INVESTIGATIONS INTO THE ACTIONS OF VASOPRESSIN, HISTAMINE, ISOPROTERENOL, PHENTOLAMINE, PHENYLEPHRINE, AND PROPRONALOL ON FLOW THROUGH PERFUSED RAT LIVERS

An abstract of a Thesis by
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The problem. The experiments were intended to elucidate any intrahepatic actions of the drugs employed as presinusoidal or postsinusoidal and to identify any antagonism of the drugs to others.

Procedure. Livers were excised and perfused at a constant rate through the portal vein with different drugs and at different concentrations. Absolute pressure and change of weight were recorded continuously.

Findings. No consistent effects were found with the methods employed. Several factors were not important to this lack of action and several others appear possibly important.

Recommendations. Further investigation should be undertaken to identify a procedure that will show actions for those drugs and oxytocin. Once a procedure is identified, any actions of the drugs should be characterized as pre- or postsinusoidal, and the adrenergic agonists, histamine, and vasopressin should be tested for antagonism of each other. Dog livers as well as rat livers and healthy as well as cirrhotic livers should be tested because they would provide important data to compare with other research.
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INVESTIGATIONS INTO THE ACTIONS OF VASOPRESSIN, HISTAMINE, ISOPROTERENOL, PHENTOLAMINE, PHENYLEPHRINE, AND PROPRONALOL ON FLOW THROUGH PERFUSED RAT LIVERS

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INTRODUCTION AND REVIEW OF THE LITERATURE

The action of vasopressin on flow through rat livers was the subject of the investigations reported here. Vasopressin is an effective drug for the treatment of hemorrhage from esophageal varices. Typically, varices are associated with portal hypertension; and both portal hypertension and varices are important complications of liver disease. The architecture of a healthy liver will undergo change as liver disease progresses, and liver disease of almost any etiology will produce the same changes. With this progression of most liver disease comes portal hypertension, which may be thought of in simple terms as increased blood pressure in the hepatic portal vein (HPV) in response to increased resistance to flow through the liver. Portal hypertension gives rise to secondary changes in the venous system. With increased HPV pressure, hepatofugal collateral veins are recruited to carry increased amounts of flow. The esophageal veins are important hepatofugal collaterals that connect the gastric veins of the portal system with the azygos veins of the caval system. When HPV pressure is high, the esophageal veins carry a great deal of flow and become enlarged and twisted. In this condition, they are referred to as esophageal varices and they often hemorrhage. Vasopressin has been used successfully to control variceal bleeding. The means by which vasopressin stops bleeding is the subject of some disagreement.
Vasopressin treatment of bleeding varices is strongly linked to several theories of the mechanism of action; thus, one needs to be familiar with all the facts before deciding on the importance of any one mechanism. Specifically, vasopressin may control bleeding by constriction of esophageal smooth muscle, or vasopressin may produce decreases in HPV pressure and flow that decreases flow through the esophageal veins enough to prevent bleeding. Furthermore, a number of theories are available to explain how vasopressin might decrease HPV pressure and flow. Vasopressin may decrease flow into the liver and HPV by constriction of the splanchnic arterioles (the most popular theory) or by closure of intestinal, submucosal arteriovenous shunts. It may also decrease pressure by HPV dilation or by decreasing the resistance to flow through the liver by dilation of the sphincters controlling intrahepatic flow. The experiments reported here hoped to determine if vasopressin has an intrahepatic action in rat livers and to identify any action as pre- or postsinusoidal.

The effectiveness of vasopressin therapy for bleeding varices and portal hypertension depends in part on aspects of variceal bleeding and portal hypertension that vasopressin cannot change. Elements of variceal bleeding unresponsive to vasopressin therapy are simple in the case of varices and more complicated for portal hypertension. Elements of variceal bleeding unresponsive to vasopressin
therapy can be altered permanently only by surgical interruption of flow into the esophageal veins. Elements of portal hypertension unresponsive to vasopressin therapy fall into two categories: those amenable to alteration by means other than vasopressin and those that are difficult to alter. Elements of portal hypertension unresponsive to vasopressin and amenable to alteration have been treated by surgery of the portal system and lymph system, and they might be treated with drug therapy directed against suta-coidal factors. The most common surgical alterations involve simple plumbing changes. Several different shunts can be created. Portacaval and mesocaval shunts do not reduce the factors originally responsible for portal hypertension, but they do decompress the portal system by connecting the small, high-pressure portal system directly to the large, low-pressure caval system and by-passing the high resistance in the liver. One of the causes of portal hypertension, increased flow into the portal system, is reduced by another plumbing change, a splenorenal shunt. This shunt diverts splenic flow into the renal vein which decreases the total flow into the HPV. Splenectomy, splenic artery ligation, and subtotal gastrectomy with gastric devascularization also attack the problem of increased flow. Venting the thoracic duct alters HPV pressure by reducing resistance to flow in the liver. Reduction in resistance results from an increase in thoracic duct drainage, giving
all but the RBC's and larger plasma proteins an expanded alternate drainage pathway out of the liver. It may be possible to employ drug therapy directed against autacoidal factors, compounds that may alter flow through the liver, most probably by action on hepatic sphincters. "Autacoid" is an old term revived by Douglas (1975a) to describe compounds that are not hormones or neurohumors but that have actions in the body similar to hormones or neurohumors. The autacoidal factors possibly involved with hepatic flow are released from dead and dying cells in the liver, immunologic and clotting reactions in the liver, and by healthy cells outside the liver that depend on the liver to inactivate compounds. The importance of autacoidal factors is a matter of speculation because presently their role is hypothetical.

All the factors contributing to portal hypertension that are unresponsive to vasopressin and difficult to alter concern resistance to flow through the liver: bulging of cells into the sinusoidal lumen; fibrous tissue deposition in the hepatic vein (HV), HPV, and the space of Disse; capillarization of the sinusoids, connective tissue contraction, compression of HV radicles by nodules of parenchymal cells encased by connective tissue capsules; intrahepatic shunting; and increased HA flow into the liver.
Physical Principles

Three physical principles are important in the study of portal hypertension and esophageal varices: Ohm's law, Poisseuille's law, and La Place's law.

Ohm's law, or more precisely the counterpart of Ohm's law in a fluid system, is the most important of the three physical principles. It describes the relationship between pressure ($P$), flow ($F$), and resistance ($R$):

$$P = F \cdot R$$

This relationship is important to all the theories of portal hypertension and vasopressin action. It describes how either increased resistance or increased flow can increase pressure and how a small increase in one can exacerbate the effect of increasing the other. Conversely, this relationship explains the hypotensive effects of decreases in flow or resistance.

Poisseuille's original relationship is a useful means for approximating the contribution of different factors to flow through blood vessels:

$$F = \frac{P \cdot k \cdot d_i^4}{L}$$

where $F$ refers to flow; $k$ is a constant dependent upon fluid viscosity; $P$ is pressure; $d_i^4$ is the fourth power of the internal diameter of the vessel, and $L$ is the length of the vessel. This equation assumes uniform flow velocity within
blood vessels. This assumption limits the applicability of the equation because laminar flow, with lowest velocity flow at the periphery of the vessel and highest velocity flow in the center, is thought to be a better description of flow within blood vessels.

Poisseuille's law can be applied to laminar flow when \( d_i^4 \) is defined in terms of radius and \( k \) is replaced with a formula reflecting laminar flow:

\[
k = \text{eta} = -(\text{tau}) \cdot \frac{dr}{dv}
\]

where \( \frac{dr}{dv} \) refers to the point-by-point description of laminar parabolic flow, \( \text{tau} \) refers to the force at each point, and \( \text{eta} \) may be thought of as viscosity. With these substitutions, Poisseuille's original formula undergoes integration to become:

\[
F = \frac{P \cdot \pi \cdot r_i^4}{8 \cdot L \cdot \text{eta}}
\]

In this new equation, \( \pi \) is the constant for circles and \( r \) is the internal radius of the vessel (Strandness and Sumner, 1975).

When studying portal hypertension, it is often more useful to think in terms of the contribution of specific factors to resistance to flow. Ohm's equation can be rearranged to reflect this thinking:

\[
P = F \cdot R
\]
\[ R = \frac{P}{F} \]

Poiseuille's integrated formula can undergo this same transformation. First it must be changed to the form of Ohm's law by rearranging:

\[ \frac{P}{F} = \frac{\pi \cdot \eta}{8 \cdot L \cdot \frac{r^4}{\pi}} \]

to:

\[ P = F \cdot \frac{8 \cdot L \cdot \eta}{\pi \cdot r^4} \]

This equation may be rearranged to reflect the factors important to resistance:

\[ R = \frac{8 \cdot L \cdot \eta}{\pi \cdot r^4} \frac{P}{F} \]

These mathematical statements describe the physical factors important to resistance, assuming that the fluid is a perfect solution and that the system corresponds to laminar flow in a non-compliant tube. Noteworthy in this relationship is the internal radius of the vessel taken to the fourth power. Small changes in the radius have large effects on resistance. In contrast to the indirect fourth power relationship with radius, resistance is directly proportional to the first power of vessel length and the first power of fluid viscosity.

La Place's law explains some of the forces responsible for variceal hemorrhage. This physical relationship explains how increased flow and pressure in the esophageal
veins can lead to rupture of the vessel wall; it explains the relationship between pressure in a blood vessel (P), the internal radius of the vessel (r), and the tension on the walls of the vessel (T):

\[ P = \frac{T}{r} \]

As collateral flow into the esophageal veins increases, they dilate. When they can dilate no more and the flow continues to increase, the pressure increases according to the dictates of Ohm's law. With a fixed radius as pressure increases, force on the wall of the blood vessel increases until varices and, later, bleeding result. Sustained pressure over long periods of time causes progressive weakening of the vessels, which leads to varices and eventual hemorrhage. Varicosities of the HPV, HA, and HV do not often occur because these vessels are well supported. The HPV and the HA both possess vascular smooth muscle and travel through the substance of the liver encased by the connective tissue of Glisson's capsule. The HV possesses a muscular coat and is surrounded by hepatocytes.

Vasopressin Side-Effects

Vasopressin is a specific nonapeptide with a disulfide bond linking two cysteines at positions one and six. (Reference to vasopressin as an octopeptide considers the two disulfide-bonded cysteines as one amino acid, cystine.)
Vasopressin possesses important antidiuretic properties; thus it is known as antidiuretic hormone or ADH. Antidiuretic action is not important to this discussion.

Vasopressin therapy has two side-effects that are significant in the management of its administration and several relatively less-important side-effects. The two major side-effects of vasopressin are on the heart and systemic vasculature. The effects on the heart are thought to result from a combination of a vasopressin-induced decrease in coronary artery flow (Green et al., 1942; Drapanas et al., 1961; Corliss et al., 1968) and reflexly-induces changes in vagal and sympathetic control of the heart (Brazeau, 1975). The most important question is whether the vasopressin administered will decrease coronary artery flow enough to create cardiac arrhythmias. The question is important because either non-fatal arrhythmias (Drapanas et al., 1961; Conn et al., 1972; Johnson et al., 1977) or fatal arrhythmias (Slotnik and Tiegland, 1951) are possible. Other important considerations concerning cardiac function are decreased cardiac output (Drapanas et al., 1961; Segel et al., 1963; Corliss et al., 1968; Barr et al., 1975; Millette et al., 1975; Thromford and Sirinek, 1975; Erwald, 1976; Sirinek et al., 1976b) and bradycardia (Segel et al., 1963; Cort et al., 1968; Conn et al., 1972; Nusbaum et al., 1974; Aronsen et al., 1975; Erwald, 1976, Sirinek et al., 1976b; Johnson et al., 1977). Vasopressin-induces smooth muscle
contractions also are responsible for increased systemic pressures, the second major side-effect. These contractions are the result of direct action on vascular smooth muscle; it is neither antagonized by adrenergic blocking agents or eliminated by vascular denervation (Brazeau, 1975). On a molecular level, vasopressin has been hypothesized to act on smooth muscle by increasing intracellular Mg\textsuperscript{++} concentrations which could either free intracellular Ca\textsuperscript{++} or change the actinomyosin directly (Altura, 1975b). A number of other side-effects are relatively less important. Abdominal cramps (Reynolds et al., 1960; Kessler, 1968; Nusbaum and Conn, 1975; Thromford and Sirinek, 1975; Bengmark, 1976; Rabøl et al., 1976), defecation (Baber et al., 1960; Reynolds et al., 1960; Kessler, 1968; Boesby and Pedersen, 1974; Nusbaum and Conn, 1975; Erwald, 1976; Palmer and De Carle, 1976), substernal pain (Thromford and Sirinek, 1975), as well as cutaneous blanching (Baber et al., 1960) may be related to smooth muscle contraction, although headaches (Baber et al., 1960) appear more likely to result from vasopressin-induced dilation of the cephalic vasculature (Aronsen and Nylander, 1966a and b) than smooth muscle contraction. In the special case of long-term vasopressin administration by superior mesenteric artery infusion, a not-infrequent complication is enteric bacteriemia (Conn et al., 1972; Bar-Meir and Conn, 1976).
Increased gut permeability as a result of splanchnic vasoconstriction decreasing blood flow to the area is blamed (Cirrincione and Francona, 1932; Evans et al., 1948, cited in Bar-Meir and Conn, 1976; Renert et al., 1972). Mesenteric vein thrombosis and small bowel infraction have also been reported as complications of superior mesenteric artery vasopressin infusion. Johnson et al. (1977) report complications from superior mesenteric artery vasopressin infusion related to the presence of indwelling catheters for long time periods and not to the effects of vasopressin. Vasopressin can also produce a significant reduction in blood loss and operating time in shunt operations for portal hypertension (Nusbaum et al., 1974; Sirinek et al., 1976a).

Vasopressin and Variceal Bleeding. Sources of flow into the esophageal veins and the locations of the esophageal veins within the esophagus are important to formation of varices, their bleeding, and vasopressin control of bleeding. Variceal bleeding further involves the lymph system, the law of La Place, and the vulnerability of the submucosal esophageal veins. Vasopressin is thought to arrest variceal bleeding indirectly by decreasing flow into the HPV and directly by constriction of esophageal smooth muscle which selectively decreases flow into the submucosal esophageal veins. Surgically, flow into all the esophageal veins can be restricted or flow may be selectively rerouted away from the submucosal esophageal veins.
Flow into the esophageal veins can come from several sources. Arterial blood arrives at the esophageal veins from five sources: direct branches off the aorta, branches of the left gastric artery, branches of the phrenic artery, branches of the thyrocervical truck of the subclavian artery, and branches of the branchial artery (Warwick and Williams, 1973). In a healthy individual, the esophageal veins arise from and get most of their flow from the left gastric vein (also called the coronary vein). Although varices accompany portal hypertension or obstruction to HPV flow the vast majority of the time, their presence is not always so easily explainable (Weinberg, 1949; Garret and Gall, 1953). In the case of portal hypertension, flow from the stomach into the esophageal veins increases as resistance to HPV drainage of the gastric veins increases with increased HPV pressure. Also, when resistance to splenic vein drainage into the HPV is high, splenic flow can be diverted into the esophageal veins by travelling through the short gastric veins (also called the vasa breva) to the stomach and on to the left gastric vein. When HPV pressure is substantial, both the left and right gastric vein (also called the pyloric vein) may carry flow from the HPV into the esophageal veins, and the right gastroepiploic vein may also carry flow from the superior mesenteric vein to the stomach, from where it can travel to the gastric veins and into the esophageal veins. Two anomalies of the portal
venous system encourage the diversion of flow into the left gastric vein. Netter and Oppenheimer (1964) report that the left gastric vein enters the splenic vein in about 24% of the population and that the left gastric vein enters the junction of the splenic vein and superior mesenteric vein in a smaller percentage of the population. These anomalies aid the diversion of splenic vein flow into the left gastric vein and, thus, into the esophageal veins. A study of flow diversion in alcoholics was conducted by Lebrec et al. (1976b). In 17 patients with gastric or esophageal bleeding and a history of alcoholism, flow studies were done within a week after an episode of bleeding. An average of 95% of splenic artery flow and 70% of superior mesenteric artery flow bypassed the liver. They also noted that a great variation existed in the amount of superior mesenteric artery shunting, from very little to total shunting, and that very little variation existed in splenic artery shunting, it was uniformly substantial. From their results, one may presume that the pattern of shunting varies among individuals and that splenic artery flow is the most likely to be diverted. On the basis of the anatomy of the venous system, flow through the esophageal veins is probably the result of diversion of splenic and gastric flows, and diversion of superior mesenteric vein flow seems likely to occur through other channels.

Increased hepatofugal collateral circulation through
the esophageal veins results in the presence of esophageal veins at three levels in the esophagus from veins normally present in only two layers. The esophagus contains an external fibrous layer, a muscle layer that consists of thick longitudinal and circular muscles, a submucosal layer connecting the muscle layer, and an inner mucous layer. This inner mucous layer is composed of a thin muscle and connective tissue layer external to a layer of stratified squamous epithelial cells (Warwick and Williams, 1973). In patients with portal hypertension, esophageal veins are found in three layers: the submucosal layer, the external fibrous layer, and the muscle layer. Bleeding esophageal varices appear predominantly in the submucosal layer, where the esophageal veins are primarily found in a healthy individual. Here, the blood vessels are poorly supported by connective tissue (Som and Garlock, 1947), explaining, in part, their tendency to bleed. Veins, referred to as periesophageal esophageal veins, are present in the external connective tissue layer in healthy individuals but are more marked in individuals suffering from portal hypertension (Som and Garlock, 1947; Aronsen and Nylander, 1966b; Yamamoto et al., 1976). A third set of esophageal collaterals arise in cases of portal hypertension within the muscle layer. A plexus appears in the muscle layer, between the circular and longitudinal muscles, connecting the submucosal and periesophageal veins (Yamamoto et al.,
Bleeding from esophageal varices is related to a number of factors. The lymph system may interact, though less directly than the arterial and venous flows, with the esophageal veins to promote portal hypertension and variceal bleeding. The lymph system probably contributes to portal hypertension and bleeding varices as its ability to drain excess extracellular fluid decreases. Increased lymph production from the liver and intestines tax the capacity for lymph drainage through the thoracic duct as high pressures in the esophageal veins from increased collateral flow increase the need for esophageal lymph drainage. Another, more direct mechanism may result as a consequence of common developmental features of the lymph system and venous system, especially the azygos system and the thoracic duct. The role of the lymph system in variceal bleeding, as well as portal hypertension, will be discussed later, in detail (pp. 53-64). Doubtless, the law of La Place plays a role in venous weakening and possibly precipitates hemorrhage, because distended veins are less able to resist pressure from additional flow. Submucosal esophageal veins receive little support or protection from connective tissue or musculature; thus they are especially sensitive to the forces of La Place's law and to trauma. Their close proximity to the esophageal lumen and the fact that they sometimes lie in the mucosal layer (Som and Garlock,
1947) contribute to their fragility. Here, they can be injured from the reflux of acid chyme, and this has been postulated to precipitate variceal bleeding (Gray and Whitesell, 1950; Netter and Oppenheimer, 1964). The danger of physical trauma from swallowing is illustrated by the report of a fatal case of variceal hemorrhage ascribed to trauma from swallowing several large A.P.C. tablets with little fluid to lubricate their passage down the esophagus (Duffy and Fraser, 1944).

Vasopressin-induced esophageal smooth muscle constriction may control bleeding from esophageal varices by interrupting the flow into the submucosal veins and rerouting the flow into the stronger, periesophageal veins. Surgically, the flow into the esophageal veins can be directly decreased; surgically, flow may also be rerouted from the submucosal veins into the periesophageal veins. Vasopressin cannot directly decrease the flow into the esophageal veins. If constriction of splanchnic arterioles reduces variceal bleeding by decreasing HPV flow which in turn reduces esophageal vein flow, as will be discussed later (p. 88), this is an indirect reduction of flow into the esophageal veins.

Vasopressin's hemostatic action on variceal bleeding is theorized to depend upon compression of the esophageal veins by constriction of esophageal smooth muscle (Aronsen and Nylander, 1966b; Nusbaum and Conn, 1975; Bengmark,
1976; Rabøl et al., 1976). Constriction of esophageal smooth muscle will reduce flow in the submucosal esophageal veins because it will compress tributary veins passing through the muscle layer. With closure of this pathway, flow into the esophageal veins is routed into the periesophageal esophageal veins, which are stronger and better protected from trauma resulting from swallowing. Strong support for this theory comes from Aronsen and Nylander (1966b). They observed dilated periesophageal veins and no trace of submucosal veins after vasopressin administration to dogs, presumably because constriction of esophageal smooth muscle blocked flow to the submucosal veins and routed it through periesophageal veins. Abdominal cramps, defecation, nausea, and substernal pain, mentioned earlier (pp. 10-11), support the idea of vasopressin-induced contraction of esophageal smooth muscle. Johnson et al. (1977) question this theory on the basis of Inglefinger's (1943) statements that posterior pituitary extract increased propulsive movements but decreased the tone of intestinal musculature in dogs and humans. One may question the applicability of work with posterior pituitary extract, that is a combination of compounds, to work employing only one of the compounds in that combination. Also, although the defecation and nausea observed with vasopressin administration could be the result of increased propulsive movements only and not of increased muscle tone,
abdominal cramps and substernal pain seem likely to be the result of increased muscle tone.

Surgery can permanently and directly interrupt flow into the esophageal veins. Interruption of flow into the esophageal veins by ligation of the arteries supplying blood and tributary veins is exemplified by the procedure of Yamamoto et al. (1976). Their procedure ligates the arteries to and removes any external varicosities in the greater and lesser curvatures and the cranial portion of the stomach. This procedure also includes the removal of the periesophageal veins from the 5 cm of the thoracic esophagus proximal to the esophagogastric junction, splenectomy, and a resection of a 5 cm sleeve of the esophagogastric junction and anastomosis of the esophagus within 2 cm of the closed gastric stump. Arterial ligation and removal of external veins decrease flow into the esophageal veins; resection of a sleeve of the esophagogastric junction also decreases flow, but interrupts flow into the submucosal esophageal veins more directly than other elements of the procedure; splenectomy removes a major source of flow into the HPV and a major source of flow into the esophageal veins in cases of portal hypertension.

Surgically, the flow into the relatively weak and vulnerable submucosal veins may be rerouted into the relatively strong and protected periesophageal veins. This redistribution of flow can be accomplished by a procedure
described by Som and Garlock (1947). They performed a mediastinotomy and packing, which causes the formation of granulation tissue between the esophagus and the surrounding prevertebral fascia and establishes new anastomotic channels between the periesophageal veins, embedded in the external connective tissue, and the posterior body wall. Because the periesophageal collaterals are better supported by surrounding connective tissue and better protected from trauma associated with swallowing, they are better able to handle the flow. They also drain into the low-pressure venous system of the posterior body wall rather than into the azygos system which has high pressures and flows in individuals with portal hypertension; thus hemodynamic advantage is gained over flow through the submucosal veins. One year post-operation, Som and Garlock (1947) report shrunken submucosal varices from esophagoscopy.

**Vasopressin and Portal Hypertension**

Some elements of portal hypertension, the major cause of variceal bleeding, can be altered by vasopressin; others cannot. Among the elements unresponsive to vasopressin therapy are some amenable to alteration by some other treatment and some not easily altered by any treatment.

Portal hypertension is created and prolonged by factors resistant to vasopressin and most other types of therapy. All relate directly to architectural changes in
the liver; therefore, the architecture of a healthy liver is important for understanding the abnormal organ. The illustrations of Netter and Oppenheimer (1964) and Elias and Sherrick (1969) facilitate the visualization of the liver's morphology.

