen consumption by the livers of laboratory rodents (*4*).

On the basis of these observations, Israel *et al.* (*5*) hypothesized that alcohol increases the oxygen requirement of the liver such that those regions most distant from the supply receive inadequate oxygen, become anoxic, and undergo a sequence of cellular changes leading to necrosis. The hypothesis is supported by the recent demonstration of a substantial (200 to 300 torr) intralobular oxygen gradient in isolated, hemoglobin-free, perfused rat liver (*6, 7*). In livers from rats treated with alcohol over extended periods this intralobular gradient increased 30 percent, in parallel with an increase in hepatic oxygen uptake (*7*). We have now shown that stable, circumscribed zones of anoxia develop in response to insufficient oxygen delivery and that such anoxia leads to centrilobular injury and necrosis.

Since liver function and oxygenation cannot be monitored with precision in the intact animal, we used livers isolated

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from female Sprague-Dawley rats (200 to 300 g). The livers, perfused with Krebs-Henseleit bicarbonate buffer (*p*H 7.4) at 37°C (*8*), displayed a faint but uniform pattern of surface pigmentation in which lighter and darker areas represented periportal and centrilobular regions, respectively (*6*). Using the surface pigmentation for orientation, we emplaced micro-light guides to monitor fluorometrically the oxidation-reduction state of nicotinamide adenine dinucleotide (NADH) and nicotinamide adenine dinucleotide phosphate (NADPH) (*6, 9*). Upon replacement of oxygen with nitrogen in the perfusion medium, there was an increase in pyridine nucleotide fluorescence in both periportal and centrilobular regions (Fig. 1). The increase in fluorescence in centrilobular regions preceded the increase in periportal regions as a consequence of the intralobular oxygen gradient (*6, 7*).

When the flow rate of perfusate entering the liver was reduced by about 75 percent, the tissue response was heterogeneous (Fig. 1). In centrilobular regions, fluorescence was elevated and the tissue was no longer responsive to anoxic perfusion. By contrast, periportal regions showed no elevation in fluorescence and responded to anoxic perfusion with an increase in fluorescence. Tissue pyridine nucleotide fluorescence is an indirect indicator of intracellular oxygen tension, and elevations in such fluorescence can be equated with the virtual absence of tissue oxygen (*10*). By this criterion, centrilobular areas became anoxic while periportal areas remained normoxic as flow rate was lowered to 1 ml/min per gram of liver. Since anoxia was confined to circumscribed, centrilobular regions and was stable over a relatively long period, it was possible to evaluate the structural changes induced by progressively longer periods of anoxia by using adjacent normoxic tissue as an internal control for nonspecific changes.

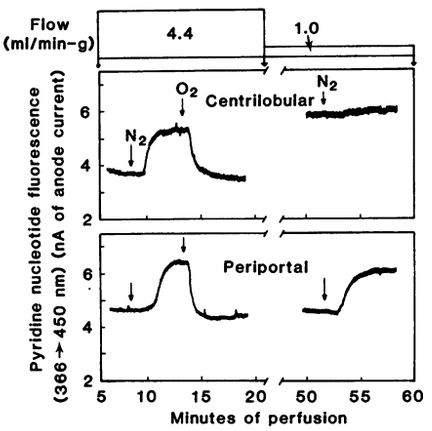

Fig. 1. Pyridine nucleotide (NADH plus NADPH) fluorescence in sublobular regions of isolated, perfused rat liver, as measured by micro-light guide tissue fluorometry (*6, 9*). Excitation light was brought to the liver surface by a single 80-μm-diameter glass optical fiber. Emission light was collected by an adjacent optical fiber and directed through a barrier filter to a photomultiplier. Individual pairs of optical fibers were placed on periportal (light) and centrilobular (dark) regions of the liver surface for simultaneous measurement of periportal and centrilobular fluorescence. Flow rates were 4.4 ml/min per gram of liver (left trace) and 1.0 ml/min (right trace). Oxygen in the perfusion medium was replaced by 95 percent nitrogen and restored where indicated by arrows.