The parenchymal cells are arranged in a complex three-dimensional structure to form sinusoidal channels which provide a pathway connecting the hepatic artery (HA) and HPV inflow tracts with the HV outflow tracts (called central veins). The HA and HPV, as well as the bile ducts and lymph channels, are enclosed by the connective tissue of Glisson's capsule within the substance of the liver. This collection of vessels and their connective tissue sheath is called the portal tract. The parenchymal cells delineate the sinusoidal channels. Typically, the parenchymal cells are one-cell thick in the dimension between the sinusoids and several cells thick in other dimensions. The sinusoids are not direct pathways between the portal tracts and the HV; intrasinusoidal sinusoids exist which connect adjacent sinusoids. The area around the portal tract has a particular architecture. The parenchymal cells form a wall surrounding the portal tract. This wall (called the limiting plate) also serves to delineate the space of Mall, which is defined as the open area between the connective tissue of Glisson's capsule and the parenchymal cells of the limiting plate. Branches of the HPV may come off the HPV and
directly pierce the limiting plate or they may travel along- side the HPV for some distance and then send channels through the limiting plate. The channels running parallel to the HPV are called the distributing veins. All the branches which pierce the limiting plate (called inlet venules) give rise to candelabra-like projections of sinusoids.

The HA supplies a capillary plexus around the bile ducts; what else and how else it supplies the human liver is a topic of disagreement. Elias and Petty (1953) state that the HA has paraportal arterioles supplying the areas adjacent to the portal tracts as well as intralobular arterioles supplying the rest of the lobule. They comment additionally that the intralobular arterioles are more numerous than paraportal ones. Also, they report the presence of veins from the biliary capillary plexus to the intralobular HPV's. Nakata and Kanbe (1966) state that the HA nourishes the portal tract and may nourish a few peripheral layers of parenchymal cells. They specifically deny the presence of paraportal or intralobular arterioles, veins from the biliary plexus, or direct HA-HPV anastomoses. Mitra (1966a), on the other hand, found HA-HPV anastomoses and small lobular arteries emptying into the periphery of the lobule, but no evidence of large, intralobular arteries. Although the structure of the HV system in the rat liver differs substantially from the structure of the human liver
(Elias and Popper, 1955), it is interesting to note that intralobular arterioles have been identified in rat livers by Kanbe (1965) and McCuskey (1966), but not observed by Nakata (1967).

Two common microscopic observations of the living livers of amphibians, primarily frogs, and mammals, primarily rats (Wakim and Mann, 1942; Seneviratne, 1949; McCuskey, 1966), are likely also to apply to human livers. To begin with, not all sinusoids are in use at any one time. Interpretation of physical data from flow studies (Brauer et al., 1953; Mitzner, 1974a) is consistent with the idea that resistance to flow may vary with the number of sinusoids recruited to handle flow. These observations coupled with descriptions of intrasinusoidal sinusoids suggest the liver perfusion can be increased and decreased. Increasing perfusion would pool blood; decreasing perfusion would result in mobilizing blood into the systemic circulation. These observations correlate with ideas about the liver's blood reservoir function, to be discussed later (pp. 98-105). A second observation is rhythmic and pulsatile flow in the sinusoids. It has been observed that the contents of the sinusoids get well-mixed. Observations of rhythmic and pulsatile flow appear consistent with reports of sphincters at the HPV and HV ends of the sinusoids (Knisely et al., 1948, cited in Elias and Sherrick, 1969). Nakata (1967) did not find sphincters for control of sinusoidal flow in
rat livers; and Gibson (1959) specifically denies the presence of muscular sphincters at the sinusoidal-central vein junction in humans.

The architecture of healthy sinusoids is primarily dependent upon endothelial cells and Kupffer cells because these cells delineate the sinusoidal lumen and the space of Disse. Kupffer cells differ from endothelial cells primarily by an ability to phagocytize. The lumen of the sinusoidal channel is defined by these cells. Since these cells and the sinusoidal lumen do not totally occupy the space between the parenchymal cells, an additional space (the space of Disse) is defined, first described by MacGilvary (1865, cited in Child, 1954). A fibrous tissue network forms a sparse basement membrane for the Kupffer and endothelial cells. Relatively thick fibres run parallel to the path of the sinusoid, and relatively thin fibres encircle the cells and the lumen they define. Communication between the sinusoidal lumen and the space of Disse is provided by fenestrations in the endothelial and Kupffer cells and gaps between the cells. Fenestrated cells are called sieve plates. At the HPV end of the sinusoid, the diameter of the fenestrations approaches 2.0 micrometers; along the length of the sinusoid, the diameter is 0.1 to 0.2 micrometers (Grisham, 1975). Many gaps may be found between the cells, and cells overlap in other places. Many of the interactions between the blood and the liver take place in the space of
Disse, into which the microvilli of the parenchymal cells extend. In rats, the fenestrations and gaps between the endothelial and Kupffer cell membranes extends for at least 90% of the length of the sinusoids. At the HPV and HV ends of the sinusoid, the lining is continuous for a short distance, apparently a continuation of the HPV or HV radicles (Yoffey and Courtice, 1970).

**Elements of Portal Hypertension Not Easily Altered That Exist Within the Architecture of a Healthy Liver.**

Three architectural changes at the sinusoidal level contribute to portal hypertension and are relatively irreversible: cells bulging into the sinusoidal lumen; fibrosis of the HV, HPV, and the space of Disse; and capillarization of the sinusoids.

The first change concerns the protrusion of fat-filled cells into the lumen of the sinusoids. In mice, swelling of fat-filled parenchymal cells has been observed after administration of a lipotrope-deficient diet (Rogers and MacDonald, 1965; Rappaport et al., 1970), and unspecified "edema" of parenchymal cells is reported after fulvine injection (Rappaport et al., 1970). In carbon tetrachloride-treated rats, an increase in the size of parenchymal cells is correlated with decreases in hepatic flow (Nakata et al., 1973). Also in carbon tetrachloride-treated rats, sinusoidal flow decreases over a period of time caused by sinusoidal occlusion have been quantitated (Nakata, 1967)
as well as correlated with an increase in cell size (Fujimoto and Nakata, 1972). In addition, swelling of fat-filled parenchymal and Kupffer cells (MacDonald, 1962) as well as fat-filled parenchymal cells only (Wada et al., 1974) have been reported in rats after carbon tetrachloride administration. Endotoxin administration a prepared rat causes hepatic congestion, yielding immediate swelling of Kupffer cells into the sinusoidal lumen (McKay et al., 1966). Hypoxia produces hydroptic swelling of Kupffer and parenchymal cells (Nakata et al., 1971), and this blockage of flow may be involved in a positive feedback system with decreased flow causing greater hypoxia and worse swelling and blockage. Rats fed a butter-rich diet developed fat-filled parenchymal cells and higher portal pressures than control rats (Latour et al., 1974); among rats on a lipotrope-deficient diet, only those livers having sufficient fat to cause distention of parenchymal and Kupffer cells developed cirrhosis (MacDonald, 1962). In dogs, Kupffer cell enlargement along with dilation of the lymphatics has been observed in in situ livers subject to ischemia of up to four hours (Grana et al., 1968). Injection of distilled water into the livers of dogs and cats produced swelling of liver cells and an increase in HPV pressure (Bainbridge and Trevan, 1917). Sundet (1975) presents data from flow studies and hypothesizes that olive oil injection into rats results in fat accumulation that increases
resistance to hepatic flow. Histologic evidence is not presented to support this hypothesis, but it does not seem unreasonable when compared to the work of others.

Swollen parenchymal and Kupffer cells have been thought to have a role in the creation of liver damage and portal hypertension in humans. Observations that cell swelling may be able to create liver damage in humans may be extended to infer support for cell swelling producing portal hypertension: perhaps swollen cells can interrupt enough flow to create portal hypertension if they can cause cell damage and necrosis and a cirrhotic liver results. Lieber (1975) established that alcoholic fatty livers can progress to cirrhotic livers. Hinsworth (1947) theorizes that parenchymal cell swelling in humans decreases flow and that the decreased flow is responsible for necrosis around the central veins. Baxter and van Slyke (1948) and Wallach and Popper (1950) support Hinsworth's (1947) theory. The presumption that cell swelling can cause liver damage is disputed by Thaler (1975). He believes cell damage and cirrhosis result, not from the cell swelling, but from whatever caused the swelling. Cell swelling in cirrhosis may be the result of fat accumulation and it may be the result of fat accumulation due to a decreased export ability (Baraona et al., 1975). Cell swelling has been theorized to be able to create portal hypertension. Moschowitz (1948) observed fat-filled liver
cells in patients with cirrhosis and postulated that they had a role in creating portal hypertension. In 1954, Madden et al. theorized that outflow blockage could be caused by cellular edema. Hales et al. (1959), writing about corrosion casts of human livers, theorized that swollen, fatty, and necrotic parenchymal cells were responsible for the decreased venous bed observed early in the cirrhotic process; they theorized that an entirely different change, contraction of fibrous tissue, was important later on. MacDonald (1962), writing about nutritional cirrhosis, described swelling of parenchymal, Kupffer, and sinusoidal cells occluding the sinusoidal lumen and postulated a concomitant obstruction to flow. Correlations have been made that firmly establish the role of swollen cells and occlusion of the sinusoidal lumen in the etiology of portal hypertension. Increases in the size of liver cells concurrent with increases in HPV pressure have been documented in a study of the effects of non-surgical therapy. Leevy et al. (1958) found that HPV pressure decreased when therapy reduced the amount of fat in the liver cells. A close reverse correlation has been observed in humans between the size of the sinusoidal lumen and pressures in a study of surgical biopsy specimens and HPV pressures (Nakata et al., 1973) and in a study of needle biopsy specimens and wedged HV pressures (Nakamura et al., 1968, cited in Nakata et al., 1973). Wedged HV pressures are recorded by occluding a HV
with a venous catheter and are believed to be a direct measure of sinusoidal pressure (Reynolds et al., 1954; Reynolds et al., 1970). The idea of cell swelling occluding sinusoids and creating portal hypertension is not plainly stated by Leevy et al. (1964) in their work with radioactively-labelled liver cells of alcoholics; however, the implication from their observations is unmistakable. They reported Kupffer cell and connective tissue proliferation accompanied by decreased flow and increased wedged HV pressures. Kupffer cell enlargement during proliferation presumably is the important factor to decreased flow, because connective tissue proliferation would not be substantial enough in a short period to block flow. These observations are similar to observations from similar later work by Leevy (1966) with both alcoholic and viral cirrhosis.

Two reports support cell swelling and perisinusoidal fibrosis as factors in the creation of portal hypertension. Russell et al. (1974) report two patients with portal hypertension and ascites whose only liver pathologies were increased connective tissue in the space of Disse and enlarged fat-storing cells (Ito cells) occluding the lumen of the sinusoids. This condition was apparently the result of chronic hypervitaminosis A as a health fad. These cases represent a good example of portal hypertension that is probably dependent on these two factors. In a report four
years earlier, portal hypertension without ascites was reported in a patient presenting perisinusoidal fibrosis and swollen Kupffer cells as the only likely causes (Kluge, 1970).

Perisinusoidal and HV fibrosis appear to be able to create portal hypertension in combination, and perisinusoidal fibrosis by itself appears to be able to create portal hypertension. A form of liver disease peculiar to the alcoholic (called sclerosing hyaline necrosis) is characterized by portal hypertension; hyaline degeneration; fibrosis, primarily of the central vein; but no swollen cells or nodular architecture. The earliest lesion is reported to be perisinusoidal fibrosis and it is associated with portal hypertension (Edmondson et al., 1963; Edmondson et al., 1967; Reynolds et al., 1969). Portal hypertension presenting only perisinusoidal fibrosis also is reported by Tandon et al. (1970).

HPV fibrosis appears to be able to create portal hypertension, and perisinusoidal fibrosis may be unrecognized or recognized but not thought to be important in the fibrosis of the HPV bed reported by several investigators (Ramalingswami et al., 1962; Mikkelsen et al., 1965; Boyer et al., 1967; Donovan et al., 1969). They report portal hypertension associated with HPV fibrosis, but not associated with any derangement of the lobular architecture. Donovan et al. (1969) cite similarities between their
patients and those of Mikkelsen et al. (1965). Donovan et al. (1969) theorize that the HPV fibrosis can be caused by increased HPV pressure from arteriovenous fistulae and that after several years of the HPV fibrosis is the primary cause of portal hypertension. Boyer et al. (1967) state that they observe a pathology similar to that observed by Mikkelsen et al. (1965), but they believe the cause is different. Boyer et al. (1967) attribute the HPV fibrosis they observe to result of the high incidence of sepsis, pyelophlebitis, and intra-abdominal disease present in children of the population from which they obtained patients (India). Boyer et al. (1967) also report the presence of perisinusoidal fibrosis, but ascribe it a secondary role in the production of portal hypertension. The possibility exists that the patients of Boyer et al. (1967) as well as Ramalingswami et al. (1962), Mikkelsen et al. (1965), and Donovan et al. (1969) all possess perisinusoidal fibrosis and that it plays a role in the creation of portal hypertension.

The third change is sinusoidal architecture that can create portal hypertension is the capillarization of the sinusoids, and it involves changes in endothelial cells and connective tissue in advanced cirrhosis. The endothelial cells lose their fenestrations, become continuous with each other, and gain a basement membrane (Shaffner and Popper, 1963). Flow into and through the space of Disse is restricted, and thus the effective radius available to handle
flow is reduced by whatever fraction is provided by free communication with the space of Disse. The implications for hepatic function are important. If sinusoidal blood cannot reach the sinusoidal membranes of the hepatocytes easily, the hepatocytes will suffer from lack of nutrition and the individual will suffer because of poor parenchymal cell interaction with substances in the blood. That the space of Disse is reported to be filled with cell debris, fibrous tissue, and various mesenchymal cells does not detract from the role capillarization plays. These other features only make matters worse. Capillarization of the sinusoids has been observed in rats treated with carbon tetrachloride (Steigner, 1966). Quinn and Higginson (1965) are not convinced that capillarization of the sinusoids is always extensive in cirrhotic livers.

Elements of Portal Hypertension not Easily Altered That Result from Changes in the Liver's Architecture. The elements of portal hypertension that are not easily altered that have been discussed up to this point are factors that operate within the architecture of a healthy liver. Four additional elements of portal hypertension that are not easily altered exist within the disturbed architecture of cirrhotic livers. An overview of the gross morphological changes of cirrhotic livers can help one understand these four elements of portal hypertension. In addition, the cirrhotic process in general is important to understand
because portal hypertension can result from liver diseases of different etiologies. The liver reacts in a similar manner to a variety of liver diseases; therefore, the end stage of cirrhosis from several different causes will involve the same kind of structure despite the fact that the etiology and early stages were entirely different (Baggenstoss, 1975).

A number of changes are common events in the development of the cirrhotic liver (a liver characterized by nodular parenchyma and divided by connective tissue at the expense of the normal architectural pattern). Although all of the following characteristics may not apply to every disease, the majority are common to cirrhosis as the result of any etiology:

1) Ductular, central vein, and portal tract inflammation and fibrosis with ductular cell proliferation as ductules, singularly or in clumps. It may be significant that connective tissue serves as the inducer for ductular cell differentiation in the embryo (Bloom, 1926; Moolten, 1943) and that ductular cells are suspected of being derived from parenchymal cells (Popper and Zak, 1958).

2) Swelling of cells in the sinusoids, especially at the HV ends, as well as the collection of inflammatory cells and fibrosis blocking flow and eventual causing necrosis.

3) Some sinusoids remain open and carry the flow from the blocked sinusoids, enlarging, fibrosing, and acquiring the continuous endothelial lining and basement membrane characteristic of extrahepatic capillaries. Portal tract destruction and central vein destruction also give rise to shunts. The HA supply to the parenchyma is important here because the fewer HA radicles traversing the parenchyma the less the likelihood that cirrhotic changes in that
region will produce HA-HV and HA-HPV shunts. Moschowitz (1948) and MacDonald (1962) have ideas about the genesis of shunts distinct from recruitment of sinusoids. Moschowitz (1948) theorizes that in cirrhosis angiogenesis occurs from within the connective tissue strands to form HA-HPV shunts and HPV-HV shunts. Angiogenesis results from fibroblastic transformation to vascular endothelium. He relates this process to the differentiation of the primitive mesenchymal cell in the embryo. MacDonald (1962) theorizes that in nutritional cirrhosis the endothelial cells proliferate to form new or larger venous and lymph channels. The new venous channels become HPV-HV shunts surrounded by connective tissue whose fibers radiate from the portal tracts and central veins and eventually connect with other fibers from similar or different origins. With time, these fibrous tracts widen into the parenchyma.

4) Phagocytosis of necrotic hepatocytes and sinusoidal cells leaves an empty connective tissue framework (called the trabecula or stroma) that eventually collapses and condenses.

5) Bands of the collapsed connective tissue framework and bands formed by shunts within connective tissue traverse the parenchyma and divide it into nodules.

6) Cells sequestered by fibrous bands lose the typical acinar/lobular structure and may either continue to prosper or die. Cell death leads to greater areas of collapsed framework and results in breaks or fissures in healthy tissue due to mechanical stress. In time, the fissures and breaks fill in with connective tissue. Continued prosperity for the cells eventually can lead to restoration of the original acinar/lobular architecture within the nodule (Popper and Zak, 1958).

Connective tissue contraction, HV compression by growing parenchymal cells encased by connective tissue capsules, as well as intrahepatic shunting and the increased contribution of HA flow to the total hepatic flow are all causes of cirrhotic portal hypertension that are
not easily altered and operate within the disturbed structure of cirrhotic livers to increase portal pressure.

Contraction of the connective tissue around intra-hepatic shunts is a straightforward mechanism to increase portal pressure (McIndoe, 1928; Moschcowitz, 1948; Hales et al., 1955; Hales et al., 1959; MacDonald, 1962; Edmondson, 1963).

Nodules of parenchymal cells surrounded by connective tissue, structures characteristic of cirrhotic livers, provide the means for compression of HV radicles, an important architectural element of portal hypertension, and two additional, less-important elements. Parenchymal cells encased in a connective tissue capsule will expand until the limits of the capsule are reached; at which time they will expand at the expense of tissue and vascular space within the capsule, often an HV radicle (Kelty et al., 1950). The HV is not protected by Glisson's capsule; therefore the HV is vulnerable to encroachment by expanding cells. Popper and Zak (1958) further theorize that the nodules expand and compress their connective tissue capsules and in turn compress other nodules. MacDonald (1962) also theorizes that connective tissue contraction may compress the HV indirectly by compressing parenchymal cells which in turn compress the HV. If parenchymal cells compress connective tissue capsules, the shunts which are found within those capsules also may suffer compression (Popper
and Zak, 1958). Quinn and Higginson (1965) offer an interesting hypothesis on the nature of the cells within the capsule; they theorize that these cells may have become neoplastic. Mice treated with carbon tetrachloride; thioacetamide; and choline-deficient, high-fat, low-protein diets all developed these nodules. Transplatable and metastasizing tumors have been induced by all three of these methods. Also, the nodules continue to grow after the stimulation is removed, and the progression of the nodules cannot be correlated with the extent of fibrosis, inflammation, necrosis, or bile duct proliferation.

Intrahepatic shunting may increase portal pressure by bypassing the pressure drop available from a capillary bed, decreasing the volume of the HPV and HV vascular beds, increasing HV pressure as the result of shunting, and increasing the HA percentage of total hepatic flow.

Shunting maintains HPV pressure at high levels because the flow is channelled through a smaller effective radius. Shunts are created when several sinusoids in a sinusoidal bed become clogged as a result of the cirrhotic process and one or two inherit flow from closed sinusoids. The flow capacity of the shunts is not great enough to compensate for the flow capacity lost with clogged sinusoids although the flow capacity of a shunt may be greater than the flow capacity of the original sinusoid. HPV-HV shunts (Moschcowitz, 1948; Popper et al., 1952; Hales et al.,
1955; Popper and Zak, 1952; MacDonald, 1962; Miyake et al., 1964; Mitra, 1966b). HA-HPV shunts (Moschcowitz, 1948; Popper et al., 1952; Mann et al., 1953; Hales et al., 1955; Miyake et al., 1964; Mitra, 1966b), and HA-HV shunts (Miyake et al., 1964; Appelman, 1972), have been reported.

The volume of the vascular bed perfused by the HPV and HV is decreased in a cirrhotic liver (McIndoe, 1928; Hales et al., 1955; Hales et al., 1959; Carter et al., 1961; Miyake et al., 1964; Mitra, 1966b) presumably as a result of cell death and the creation of shunts. This results in a decrease in the effective radius to handle the HPV inflow and HV outflow which increases portal pressure.

HPV-HV and HA-HV shunting delivers blood to the HV at higher pressures and results in an increased HV pressure. Increased HV pressure results in an increased resistance to hepatic flow. A cirrhotic liver is less able to absorb increased HV pressures and not pass them on to the HPV because of the simultaneous increase in the number of shunts, decrease in the capillary bed, and a decrease in lymph drainage capacity, which will be discussed later (pp. 53-63).

The HA component of total hepatic flow increases in cirrhosis and increases portal pressure because HA flow enters the liver at arterial flow rates and pressures. The classic experiments of Herrick (1907) demonstrate this in cirrhotic livers. Herrick (1907) perfused healthy and
cirrhotic human livers at autopsy through the HPV and HA and monitored the pressures in each channel. In a healthy liver, a forty millimeter increase in HA pressure was necessary to increase HPV pressure one millimeter; in a cirrhotic liver, only six millimeters of increase in HA pressure was required to increase HPV pressure one millimeter. Herrick's (1907) results have been reproduced by others (Dock, 1942; Hales et al., 1959; Miyake et al., 1964; Takahashi and Tezuka, 1974).

The increased HA contribution to hepatic flow results from three changes in a cirrhotic liver. First, the HA bed is greater in a cirrhotic liver than in a healthy liver (McIndoe, 1928; Hales et al., 1955; Popper and Zak, 1958; Hales et al., 1959; Carter et al., 1961; Miyake et al., 1964; Mitra, 1966b). Secondly, HA-HPV shunts bypass the pressure drop offered by the capillary bed and send arterial flow more directly into the HPV system. Thirdly, the HA autoregulatory system may go awry. Evidence of an autoregulatory system for the HA decreasing HA flow as HPV flow increases has been observed in calf livers (Condon et al., 1962) and dog livers (Torrance, 1961; Hanson and Johnson, 1963; Hanson, 1964; Hanson and Johnson, 1966; Takeuchi et al., 1969). Geumi et al. (1968) did not observe HA autoregulation in dog livers. The presence of autoregulation of flow is ordinarily deduced from a change in resistance calculated from measured flows and pressures or from changes in the
slope of flow-vs.-pressure graphs (resistance is the slope of the line which demonstrates the relationship between the two). In cirrhotic livers, autoregulation may be interferred with and result in an increased HA flow and a subsequent increase in portal hypertension. HPV autoregulation may exist, and HPV pressure is reported to regulate splanchnic resistance. Although these two regulation systems do not provide direct proof of HA autoregulation, their presence makes HA autoregulation appear more plausible. HPV autoregulation has been reported by some investigators in calves (Condon et al., 1962) and dogs (Torrance, 1961; Hanson, 1964; Hanson and Johnson, 1966), and found to be absent by other investigators in dogs (Takeuchi et al., 1966) and rats (Brauer, 1964). Brauer (1964) states that almost no HPV-HA reciprocity of flow exists in calf livers and only a small amount of HPV-HA reciprocity of flow exists in dog and rat livers. Splanchnic resistance has been shown to be a function of HPV pressure in healthy dogs, and splenectomy increases the dependence of splanchnic resistance on HPV pressure (Mitzner, 1974b).

Retrograde flow through the HPV is theoretically possible when increased hepatic outflow resistance accompanies increased HA flow. This phenomenon has been observed in humans (Hales et al., 1959; Warren and Muller, 1959; Mikkelsen et al., 1962; Johnson et al., 1966; Smith et al., 1971; Nusbaum et al., 1974; Galambos et al., 1976;
Elements of Portal Hypertension that can be Altered by Treatments Other than Vasopressin. Four elements of portal hypertension that are not responsive to vasopressin therapy can be altered by various treatments. Increased flow into the portal system is commonly altered by surgery. Increased blood volume is also commonly altered, and it is attractive because of the relative ease with which it can be accomplished with diuretics. Increasing lymph drainage through the thoracic duct is not a popular procedure for the treatment of portal hypertension, ascites, or bleeding varices, but it has both a theoretical basis and clinical results to support it. The role of autacoidal factors is not well-understood, but if they do play a role, they could be sensitive to specific treatments. Autacoids important to portal hypertension may be thought to include those substances released by healthy, unhealthy, and dead cells; immunological reactions; and clotting reactions that have vasoactive properties. Presently, their role is not established; consequently, this approach to portal hypertension, ascites, and bleeding varices is not an important one.

Increased Flow. Increased flow into the HPV can increase portal pressure according to the dictates of Ohm's law even though four differences exist between a rigid tube and the HPV and hepatic vascular beds. Uncomplicated cases
of increased flow and cases of increased flow in combination with increased resistance have been reported to be responsible for portal hypertension. Increased flow into the HPV other than from a major arteriovenous fistula is reported to come primarily from the spleen and stomach. Alterations of flow are primarily surgical and are related to either a splenectomy or an anastomosis of a portion of the portal system to a portion of the caval system.

To the extent that the HPV and the hepatic vascular beds resemble a set of rigid tubes, pressure will increase with flow according to Ohm's law; to the extent that they differ, an increase in flow can be compensated for by other factors and not result in increased pressure. The HPV and the hepatic vascular beds possess four differences in comparison with a theoretical rigid tube: vascular and hepatic compliance, architectural changes, lymph drainage, and the availability of hepatofugal collateral circulation. Each of these factors has its individual effects on pressure and may also interact with the other factors. Some flow increases will be absorbed with minimal increase or no increase in back pressure by the expansion of vessels within the portal system and the liver. Human livers, if similar to rat livers, have second compliance mechanism by virtue of their ability to mobilize unused sinusoids at higher pressures (Brauer et al., 1953; Mitzner, 1974a). The effect of this vascular and hepatic compliance is to change the
system by increasing the effective radius to handle flow. The second difference, architectural alterations brought about by progressing liver disease, increase the resistance of the system by decreasing the effective radius available to handle flow. The lymph system is able to drain excess extracellular fluid away from the tissues and is the third difference. According to Starling's capillary hypothesis, when all other factors are equal, increased capillary pressure will put more fluid into the extracellular spaces from the capillaries. By removing extracellular fluid, lymph drainage can drain some extra flow and absorb pressure. Lymph production will be discussed in detail, later (pp. 53-64). The creation and expansion of collateral circulation is fourth difference between a hypothetical rigid tube and the HPV and hepatic vascular beds. Alternative vascular channels are created and expand when flow in one channel becomes difficult or blocked. McIndoe (1928) classified the collateral channels resulting from portal hypertension on embryological grounds. His first group results from the union of glandular epithelium from the gastrointestinal tract with squamous epithelium of what was once ectoderm. The esophageal veins fall into this category as do the superior hemorrhoidal veins connecting the portal system with the middle and inferior hemorrhoidal veins of the caval system. McIndoe's (1928) second group is composed of the remnants of the fetal circulation. The
umbilical and paraumbilical veins conduct HPV blood to the anterior abdominal wall and cause the characteristic caput medusae of visible, enlarged veins on the abdomen of some patients with portal hypertension. His third group consist of vessels formed where abdominal viscera become adherent to the abdominal wall, primarily retroperitoneally. These veins (called the veins of Retzius) include veins arising from adhesions due to pathologic or surgical processes.

Increased flow into the HPV can increase HPV pressure both by itself and by working in concert with increased resistance. Cases of portal hypertension that result solely from increased flow are ordinarily the result of substantial arteriovenous fistulae. Increased flow into the portal system also can occur without an arteriovenous fistula, and the stomach and spleen are reported to be the primary sources of this increased flow. This kind of increased flow must work together with an increase in resistance to overcome the body's adaptive responses and create portal hypertension.

Increased flow with or without the help of increased resistance must overcome the body's adaptive responses in order to create portal hypertension. The adaptive responses of humans and Macaca mulatta monkeys (Child et al., 1950; Child et al., 1952) to total HPV blockage provide good examples of the interplay of vascular compliance, lymph
flow, and collateral circulation in response to increased resistance and pressure. Veins dilate, lymph flow increases from the region, and collateral veins (called the veins of Sappey) expand and develop to bypass the blockage and conduct blood to the liver. In the Macaca mulatta monkey, HPV pressure returned to normal within a week after the total blockage of flow. In cirrhosis, hepatofugal collaterals develop to bypass the blockage in the liver and conduct blood back to the inferior vena cava (IVC), but the responses are similar: veins dilate, HPV system lymph production increases with increased HPV pressure and hepatic lymph production increases as postsinusoidal blockage increases, and collaterals expand and develop (as discussed by McIndoe (1928)) to bypass the blockage in the liver. To create portal hypertension, one must overwhelm the body's compensatory responses. According to the theory of C. L. and M. H. Witte (Witte et al., 1974a; Witte and Witte, 1975; Witte et al., 1976a), portal hypertension is rarely the result of uncomplicated increased flow or increased resistance; they believe it is ordinarily the result of the two working together. The multiplicative effect on pressure of simultaneous increases of flow and resistance detailed by Ohm's law explains how simultaneous increases of flow and resistance can be powerful enough to overwhelm the body's substantial adaptive responses.

Increased flow from an arteriovenous fistula can
create portal hypertension without the help of increased resistance. Uncomplicated extrahepatic HA-HPV fistulae (Strickler et al., 1952; Madding et al., 1954; Wheeler and Warren, 1957; Boyer et al., 1967) and uncomplicated splenic arteriovenous fistulae (Cassel et al., 1957; Murray et al., 1960; Stone et al., 1965; Berman et al., 1968; Donovan et al., 1969; Nissan and Bar-Maor, 1971) have been reported to cause portal hypertension.

Splenic and gastric arteriovenous shunts are considered the primary causes of increased splenic and gastric flow into the portal system other than major arteriovenous fistulae in cirrhotic portal hypertension. Splenic and gastric shunting is not one large anastomosis or a few smaller ones; rather, it is a diffuse collection of many very small shunts. Clinical signs of arteriovenous shunting can be observed in many cases of cirrhotic portal hypertension.

Increased splenic flow due to intrasplenic arteriovenous shunting is observed in many cases of cirrhotic portal hypertension presenting enlarged spleens, and reciprocal reticuloendothelial system function is thought to provide the stimulus for this increased splenic flow. The condition is called hypersplenism. The enlarged spleen found in cirrhotic portal hypertension is the result of a great number of open arteriovenous shunts, increased flow through the slower-moving channels of the spleen, and a
great deal of connective tissue (Tumen, 1970). Womack and Peters (1961) and Dumont et al. (1970b) believe the arteriovenous shunting is the primary cause of the increased flow. Dumont et al. (1970 b and c) theorize that splenomegaly and increased splenic flow result from an increase in splenic reticuloendothelial system activity reciprocal with a decrease in hepatic reticuloendothelial system function. Dumont et al. (1970 b and c) attribute the decreased hepatic reticuloendothelial system activity to the decrease in the number of healthy cells in the liver and the increase of capillarization of the sinusoids; extra- and intrahepatic shunting seem likely to be additional, important factors decreasing hepatic reticuloendothelial system activity. C. L. and M. H. Witte (1975) expanded the theory of reciprocal reticuloendothelial system activity. They theorize that splenomegaly and increased splenic flow are the result of splenic reticuloendothelial system hyperplasia in response to depression of the hepatic reticuloendothelial and/or a decrease in transhepatic flow. They support their theory with observations of splenomegaly and increased splenic flow in cases of: 1) the production of abnormal erythrocytes in hereditary spherocytosis, 2) the accumulation of macromolecular cerebrosides in Gaucher's disease, and 3) primary disorders of the reticuloendothelial system and blood-forming organs such as myeloid metaplasia and disproteinemias. Increased splenic reticuloendothelial system
activity can explain the observation of increased flow through the slower-moving channels. Splenic fibrosis can be explained by the presence of increased portal pressures and the theory of Thoma (1920, cited by Moschcowitz, 1948). As portal pressure increases, pressure in the spleen will increase. Thoma (1920, cited by Moschcowitz, 1948) states that the caliber of the blood vessel lumen increases with increases of the flow through it and that the thickness of vessels increases with increases of the pressure in them. In the case of the spleen, portal hypertension promotes splenic fibrosis.

The theory of increased splenic flow due to the reciprocal nature of the hepatic and splenic reticuloendothelial systems is consistent with the theories of Ravenna (1940) and the observations of Mitzner (1974b). Ravenna (1940) theorized that the spleen is a major regulator of flow into the HPV; Mitzner (1974b) observed that splenectomy in dogs resulted in a closer relationship between splanchnic resistance and HPV pressure than before the splenectomy. Both Ravenna's (1940) theory and Mitzner's (1974b) observations suggest that splenic and splanchnic flows are reciprocal to some extent, and such reciprocity would be expected if the activities of the splenic and hepatic reticuloendothelial systems were reciprocal. Ravenna (1940) also further theorized that splenomegaly in cirrhotic portal hypertension is the result of the spleen's role as a
regulator of hepatic flow. This additional theory of Ravenna's (1940) needs only to further specify the reticuloendothelial system as the controlling element to become the theory of Dumont et al. (1970 b and c) and the Wittes (1975).

Clinically, hypersplenism is manifest by anemia and leukocytopenia resulting from splenic sequestration, nutritional and metabolic disturbances associated with increased RBC formation, and the presence of an enlarged spleen (Blaustein and Diggs, 1963; Tumen, 1970).

Increased gastric flow into the HPV is thought to be created by humoral factors dilating the sphincters that control flow through gastric arteriovenous shunts (Ravenna, 1940; Peters and Womack, 1961; Johnson, 1971). The shunts are thought to remain open as forces explained by the law of La Place overcome the ability of the sphincters to close and eventually overwhelm the sphincter muscles (Peters and Womack, 1961).

No clinical manifestations are peculiar to gastric arteriovenous shunting; however, both splenic and gastric arteriovenous shunting can produce a clinical picture similar to that observed with systemic arteriovenous fistulae: a hyperdynamic cardiovascular state characterized by increased cardiac output, decreased peripheral resistance, and increased blood volume. Because the fistula offers a low-pressure channel, it captures some of the
arterial circulation and diminishes circulation to the rest of the body. In compensatory response to decreased peripheral perfusion, peripheral resistance decreases and cardiac output increases (Strandness and Sumner, 1975). Blood volume increases because of two factors. One is increased sodium retention as decreased flow to the kidneys accompanying the generalized decreased peripheral perfusion produces increased aldosterone secretion. The second is the increase in the volume of the vascular bed due to the volume of the fistulae (Strandness and Sumner, 1975). The hyperdynamic cardiovascular state involving increased cardiac output, increased blood volume, and decreased peripheral resistance has been observed in cirrhosis (Kowalsky and Abelman, 1953; Abelman et al., 1955; Murray et al., 1958; Murray et al., 1960; Womack and Peters, 1961; Johnson et al., 1966; Van Way et al., 1971; Johnson, 1971; Siegel et al., 1974). In addition, warm flushed extremities (liver palms), pounding pulses, capillary pulsations, and clubbing of the fingers are also observed and may be attributed to the effects of the hyperdynamic cardiovascular state (Murray et al., 1958; Peters and Womack, 1961).

HPV flow can be altered two ways. Increased flow into the HPV from the spleen may be selectively eliminated by splenectomy or splenic artery ligation. Also, some part of the portal system may be anastomosed to some part of the caval system to decompress the portal system by diverting
Splenectomies or splenic artery ligations decrease portal hypertension and varices bleeding because they decrease HPV flow. The spleen ordinarily provides 40% of the HPV flow (Dumont et al., 1970b). Splenectomies or splenic artery ligations are the operation of choice when portal hypertension can be identified as primarily the result of increased splenic flow (Peters and Womack, 1961; Berman et al., 1968; Dumont et al., 1972; Witte et al., 1974a and b; Witte and Witte, 1975). In addition, splenectomy offers relief for many patients with unspecified portal hypertension (Gray and Whitesell, 1950; Dumont and Witte, 1966a); although the importance of increased splenic flow may have been unrecognized in these patients. Lebrec et al. (1976b) describe almost total splenic artery shunting in cirrhosics, and one may assume that most of this flow is shunted away from the liver via the esophageal veins; thus, much of the relief offered by splenectomy or splenic artery ligation for bleeding esophageal varices may result from decreasing esophageal vein flow. This would decrease HPV pressure by allowing more HPV flow to bypass the liver through the esophageal veins and further decrease flow into the liver. Splenic artery ligation may be preferable to splenectomy because some splenic function remains by virtue of collateral arterial channels into the spleen (Witte et al., 1976b; Witte 1976). Any benefit from splenic artery
ligation must be balanced against the risks from splenic infarction. The effectiveness of splenectomies and splenic artery ligations support the idea of a role for increased splenic flow in the creation of portal hypertension.

Anastomosing a portion of the portal system to a portion of the systemic caval circulation decreases HPV flow by diverting some of the high-pressure portal flow into the low-pressure caval circulation. The relative merits of gastrocaval, portacaval, mesocaval, and splenorenal shunts, their variations, and other shunting procedures are being actively discussed and studied now, and for the most part they are beyond the province of this discussion. Encephalopathy and diversion of hepatotrophic substances away from the liver are side-effects of surgical shunting of blood away from the liver as well as of shunting the result of pathological process.

The exact etiology of encephalopathy is not understood, but it involves the gut, gut bacteria, and substances of nitrogenous origin (Fischer, 1974; Fischer and Baldessarini, 1976). Encephalopathy begins as confusion and disorientation, and it progresses through asterixis and aphasia to coma. Ammonia and false neurotransmitters have been theorized to be responsible for encephalopathy. Papenberge et al. (1975) believe ammonia creates encephalopathy by interfering with cerebral oxidative metabolism. Arterial, venous, and cerebrospinal fluid levels of ammonia
correlate poorly to the degree of encephalopathy observed (Fischer and Baldessarini, 1976). False neurotransmitters are believed to create encephalopathy by virtue of competition for pre- and postsynaptic receptor sites with regular neurotransmitters, because they do not cause depolarization when they bind to the postsynaptic membrane. When enough false neurotransmitters are sequestered within the synaptic vesicles, the vesicles empty upon stimulation but do not depolarize the postsynaptic membrane; thus, the impulse stops at that junction. This interruption of nerve impulses is thought to cause encephalopathy (Fischer, 1974; Faraj et al., 1976; Fischer and Baldessarini, 1976). Candidate false neurotransmitters are primarily analogues of epinephrine and norepinephrine and are thought to result from altered synthetic and destructive pathways for epinephrine and norepinephrine (Fischer, 1974; Faraj et al., 1976; Fischer and Baldessarini, 1976). Octopamine is thought to be an important false neurotransmitter (Fischer, 1974; Faraj et al., 1976; Fischer and Baldessarini, 1976).

Substances in the portal blood of rats (Fischer et al., 1971) and dogs promote hepatic regeneration. In dogs, some of the substances involved have been identified. It is believed that insulin and other factors from the proximal portion of the portal bloodstream promote hepatic regeneration in dogs (Starzl et al., 1973; Starzl et al., 1975 a and b; Starzl et al., 1976). Glucagon has no regenerative
influence (Starzl et al., 1975b; Starzl et al., 1976) and appears to reduce insulin's effectiveness (Starzl et al., 1976). Rat livers, too, regenerate in response to insulin (Younger et al., 1966). In addition, lymphocytes from healthy partially-hepatectomized rats have the capacity to cause hepatic regeneration (Sakai et al., 1975). Substantial pathologic or surgical shunting diverts hepatotrophic substances away from the liver and therefore reduce the stimulus to regenerate and creates a self-sustaining insufficiency.

**Blood Volume.** The second cause of portal hypertension which is amenable to alteration by treatment other than vasopressin is blood volume. Portal pressure has been positively correlated with blood volume (Lieberman and Reynolds, 1967; Zimmon and Kessler, 1974). Zimmon and Kessler go so far as to state that diuretic therapy can reduce HPV pressure without impairing tissue perfusion in a majority of cirrhotics. Increased blood volume can result from the hemodynamic effects of portal-systemic shunting, the loss of hepatic control of venous return, and from increased aldosterone concentrations in the blood. Portal-systemic shunting is thought to act like arteriovenous fistulae (p. 48) in the production of portal hypertension (Lieberman and Reynolds, 1967). A healthy liver in man and dogs acts like a blood reservoir during inspiration and empties during expiration (Moreno, 1964; Moreno et al.,
1967). This regulation is absent in a cirrhotic liver and contributes to the hyperdynamic cardiovascular state of cirrhotics (see p. 48) and to hypervolemia by increasing venous return (Moreno, 1964). Shunting and hepatic injury combine to decrease aldosterone inactivation in a cirrhotic (Lieberman and Reynolds, 1967; Rosoff et al., 1975). Hepatofugal collateral circulation recruits the adrenals as a drainage pathway, and this backward flow could increase aldosterone secretion (Sasano et al., 1974). Increased intrahepatic pressure may be a stimulus for aldosterone secretion (Lieberman and Reynolds, 1967; Orloff et al., 1968; Bosch et al., 1976). Too, the kidneys may be involved in increased aldosterone secretion due to an increased sodium absorption by the proximal convoluted tubules decreasing sodium delivery to the distal convoluted tubules (Rosoff et al., 1975).

The Lymph System. The lymph system does not actively produce portal hypertension and esophageal varices; rather, it becomes a factor only when lymph production can no longer increase to oppose pressure increases in the HPV and the esophageal veins. Lymph of different protein concentrations is produced by the splanchnic bed, a healthy liver, and a cirrhotic liver. Ascites may be thought of as lymph; in particular, the quantity of lymph produced that is in excess of the drainage capacity of the lymph system. Five general restrictions to lymph drainage influence both portal
hypertension and bleeding varices. Blockage of the right lymphatic duct close to the liver can interfere with hepatic lymph drainage and lymphovenous communications in the area of the esophagus may be important to variceal bleeding. Restriction of lymph drainage has deleterious ramifications in the respiratory system. Operations that increase thoracic duct flow to combat portal hypertension, variceal bleeding, and ascites have been reported. Ascites has been theorized to result from surgical transection of lymphatics, from hepatic outflow blockage, and from increased blood volume. A number of surgical procedures have been used to reduce ascites.

Splanchnic and hepatic lymph production can dampen HPV pressure increases. Capillary pressure increases result in increased movement of fluid from the capillaries to the extracellular spaces and therefore increased lymph production. Increased HPV pressure results in increased splanchnic lymph production because the increased HPV pressure will increase pressure in the splanchnic capillaries; increased splanchnic lymph production thus dampens HPV pressure increases. Extrahepatic HPV occlusion in humans and Macaca mulatta monkeys results in increases of splanchnic lymph production until enough collateral channels were present to bypass the blockage and return pressure to normal; in the monkeys, this adaptation requires only a week (Child et al., 1950; Child et al., 1952). Increases in
postsinusoidal resistance raise the sinusoidal pressure, and hepatic lymph production can increase enough to block the transmission of increased pressure from the liver to the HPV in healthy dogs (Grindlay et al., 1948; Nix et al., 1951; Hyatt et al., 1955). In the case of cirrhotic portal hypertension, splanchnic lymph production can be increased by HPV pressure increases related to increased flow and related to increases of presinusoidal resistance the result of the cirrhotic process. Increases in postsinusoidal resistance the result of cirrhosis can increase hepatic lymph production and increase of splanchnic lymph production when the cirrhotic liver does not totally absorb all the pressure increase resulting from the increase of postsinusoidal resistance. Intrahepatic shunts contribute to this transmission of pressure into the HPV because the shunts are less permeable to plasma than the sinusoids that they replace (Dumont et al., 1975).

Lymph drainage from the area of the esophageal veins opposes pressure increases and, therefore, varix formation and hemorrhage. Increased hepatofugal collateral flow brings increased pressure to the esophageal and azygos veins, and lymph production increases from this area, as it does in other areas of the body, to dampen some of the pressure increases.

Lymph from a healthy liver has a higher protein
concentration than lymph from a cirrhotic liver or lymph from the splanchnic bed because the sinusoids in a healthy liver are more permeable than shunts (capillarized sinusoids) in a cirrhotic liver and ordinary capillaries. The gaps in and between endothelial cells are responsible for the ease of movement of large proteins from the plasma into the hepatic lymph. The high permeability of sinusoids is demonstrated by the work of Mayerson (1963) with dog livers. He measured the permeability of sinusoids to dextrans of different molecular weight against the permeability to 70,000 mw albumins and found that his largest dextrans (412,000 mw) diffused only a little slower than the albumins. The work of Bauer et al. (1959) with rat livers also demonstrates the high permeability of the sinusoids. He found that the protein concentration of the hepatic lymph was approximately equal to that of the plasma at a pressure difference of 4-5 cm of water between the HV and the hilum of the liver. Lymph from the splanchnic bed and a cirrhotic liver has a lower protein concentration than lymph from a healthy liver (Witte et al., 1968; Witte et al., 1969; Witte et al., 1971; Dumont et al., 1975; Ismail and Aboul-Enein, 1976; Sadek et al., 1976) because the splanchnic capillaries and capillarized sinusoids of a cirrhotic liver have a similar histologic structure that is less permeable to plasma than the sinusoids of a healthy liver. Both splanchnic capillaries and capillarized
sinusoids (Shaffner and Popper, 1963) possess a thick basement membrane and continuous endothelial cells typical of capillaries and different from healthy sinusoids. Courtice et al. (1962) cite experiments with rabbits to support the view that some additional factor operates to transport proteins from the plasma to the lymph, but they offer no speculation on what the mechanism might be.

The thoracic duct provides the primary lymph drainage for the portion of the body caudal to the heart (Warwick and Williams, 1973). The thoracic duct is larger than the right lymphatic duct (Warwick and Williams, 1973), and the liver provides half of the flow in the thoracic duct (Dumont and Witte, 1966b). Some lymph drainage is provided for the right side of the body caudal to the heart by the bronchomediastinal trunk of the right lymphatic duct; specifically, the right lymphatic duct drains the right side of the thorax, the right lung, and the convex surface of the right side of the liver (Yoffey and Courtice, 1970). The right lymphatic duct empties through one or several one-way valves into the notch made by the junction of the right subclavian vein and the right jugular vein. The thoracic duct empties through a similar valve or valves into the same spot on the left side. Several anastomoses between the right lymphatic duct and the thoracic duct occur in man (Yoffey and Courtice, 1970); therefore, blockage of the right lymphatic duct is not likely to
create major problems unless the block is below the first communication with the thoracic duct. Because the right side of the liver is at the end of the right lymphatic duct, blockage before the first communication with the thoracic duct could result in stasis of flow in portions of the right side of the liver.

Three restrictions of thoracic duct drainage (the size of the ampulla at the lymphovenous junction, the loss of rhythmic pulsations of the thoracic duct, and increased venous pressure at the lymphovenous junction) can promote portal hypertension and varix formation and hemorrhage by restricting increases of lymph drainage. Blockage of the right lymphatic duct can create resistance to hepatic lymph drainage, and special lymphovenous communications in the esophageal area may promote varix formation and hemorrhage.