After 15, 30, or 45 minutes of perfusion at low flow rates (0.8 to 1.0 ml/min), the livers were fixed by adding 2 percent glutaraldehyde and 2 percent paraformaldehyde to the perfusion medium. After 5 minutes, the tissue was transferred into a secondary fixative consisting of cold 2 percent glutaraldehyde and 0.1*M* sodium phosphate buffer (*p*H 7.4). Then the tissue was cut into 1-cm^3 blocks and stored in secondary fixative until the following day, when it was dehydrated in acetone and critical point–dried. Tissue to be viewed by scanning electron microscope (JEOL 35 at 25 kV) was cut with a razor blade and coated with gold and palladium. Tissue to be viewed by transmission electron microscope (JEOL 100CX at 60 kV) was cut into 1-mm^3 blocks after the initial fixation and placed in the secondary fixative for an additional 2 hours. Subsequent steps of postosmication, dehydration, embedding in Epon 812, sectioning, and staining with uranyl acetate and lead citrate were performed by standard techniques.

Scanning electron micrographs of periportal tissue were normal in appearance even after 45 minutes of hypoxia (Fig. 2a) (*11*). Bile canaliculi were well defined and contained microvilli projecting into the lumen. Intercellular borders were regular except for the presence of peg-like interdigitations. The sinusoidal endothelium was interrupted by large (~ 2 μm) and small (~ 0.15 μm) fenestrations. The small fenestrations occurred in clusters whose overall dimensions equaled the size of the large fenestrations. The subendothelial surfaces of the hepatocytes were covered by microvilli, which were clearly seen within the larger fenestrations. Microvilli rarely protrud-

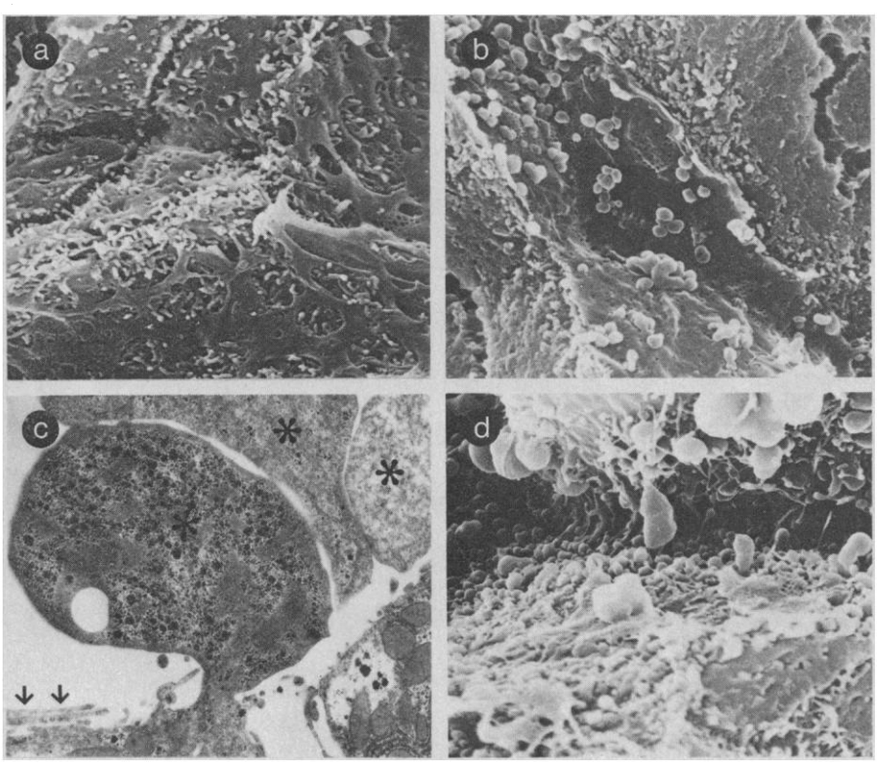

Fig. 2. (a) Scanning electron micrograph of periportal tissue after 45 minutes of hypoxia (flow rate, ~ 1 ml/min). Periportal regions after shorter periods of hypoxia were virtually indistinguishable from both periportal and centrilobular regions of livers perfused at high flow rates (×4600). (b) Scanning electron micrograph of centrilobular tissue after 15 minutes of hypoxia (×4600). (c) Transmission electron micrograph of centrilobular tissue after 30 minutes of hypoxia. Asterisks indicate portions of three bleb-like protrusions of hepatocyte plasma membrane and cytoplasm. Arrows indicate the sinusoidal endothelium (×7200). (d) Scanning electron micrograph of centrilobular tissue after 45 minutes of hypoxia (×4600).

ed very far into the sinusoidal lumen itself.