The size of the ampulla draining the thoracic duct into the venous system is thought to be a limiting factor for lymph drainage (Dumont and Mulholland, 1963; Dumont, 1964; Dumont and Mulholland, 1965; Dumont and Witte, 1966a; Bradham and Takaro, 1968; Bhalerao et al., 1971; Witte et al., 1971; Dumont, 1975). The round or oval shape of the actual orifice becomes slit-like when the thoracic duct is distended, and this further decreases flow (Naitove, 1965, cited by Dumont and Mulholland, 1965). However, Warren et al. (1968) challenge the size of the orifice as a factor limiting lymph flow. Lymph moves through the thoracic duct
by rhythmic muscle contractions (Kinmonth and Taylor, 1956; Hall, 1969). If muscles contact in response to increased lymph pressure, sustained and abnormally high pressure over a period of time might reduce flow by exhausting the muscles (Hall, 1969). Increased venous pressure at the lymphovenous junction may reduce lymph drainage (Yoffey and Courtice, 1970). Thoracic duct resistance increases with increased left inominate vein pressure (Wégria et al., 1963) and also with left jugular vein pressure (Szabó and Magyar, 1967); however, left subclavian vein pressure is reported to be normal in cirrhotics (Dumont, 1964; Dumont et al., 1971). Stasis of flow in the right lymphatic duct due to a blockage before the first anastomosis with the thoracic duct has been reported in humans (Magnenat and Delaloye, 1964, cited in Dumont and Witte, 1966a). Such a blockage has the potential to be important to portal hypertension because 20% of hepatic lymph drains through the right lymphatic duct (Dumont and Witte, 1966a). Right-side stasis of lymph flow may have gone unnoticed in several cases where thoracic duct cannulation, a surgical procedure to be discussed later (p. 62), failed to control bleeding (Dumont and Witte, 1966a). Special lymphovenous communications in the esophageal area may promote varix formation and hemorrhage. Lymphovenous communications are present throughout the body (Warwick and Williams, 1973). Special lymphovenous communications in the esophageal area
have an embryological basis because the thoracic duct and
the azygos vein have the supracardinal vein as a common
primordium (Dumont and Witte, 1966a and b; Dumont et al.,
1970a). As hepatofugal collateral circulation through the
esophageal veins increases, flow and pressure in the
azygos vein increases and may be transmitted to the
thoracic duct and the local lymph system by these special
lymphovenous communications. The esophageal veins are
thought to be particularly susceptible to hemorrhage
because their greatly increased flow demands increased
lymph drainage and subjects them to great pressure while
their lymph system is exposed to extra demands because it
is subject to the pressures from the special lymphovenous
communications (Dumont and Witte, 1966a and b; Dumont et
al., 1970a). An increase in resistance to lymph flow
through the thoracic duct generally would also be expected
because of these special lymphovenous communications.
Ludwig et al. (1968) and Dumont et al. (1970a) report
retrosternal lymphovenous communications in cirrhotics,
and Dumont and Witte (1966a and b) and Dumont et al. (1970a)
report that RBC's in the thoracic duct of cirrhotics come
from the azygos vein. Lymphovenous communications serve
an important homeostatic purpose: they allow lymph produc-
tion in excess of the carrying capacity of the thoracic
duct to be drained into the venous system (Dumont, 1975).
Warwick and Williams (1973) report that total blockage of
the thoracic duct in a healthy individual does not result in back-up of lymph because of the presence of lymphovenous communications.

Increased resistance to lymph drainage and increased azygos and pulmonary vein pressures are thought to decrease pulmonary function because they produce pulmonary edema. This edema is theorized to create a fluid barrier to resist oxygen diffusion across the alveolar-capillary membrane (Ruff et al., 1971; Siegel et al., 1974; Arndt et al., 1975), mechanically compress small airways (Ruff et al., 1971), and create prolonged increased pressures that cause a thickening of arterial and capillary membranes (Siegel et al., 1974; Arndt et al., 1975). Poor pulmonary lymph drainage due to increased flow and pressure in the thoracic duct is an important reason for this (Ruff et al., 1971; Siegel et al., 1974; Arndt et al., 1975). Increased azygos vein pressure due to hepatofugal collateral circulation is thought to be another important reason for the pulmonary edema (Ruff et al., 1971; Siegel et al., 1974; Arndt et al., 1975) because much of the thorax is drained by the azygos vein. Increased pulmonary vein pressure causes pulmonary edema (Dawson et al., 1965), and increased pulmonary vein pressure from azygos vein-pulmonary vein shunting is thought to be important to pulmonary edema in cirrhotics (Ruff et al., 1971). This kind of shunting has been reported (Shaldon et al., 1961b; Nakamura et al., 1965). Nakamura
et al. (1965, cited in Nakata et al., 1973) report that not enough flow passes through these shunts to account for the oxygen desaturation observed.

Increasing thoracic duct drainage by cannulation or construction of a lymphovenous anastomosis can reduce ascites (Dumont and Mulholland, 1962; Dumont and Witte, 1966a and b; Steigman et al., 1967), stop variceal bleeding (Dumont and Mulholland, 1962; Bowers et al., 1964; Dumont and Witte 1966b; Cuento and Currie, 1967; Dumont, 1969; Dumont et al., 1970b; Bhalerao et al., 1971; Dumont et al., 1972), and decrease portal pressure (Dumont and Mulholland, 1962; Cuento and Currie, 1967) and wedged HV pressure (Warren et al., 1968). Thoracic duct flow may be diverted either into the digestive tract or into the venous system. When thoracic duct flow is diverted into the digestive tract, immunologic activity is suppressed presumably due to the loss of the lymph proteins (Dumont and Mulholland, 1962; Mayer and Dumont, 1963; Dumont et al., 1964). Lymphovenous anastomoses have had limited surgical trials (Bhalerao et al., 1971; Dumont et al., 1972; Serényi et al., 1976). The procedure is effective for ascites but gives inconsistent results with variceal bleeding. The results of surgery to change the lymph drainage system are mixed—perhaps reflecting the inability to identify individuals with portal hypertension and bleeding varices resulting primarily from lymph drainage deficiencies.
Ascites may be thought of as that quantity of lymph produced that is in excess of the lymph system's drainage capacity, and its presence may be thought to signify that changes greater than the body's ability to adapt have occurred. Baggenstoss and Wollaeger (1956) theorize that ascites develops after uncomplicated HPV blockage only in those individuals without well-developed collateral circulation. Hepatic outflow blockage and increased blood volume are thought to be major factors overwhelming the body's adaptive capacities in cirrhotic portal hypertension. Autacoid-induced outflow blockage also may be important. Transection of hepatic lymphatics during construction of an end-to-side portacaval anastomosis can create ascites (Witte et al., 1971), but this means is in an unrelated class by itself. Architectural changes have been mentioned which create hepatic outflow blockage. Madden et al. (1954) theorize that reversible ascites, the result of edema of the HPV and HV beds, and irreversible ascites, the result of fibrotic obliteration of the HV bed, exist. Zimmon and Kessler (1974) state that severe outflow resistance may prevent a satisfactory response of decreased blood volume in response to diuretic therapy in cirrhotics with ascites. Autacoids may create outflow blockage; thus they have the potential to create ascites. Candidates autacoids and their possible roles will be discussed in the next section. Ascites from increased blood volume may result
from a combination of decreased aldosterone inactivation and increased secretion (see p. 53) and in particular response to increased secretion by the distal convoluted tubules in response to increased sodium absorption by the proximal convoluted tubules (Rosoff et al., 1975).

Ascites may be reduced by medical therapy directed against hypervolemia or one of a number of surgical procedures. Side-to-side portacaval anastomoses control ascites well because they offer decompression for both the portal and sinusoidal beds (Witte et al., 1971). Both omentopexy (suturing the omentum to the liver and the abdominal wall to promote collateral circulation) (Pemberton and Kiernan, 1945; Whipple, 1945; Garrido et al., 1976) and ileoenterectomy (eversion of a portion of the terminal ileum to absorb ascitic fluid) Neumann et al., 1957) have been reported to control ascites. Peritoneojugular shunts to drain ascitic fluid from the peritoneum into the venous system are a recent development, and they have been successful in several initial trials (LeVeen et al., 1974; Sampliner et al., 1976; Wapnik et al., 1977).

Autacoids. The fourth alterable factor that contributes to portal hypertension, the effects of autacoidal factors on flow through the liver, is presently hypothetical. "Autacoid" is a broad term intended in this case to include any agents that disrupt flow through the liver as a result of vasoactive properties and that resemble but are
not humoral or neurohumoral factors (Douglas, 1975a). Agents released by immunologic reactions; clotting reactions; and from dead, injured, and healthy cells are potential causes of increased resistance to flow. Serotonin, kinins, and histamine are obviously suspect, but they are not the only compounds with vasoactive potential. Bacterial endotoxins are not autacoids, but they apparently cause the release of autacoids.

Local flow through the human liver is controlled at several places, each a possible site for the action of autacoids. Inflow as well as outflow sphincters may be involved because the contents of the sinusoids appear to get well-mixed (p. 22). Knisely et al. (1948, cited in Elias and Sherrick, 1969) describe inlet sphincters at the point where inlet venules branch out in a candelabra-like pattern of sinusoids. Märck (1951, cited in Elias and Sherrick, 1969) describes HA sphincters within the portal triad where the HA branches follow the inlet venules. Elias (1949a and b, cited in Elias and Sherrick, 1969) describes sphincters at the entrance of both paraportal and intralobular arterioles into the sinusoids. Control of hepatic outflow has been reported at five sites: bulging Kupffer cells (Rüttner and Vogel, 1957, cited in Elias and Sherrick, 1969); outlet sphincters at the sinusoidal-central vein junction (Knisely et al., 1948, cited in Elias and Sherrick, 1969), although Gibson (1959) specifically denies
the presence of muscular sphincters in this location; constriction of the central veins at the entrance to larger sublobular veins (Popper, 1931a and b, cited in Elias and Sherrick, 1969; Elias and Popper, 1955; Gibson, 1959); constriction of the smaller sublobular veins at the entrance to the larger sublobular veins (Elias and Popper, 1955; Gibson, 1959); and annular rings of muscles at the HV-IVC junction (Elias and Feller, 1926, cited in Bauer et al., 1932; Elias and Feller, 1931, cited in Bauer et al., 1932; Popper, 1931a and b, cited in Elias and Sherrick, 1969; Snyder, 1942; Gibson, 1959).

Immunologic reactions offer potential for the production of autacoids because of the compounds they release and because they may precipitate clotting reactions and the release of compounds associated with it. Substantial enough immunologic activity can be present in a liver to support the presumption that enough autacoids can be released to produce an effect, and glomerular lesions may be a side-effect of this activity. Antibodies may be produced directly to liver antigens; antibodies may also be produced to intestinal antigens made available by circumvention of the Chase-Sulzberger effect and cross-react secondarily with hepatic antigens. The portal hypertension of primary biliary cirrhosis may be related to autacoids from immunologic reactions.

The magnitude of immunologic reactions in a liver
can be substantial, and a substantial amount of immunologic reactions provides the means for release of enough autacoids to influence flow. The inflammation in the livers of rats given oral doses of carbon tetrachloride can involve so great an aggregation of macrophages that portal hypertension is hypothesized to result in part from sinusoidal clogging (Nakata and Higaki, 1969). Kater et al. (1976) concluded that a continuous perisinusoidal pattern of IgA immunoglobins is strongly suggestive of alcoholic liver disease. They arrived at this conclusion after treating liver sections from 320 individuals with various forms of liver disease with fluorescent anti-IgA antisera, and their conclusion is supported by other observations about the role of IgA in alcoholic liver disease. Increased IgA levels in cirrhotic alcoholics are reported elsewhere (Zetterman and Leevy, 1975; Zinneman, 1975), and IgA is reported to be the immunoglobin class most increased in alcoholic cirrhosis (André and André, 1976). Reports of IgA binding to alcoholic hyaline (Zetterman and Leevy, 1975; Zinneman, 1975) are supportive because hyaline accumulation in parenchymal cells is characteristic of alcoholic liver disease. The increased incidence of glomerular lesions in cirrhotics may be a side-effect of increased IgA formation and subsequent antigen-antibody complex formation. Bradfield (1974) and André and André (1976) report glomerular lesions and deposition of IgA antigen-antibody complexes on
the glomeruli. Bradfield (1974) theorizes that the IgA antigen-antibody complex deposition is the result of decreased phagocytosis due to Kupffer cell bypass, injury, and death and a decreased complement system; he also theorizes that the glomerulitis associated with hyperlipoproteinemia is the result of antigen-antibody complex deposition on the glomeruli because the lipoproteins overwhelmed the phagocytic capacity of the liver.

Antibodies may be produced directly in response to hepatic antigens; they also may be produced in response to intestinal antigens as a result of circumvention of the Chase-Sulzberger effect, and these antibodies may then cross-react secondarily with hepatic antigens. Antibodies to specific hepatic antigens have been identified in cirrhosis of several etiologies: alcoholic hyaline (Zetterman and Leevy, 1975; Zinneman, 1975; Zetterman et al., 1976); possibly to bile ducts (Miller et al., 1974); paraportal and intralobular ductules, particularly in primary biliary cirrhosis; liver-cell nuclei (Zinneman, 1975); mitochondria (Dawson et al., 1973); and altered albumin (Zetterman and Leevy, 1975)—production of altered albumin is a possible result of disturbed parenchymal cell function due to the toxic effects of ethanol (Zetterman and Leevy, 1975). Antigen-antibody activity in the liver may also result from the cross-reactivity between hepatic antigens and antibodies to the intestinal antigens made
available by the circumvention of the Chase-Sulzberger effect. Cross-reactivity has been demonstrated between antibodies to intestinal antigens and hepatic antigens in rabbits (Rabin and Rogers, 1976). In cirrhotics, cross-reactivity of antibodies to enteric bacterial antigens is suggested in the reaction to tissue (Simjee et al., 1975; Sybran et al., 1975), RBC's (Wybran et al., 1975), rheumatoid factor (Christian et al., 1965), and nuclei (Simjee et al., 1975).

In a healthy individual, antigens first presented to the body through the digestive tract will elicit a decreased response from the immune system when subsequently presented by another means (intravenously or subcutaneously, e.g.). This is known as the Chase-Sulzberger effect. It has been convincingly demonstrated in dogs (Cantor and Dumont, 1967) and rats (Thomas et al., 1976) and less certainly in rabbits (Rabin and Rogers, 1976). Antibodies to enteric bacteria (Christian et al., 1965; Triger et al., 1972; Wybran et al., 1975; Simjee et al., 1975), bacterial endotoxin (Simjee et al., 1975), and dietary antigens (Triger et al., 1972) have been reported in cirrhotics, and these observations strongly suggest the Chase-Sulzberger effect has been bypassed and offers the potential for cross-reaction with hepatic antigens.

Seven changes and conditions likely in cirrhosis contribute to the circumvention of the Chase-Sulzberger
effect. First, intrahepatic shunts; cell injury and death; perisinusoidal fibrosis; general cell debris, antigen-antibody complexes, and lymphocytes in the sinusoidal lumen and the space of Disse; capillarization of the sinusoids; and hepatofugal collateral circulation all contribute to a decreased number of and access to cells available for phagocytosis and thereby increase the availability of bacterial and intestinal antigens and bacterial endotoxin to the immune system. A second change, increased demand for phagocytosis, detracts from the liver's normal phagocytic functions (Cantor and Dumont, 1967; Bradfield, 1974; Bjørneboe and Prytz, 1975; Simjee et al., 1975; Zinneman, 1975). A third change, intestinal ischemia, increases intestinal permeability (Cirrincione and Francona, 1932; Evans et al., 1948, cited in Bar-Meir and Conn, 1976; Renert et al., 1972; Conn et al., 1972; Bar-Meir and Conn, 1976); therefore bacteria, their endotoxin, and other intestinal antigens could be presented to the portal system and the liver in greater-than-normal amounts, and the need for phagocytosis is greater. The toxic effects of endotoxin present in greater-than-normal quantities are a fourth change circumventing the Chase-Sulzberger effect. *Salmonella enteriditis* endotoxin exerts a direct toxic effect on reticuloendothelial function of rat livers (Ruggiero et al., 1976); thus other endotoxins may reduce phagocytic ability. Also, endotoxin is thought to have
adjuvant properties because it is a lipopolysaccharide (Simjee et al., 1975; Thomas et al., 1976). The adjuvant properties of endotoxin are a fifth change. Thomas et al. (1976) speculate that the Chase-Sulzberger effect results from depressed T-cell activity that yields little B-cell stimulation and subsequent antibody formation. The adjuvant nature of endotoxin is thought to permit bypass of T-cell mediation and to stimulate B-cells directly. The question of T-cell and B-cell stimulation may not be quite as simple as Thomas et al. (1976) suggest, however.

Sanchez-Tapias et al. (1976) describe an increased presence and activation of only T-cells in alcoholic hepatitis and increased presence and activation of both T-cells and B-cells in patients with chronic, active hepatitis and primary biliary cirrhosis. Direct ethanol potentiation of lymphocytes is a sixth change. In vitro lymphocyte transformation in blood from patients with chronic active hepatitis or alcoholic hepatitis is potentiated by small, non-toxic amounts of ethanol and acetaldehyde (the breakdown product of ethanol whose subsequent transformation to acetic acid is the rate-limiting step in human ethanol metabolism). When clinical and laboratory evidence of alcoholic hepatitis subsides, so does the ethanol and acetaldehyde potentiation. Potentiation does not occur with lymphocytes from patients with fatty livers, acute viral hepatitis, or inactive cirrhosis, however (Zetterman
These observations suggest that ethanol is causing liver damage and simultaneously may be interfering with the Chase-Sulzberger effect by action on the lymphocytes.

Primary biliary cirrhosis is primarily a disease of older women (Baggenstoss et al., 1964; Kew et al., 1971; Shaffner, 1975), and presents a perplexing problem to anyone who studies portal hypertension. Autacoids offer a possible explanation for the portal hypertension observed. Only in advanced stages does it resemble cirrhosis; in all but the late stages, the evident lesion is the destruction of bile ducts. Inflammation and fibrosis of the paraportal and intralobular bile ductules, as well as the whole portal tract, occurs with proliferation of bile ductules and ductular epithelial cells within and outside the bile ducts in the portal tracts. Often, portal hypertension is present at this early stage (McMahon, 1931; Popper and Zak, 1958; Baggenstoss et al., 1964; Zeegan et al., 1969; Dawson et al., 1973; Shaffner, 1975; Popper and Kent, 1975; Bauer et al., 1976). In the late stages of the disease, the liver becomes cirrhotic following necrosis of the inflamed proliferating ductules and the production of septa which radiate from the portal tracts to connect the HPV's and HV's. It is surprising that portal hypertension appears before major cirrhotic changes (Sherlock, 1959; Hoffbauer, 1960; Zeegan et al., 1969; Kew et al., 1971; Dawson et al.,
1973; Popper and Kent, 1975; Bauer et al., 1976; Lebrec et al., 1976a). Portal hypertension may depend upon inflammation of the portal tracts, and this inflammation could disrupt the prebiliary HA plexus and create HA-HPV anastomoses (Hoffbauer, 1960) or compress and destroy portal tracts (McMahon, 1931; Zeegan et al., 1969; Bauer et al., 1976). However, patients with portal hypertension are seen without portal tract damage, HV obstruction, or sinusoidal obstruction (Kew et al., 1971; Lebrec et al., 1976a). The early appearance portal hypertension in primary biliary cirrhosis has not been adequately explained and autacoids may be involved. Primary biliary cirrhosis presents a picture of active generalized inflammation and antigen-antibody reactions, and the course of the disease is not correlated with biochemical or immunologic factors (Geubel et al., 1976). Geubel et al. (1976) suggests that the lack of correlation is because immunologic reactions are responses to the disease process and not to the etiological agent. Whether the immunologic reactions are the primary cause of the disease or a reaction to the disease process is not important because autacoids would be released by the immunologic reactions.

Endotoxin may promote portal hypertension indirectly through autacoids whose release it causes. Some of the hepatic manifestations of endotoxin shock in hyperlipemic rats result from constriction of HPV inflow, mediated by
the alpha-adrenergic receptor (Latour et al., 1974). Similar results, although not as precisely defined in terms of location and means, were obtained by Nolan and Ali (1975) for endotoxin shock in rats with fatty livers. McKay et al. (1966) observed swelling of Kupffer cells one hour after injection of endotoxin in pregnant rats, and this swelling might be the result of anoxia resulting from HPV inflow blockage. In rabbits, radioactively-labeled endotoxin is sequestered in the liver, spleen, platelets, and leukocytes (Braude et al., 1955); thus three parts of the body other than the liver have an opportunity to interact with endotoxin and release vasoactive substances, and one, the spleen, is upstream from the liver. Endotoxin causes aggregation of rabbit platelets and release of serotonin (Des Prez et al., 1961). In addition, Kobold et al. (1962) report that when endotoxin is mixed with dog blood, serotonin (pp. 75-78) and histamine (pp. 78-88), two vasoactive substances discussed later, as well as an unidentified vasoactive substance are released. This latter substance is important to endotoxin shock. Its actions are not blocked by antagonists alpha-adrenergic, H1-antihistamine, or serotonin. Its properties resemble those of several kinins, a class of vasoactive substances also discussed later (pp. 75-78). Filkins (1969b) reports that a combination of plasma, platelets, and endotoxin are necessary for the decreased flow seen in response to endotoxin.
administration to rats. Filkins' (1969b) findings are consistent with findings of Nies et al. (1968). Nies et al. (1968) report that in different experimental animals endotoxin shock is: 1) monophasic, with increases in concentrations of histamine parallel with the increases in the severity of shock, and 2) biphasic, with an initial drop in pressure which is correlated to the blood levels of kinin.

The gut can become ischemic from changes in pressure and flow and may release endotoxin, serotonin, histamine, and a vasodilator substance consequent to these factors which create portal hypertension; these compounds could disrupt hepatic flow. The portal circulation in portal hypertension is drastically abnormal and probably results in gut ischemia. Portal pressure can rise so much that the mesenteric veins become much enlarged, and the veins in the anterior abdominal wall may carry so much collateral flow that they bulge out in a striking and characteristic caput medusae. The esophageal veins are recruited to carry much of the splenic and gastric venous flow, and they become enlarged, twisted, and vulnerable to hemorrhage. With this much pressure in the venous drainage system of the gut and the likelihood of open arteriovenous shunts in its vascular bed, some portions of the gut can be expected to have poor flow and be a little ischemic. Considering the dynamic nature of blood flow, the whole gut might be a little ischemic; certainly portions with significantly reduced flow at any one moment would be in dire
need, actively releasing vasodilator substances. Much of the body's serotonin is found in the gut (Douglas, 1975c). In an ischemic gut, histamine release also seems probable as it is found in the intestinal mucosa (Douglas, 1975b), and is synthesized by intestinal bacteria (Douglas, 1975b). It is suspected to be a vasodilator substance active in control of microcirculation (Schayer, 1962; Douglas, 1975b; Beaven, 1976a). Kobold and Thal (1963) have identified a vasoactive polypeptide released by an ischemic gut which is present in the lumen of the intestine and dependent upon bacterial activity and pancreatic proteolytic enzymes. The action of this substance is similar to that of bradykinin and in addition to the actions of histamine and serotonin. Their work also documents the release histamine, serotonin, and kinins. Their experiments do not eliminate the effects of bacterial endotoxin; thus increased permeability to endotoxins may evoke the release of histamine, kinins, and serotonin through endotoxin-blood interactions.

The creation and release of serotonin and kinins appears to be a likely result of antigen-antibody reactions, generalized inflammation, and blood clotting, and because these three conditions seem likely to be present in a cirrhotic liver, this supports the possibility that serotonin and kinins are created and released in the liver and contribute to local interruption to flow and portal hypertension. Leukocytes (Melmon and Kline, 1967) as well as
antigen-antibody reactions (Nies et al., 1968) and activation of the complement system (Douglas, 1975c) can generate kinins. Blood clotting is observed in a cirrhotic liver as a result of cell injury and slow or stagnant blood flow. The cascade of reactions in blood clotting is similar to and interfaces with some of the reactions of kinin production (Douglas, 1975c). Kinin production and endotoxin shock both activate the Hageman factor (XII) of blood clotting (Douglas, 1975c). Kinin production and clotting may lead to serotonin release because platelets contain serotonin (Douglas, 1975b).

A large number of polypeptides with similar pharmacological properties are called kinins (Douglas, 1975c), and kinins have the potential to be important autacoids on the basis of their action in the liver, their production, and the fact that the liver is not responsible for their breakdown. Bradykinin decreases flow in an isolated, perfused rat liver (Filkins, 1969a) and is strongly implicated in the initial response of the biphasic response to endotoxin shock in monkeys (Nies et al., 1968). In humans, intravenous bradykinin is associated with decreased splanchnic resistance and no change in free or wedged HV pressures (Feruglio et al., 1964a). It is interesting that Lukjan (1975) found a decrease in levels of kininogen (the kinin precursor) and an increase in kininase activity in cirrhotic patients. Cell lysis results in the release of
kininases (Douglas, 1975c). Moreover, activated Hageman factor (XII) can initiate endotoxin shock in a prepared animal (Latour et al., 1974) as well as initiate kinin production (Soltay et al., 1971). Kinins have a half-life of about fifteen seconds; they are broken down by enzymes in the blood and lungs but not the liver (Douglas, 1975c). The short half-life, independent of good or poor liver function, raises doubts about the suitability of kinins as autacoids which mediate portal hypertension; however, because hepatic inactivation is not important, any kinins produced in the liver would survive at least fifteen seconds—presumably enough time to exert their vasoactive effects.

Serotonin (5-hydroxytryptamine) may be an autacoid. Approximately 90% of the body's serotonin is found in the gut, and most of the remaining 10% is found in the platelets. Metabolism in the liver and lungs is important to its disposal (Douglas, 1975b). Serotonin is released from the plasma by endotoxin (Kobold et al., 1962) and clotting (Douglas, 1975b). It decreases hepatic blood flow in rat livers (Levine et al., 1964) and constricts the HPV and HA, but not the HV in dog livers (Andrews and Butterworth, 1958). It also decreases HA and HPV flow in dogs (Mahfouz and Aida, 1967). Cirrhotic rat livers show a decreased uptake of serotonin (Ahtee et al., 1974). In cirrhotics, platelets have a decreased content and uptake of serotonin,
and serotonin binding to "tissue" is defective (Ahtee et al., 1974). Chiandussi et al. (1963) increased blood concentrations of serotonin in cirrhotics by intravenous serotonin administration, but this produced only a small decrease in hepatic blood flow; pulmonary metabolism may have reduced serotonin's effect in this case. Serotonin is most likely to be released in a cirrhotic liver as a result of clotting, but release by endotoxin and an ischemic gut appear to be likely possibilities too.

**Histamine.** Histamine may have actions in the liver, the splanchnic bed, and the esophagus to promote portal hypertension and bleeding varices. These hypotheses are little more than speculation presently, because very little experimental work has been done to investigate them. Histamine is readily available from hepatic tissue damage and the stomach. The muscles around medium-sized sublobular HV's and the HV-IVC junction are likely locations for histamine action in the liver, and histamine can easily be available to these HV's in higher-than-normal concentrations in a cirrhotic liver. Increased blood concentrations of histamine also may increase gastric and splanchnic flows and increase flow through the submucosal esophageal veins from dilation of esophageal smooth muscle. As a result of these actions and several others, anti-histamines may have important therapeutic roles.

Histamine is available to a cirrhotic liver, the
splanchnic bed, and the esophagus, from several sources. Histamine is recognized as an important mediator of gastric secretion, but its precise role is still under investigation (Douglas, 1975b; Beaven, 1976a). Regardless of its exact interactions, it appears in the HPV during digestion in substantial quantities. Trauma to hepatic tissue causes histamine release from the mast cells, and the presence of bile salts also causes histamine release (Douglas, 1975b). As tissue injury and the presence of bile salts are to be expected in a cirrhotic liver, local production of histamine seems inevitable. As has been previously noted (p. 70), intestinal ischemia increases the permeability of the gut to intestinal contents, and this may introduce bacterially-produced histamine. Also, histamine is stored in the intestinal mucosa (Douglas, 1975b) and may be released as a result of intestinal ischemia. Intestinal ischemia releases histamine indirectly because of the gut's increased permeability to endotoxin, which in turn causes histamine release. Antigen-antibody reactions, generally (Douglas, 1975b), as well as IgE reactions that take place on the surface of mast cells in hypersensitivity, specifically (Beaven, 1976b), are also known to release histamine. Seratonin has been shown to cause histamine release from dog blood (Moore et al., 1963) and cat blood (Feldberg and Smith, 1953).

Humans possess HV musculature in enough quantity
around medium-sized (diameter greater than 400 microns) sublobular veins and at the HV-IVC junction to support speculation that HV constriction can interrupt hepatic flow. Humans do not possess (Miyake, 1929, cited by Bauer et al., 1932; Bauer et al., 1932; Snyder, 1942; DuMais, 1944; Elias and Popper, 1955; Gibson, 1959) the spiral muscles present in dogs (Arey, 1941; DuMais, 1944; Thomas and Essex, 1949; Elias and Popper, 1955) and rats (DuMais, 1944; Thomas and Essex, 1949; Elias and Popper, 1955), and this spiral musculature accounts for the great sensitivity of dog livers to histamine (Arey, 1941; Thomas and Essex, 1949; Elias and Popper, 1955; Gibson, 1959). Humans have not been thought to have enough HV musculature to interrupt hepatic flow (letter dated 12 October, 1976, from A. H. Baggenstoss, M.D., Department of Pathology, Mayo Clinic, Rochester, MN; letter dated 13 December, 1976, from T. B. Reynolds, M.D., Department of Internal Medicine, University of Southern California School of Medicine, Los Angeles, CA); however, Miyake (1929, cited by Bauer et al., 1932) describes "substantial" longitudinal HV musculature, Snyder (1942) describes "much" longitudinal and ring HV musculature, and Gibson (1959) reports longitudinal and ring HV musculature and observes that medium-sized sublobular HV's possess more musculature than HPV's of similar diameter. Wallach and Popper (1950) describe central vein-sublobular vein and sublobular vein-sublobular vein junctions and theorize that
constriction of the musculature around sublobular veins at these locations increases resistance to flow and acts as an important element of central necrosis in the cirrhotic process. Gibson (1959) concurs with the observations of Elias and Popper (1955). Gibson (1959) observes that HV musculature thins out from the HV-IVC junction and stops abruptly with medium-sized sublobular veins and speculates that these muscles at these junctions are the chief HV sphincter mechanism. Muscular rings at the HV-IVC junction are observed in humans (Bauer et al., 1932; Snyder, 1942; Gibson, 1959; Walker et al., 1960; Moreno et al., 1962). These muscles in dogs are sensitive to histamine and provide a model for possible histamine action in humans. Walker et al. (1960) report constriction of the terminal 1.5-2.0 cm of the HV in dogs in response to histamine and hepatic trauma. Bauer et al. (1932) report abolishment of epinephrine- and histamine-induced hepatic outflow blockage after removal of the terminal 5 cm of the intrahepatic HV in dogs. Because the HV spiral muscles of dogs are continuous with the HV-IVC sphincter muscles (Bauer et al., 1932), the lack of histamine sensitivity of the spiral muscles after HV-IVC sphincter muscle excision may be related to trauma from the removal. Moreno et al. (1962) report constriction of HV-IVC sphincter muscles in dogs after intrahepatic parenchymal injection of radiopaque dye preceeded by procaine injection. This constriction was not
the result of dye irritation of the HV vasculature, and they postulated that the HV-IVC sphincters were stimulated to constrict by a substance released from a "histamine-like" reaction evoked by local damage at the injection site.

A blood reservoir function of the liver strengthens the argument in favor of the potential for some kind of outflow blockage. A blood reservoir capacity has been demonstrated in cats (Griffith and Emery, 1930; Greenway et al., 1969; Lautt and Greenway, 1976), dogs (Bauer et al., 1932; Katz, 1938; Moreno, 1964; Moreno et al., 1967), and in humans (Moreno et al., 1964). In portal hypertension, the blood reservoir controls would be pushed far beyond their normal limits by autacoids. The probable results would be recruitment of intrasinusoidal sinusoids and increased lymph flow. When the intrasinusoidal sinusoids are filled and the lymph drainage capacity is surpassed, ascitic fluid will weep from the surface of the liver and HPV pressure will increase.

Histamine concentrations at the HV radicles of cirrhotic livers and systemically may be greater in cirrhotics than in healthy individuals. Histamine presumably can create an outflow blockage because it escapes hepatic inactivation and arrives at the HV musculature. In humans, the liver is the principle site of histamine inactivation (Eiseman, 1962; Douglas, 1975b), the lungs are an important secondary site (Douglas, 1975b), and enzymes
are present in the blood (Beaven, 1976a). A histamine concentration 50 times greater than normal can enter the liver through the HPV and result in no increase in the HV histamine concentration in healthy dogs (Drapanas et al., 1965). Histamine escapes hepatic metabolism in cirrhotics because of decreased hepatic function from cell injury and death and because of extra- and intrahepatic shunting (Eiseman, 1962). Hepatofugal collaterals and intrahepatic HPV-HV shunts contribute to histamine appearance in the HV bed in different ways. Extrahepatic shunting bypasses the HV's entirely; however, intrahepatic shunting provides a means for histamine to escape hepatic inactivation and reach the HV system. Moreover, as the systemic circulation acquires greater concentrations of histamine, the HA will deliver more histamine into the liver and the HA-HV and HA-HPV shunts will become more important.

The contribution of histamine to portal hypertension and bleeding varices may go beyond that of an autacoid constricting HV musculature. Histamine may increase flow into the portal system and esophageal veins. Histamine opens the human gastric capillary bed and increases flow (Peters and Womack, 1961), as one might expect from a gastric secretagogue. Increased gastric flow may be a significant factor in the total increased flow in portal hypertension. Ercan and Türker (1976) describe a means by which histamine can increase intestinal flow. They observed
a small pressure drop followed by a pressure increase in the cat mesenteric circulation in response to histamine administration. $H_2$-antihistamines blocked the pressure rise. Histamine causes a slight arteriolar constriction in cats and arteriolar dilation in dogs, monkeys, and humans (Douglas, 1975b). The selective antagonism reported by Ercan and Türker (1976) is supported by Chipman and Glover (1976) for human peripheral circulation although they found the actions of $H_1$ and $H_2$ antagonism reversed. DeCarle and Glover (1974) and DeCarle et al. (1976) report that histamine relaxes the esophageal smooth muscle of the oppossum, and DeCarle and Glover (1974) report that histamine relaxes the esophageal smooth muscle of monkeys. Cohen and Snape (1975) report that the $H_2$-antihistamine metiamide increases the lower esophageal sphincter pressure in normal, anesthetized oppossums. Submucosal esophageal varices are the veins varices most likely to hemorrhage because they have the poorest support and are the most vulnerable to trauma. The reports cited above suggest how histamine is important to variceal bleeding: Increased histamine concentrations in the esophageal veins relax esophageal muscle; this relaxation increases flow through the relatively weak and vulnerable submucosal esophageal veins. The greater the histamine concentrations, the greater the esophageal muscle relaxation and the greater the flow passing through to the deep submucosal veins.
A number of observations from experiments unrelated to control of hepatic outflow lend indirect support to a role for histamine in portal hypertension and variceal bleeding. Cirrhotics have an increased incidence of ulcers and exacerbation of ulcers (Clarke et al., 1958; Douglas, 1975b). Patients who have undergone shunt surgery have increased systemic histamine concentrations (Stopik et al., 1976). Work with dogs indicates that increased systemic histamine concentrations are responsible for gastric hypersecretion after HPV obstruction (Gregory, 1957; Gregory, 1958) and portacaval shunts (Clark et al., 1959; Silen and Eiseman, 1959; Silen and Eiseman, 1961; Eiseman, 1962; Peters and Carter, 1966). Bypass of hepatic histamine inactivation could be the common denominator between HPV obstruction and portacaval shunting. If enough histamine is present in the systemic circulation to create gastric hypersecretion, two ideas seem appropriate: The increased systemic histamine concentrations may contribute to the decreased peripheral resistance observed as a part of the hyperdynamic cardiovascular state present in cirrhotics. Also, if a good deal of histamine is escaping inactivation, histamine concentrations in the HV may be elevated and of importance to portal hypertension. Bailey et al. (1976) report success in the control of hemorrhage from gastric and esophageal erosions of patients in fulminant hepatic failure by treatment with intravenous H₂-antihistamines.
(cimetidine and metiamide). They ascribe this success to a
decrease in gastric acid secretion, which they believe pre-
cipitated the bleeding by erosion. However, their report
does not provide critical evidence that decreased acid
secretion decreased the bleeding, and this raises the
possibility that the antihistamines decreased bleeding by
antagonism of histamine-induced relaxation of both gastric
and esophageal smooth muscle.

Histamine and antihistamines may have a number of
actions important to livers and esophageal varices. If
histamine plays an important role in the production of
portal hypertension and variceal bleeding, antihistamines
may have therapeutic value. Antihistamines, in general,
may work in the liver to decrease hepatic resistance and
cause constriction of esophageal muscle. H₂-antihistamines,
specifically, may selectively block dilation of the gastric
and intestinal vascular beds if the observations of Ercan
and Türker (1976) and Chipman and Glover (1976) apply.
Work with insulin (p. 52) raises the possibility that one
day cirrhosis may be treated with HA and HPV infusions of
hepatotropic substances. If histamine, or any other
autacoid, contributes significantly to decreased hepatic
flow, antihistamines, or other specific antagonists, would
be valuable adjuncts to infusion therapy because they would
open outflow and increase hepatic perfusion. In the growth
of other tissues histamine may be a hepatotropic substance
(Douglas, 1975b). If so, this would contraindicate anti-histamine therapy. Different histamine receptors might be responsible for HV constriction and cell growth, however; thus, the possibility exists for selective blockade and stimulation. The role of anti-histamines might be extended to include liver transplants. Grana et al. (1968) suggest that ischemic dog livers generate toxic products (autacoids) that increase resistance to hepatic drainage. If histamine is released by an ischemic liver, anti-histamines in the liver perfusate would open up the hepatic circulation and limit damage. The action of vasopressin may be related to histamine actions in the liver (Režabek, 1967, p. 64).

**Portal Hypotensive Actions of Vasopressin.** Vasopressin decreases HPV pressure in humans (Davis et al., 1957; Schwartz et al., 1959; Heimburger et al., 1960; Shaldon and Sherlock, 1960; Schenck et al., 1962; Nusbaum et al., 1968; Barr et al., 1975; Conn et al., 1975; Thromford and Sirinek, 1975; Sirinek et al., 1976a and b). The portal hypotensive action of vasopressin results from a decrease in portal flow and also may result from decreases of HPV system and hepatic resistance.

Vasopressin decreases flow into the portal system by constriction of splanchnic arterioles and may decrease flow by closure of submucosal arteriovenous shunts. Constriction of the splanchnic arterioles is the mechanism most often cited to explain vasopressin's portal hypotensive
action. Splanchnic arteriolar constriction reduces portal pressure because it decreases flow into the portal system, and it reduces variceal bleeding because it reduces hepatofugal collateral circulation. A vasopressin-induced decrease in HPV flow has been observed in dogs (Holtz, 1932; Wiggers et al., 1946; Heimburger et al., 1960; Drapanas et al., 1961; Butz et al., 1962; Peters et al., 1962; Feruglio et al., 1964b; Shoemaker, 1964; Texter et al., 1964; Mahfouz and Aida, 1967; Cort et al., 1968; Nusbaum et al., 1968; Hanson, 1970; Fingeroth et al., 1973; Skivolocki and Thromford, 1973; Wilson et al., 1976), cats (Clark, 1928; Holtz, 1932; McMichael, 1932; Cohen et al., 1970; Krarup, 1975), and humans (Davis et al., 1957; Shaldon et al., 1961a; Schenck et al., 1962; Feruglio et al., 1964b; Nusbaum et al., 1968; Barr et al., 1975; Erwald et al., 1976). Vasopressin also is thought to decrease flow into the portal system by closure of splanchnic submucosal arteriovenous shunts (Eisman et al., 1959; Johnson et al., 1960; Edmunds and West, 1962). The observations of Nusbaum et al. (1968) and Wilson et al. (1976) are consistent with this theory.

Vasopressin is thought to decrease resistance in the portal system and the liver. Vasopressin has been reported to decrease resistance in the portal system (Shoemaker, 1964; Texter et al., 1965) and this will decrease portal pressure if flow does not increase.
Aronsen and Nylander (1966b) present angiograms of dogs to substantiate their claim that vasopressin dilates the extra-hepatic HPV. That vasopressin may decrease intrahepatic resistance is suggested without experimental evidence by some authors and in conjunction with experimental evidence by others. Butz et al. (1962) transposed the HV and IVC in dogs so that the splanchnic drainage did not flow through the liver. In these dogs, hepatic resistance decreased in livers treated with vasopressin. Reduction of resistance could result from HPV dilation; alternatively, it could involve a mechanism suggested by the work of Holtz (1932). Holtz (1932) observed a decrease in hepatic resistance in dog livers treated with either vasopressin or oxytocin. When the 5 mm of the HV closest to the IVC was excised, effects were abolished. McMichael (1932) observed a vasopressin-induced decrease in HPV pressure in cats with the superior mesenteric artery clamped and concluded that intrasinusoidal resistance had decreased. He speculated that presinusoidal arterioles which govern the flow into the HA-HPV shunts had been constricted and decreased flow into the HPV. Although the prebiliary capillary plexus is a suitable vascular bed for his theory, his conclusions must be considered tentative because he measured only carotid artery HPV pressures, and a clamp on the superior mesenteric artery did not allow him clean separation of splanchnic and hepatic actions. Eiseman et al. (1959)
suggest that vasopressin relaxes contractile elements in the outflow control of the liver but offer no experimental data or citations. Because Holtz (1932) is cited elsewhere in the paper, the lack of a citation may be an omission. Kessler (1968) and Rabøl et al. (1976) worked with cirrhotics. Both state, without explanation, that vasopressin decreases hepatic resistance. Rabøl et al. (1976) refer to "transsinusoidal resistance" and Kessler (1968) states that vasopressin works on the hepatic vasculature. Hanson (1970) states that vasopressin reduces hepatic resistance and cites Kestens and Haxhe (1966). Vasopressin also could decrease intrahepatic resistance through antagonism of histamine, if histamine plays a role in portal hypertension. Režabek (1967) observed both therapeutic and prophylactic antagonism of histamine-induced hypotension by intravenous vasopressin infusions in rats and guinea pigs. Vasopressin may antagonize the actions of histamine, because it acts directly on the contractile elements of smooth muscle (Brazeau, 1975). On the other hand, Mahfouz and Aida (1967) report a vasopressin-induced increase in hepatic resistance in dog livers.

_Intrahepatic Actions of Adrenergic Agonists._

Hepatic inflow appears to be under alpha-adrenergic control. Work with isoproterenol, a beta-adrenergic agonist, and epinephrine suggests that hepatic outflow is under beta-adrenergic control. Both isoproterenol and vasopressin
appear to be able to decrease hepatic outflow resistance.

Hepatic inflow appears to be under alpha-adrenergic control. Greenway et al. (1967) observed that hepatic nerve stimulation in cats increased HPV pressure and was blocked by the alpha-adrenergic antagonist phenoxybenzamine. Latour et al. (1974) found increased HPV pressures in hyperlipemic rats subjected to endotoxin shock by injection. When the livers were sectioned after India ink injections, Latour et al. (1974) found carbon particles only in the portal tracts. Pretreatment of the rats with phenoxybenzamine resulted in decreases of HPV pressures before endotoxin injection and prevented endotoxin-induced HPV pressure increases; moreover, carbon particles could be found throughout the liver. Friedman et al. (1951) report that dibenamine, another alpha-adrenergic antagonist, dilates canine HPV and HV systems constricted in response to hemorrhagic shock. Daniel and Pritchard (1951b) observed constriction of intrahepatic HPV branches in response to epinephrine and hepatic nerve stimulation in dogs, cats, rats, and monkeys in angiograms. Their observations coupled with the observations of Greenway et al. (1967) strongly suggest that the alpha-adrenergic receptor controls HPV constriction in the cat. The work of Latour et al. (1974) strongly implies that rat HPV systems have similar nervous controls. Because HPV constriction has been observed to be related to alpha-adrenergic action in
several animals by different investigators, one is lead to suspect that the same controls exist in humans.

Isoproterenol appears to decrease hepatic resistance, particularly hepatic outflow resistance. Didichen and Schenck (1970) report a decrease in hepatic resistance concurrent with an increase in mesenteric blood flow in dogs given isoproterenol intravenously. Butz et al. (1962) report an isoproterenol-induced decrease in hepatic resistance in dogs subjected to an infrahepatic portacaval transposition. Schon and Labat (1971) perfused isolated dog livers with isoproterenol at constant pressure and observed a statistically significant increase in HA and HPV flows and a statistically significant decrease in lymph production. The decrease in liver weight was below the level of statistical significance (T-test, p 0.8); however, they perfused the livers in a plastic bag and weighed them on a tray suspended from a balance. Fluid trapped in the bag or on the tray may have influenced their results. In view of the nearly significant values reported and the suspected imprecision of weight determination, one cannot conclude that isoproterenol did not decrease liver weight.

Taken collectively, the increases in flows and decreases in lymph production, and probably weight, strongly suggest that isoproterenol reduces outflow resistance.

Epinephrine appears to be able to decrease hepatic outflow resistance and dilate the sphincters at the HV-IVC
junction. Epinephrine pretreatment prevented constriction of the HV-IVC sphincter observed by Moreno et al. (1962, p. 58) and allowed unrestricted drainage. In addition, in two dogs subjected to some extra "stress", drainage of the dye was unrestricted. Their postulation of a "histamine-like" reaction (p. 83) prompts comparison of their observations with the observation of Andrews et al. (1955) that epinephrine antagonizes the action of histamine in dog livers. Bauer et al. (1932) observed that a low concentration of epinephrine decreased HPV pressure and liver volume concurrent with an increase in outflow. After removal of the 5 mm of the HV closest to the IVC, similar concentrations caused a weak increase in HPV pressure and an increase in liver volume. A high concentration of epinephrine increased HPV pressure and liver volume while decreasing outflow. An intermediate concentration increased portal pressure and decreased liver volume. In cat and rabbit livers, low concentrations of epinephrine decreased liver volume and increased outflow, and high concentrations increased liver volume and decreased outflow (Deysach, 1941). Andrews et al. (1955) reported reduced HV outflow in dog livers perfused with high concentrations of epinephrine and low concentrations either reduced or increased flow. Liver volume increased with high concentrations and decreased with intermediate and low concentrations.

HV constriction in response to beta-adrenergic
stimulation does not appear to be of major importance in dogs. Constriction becomes important only with high epinephrine concentrations, for the contractions in response to small and intermediate concentrations are not evident until the action on the HV-IVC sphincter is eliminated. Both Bauer et al. (1932) and Andrews et al. (1955) reported HV dilation with small and intermediate concentrations and HV constriction with high concentrations. The contractions of the HV system in response to low and intermediate concentrations of epinephrine presumably are overshadowed by the dilation of the HV-IVC sphincters (Bauer et al., 1932). This suggests that the muscles that constrict in response to low concentrations are found deep to the HV-IVC sphincters, contract weakly, and do not control resistance ordinarily. Longitudinal muscles around the larger HV channels seem suitable candidates, and they are also found in human livers (Miyake, 1929, cited by Bauer et al., 1932; Snyder, 1942; Gibson, 1959).