After only 15 minutes of hypoxia, striking alterations in cell structure were observed in centrilobular regions (Fig. 2b). Most prominent were bleb-like protrusions of hepatocyte plasma membrane and cytoplasm into the lumen of the sinusoids through fenestrations in the endothelium. Bile canaliculi were marginally more distended, and intercellular borders showed a general flattening of larger topological features and a fine surface granularity.

The projecting blebs remained connected to hepatocytes by slender necks, as demonstrated by thin-section electron microscopy (Fig. 2c). Bleb contents were variable. In most blebs, only amorphous granular material was observed. Others contained endoplasmic reticulum and glycogen rosettes; larger organelles, such as mitochondria and lysosomes, were absent. After 45 minutes of hypoxia, blebs covered both the sinusoidal and the intercellular surfaces of the hepatocytes, grossly distorting them (Fig. 2d). In addition, the endothelium was torn and fragmented.

Clearly, perfusion of isolated, hemoglobin-free liver at low flow rates produces anoxic stress only to that portion of the lobule near the central vein. In experiments in which flow was reduced further, the amount of injured tissue increased. However, the area most proximal to the terminal portal venule was consistently spared.

Plasma membrane alterations have been described in a number of organs, including liver, following cellular injury from anoxia and other causes (*12*). Formation of the membranous blebs closely resembles the structural changes produced in isolated hepatocytes by the cytoskeletal disrupters cytochalasin B and phalloidin (*13*). Thus, the structural perturbations observed in hypoxia may represent a failure of the cytoskeleton to maintain cell shape and volume. It is also possible that the blebs formed during hypoxia are in the process of budding off and being released into the circulation. This could represent the well-known release of enzymes by injured hepatic tissue.

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Intrinsic Birefringence Signal Preceding the Onset of Contraction in Heart Muscle

Abstract. *An intrinsic birefringence signal with two components occurring before sarcomere shortening was measured in mammalian cardiac muscle. The second component was sensitive to the inotropic state of the muscle as affected by external calcium concentration and epinephrine but not by changes of resting length. The second component was absent in frog heart. These results suggest that the second component of the birefringence signal reflects the activity of the sarcoplasmic reticulum related to excitation-contraction coupling processes occurring prior to onset of contraction in mammalian cardiac muscle.*

Intrinsic birefringence signals with a time course similar to an action potential were first recorded in electrical eel electric organ and squid giant axon (*1, 2*). In skeletal muscles, in addition to the component of the birefringence signal accompanying the action potential, a second component occurred immediately after the action potential and prior to the onset of contraction (*3, 4*). This second component has been attributed to the activity of the sarcoplasmic reticulum (SR) prior to activation of contraction. In intact skeletal muscle fibers the time course of the second component of the birefringence signal is similar to signals obtained by using voltage-sensitive dyes (*5, 6*) or the metallochromic calcium-indicator dye arsenazo III (*7*).

In these studies we have investigated birefringence signals in two different types of hearts; one with extensively developed SR (rat, guinea pig, and cat) and the other with much smaller content of SR and no T-tubular system (frog atrium and ventricle). In rat, guinea pig, and cat ventricular muscle, we found two distinct components of an intrinsic birefringence signal which preceded the development of tension. The first component occurred simultaneously with the upstroke of the action potential. The second component began after a delay with respect to the upstroke and was much larger and slower than the first. Both components preceded the onset of sarcomere motion as measured by scattered incandescent light or laser-diffraction pattern, or both. The amplitude and rate of rise of the second component was related to the inotropic state of the muscle as regulated by the external calcium concentration, $[Ca]_o$, and epinephrine, but not by variations of resting muscle length. In frog atrial and ventricular muscle the birefringence signal showed only the action potential–related first component, under all experimental conditions including variations of $[Ca]_o$ and addition of epinephrine or caffeine. We conclude that the second component of the birefringence signal represents a step in activation of contraction that is probably related to the Ca^{2+}-release process of the SR in mammalian heart muscle.

Papillary muscles or trabeculae carnae (60 to 300 μm in diameter by 0.75 to 1.2 mm long) were dissected from the right ventricle of rat and guinea pig hearts and from the right ventricle and atrium of cat

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