Isoproterenol and vasopressin both may decrease hepatic outflow resistance. Isoproterenol and vasopressin have been used in combination to decrease HPV pressure in dogs (Skivolocki and Thromford, 1973; Sirinek and Thromford, 1974; Sirinek et al., 1976b) and in humans (Sirinek and Thromford, 1975). This combination is particularly interesting because HPV pressure is selectively decreased while the cardiovascular side-effects of both appear to be
cancelled. Vasopressin alone decreases cardiac output and increases peripheral resistance, and isoproterenol alone increases cardiac output and decreases peripheral resistance. That these cardiovascular side-effects are cancelled is significant. It is even more significant that a decrease in HPV pressure occurs while an isoproterenol-induced increase in mesenteric flow is in competition with a vasopressin-induced decrease in mesenteric flow. If isoproterenol and vasopressin both decreased intrahepatic resistance, this could account for the decrease in HPV pressure.

The observations of the actions of the isoproterenol-vasopressin combination and the work of Holtz (1932) suggest vasopressin dilates the HV-IVC sphincter. Holtz (1932) observed that pituitary extract, a combination of vasopressin and oxytocin, decreased HPV pressure and liver volume and increased outflow in dog livers. The effect was abolished when the 5 mm of the HV closest to the IVC was removed. Curiously, Holtz (1932) found that oxytocin was 20 times more potent than vasopressin in this respect. Oxytocin therefore dilates a sphincter that also may be dilated by epinephrine and very possibly by beta-adrenergic action. It is puzzling that Holtz (1932) found oxytocin a more potent element of pituitary extract than vasopressin because vasopressin dilation of the HV-IVC sphincter is suggested by other work. Additional experiments identifying
the intrahepatic actions at vasopressin and oxytocin would appear to be profitable because vasopressin dilation of the HV-IVC sphincters could be a common denominator between vasopressin and isoproterenol and could antagonize the action of histamine at this location. Other HV segments also may be dilated by vasopressin and isoproterenol.

If vasopressin and isoproterenol have similar effects on the same anatomic structure, they also might have a common biochemical mechanism, but neither glycogenolysis nor the intracellular intermediate 3'-5' cyclic adenosine monophosphate appear to be potential common mechanisms for isoproterenol and vasopressin. The beta-adrenergic receptor is believed to be responsible for glycogenolysis in the liver, but the evidence is incomplete (Innes and Nickerson, 1975). Vasopressin stimulates hepatic glycogenolysis in dogs (Bergen et al., 1960) and rats (Kirk and Hems, 1975; Keppens and DeWulf, 1975). The ability of glycogenolytic substances to dilate portal venules, sinusoids, sphincters, central veins, and hepatic arterioles in the rat is directly related to glycogenolytic activity (McCuskey, 1966). Speculation about glycogenolysis and HV-IVC sphincters dilation seems inappropriate in view of the actions of serotonin. It promotes glycogenolysis but also increases hepatic resistance to flow in rats (Levine et al., 1964). 3'-5' cyclic adenosine monophosphate does not appear to be a common denominator for isoproterenol and
vasopressin either. 3'-5' cyclic adenosine monophosphate has been postulated as a common denominator for vasopressin and isoproterenol actions in rat kidneys (Levi et al., 1971). In humans, 3'-5' cyclic adenosine monophosphate has been linked to the ADH action of vasopressin (Brazeau, 1975) and to the action of the beta-adrenergic receptor (Koelle, 1975). In addition, adenosine nucleotides, especially 5'-adenosine triphosphate (a breakdown product of 3'-5' cyclic adenosine monophosphate via the enzyme phosphodiesterase) and 5'-adenosinediphosphate have been shown to dilate hepatic arterioles in rats (McCuskey, 1966). However, Jard and Bockaert (1975) state that the production of 3'-5' cyclic adenosine monophosphate and biological activity are not always parallel in mammals, and two studies have shown that vasopressin does not produce an increase in 3'-5' cyclic adenosine monophosphate when perfused through rat livers (Kirk and Hems, 1974; Keppens and DeWulf, 1975).

Control of the Hepatic Blood Reservoir. Alpha-adrenergic mediation of strong constriction of the HPV and weak constriction of the HV and beta-adrenergic mediation of strong dilation of the HV suggest different functions for different hepatic sphincters and a control system for a hepatic blood reservoir. The controls hepatic blood reservoir suggested would be beneficial in times of hemorrhage and stress and be able to account for the regulation of venous return in conjunction with breathing that is
observed. Moreno (1964) and Moreno et al. (1967) demonstrate and discuss the liver's role in the regulation of venous return in healthy humans and dogs (p. 52). Gibson (1959) states the human HV-IVC sphincters appear substantial enough to control venous return during heavy breathing but adds, "...the amounts seem smaller than would be expected if their actions played a part in the daily economy of the body." The controls discussed also would be beneficial in times of hemorrhage and stress because they would reduce the relatively non-essential splanchnic and HPV flows, mobilize blood in the liver for the rest of the body from the intrahepatic HPV and HV systems, drain some of the fluid from the extracellular spaces, and possibly reduce circulation to the periphery of the liver. These controls are consistent with the hypothesis of Moreno et al. (1962) that epinephrine opens the liver for the release of blood.

HPV constriction decreases hepatic flow indirectly through a feedback system that influences splanchnic resistance, and this can work in concert with the hypothesized controls of the hepatic blood reservoir. Portal resistance is so small in comparison to splanchnic resistance that splanchnic resistance should be considered the regulator of hepatic inflow in dogs (Mitzner, 1974b), cats (Greenway et al., 1967), and presumably humans. Mitzner (1974b) reported that increased HPV pressure in dogs increases splanchnic
resistance. His hypothesis for control of HPV flow can be blended with the observation of the actions epinephrine and hepatic nerve stimulation: In times of hemorrhage or stress, when epinephrine release and hepatic nerve stimulation may be assumed to be present, HPV constriction from epinephrine or nerve stimulation will reflexly discourage flow through the relatively non-essential digestive tract. HPV flow decreases with epinephrine-induced increases in HPV pressure have been observed in dogs (Bauer, et al., 1932; Hoffbauer and Bollman, 1950; Daniel and Pritchard, 1951b; Shoemaker, 1964) and cats (McMichael, 1932). Epinephrine-induced increases in HPV resistance have been reported in dogs (Bauer et al., 1932; Butz et al., 1962; Shoemaker, 1964), and the hepatic nerve-induced increases in pressure have been reported in dogs (MacLeod and Pierce, 1914; Edmunds, 1915; Bauer et al., 1932; Daniel and Pritchard, 1951b; Greenway et al., 1967; Greenway et al., 1969). Increased HPV pressure, which reflexly increases splanchnic resistance and decreases HPV flow, also can mobilize fluid from the extracellular spaces and blood from the sinusoids. The reflexly decreased HPV flow should lower sinusoidal pressure and effectively drain some of the extracellular fluid; thus stored plasma is mobilized to return to the systemic circulation. Also, a decreased capillary pressure may decrease the number of sinusoids recruited to handle flow (Brauer et al., 1953, p. 29; Mitzner, 1974a, p. 29);
this, also effectively drains blood from the liver. The result of HPV constriction for the cardiovascular system is an increase in blood volume, a partial bypass of the splanchnic bed, and increased venous return.

Regulation of hepatic flow by a feedback on splanchnic resistance rather than through changes in hepatic resistance seems probable for three reasons. Because of the substantial surface area of the splanchnic bed, a small drop in pressure could move a great quantity of fluid out of the capillaries into the systemic circulation thus pressure-maintenance mechanisms are required (Mitzner, 1974b). If hepatic resistance controlled hepatic flow, increased hepatic resistance without feedback on splanchnic flow could pool a great quantity of blood in the splanchnic bed, because no major alternative routes exist for bypassing the liver in a healthy individual (Mitzner, 1974b). Observations of reciprocal reticuloendothelial system function between the spleen and the liver fit with Ravenna's (1940) theory and Mitzner's (1974b) report about the interplay between HPV pressure, splenectomy and splanchnic resistance (p. 46). Esophageal varices and the collateral circulation may be the partial result of enough increase in hepatic resistance to influence hepatic flow and to overload the lymph system.

A decrease in hepatic outflow resistance can mobilize fluids from the extracellular spaces, mobilize
blood from the sinusoids, and provide a more direct route to the systemic circulation than the lymph system for glucose and plasma proteins during times of hemorrhage or stress. Epinephrine decreases liver volume in dogs (Edmunds, 1915; Bauer et al., 1932; Andrews et al., 1955) and cats (Clark, 1928; McMichael, 1932). Decreased liver volume is also observed in response to hepatic nerve stimulation in dogs (Bauer et al., 1932) and cats (Griffith and Emery, 1930; Greenway et al., 1967; Greenway et al., 1969). It is reasonable to assume that epinephrine and hepatic nerve stimulation have similar actions on the HV system because they act similarly in the HPV system. Reduced outflow resistance and consequently sinusoidal pressure result in a net movement of extracellular fluid back into the sinusoids and, therefore, back into the systemic circulation. Also, a decreased pressure may decrease the number of sinusoids employed to handle the flow (Brauer et al., 1953, p. 29; Mitzner, 1974a, p. 29) and thus the volume of blood in the liver. Glucose from epinephrine-induced hepatic glycogenolysis and plasma proteins can be important as osmotically-active compounds available to maintain plasma volume; they also play important roles individually: Glucose as an energy source and plasma proteins in immune responses and clotting. As decreased sinusoidal pressure presumably increases HV flow at the expense of lymph flow, more glucose and plasma
proteins reach the systemic circulation via venous drainage, a quicker pathway than lymph drainage. The consequence of decreased outflow resistance for the cardiovascular system is an increased cardiac output subsequent to increased venous return and blood volume from the mobilization of fluid and proteins from the extracellular spaces and of blood from sinusoids.

Blood may be mobilized from intrahepatic blood vessels by the constriction of muscles around capacitance vessels of the HPV and HV systems. Greenway et al. (1967) suggested that hepatic nerve stimulation decreases liver volume by constriction of capacitance vessels. Friedman et al. (1951) report that dibenamine, an alpha-adrenergic antagonist, dilates canine HPV and HV systems constricted in response to hemorrhagic shock. This is exactly the response one would predict from this hypothesis of hepatic flow control. Two previously discussed studies support these observations (Bauer et al., 1932, p. 66; Daniel and Pritchard, 1951a, p. 65). Constriction of HPV and HV capacitance vessels, both HPV and HV, provide the cardiovascular system with an increased venous return and blood volume, which increases cardiac output. The HPV constriction may increase HPV pressure and reflexly increase splanchnic resistance, resulting in a partial bypass of splanchnic perfusion.

In rats, cats, and monkeys, hepatic nerve
stimulation occasionally create a restricted intrahepatic circulation (Daniel and Pritchard, 1951b). Serial antograms show a marked decrease in peripheral hepatic perfusion and a shorter transit time. This streamlined hepatic flow, presumably available to the liver in times of hemorrhage and stress, maintains high HPV pressure. This keeps splanchnic resistance high, which diverts flow away from the relatively nonessential splanchnic vascular bed and decreases the time and volume of blood involved in hepatic perfusion. The cardiovascular system receives an increase in venous return and responds with an increased cardiac output. Gibson (1959) discounts the importance of this restricted circulation on an anatomical basis.

Different functions for different portions of the HV system are suggested by the observations of several investigators and the hypotheses of control of the hepatic blood reservoir. Apparently, the sublobular veins that control drainage from central veins and other smaller sublobular veins control sinusoidal drainage and pressure. The larger sublobular veins, the collecting veins, and the HV's are capacitance vessels, and their capacity is regulated in part by their longitudinal muscles. The HV-IVC sphincters control the drainage from the capacitance vessels. During hemorrhage or trauma, one would expect the smaller sublobular vein muscles to dilate in response to initial low concentrations of epinephrine and mobilize fluid
from the extracellular spaces and blood from the sinusoids. Constriction at higher concentrations of epinephrine and greater levels of nerve stimulation (Bauer et al., 1932, p. 94; Deysach, 1941, p. 94; Andrews et al., 1955, p. 94) should increase sinusoidal and HPV pressure. The observations of central necrosis as an agonal change (Popper, 1948) and sublobular vein constriction as its cause (Wallach and Popper, 1950) are consistent with the hypothesis that sublobular vein musculature can control sinusoidal pressure. Weak constrictions of the larger sublobular veins, the collecting veins, and HV's are well-suited to the task of mobilization of stored blood without an increase intrasinusoidal pressure (Bauer et al., 1932, p. 94). In times of hemorrhage and stress, weak contractions would increase outflow without interfering with the decrease in sinusoidal pressure necessary to recruit plasma and blood from the sinusoids. During the initial stages of hemorrhage and stress, low concentrations of epinephrine and low levels of hepatic nerve stimulation would dilate the HV-IVC sphincters, constrict the HV capacitance vessels (Bauer et al., 1932, p. 94), and thus facilitate the mobilization of hepatic blood. As epinephrine concentrations and the level of nerve stimulation increased, the HV-IVC sphincters would constrict; they could constrict enough to influence HPV pressure (Bauer et al., 1932, p. 94). It would be advantageous to have the HV-IVC sphincters able to influence
HPV pressure. The immediate post-sinusoidal musculature would be subject to locally high concentrations of vaso-dilator autacoids released by ischemic cells. Autacoids in lower concentrations would reach the HV-IVC sphincters due to dilation. As a result, the HV-IVC sphincters would be less sensitive to ischemia and better able to maintain high HPV pressure in the face of a prolonged need to divert flow from the splanchnic circulation.

The hypotheses of alpha-adrenergic control of HPV constriction and weak constriction of the HV, and beta-adrenergic control of the dilation of the HV system are not consistent with everything that is known about the control of hepatic flow. Innes and Nickerson (1975) report that epinephrine increases splanchnic flow in humans. This contradiction to the hypothesis presented may be the result of some elemental disagreement between the hypothesis and fact, or the contradiction may be the result of a particular concentration of epinephrine and really no contradiction at all. Also, any hypothesis of hepatic flow control that involves only adrenergic neurotransmitters offers a simplistic explanation at best.

Vasopressin Research. Research with vasopressin is concerned with the search for a vasopressin analogue that possesses all the desirable actions and no undesirable side-effects and with the techniques of vasopressin administration. Altura et al. (1975a and b) report different vasopressin and
oxytocin receptors in different areas of the rat's body, and this is the theoretical basis for research with vasopressin analogues.

Amino acid-substituted vasopressin analogues offer an altered three-dimensional compound. The prototype of a vasopressin analogue with an altered structure and different actions is oxytocin, which has quite different actions from vasopressin but differs only by substitution of two amino acids. At the number three position, vasopressin has phenylalanine, an amino acid with an aromatic ring attached to the backbone structure by a CH₂, while oxytocin has isoleucine, with a four-carbon side-chain in an isoconfiguration. At the number eight position, oxytocin has leucine, which possesses a four-carbon side-chain different from isoleucine only in the configuration of its side-chain, while vasopressin has arginine, a basic amino acid with a long side-chain of carbon, hydrogen, and nitrogen molecules.

The three-dimensional structures of oxytocin and vasopressin are similar. According to Jard and Bockaert (1975), oxytocin and lysine vasopressin have two beta-turns. One beta-turn includes the number seven through number nine amino acids; the second beta-turn is within the ring and includes the number two through number five amino acids. Jard and Bockaert (1975) also state that in oxytocin and vasopressin hydrogen bonds exist between the nitrogen
of the number nine amino acid and the carbonyl oxygen of the number six amino acid, and between the nitrogen of the number five amino acid and the carbonyl oxygen of the number two amino acid. Additionally, in oxytocin, a third hydrogen bond exists between the nitrogen of the number eight amino acid and the carbonyl oxygen of the number five amino acid, and the phenyl group of the number two amino acid (tyramine) is folded over to lie in the same plane as the ring of amino acids. Jard and Bockaert (1975) acknowledge that the three-dimensional descriptions presented above may not be present in the body because they were determined in test solutions different from the solutions encountered in the blood. (The three-dimensional structure of proteins can be different in solutions of different ionic composition.) Brazeau (1975) states that the disulfide bond and the exact size of the ring are essential to the activity of the vasopressin nonapeptide. Jard and Bockaert (1975) state that the disulfide bond, the two beta-turns, and the hydrogen bonding between the amino acids at position five and position nine are important to the activity of oxytocin and vasopressin.

The effects of a number of different amino acid substitutions have been studied. Brazeau (1975) states that removal of the number nine amino acid, glycine, totally inactivates vasopressin. He also states that deamination of that glycine results, not in inactivation, but in
increased ADH activity; that the basistry of the number eight amino acid is important to the ADH activity; and the length of the side-chain on the number eight amino acid, but not its composition, is an important factor to oxytocin. Altura (1975a) states that for vasopressin's pressor activity and binding, an aromatic side-chain, rather than an open-chain compound, is needed on the number three amino acid. He further states that the number two position needs a phenol side-chain, especially its hydroxyl group, for binding and pressor activity. Jard and Bockaert (1975) state that the hydroxyl group on the number two amino acid in vasopressin is fundamental to its binding ability but less important to its ability to stimulate. They also state that, in the number eight position in vasopressin, the d-isomers of lysine and arginine have greater ADH and pressor activities than their respective l-isomers and that substitution of the amion acid threonine for glutamine at the number four position in vasopressin and oxytocin increases ADH activity and decreases pressor activity.

No vasopressin substitution analogue has been able to control HPV pressure and bleeding without deleterious cardiovascular side-effects. The vasopressin analogue phenylalanine$^2$-lysine$^8$ vasopressin (called felypressin [Brazeau, 1975] or octapressin [Feruglio, 1964b]) is an example of a vasopressin substitution analogue. It has a
greater pressor activity than ADH activity (Tsakaris et al., 1964; Schwartz, 1970; Brazeau, 1975). Tsakaris et al. (1964) report no deleterious cardiovascular side-effects with it. Feruglio et al. (1964b) report increased systemic blood pressure; and, Segel et al. (1963) found increased systemic blood pressure and decreased cardiac output, heart rate, and stroke volume as well. Brazeau (1975) reports no cardiac effects.

Triglycyl vasopressin is an example of the other major form of vasopressin analogue under study, amino acid-addition analogues. Triglycyl vasopressin is a vasopressin nonapeptide with a triglycyl peptide attached to the number one amino acid. This tripeptide endows the compound with prolonged action (Režabek, 1967; Cort et al., 1968; Kynčl et al., 1974; Aronsen et al., 1975) by virtue of the slow release of the vasopressin nonapeptide by enzyme action (Aronsen et al., 1975). In dogs treated with triglycyl vasopressin, no cardiac arrhythmias or decreases in cardiac output were observed; in fact, cardiac output increased presumably due to increased venous return (Cort et al., 1968). Reports with human subjects sound promising. Cirrhotics with bleeding varices have been treated successfully with triglycyl vasopressin (Aronsen, 1972) and triglycyl-lysine vasopressin (Aronsen et al., 1975), and cardiac arrhythmias were not observed.

Because no vasopressin analogues have been reported
to have significantly different actions and to minimize confusion, all compounds used in research cited in this paper are referred to as vasopressin, whether the compound was vasopressin, a crude extract thought to be vasopressin, or a vasopressin analogue.

The techniques of vasopressin administration also have been studied. The choice of bolus injection or continuous infusion, intravenous versus superior mesenteric artery infusion, and administration of high or low concentrations of vasopressin have been studied. Thromford and Sirinek (1975) compared the responses of patients to a bolus injection of 15 units of vasopressin over five minutes to the responses of patients given 40 units of vasopressin intravenously over the course of an hour. Curiously, in the first 15 minutes, less vasopressin had been administered by continuous infusion than by bolus injection, but the reduction in HPV pressure was greater. Continuous infusion also reduced HPV pressure for a longer time, reduced cardiac output for a shorter time, and caused less abdominal pain and substernal discomfort. Barr et al. (1975) tested two different and important ideas about vasopressin therapy. In dogs, superior mesenteric artery and intravenous infusions were compared with a standard dose, similar to a human dose, and one-fifth of the standard dose. Interestingly, no difference was found between the results of the superior mesenteric artery and intravenous infusions on
cardiac output, systemic blood pressure, HPV pressure, HPV flow, and superior mesenteric artery flow. Even more interesting is the observation that a reduction of the dose to one-fifth the original cuts the deleterious cardiovascular effects by a greater amount than the decrease in superior mesenteric artery flow and the decrease in HPV pressure. Johnson et al. (1977) conducted a prospective, randomized clinical trial of intravenous and superior mesenteric artery vasopressin administration to alcoholic cirrhotic patients for control of variceal bleeding. They observed no significant difference between the ability of the two methods of administration to control bleeding but did observe a significantly greater number of complications (catheter-related and cardiac) among the patients treated by superior mesenteric artery infusion. On the basis of their observations, they conclude intravenous vasopressin is a preferable method of administration to superior mesenteric artery infusion.

Nusbaum and Conn (1975) hypothesized about the creation of the side-effects of vasopressin administration. After analyzing the data presented in two other papers (Millette et al., 1975; Sirinek and Thromford, 1975), they suggested that individuals respond to vasopressin by either large portal effects relative to cardiovascular effects or vice versa. These two responses occur in a ratio of 1:1. Acknowledging it as a "flimsy hypothesis"
needing investigation, they propose that the amount of liver bypass through portal-systemic shunting is an important factor determining the relative amount of cardiovascular response to vasopressin—the greater the proportion of shunting, the greater the proportion of cardiovascular effects to portal effects. Nusbaum and Conn (1975) did not mention intrahepatic shunting, capillarization of the sinusoids, and liver cell damage, but these factors also would appear to be important. This hypothesis of Nusbaum and Conn (1975) may involve a mechanism other than splanchnic arteriolar constriction for vasopressin action on HPV pressure. Hepatic bypass from portal-systemic shunting may be important primarily because it bypasses intrahepatic effects of vasopressin. The result is the same, relatively increased cardiovascular effects, but the reason is because fewer intrahepatic actions are possible. Both hypotheses lack experimental data and need testing.

Conclusions. Conclusions can be made about the role of different factors in portal hypertension, the role of different factors in esophageal variceal formation and hemorrhage, the various possibilities for histamine action in portal hypertension and variceal bleeding, and about the different actions of vasopressin.

Different factors that promote portal hypertension are important at different times during the progression of portal hypertension in cirrhosis, and all of these factors
are not necessarily present in every case of cirrhosis. Three time periods can be hypothesized. In the earliest stage, portal hypertension is promoted by three factors that operate within the normal hepatic architecture. An intermediate stage is recognizable by the emergence of factors that operate above the sinusoidal level. At this intermediate stage, factors of local importance work with factors that act on a larger scale to create resistance to hepatic flow, but no major changes are present in the hepatic architecture. The final stage can be characterized morphologically from major interruptions of the liver architecture (isolation of healthy cells within broad bands of connective tissue). These changes mark the emergence of intrahepatic shunting and the compression of HV radicles as major factors in the production of portal hypertension.

The earliest stage of portal hypertension is the result of swollen cells, RBC's, leukocytes, and clotting occluding the sinusoidal lumen and local constriction of sphincters. Swollen parenchymal, Kupffer, and endothelial cells are often present and are known to be capable of increasing HPV pressure. Cell swelling can be due to accumulation of substances in healthy cells or occur just before cell death. As the lumen of the sinusoids narrows, RBC's and leukocytes may stack up and further block flow. Cell death can also draw leukocytes to the area. Clotting seems a probable accompaniment to stagnant flow and cell
death. Cell injury and death, leukocyte-cell interactions, and clotting may release autacoids that could cause local sphincter constriction and further flow decreases. Increases in pressure in this early stage are probably dampened by local increases in hepatic and HPV system lymph production, recruitment of unused sinusoids, and HPV vascular compliance both intrahepatically and extrahepatically. The changes of the early stage are important to portal hypertension because they reduce the liver's capacity to compensate for the effects of other factors, later. In addition, local degeneration of sinusoids will give rise to the later major architectural changes.

Although these cases are rare, full-blown cases of portal hypertension and esophageal varices can occur without changes in the hepatic architecture as a result of cell swelling, fibrous tissue deposition, a combination of the two, or from increased flow into the HPV. This kind of cell swelling or fibrous tissue deposition differs from that which works in concert with other factors because it occurs diffusely throughout the liver and not in patches as it does in cirrhosis. Increases in HPV flow alone may result in portal hypertension and varices; usually this is the result of an arteriovenous fistula draining directly into the HPV. This, too, does not qualify as an ordinary case of portal hypertension and varices. Autacoids could be working unrecognized in concert with cell swelling and
fibrous tissue deposition when they produce portal hypertension. Autacoids may also work alone to produce portal hypertension, as was hypothesized earlier (pp. 72-73) for the portal hypertension of primary biliary cirrhosis.

In the intermediate stage of portal hypertension of cirrhosis, factors other than local ones become important, and the changes of the final stage are not yet prominent. The important factors are intrahepatic shunting, the inability of the lymph system to increase hepatic drainage, autacoidal factors, and increased flow into the HPV. Intrahepatic shunting increases HPV pressure by bypassing the pressure drop offered by capillaries (HA-HPV, HA-HV, and HPV-HV); increasing the HV pressure (HA-HV and HPV-HV) and providing easy communication for the increased HV pressures to the HPV (HPV-HV); and from contraction of the connective tissue surrounding shunts—connective tissue which arises from recent synthesis and from the collapse of the stroma around shunts. Lymph flow becomes a factor in the intermediate stage, not because it actively contributes to portal hypertension, but because it has reached the limit of its capacity to oppose it. Later, ascites will signal the fact that sinusoidal pressure has increased lymph production to such a level that lymph drainage is insufficient and lymph is forced through Glisson's capsule. If histamine and other autacoids have a role in the production of portal hypertension through constriction of sphincters, especially
HV sphincters, they would become important at this time. Histamine and other autacoids will probably be released by the interactions in sinusoids lined with dead and injured cells; in which antigen-antibody reactions are taking place; and in which RBC's, leukocytes, and clots are collecting. As conditions worsen, more autacoids will pass unaltered through sinusoids and shunts to come in contact with HV sphincters. As the concentration of autacoids increases in the HV system their effects will no longer be localized because larger HV's will be constricted, affecting larger areas of the liver. Increased flow should become a factor at the intermediate stage. It results from changes in splenic, gastric, and intestinal flow controls. Increased gastric flow may be a result of the extended presence of autacoids that open arteriovenous shunts; if so, this is the time when the effects would appear because collateral channels would have appeared and intrahepatic shunting would be available to allow the blood concentrations to increase. Of the four factors important at the intermediate stage, intrahepatic shunting is the most important element of portal hypertension. It not only increases pressure directly, but it advances liver disease by robbing liver cells of blood flow, promotes the creation of autacoids, and provides a pathway for those autacoids to reach the HV system unaltered.

The final stage of portal hypertension occurs when
intrahepatic shunting and HV compression by nodules of parenchymal cells become important. The distinction between the intermediate stage and the final stage is most easily recognized morphologically by the change from an essentially normal morphology to micronodular or macronodular morphology. The distinction between the intermediate and final stages of the portal hypertension of cirrhosis can also be drawn by functional criteria: Restricted lymph drainage, autacoids, and increased flow into the HPV continue to be important, but intrahepatic shunting and HV compression from parenchymal cells encased in connective tissue capsule rise in importance, and the final stage is reached when intrahepatic shunting and HV compression dominate the situation.

Esophageal varices are created and hemorrhage because of increased hepatofugal collateral circulation and the vulnerability of the submucosal esophageal veins, but the contributions of the lymph system and histamine to variceal formation and hemorrhage have yet to be assessed. Because the majority of splenic vein flow is diverted into the esophageal veins, increased flow probably is a major element of the formation and hemorrhage of esophageal varices. The lack of support from connective tissue and muscle plus the proximity to the esophageal lumen combine to make a strong case for the vulnerability of the submucosal esophageal veins as an element of variceal formation and
hemorrhage. The role of the lymph system in variceal bleeding is not completely understood. If substantial lymphovenous communications exist between the azygos veins and the thoracic duct, this is an important factor because the veins will be less able to cope with increased flow and more likely to hemorrhage. Histamine relaxation of esophageal muscle may also contribute because wholesale rerouting of gastric drainage through the esophageal veins provides substantial quantities of histamine to the area. Relaxation of esophageal muscle results in increased flow through the vulnerable and poorly-supported submucosal esophageal veins. From present knowledge, histamine appears to have more potential importance to variceal bleeding than the lymph system, but not enough is known about the actions of either to make a definite judgment possible.

Histamine has three possible actions favoring portal hypertension and variceal bleeding that appear to be both probable and potentially important. Substantial extrahepatic shunting and the potential for gastric drainage to bypass the liver via the esophageal veins both would permit histamine to reach the proper locations in adequate quantities. If the increased incidence and exacerbation of ulcers in cirrhotics is due to elevated blood concentrations of histamine, it suggests that histamine may cause increased gastric and possibly intestinal flows into
the HPV. Second, substantial intrahepatic shunting favors 
histamine as an agent responsible for HV constriction as 
does the liver's downstream location from the stomach and 
the probability of histamine release from a sick liver. In 
the absence of a blood reservoir function in humans, the 
possibility of histamine constriction suffers for the want 
of sufficient HV musculature. If a blood reservoir func-
tion does exist, the likelihood that sufficient HV muscu-
ture exists is greatly enhanced. Thirdly, if histamine 
does dilate esophageal muscle, an important result would be 
an increase in flow through the submucosal esophageal veins 
because their tributary channels must pass through the 
relaxed muscle layers. All three of these hypotheses of 
histamine action lack experimental evidence.

Vasopressin appears to have two likely actions that 
 oppose portal hypertension and variceal bleeding and three 
actions that are uncertain. First, vasopressin certainly 
decreases flow into the HPV system by constriction of 
splanchnic arterioles, and a decrease in flow from selective 
closure of splanchnic submucosal arteriovenous shunts does 
not seem unlikely. Although the evidence is not as strong 
because of the paucity of experimental work, esophageal 
smooth muscle constriction is a second likely action of 
vasopressin. Support for this idea comes from a small 
amount of experimental work, obvious theoretical potential, 
and clinical observations of abdominal and substernal
cramping. HPV dilation is an uncertain vasopressin action. Hepatic outflow resistance may be greater than HPV resistance in most cases of portal hypertension; therefore HPV dilation may not have an important effect on HPV pressure. A decrease in intrahepatic resistance is a second uncertain action of vasopressin. Vasopressin may dilate hepatic sphincters and thereby decrease intrahepatic resistance. This hypothesis of vasopressin action suffers from lack of evidence detailing presence of and the capacity for action. If vasopressin is important as an autacoid antagonist, the implication for portal hypertension and liver disease is that a major element, the contribution of autacoids, has been overlooked in the past. The lack of experimental evidence makes this idea speculative at the moment. That a major element of portal hypertension and liver disease has been overlooked seems doubtful enough to make one hesitant to assign much importance to it. Specific histamine antagonism to vasopressin's portal hypotensive action.Obviously, this hypothesis must face the uncertainties of histamine action as well as histamine antagonism. However, because an antihistamine role for vasopressin has been reported and vasopressin is thought to work directly on contractile elements, providing a mechanism for histamine antagonism, this speculation does not seem too far-fetched.

Introduction to the Experiments. The experiments carried out in this study were designed to determine if
vasopressin has an intrahepatic action in the rat liver and to assign any action found to inflow or outflow control. The work of Schon and Labat (1971) is an obvious model. Their method was modified by the use of a constant flow with continuous pressure measurement rather than constant pressure with flow measurement because flow measurement without flowmeters is more cumbersome and less accurate than direct readouts of perfusion pressure. Continuous pressure measurements also can be easily correlated with continuous readings of weight. Another modification was the perfusion of livers on a wire weighting tray, which allowed free drainage of fluid. If changes in pressure were not accompanied by weight changes, it was assumed that changes in resistance had occurred presinusoidally, and it was assumed that changes in pressure associated with weight changes were the result of changes in post-sinusoidal resistance. In all experiments, an alpha-adrenergic agonist, phenylephrine, was used to provide a model of inflow constriction and a beta-adrenergic agonist, isoproterenol, was used to provide a model of outflow dilation. In later experiments, histamine was tested in order to provide a model of outflow constriction and in the hope of later being able to test for antihistamine properties of vasopressin and the adrenergic agonists.
MATERIALS AND METHODS

Rat livers were perfused with saline and with several concentrations of different drugs while the absolute pressure and weight changes were recorded. Pressure and weight changes were used to identify the location of action as pre- or post-sinusoidal. Because the perfusions were done at a constant flow rate, changes in pressure were presumed to reflect changes in resistance. The group A livers proved unrewarding as an experimental series, and the group B livers were run afterwards as a pilot series. Both Group A and B livers were perfused with saline and with several concentrations of different drugs in saline. Vasopressin was used to determine if it has an intrahepatic action and to identify any action as pre- or post-sinusoidal.

Phenylephrine, an alpha-adrenergic agonist, and phentolamine, and alpha-adrenergic antagonist, were used to identify the role of alpha-adrenergic action and to provide a model of increased pre-sinusoidal resistance. Isoproterenol, a beta-adrenergic agonist, and propranolol, a beta-adrenergic antagonist, were used in order to identify the role of beta-adrenergic action and to provide a model of decreased post-sinusoidal resistance. Histamine, an autacoid, was used to provide a model of increased post-sinusoidal resistance, and it was hoped that later it could be used to identify any interactions between it and the actions of alpha-adrenergic and beta-adrenergic agonists and vasopressin.
In the group A series, livers were taken from 350-400g white, male rats (Sasco, Inc., Omaha, NB). Two rats were housed per cage without care and fed Purina Rat Chow (Ralston Purina, Checkerboard Square, St. Louis, MO) and tap water ad lib. Heparin (Sigma Chemicals, St. Louis, MO; #H-3125), isoproterenol (Sigma Chemicals, St. Louis, MO; #I-5627), phenylephrine (Sigma Chemicals, St. Louis, MO; #P-6126), propranolol (Sigma Chemicals, St. Louis, MO; #P-0884), phentolamine (Ciba Pharmaceutical Co., Summit, NJ; Regitine Mesylate injection), and vasopressin (Sigma Chemicals, St. Louis, MO; #V-2875) were employed. Three liters of both 10^{-6} m phentolamine and 10^{-6} m propranolol were prepared in advance and stored in Pyrex flasks between 0° and 5° C during the three weeks of experimentation. Enough 100 ml quantities of the drugs solutions were prepared each day from frozen stock solutions and stored between 0° and 5° C. Large quantities of saline for drug solutions and perfusions was prepared in advance and stored in half-gallon bottles of non-chemical resistant and non-heat resistant glass between 0° and 5° C. The recipe for saline was a modified Krebs solution (Prosser, 1973), adjusted to pH 7.4, with 0.01g/liter of ascorbic acid added to stabilize the drugs. The water was from the Olin Hall distilled water taps, and it was further deionized in a deionizing column (Barnstead Still and Sterilizer Co., Boston, MA; #0802-Standard) three times. The water was
stored in five-gallon jugs of non-heat-resistant and non-chemical-resistant glass. The 100 ml quantities of saline and drug solutions were kept in 250 ml Pyrex flasks and stored between 0° and 5° C until used.

The perfusion apparatus consisted of Tygon tubing from a 37° C water bath, through a finger pump (Sigma Motor Co., Middleport, NY; model T-8), to a wire mesh weighting tray suspended from a force transducer (Grass Instrument Co., Quincy, MA; #FT 03 B). Two lines led off the main line, close to the finger pump. The line closest to the pump led to a manometer used to calibrate the pressure transducer. This line was flooded and the manometer was closed during liver perfusions. The second line led to the pressure transducer (Stratham Laboratories, Inc., Hato Rey, Puerto Rico; Model P 23AA). Pressure and weight measurements were recorded continuously on a Beckman Dynograph (Beckman Instruments, Inc., Schiller Park, IL; type R-411). Absolute pressures and changes of weight were recorded. The tubing that emerged from the finger pump, the base of the water monometer, the line into the pressure transducer, and the open end of the tubing secured to the weighting tray were approximately at the same height in order to minimize static pressure differences. The perfusion rate was between 15 and 16 ml/min and constant during any one day. Transit time through the system was approximately 60 seconds.
The sequence of events for each liver perfusion began with an intraperitoneal injection of 1,000 USP-JA units of heparin. Ten minutes passed before the rat was swung by its tail and killed by concussion. Within that ten-minute interval, saline was placed in the water bath to allow it ten minutes to come to 37° C. All drug solutions had a ten-minute warm-up prior to infusion to bring them to 37° C. Through a midline incision, the HPV was cannulated with plastic tubing of a diameter close to that of the HPV. The liver was removed by cutting the ligaments and the HPV without concern for the proximity to the HPV or the cannula, but care was taken to cut the HV close to the substance of the liver. The cannulated liver was placed on a dissecting tray; approximately 50 ml of 37° C saline was poured over it to facilitate manipulation; the lobes of the liver were arranged in an orderly fashion; and the liver was transferred to the weighing tray, and the cannula was plugged into the perfusion apparatus. The liver rested on the tray with the caudal portion up. On most occasions, the liver and cannula were manipulated in order to achieve a minimum perfusion pressure, and once this pressure was established, the liver and perfusion apparatus were not disturbed. Less than ten minutes passed between the death of each rat and the beginning of perfusion. Each liver was perfused with saline until steady pressure and weight readings were observed; typically this required approximately ten minutes.
Two ten-minute perfusion periods followed. Vasopressin, at a concentration of 10.0, 1.0, or 0.1 units/liter; isoproterenol at $10^{-4}$ m, $10^{-6}$ m, or $10^{-8}$ m; or phenylephrine at $10^{-5}$ m and $10^{-9}$ m were infused during the first ten-minute period. Either phentolamine or propranolol were infused during the second ten-minute period. Four livers were run at each concentration of isoproterenol, phenylephrine or vasopressin, two of them with phentolamine and two of them with propranolol. The order of isoproterenol, phenylephrine, and vasopressin experimentation, as well as the order of concentrations within each drug series and the order of blocking agents was randomized. At least one saline control was run each day. The flow rate was varied with several control livers to verify change of pressure and weight with change of flow rates. The open end of the tubing at the water bath was dipped in saline between removal from one flask and placement into another when perfusion solutions were changed. The tubing was flushed with distilled water at the end of each day and the beginning of each day.

The flow rate of 16 ml/min was chosen on the basis of comparison between published estimates of HPV flow in healthy rats and the data from the work of Sundet (1975), which employed a similar perfusion apparatus and methods. Sundet's (1975) work suggests that resistance to flow varies with flow rates in healthy livers. Sixteen ml/min is a flow rate within a range of estimates of normal flow and
is within the range of flow rates at which resistance is not changing according to Sundet (1975). Phentolamine was chosen as an alpha-adrenergic antagonist because it offered competitive inhibition. Because a histologic difference, though small, was noticed between damage to dog livers ischemic for 45 minutes and those ischemic for longer periods (Grana et al., 1968), the period from the death of the rat to the end of the perfusion was kept to less than 45 minutes in order to minimize the effects of ischemia. The infusion sequence of saline; then isoproterenol, phenylephrine, or vasopressin; then phentolamine or propranolol was intended to provide a baseline for pressure and weight for each rat and to offer models for pre- and post-sinusoidal resistance and of competitive antagonism of drug action against which to evaluate the action of vasopressin.

Because the trials with the group A series of livers failed to show any reproducible responses to drug infusions, a number of changes in the methods were instituted and the group B trials were run in the hope of identifying a method and a range of concentrations which would show responses to drug infusions. The data from the group A series of livers contained no reproducible results; and the group A series was almost totally without response to drug infusions. The trial of $10^{-3}$ m isoproterenol in the last group A liver was done under different conditions than the other
drug trials, and the response to it and its antagonist suggested that a change in the methods would result in reproducible responses to drug infusions. A number of changes in the methods were instituted to eliminate elements of the methods which were thought to interfere with responses to drugs. Changes were made in the protocol of the preparation of saline and drug solutions to minimize the chances for contamination and the time the drugs spent at 37° C. The perfusion apparatus was changed to eliminate unnecessary portions and to shorten the transit time of solutions through the apparatus. A bubble-catcher was installed to catch bubbles which otherwise would have passed into the liver and blocked perfusion. Nembutal pre-treatment was employed to eliminate the trauma from concussion death and to allow surgery on a live rat. Surgery on a live rat decreased the time of hepatic anoxia prior to perfusion.

Conditions for the group B series were identical to those of the group A series except for the following changes: Rats premedicated with Nembutal were injected intraperitoneally with 0.04 mg in saline five minutes before the heparin injection, and the surgery was performed on live rats. The HPV distal to the cannula was clamped to minimize blood loss. The liver spent between five and eight minutes without HPV flow and less than three minutes without HA flow. The perfusion apparatus differed in several respects. The pressure transducer was calibrated separately
from the system, and the line to the manometer was removed. A closed, flooded, vertical tube was placed on the main line between the line to the pressure transducer and the weighing tray to catch bubbles in the saline. Some tubing was eliminated from the earlier apparatus, which shortened the transit time to approximately 40 seconds.

The saline for saline infusions and drug solutions was made up as needed from stock solutions of ions stored between 0° and 5° C. The saline for the saline washes and for the drug infusions was often measured cold and the 100 ml aliquots placed in the water bath in Pyrex flasks to warm to 37° C; about as often the saline was placed in a 600 ml Pyrex beaker, and the beaker was placed in a drying oven until it warmed to above 37° C and then placed in the water bath to cool to 37° C. This second method was employed because it required less time. Stock solutions of all drugs were kept frozen and new solutions were used each day. Drug solutions were mixed from stock solutions, stored between 0° and 5° C, approximately 30 seconds prior to infusion. In the group B series of livers, histamine (Sigma Chemicals, St. Louis, MO; #H-7250) was tested in addition to all the drugs tested in the group A series. The group B series of livers is divided into two groups: those livers from rats not pretreated with Nembutal and those from rats pretreated with Nembutal.

The sequence of drugs was different for the group B
series. A perfusion period to establish steady baselines, typically ten minutes, was followed by alternating infusions of a drug solution for five minutes and saline for eight minutes, to a limit of three combinations per liver. Three combinations were run because the baselines of saline-perfused livers remained steady at least that long and the overall time between hepatic ischemia from surgery and the end of perfusion was kept close to 45 minutes. Histamine was tested in concentrations of $10^{-2}$ m through $10^{-7}$ m in non-Nembutal-treated livers and in concentrations of $10^{-5}$ m through $10^{-7}$ m in Nembutal-treated livers. Isoproterenol was tested in concentrations of $10^{-2}$ m through $10^{-7}$ m in non-Nembutal-treated livers and in concentrations of $10^{-3}$ m through $10^{-5}$ m in Nembutal-treated livers. Vasopressin was tested in concentrations of 0.01 through 100.0 units/liter in non-Nembutal-treated livers and in concentrations of 0.1 through 10.0 units/liter in Nembutal-treated livers. Phenylephrine was tested in concentrations of $10^{-3}$ m through $10^{-8}$ m in non-Nembutal-treated livers; phentolamine was tested in concentrations of $10^{-6}$ m through $10^{-9}$ m in non-Nembutal-treated livers; and propranolol was tested in concentrations of $10^{-5}$ m through $10^{-8}$ m in non-Nembutal-treated livers. Thirty-five non-Nembutal-treated experimental livers were run and one non-Nembutal-treated liver was perfused with saline as a control. Fourteen Nembutal-treated experimental livers were run and three
Nembutal-treated livers were perfused with saline as controls.

In the analysis of the methods, a range of variation was defined as ordinary from observations of trials with drug solutions and saline solutions. A pressure variation of 0.5 cm of water and a weight variation of 0.05g was observed in response to drug solutions that did not create any greater change and in response to saline infusions. Ten livers were perfused with saline under the conditions of group A livers, one liver was perfused with saline according to the dictates of the non-Nembutal-treated group B series, and three livers were perfused under the conditions of Nembutal-treated group B livers to establish the action of saline infusions.

The criteria to recognize a change were set from the data. To be counted, a change needed to be greater than 0.5 cm of water or 0.05 g, and it needed to be abolished by perfusion with saline or antagonized by the specific blocking agent. In cases where the changes were not completely removed or antagonized, only the portion reduced or antagonized was counted as a change. In those cases where the saline wash over-compensated for the change induced by the drugs, only the amount of the initial, drug-induced change was counted as a change.
DATA

The results from the trials with the group A and group B livers are presented in Tables 1-6. In addition, some observations on the experiments are presented.

A number of observations were made during the perfusions. These observations concern blood loss during surgery, appearance of the abdominal viscera and the HPV, blanching of the livers during perfusions, the action of the bubble-catcher on pressure, the patterns of pressure and weight as they stabilized at the beginning of each perfusion, and the lack of a difference in the variations observed during periods of drug infusion and periods of saline infusion. Little bleeding was observed from Nembutal-treated rats during surgery. Variation in the amount of blood loss during surgery without Nembutal pretreatment resulted from cerebral, thoracic, and/or abdominal hemorrhage produced by concussion, and a small contribution resulted from HPV bleeding. Blood loss in Nembutal-treated rats was a small and consistent amount much less than the blood loss from surgery on non-Nembutal-treated rats. In rats given Nembutal, the abdominal viscera were much redder (a bright red) and the HPV was much larger than in rats not pretreated with Nembutal. In addition, patches of liver containing blood were much less frequent in livers from Nembutal-treated rats than in the livers from non-Nembutal-treated rats.
Table 1. The number of trials that exhibited changes or did not exhibit changes and the changes of perfusion pressure and weight observed during the perfusion of isoproterenol, phenylephrine, and vasopressin into livers from group A rats. Changes less than 0.5 cm of water or 0.05 g are not counted as changes.

<table>
<thead>
<tr>
<th>Isoproterenol</th>
<th>Trials</th>
<th>Changes Observed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(cm of water, g)</td>
</tr>
<tr>
<td></td>
<td>No Change</td>
<td>Change</td>
</tr>
<tr>
<td>$10^{-3}$</td>
<td>0</td>
<td>1*</td>
</tr>
<tr>
<td>$10^{-4}$</td>
<td>4</td>
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<tr>
<td>$10^{-6}$</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>$10^{-8}$</td>
<td>4</td>
<td>0</td>
</tr>
</tbody>
</table>

*Perfused under different conditions than other group A livers.

<table>
<thead>
<tr>
<th>Phenylephrine</th>
<th>Trials</th>
<th>Changes Observed</th>
</tr>
</thead>
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<td></td>
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<td>(cm of water, g)</td>
</tr>
<tr>
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<td>Change</td>
</tr>
<tr>
<td>$10^{-5}$</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>$10^{-9}$</td>
<td>4</td>
<td>0</td>
</tr>
</tbody>
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<table>
<thead>
<tr>
<th>Vasopressin</th>
<th>Trials</th>
<th>Changes Observed</th>
</tr>
</thead>
<tbody>
<tr>
<td>units/l NO</td>
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</tr>
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<td></td>
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</tr>
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</tr>
<tr>
<td>1.0</td>
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</tr>
<tr>
<td>0.1</td>
<td>4</td>
<td>0</td>
</tr>
</tbody>
</table>
Table 2. The number of trials that exhibited changes or did not exhibit changes and the changes of perfusion pressure and weight observed during the perfusion of histamine and propranolol into livers from non-Nembutal-treated group B rats. Changes less than 0.5 cm of water or 0.05 g are not counted as changes.

<table>
<thead>
<tr>
<th>Histamine</th>
<th>Trials</th>
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</tr>
</thead>
<tbody>
<tr>
<td>m</td>
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<td>Change</td>
</tr>
<tr>
<td>$10^{-2}$</td>
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<td>1</td>
</tr>
<tr>
<td>$10^{-4}$</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>$10^{-5}$</td>
<td>5</td>
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<tr>
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<td>1</td>
</tr>
<tr>
<td>$10^{-7}$</td>
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<table>
<thead>
<tr>
<th>Propranolol</th>
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<tr>
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<tr>
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</tr>
<tr>
<td>$10^{-6}$</td>
<td>2</td>
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<tr>
<td>$10^{-7}$</td>
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<tr>
<td>$10^{-8}$</td>
<td>1</td>
<td>0</td>
</tr>
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</table>
Table 3. The number of trials that exhibited changes or did not exhibit changes and the changes of perfusion pressure and weight observed during the perfusion of phentolamine, phenylephrine, and propranolol into livers from non-Nembutal-treated group B rats. Changes less than 0.5 cm of water or 0.05 g are not counted as changes.

<table>
<thead>
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<th>Phentolamine</th>
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</tr>
<tr>
<td>$10^{-6}$</td>
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<td>1</td>
</tr>
<tr>
<td>$10^{-7}$</td>
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<tr>
<td>$10^{-8}$</td>
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<td>2</td>
</tr>
<tr>
<td>$10^{-9}$</td>
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<td>0</td>
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<table>
<thead>
<tr>
<th>Phenylephrine</th>
<th>Trials</th>
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</tr>
</thead>
<tbody>
<tr>
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<td>Change</td>
</tr>
<tr>
<td>$10^{-3}$</td>
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<td>0</td>
</tr>
<tr>
<td>$10^{-4}$</td>
<td>5</td>
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<td>$10^{-7}$</td>
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<td>0</td>
</tr>
<tr>
<td>$10^{-8}$</td>
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<table>
<thead>
<tr>
<th>Propranolol</th>
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<tbody>
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<td>m</td>
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</tr>
<tr>
<td>$10^{-5}$</td>
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</tr>
<tr>
<td>$10^{-6}$</td>
<td>2</td>
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<tr>
<td>$10^{-8}$</td>
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</table>
Table 4. The number of trials that exhibited a change or did not exhibit changes and the changes of perfusion pressure and weight observed during the perfusion of isoproterenol and vasopressin into livers from non-Nembutal-treated group B livers. Changes less than 0.5 cm of water or 0.05 g were not counted as changes.

<table>
<thead>
<tr>
<th>Isoproterenol</th>
<th>Trials</th>
<th>Changes Observed</th>
</tr>
</thead>
<tbody>
<tr>
<td>m</td>
<td>No Change</td>
<td>Change</td>
</tr>
<tr>
<td>$10^{-2}$</td>
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<td>5</td>
</tr>
<tr>
<td>$10^{-5}$</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

( ), #( ) Trials on the same day on consecutive livers.

<table>
<thead>
<tr>
<th>Vasopressin</th>
<th>Trials</th>
<th>Changes Observed</th>
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<tr>
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<tr>
<td>.01</td>
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</table>
Table 5. The number of trials that exhibited changes or did not exhibit changes and the changes of perfusion pressure and weight observed during the perfusion of histamine and vasopressin into livers from Nembutal-treated group B rats. Changes less than 0.5 cm of water or 0.05 g are not counted as changes.

### Histamine

<table>
<thead>
<tr>
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<th>Trials</th>
<th>Changes Observed (cm of water, g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No Changes</td>
<td>Change</td>
</tr>
<tr>
<td>$10^{-5}$</td>
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<tr>
<td>$5 \times 10^{-6}$</td>
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### Vasopressin

<table>
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</tr>
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<tr>
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</tr>
<tr>
<td>.5</td>
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<tr>
<td>.1</td>
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</table>
Table 6. The number of trials that exhibited changes or did not exhibit changes and the changes of perfusion pressure and weight observed during the perfusion of isoproterenol into livers from Nembutal-treated group B rats. Changes less than 0.5 cm of water or 0.05 g are not counted as changes.

<table>
<thead>
<tr>
<th>Isoproterenol</th>
<th>Trials</th>
<th>Changes Observed (cm of water, g)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>No Change</td>
<td>Change</td>
</tr>
<tr>
<td>$10^{-3}$</td>
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<td>2</td>
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<tr>
<td>$5 \times 10^{-4}$</td>
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</tr>
<tr>
<td>$10^{-4}$</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>$5 \times 10^{-5}$</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>$10^{-5}$</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

( )#, ( )# Trials on the same day on consecutive livers.
All livers blanched dramatically in the first few moments. Both the livers from Nembutal-treated and the non-Nembutal-treated rats had patches that did not disappear but partially blanched with perfusion and patches that disappeared during the course of the perfusion. The bubble-catcher decreased the pulse pressure without changing the average pressure as bubbles collected in it. It was also observed that as the volume of gas trapped increased the decrease in pulse pressure increased. The livers were typically perfused for approximately 10 minutes before the pressure and weight reached stable levels. The perfusion pressure dropped to stable value in all but three group A livers; and in these, pressure increased in one and remained stable in the other two. Pressure dropped to a stable value in all non-Nembutal-treated group B livers. Pressure dropped to a stable value in all but one Nembutal-treated group B liver, which had a rise in pressure to a stable value. Weight changes prior to a stable value varied; often weight leveled before the Dynograph was adjusted to its range of fluctuation. When it could be observed, weight usually decreased to a stable level. No greater variation in pressure and weight was observed during periods of drug infusion that produced no major changes than during periods of saline infusion or during the perfusion of saline control livers.
DISCUSSION

The experiments reported here offer few conclusive results; therefore they raise doubts about the adequacy of the methods to demonstrate the actions of the drugs and raise questions about possible variables and their importance. Some observations made during the experiments give rise to speculation about the mechanisms controlling the washout of blood from the sinusoids.

The action of the drugs tested on livers in groups A and B were infrequent, inconsistent, and rarely according to expectations. Differences in drug actions can be gleaned between the livers of group A and group B, and between Nembutal-treated and non-Nembutal-treated livers in group B. From these results, the conclusion can be drawn that the methods were not adequate to demonstrate the hepatic actions of the drugs.

Vasopressin produced only a few inconsistent responses (Tables 1, 4 and 5). Knowledge about vasopressin's actions in a rat liver was the primary goal of these investigations. Vasopressin was tested to determine if it possessed an intrahepatic action and to locate that action as pre- or post-sinusoidal if it did occur. During the group B trials, it was hoped that the opportunity would arise to determine if it antagonized the action of histamine. The work of Režabek (1967) suggests that vasopressin has the ability to antagonize the action of histamine.
Vasopressin is often used to treat variceal bleeding, and a decrease in portal pressure is believed to be an important element of its hemostatic action. An intrahepatic action could be important to vasopressin's portal hypotensive action, and little work has been done on this aspect of its action.

Previous work suggests that vasopressin decreases portal pressure by dilation of the HV-IVC sphincter; therefore, one would expect a decrease in pressure and a decrease in weight from drainage of fluid from the extracellular spaces. Only in the non-Nembutal-treated group B livers (Table 4) were responses to vasopressin observed, and only at a concentration of 1.0 unit/liter did a substantial number of changes occur. No pattern of change in the magnitude of responses in concert with change of concentration was observed. At a concentration of 1.0 unit/liter, only four of eight trials had any response to vasopressin infusion. Among these four trials only two had pressure changes and both increases and decreases of weight were observed among these four trials. In addition, only one change, a 0.15 g weight decrease, was greater than 1.0 cm of water or 1.0 g. Changes of 1.0 cm of water or 0.1 g are little greater than the observed variation, and they are suspect because they are small. Since the changes in response to vasopressin were few, of small magnitude, and of inconsistent type, one cannot conclude that vasopressin
does or does not have a hepatic action, much less speculate on its location or compare these responses to expectations. Isoproterenol, the beta-adrenergic agonist, produced the most responses of any drug tested, but its responses were often of small magnitude and inconsistent type. Isoproterenol was employed to provide a model of a decrease in post-sinusoidal resistance, and it was hoped to be able to experiment later with the role of beta-adrenergic action in comparison with the actions of vasopressin and histamine. Previous work suggests that isoproterenol decreases post-sinusoidal resistance by dilation of the HV-IVC sphincter and/or a generalized HV dilation; therefore, one would expect a decrease in perfusion pressure and a decrease in weight from drainage of the extracellular spaces. HV dilation could result in no change in weight if the effects of decreased capillary pressure were offset by increase in the volume of HV capacitance vessels. Among the group A livers, (Table 1) the one trial among the thirteen run in which isoproterenol elicited a response was run under conditions different than the other twelve. That no response was observed among these twelve trials argues against their methods as adequate to produce responses. The one successful trial resulted in a 36 cm of water decrease in pressure and a 0.2 g decrease in weight. The effect seems likely to be the result of isoproterenol because propranolol, a beta-adrenergic antagonist, abolished all of the pressure
decrease and 0.1 g of the weight decrease, because the size of the pressure decrease appears too large to be the result of chance, and because decreases in perfusion pressure and weight changes are consistent with expectations. Non-Nembutal-treated group B livers showed few pressure changes, only one of which was greater than 1.0 cm of water. A number of weight changes were greater than 0.1 g, but some were weight increases and some were weight decreases. No pattern of responses to increasing concentrations was observed and inconsistent pressure changes and conflicting weight changes were observed in response to similar concentrations on the same day in consecutive livers. Nembutal-treated group B livers (Table 6) showed consistent pressure changes that were small and contrary to expectations; few changes in weight that were substantial but of different types; no pattern of responses to increasing drug concentrations; and different pressure and weight changes in response to identical concentrations on the same day in consecutive livers. Nine of ten trials run at concentrations of $10^{-3}$ m and $10^{-4}$ m produced increases in pressure, but only three of the nine increases were greater than 1.0 cm of water and the attendant weight changes were few, substantial, and contradictory. The only consistent effect of isoproterenol in the group B series (Tables 4 and 6) was a small pressure increase at concentrations of $10^{-4}$ m and higher in the Nembutal-treated group B livers, and this is in direct
conflict with the large pressure decrease observed in response to $10^{-3}$ m isoproterenol in the group A series. On the basis of these results, the methods for groups A and B do not appear to be adequate to allow substantial, reproducible responses to isoproterenol. Also, because the results do not allow one to define a specific response to isoproterenol, it is not appropriate to judge the results on the basis of conformity to expectations.

Phenylephrine, an alpha-adrenergic agonist, did not consistently produce any changes, and the few changes it did create were inconsistent. Phenylephrine was used to provide a model of an increase in pre-sinusoidal resistance. Expectations for the action of phenylephrine arise indirectly from the results of work with epinephrine, nerve stimulation, and alpha-adrenergic antagonists. Increased perfusion pressure from HPV constriction was expected as was a decrease in weight from the decreased capillary pressure that followed decreased flow from constriction of the small HPV branches. Constriction of the HPV system might be expected to reroute flow into the larger channels of the HPV and the liver because of the constriction of small HPV branches. Only one of eight group A trials (Table 1) showed a response, a large increase of pressure and a moderate increase of weight. Propranolol, the beta-adrenergic antagonist, was scheduled for this trial; therefore, no data are available about the effect of
alpha-adrenergic blockade on this response. This response occurred at a $10^{-5}$ m concentration, a relatively high concentration, and the weight increase could have resulted from enough constriction of the HV capacitance musculature to influence post-sinusoidal resistance. Although one may suspect that this response was related to phenylephrine, the response from this lone group A liver must be considered suspect without the knowledge of its reaction to alpha-adrenergic blockade. Only non-Nembutal-treated group B (Table 3) livers received phenylephrine, and they showed only occasional responses. Of the two pressure increases, only one was greater than 1.0 cm of water. The two weight changes observed were substantial, but one was an increase and the other was a decrease; also, only one weight change occurred in a trial with a pressure change. The great number of trials without changes in both group A and the non-Nembutal-treated group B livers provide the strongest argument against phenylephrine as active under these conditions. These results make comparison with expectations an unrewarding task.

Infusion of histamine, a potentially important hepatic vasoactive autacoid, did not produce any dependable changes. The few pressure changes that were observed were small, and the weight changes were contrary to expected results. Histamine was used in the group B livers to provide a model of an increase in post-sinusoidal resistance.
and in the hope of conducting future experiments about the interactions among vasopressin, beta-adrenergic action, and histamine. Histamine was expected to increase perfusion pressure by constriction of the HV spiral musculature, possibly by a general HV system constriction, and by constriction of the HV-IVC sphincters. Weight was expected to increase as capillary pressure increased. Constriction of HV capacitance vessels might cause a weight decrease.

Trials in non-Nembutal-treated group B livers (Table 2) did not often result in changes. Only two of the four trials had pressure changes, and these changes were decreases of only 1.0ccm of water. All four trials had weight decreases, two of which were only 0.1 g. These few weight decreases, contrary to expectations, are puzzling because histamine-induced HV constriction is the best-documented action among the actions expected for all the drugs employed. Only one change was observed in response to histamine in all of the trials employing Nembutal-treated group B livers (Table 5) and it was small. One must conclude that histamine did not have any reproducible effects under these conditions.

Phentolamine, the alpha-adrenergic antagonist, was without effect in group A livers and created a few, conflicting responses in non-Nembutal-treated group B livers (Table 3). Phentolamine was used to provide competitive alpha-adrenergic blockade. It was expected to antagonize the action of phenylephrine and to mimic its actions in
high concentrations. It did not produce any responses in group A livers. It produced substantial, but inconsistent, changes of both perfusion pressure and weight in a few trials, and it produced no patterns of changes in responses to increasing concentrations. By virtue of its few effects and the inconsistency of the few changes that it did produce, one may conclude that it did not produce reproducible effects under these circumstances.

Propranolol, the beta-adrenergic antagonist, had an effect on one group A liver and was without effect on any of the non-Nembutal-treated group B livers tested (Table 2). Propranolol was used to provide competitive beta-adrenergic antagonism. One would expect it to antagonize any response to isoproterenol and high concentration to mimic isoproterenol actions. Because it did not elicit a response from group A livers when it was administered after other drugs and it did not elicit responses from non-Nembutal-treated group B livers, one may conclude that it was without effect under these circumstances. Its antagonism of isoproterenol in the final group A trial seems likely to be real and to result from the peculiar conditions of that one trial.

Differences between the responses of the group A and group B livers can be seen in the data as can differences between the non-Nembutal-treated and Nembutal-treated group B livers. Two differences are apparent between the group A series and the group B series. One
difference is the great lack of response in the group A livers compared to the numerous changes, though small and inconsistent, in the group B livers. The second difference is contradictory to the first difference and concerns the magnitude of the pressure changes observed in these two groups. The two pressure changes observed in the group A series were 26 cm and 36 cm of water and the changes in group B livers were never more than 5 cm of water. Histamine infusions offer differences between the Nembutal-treated and non-Nembutal-treated livers of the group B series. Four of thirteen trials in non-Nembutal-treated livers showed changes; although only one of nine trials in Nembutal-treated livers showed evidence of a change. Results from vasopressin infusions also provide a difference between the two group B series of experiments. Six of 24 trials with non-Nembutal-treated livers registered a change and none of the Nembutal-treated livers registered a change. The results from isoproterenol infusions provide three differences between the responses of non-Nembutal-treated and Nembutal-treated group B livers. First, pressure changes were consistent elements of the changes that occurred in trials with Nembutal-treated livers, although pressure changes were not consistent elements of the changes which occurred in trials with non-Nembutal-treated livers. All eight trials showing changes in Nembutal-treated livers had a pressure change. Only three of the eight trials showing change in
non-Nembutal-treated livers had pressure changes. Even when only the pressure changes greater than 1.0 cm of water are counted, the difference stands: four of four trials in Nembutal-treated livers and one of seven trials with non-Nembutal-treated livers. Second, weight changes were consistently an element of the changes in trials with non-Nembutal-treated livers, but not in trials with Nembutal-treated livers. All eight of the trials showing change in non-Nembutal-treated livers had a weight change. Only two of the eight trials showing change in the Nembutal-treated livers had a weight change. As with the pressure changes mentioned above, if only changes greater than 0.1 g are counted, the differences remain: seven of seven trials within non-Nembutal-treated livers and two of four trials in Nembutal-treated livers. Third, at a $10^{-3}$ m concentration of isoproterenol, both trials with Nembutal-treated livers showed a change, but only one of the five trials with non-Nembutal-treated livers showed a change. This observation could be questioned because the two changes observed in Nembutal-treated livers were the result of only 1.0 cm of water pressure increases.

One may conclude from this work and the work of others that the methods were not adequate to demonstrate the hepatic actions of the drugs tested. The results from trials with group A and group B livers are inconclusive. In all but the isoproterenol trials at concentrations
around $10^{-4}$ m in both categories of group B livers, very few livers responded to the drugs at all. In the case of these isoproterenol trials, the responses were contradictory and often of small magnitude. The trial of $10^{-3}$ m isoproterenol in a group A liver is a legitimate class of one because of its methods, and the inability to reproduce it is confusing. The one response to phenylephrine in group A livers adds to the confusion. Without knowledge of the reaction to alpha-adrenergic blockade, one must question the authenticity of this phenylephrine response, but the magnitude of the pressure change and its similarity to the isoproterenol response suggest it is real. The total record of inconsistent and contradictory responses to drugs and the observations by others that these drugs do have hepatic actions suggest that the methods were inadequate to allow the drugs to have an action.

After the addition of isoproterenol and propranolol at the end of the last group A trial produced such impressive results, a number of changes in the methods were instituted, and later, another change, Nembutal pretreatment was added. The protocol and apparatus for the group B series was designed to eliminate some variables thought to be important. The results from group B livers suggest that not all the important variables were considered. A number of variables are possibilities and several of these appear capable of interference.
A number of changes in the protocol and methods were instituted to control previously uncontrolled variables. The protocol for group B had saline warmed to 37° C in beakers in the water bath without drugs. Approximately 30 seconds before infusion, a small aliquot was removed from the 100 ml of saline and replaced by an equal amount of a concentrated drug solution. Because the aliquot of concentrated drug solution was typically one milliliter or less, several swirls of the flask after its addition could be expected to mix it and maintain the temperature of the 100 ml solution at 37° C; this minimized the time the drug spent in contact with the saline and the time it spent at 37° C. The hope was to minimize any deleterious effects from contact with the saline and to avoid the thermal degradation suggested by the results from the final trial of the group A series. Saline was prepared in three-liter batches as needed and rarely was stored for more than a day. The hope was to minimize any deleterious effects from bacterial contamination. The perfusion apparatus underwent modification. A bubble-catcher was installed close to the weighing tray to catch any bubbles which might appear during the time in the tubing and lodge in the liver. Some tubing was removed from the system, which shortened the transit time, in order to decrease the time the drugs spent at 37° C before they reached the liver. Also, the line to the manometer was eliminated. A shorter and smaller system
should dampen pressure fluctuations to a lesser extent, and although this had not seemed to be a problem, effort was made to decrease any effect further. Nembutal pretreatment was begun after a number of trials were run (the non-Nembutal-treated group B trials) in the hope that spinal cord, cerebral, and generalized trauma; the hypovolemic shock from cerebral, pleural, and occasional visceral hemorrhage; and ischemia for the period of surgery were important variables. Nembutal offers anesthesia; no need for concussion killing; and surgery on a live rat, which reduces the period of ischemia because HA perfusion could be maintained during most of the surgical period.

A conclusion that the drugs did not show reproducible effects because the methods were inadequate leads one to speculation about further variables. Likely variables may be grouped into three general categories: variables due to physical conditions, variables due to chemical interactions, and variables due to trauma and nervous system activity. It seems reasonable that different factors could be operating in different series of livers and that several factors could be at work at one time. From the list of likely variables, one may assemble two additional different, categories: those variables thought to be important and those variables not thought to be important.

Four ideas about physical conditions were considered to be important: leaks in the system, limitations of the
equipment, cannula placement, and drainage from the weighing tray. Leaks in the system were not observed, and checks were made throughout the periods of experimentation. Observations of control livers run with saline infusions, livers perfused at different flow rates, of group A and B perfusions, and of control studies done on the equipment suggest that the only limitation involved erratic periods of baseline drift the result of the Dynograph. The equipment was sensitive enough to pick up smaller changes of pressure and weight than were necessary. Variations during perfusion were within 0.5 cm of water and 0.05 g. Suspicions extend to those changes which were 1.0 cm of water or 0.1 g; this suspicion arises from the size of the changes, not observation of control studies. Although the angle of the cannula into the HPV sinus at the hilum of the liver was adjusted to minimize pressure, it is possible that it could have created artificially high pressures that could have masked pressure changes. It is possible that fluid pooled on the weighing tray, masking weight changes. Drainage from the weighing tray was checked often, and both the wire mesh and the hole in its center were observed to drip regularly once steady pressure and weight were established. Also, care was taken to see that the liver rested smoothly on the wire so pooling would be discouraged.

A number of sources are possible for chemicals which
could interfere with the drugs tested or the livers employed. Heparin and Nembutal could liberate chemical compounds, interfere with others, or act as chemical compounds themselves. The saline could have been contaminated with compounds from the glass jugs in which it was stored, and compounds could have been created and/or released by digestion, ischemia, anoxia, or the osmotic pressure created by perfusing with saline that does not have adjustments for plasma proteins. Heparin and Nembutal could act alone or react with each other or with effects from shock, trauma, or nerve stimulation, to interfere with drug action or hepatic blood flow by release of compounds, interference with other compounds, or on their own as compounds. Without a lengthy review of their known actions, a judgment on these actions is not meaningful. It is possible that the changes in the parenchymal cells induced by Nembutal (Harvey, 1975) could cause interference with or potentiate drug actions. Ions or compounds may have contaminated the saline as a result of water storage in glass jugs which were not made of chemical-resistant and heat-resistant glass or as a result of bacterial contamination of the saline. The primary change in the methods from group A to the non-Nembutal-treated group B livers was the elimination of the warming of drug solutions at 37°C for any great length of time prior to the beginning of perfusion. Warming the saline also could create compounds which could interfere,
especially if contaminants were present. It is by no means certain, because records were not kept, but one important element of the methods may have been the heating of saline in the drying oven. A trace of scum was often noticed floating at the top of the beaker, and one beakerful was discarded because of the amount of scum. The rats were not fasted before their death in hopes of maintaining an HPV large enough to cannulate. Compounds released from the digestive process and from blood loss due to concussion or surgery could have interfered with drug actions or acted on the liver. Ischemia, anoxia, and/or the osmotic pressure from a saline solution without allowances for plasma proteins could damage cells or cause the release of compounds which could interfere with drug action.

A number of sources of trauma to the liver and of nervous system activity were not well controlled. Although the perfusion fluids were kept at 37° C, fluids from all experiments spent approximately 40 seconds at room temperature in the tubing of the perfusion apparatus. A large enough drop in the temperature may have occurred in this 40 seconds to traumatize the liver and to decrease the activity of the drugs to a level below which they would have a discernible effect. Although no record was kept, the room temperature varied widely, from approximately 22° to 29° C. Concussion was not a precision task, and study was not done on the precise damage to the nervous system.
Damage may have varied, and certain kinds of spinal cord or brain damage may be able to influence liver flow and the release of compounds which could either interfere with drug action or hepatic flow. The concept of interference with hepatic blood flow from damage to the brain and spinal cord is supported by Wakim (1942). He found that the livers of living frogs and rats after pithing or CNS damage have a decreased number of active sinusoids as a result of constriction. Trauma to hepatic nerves from transection of the HA, HPV, HV, and the surrounding tissue would seem likely to be a fairly reproducible process because all the nerves are small, they must all be sectioned to remove the liver, and the sequence of operations in the surgery was very similar for each operation. Potential does exist for variation in the order in which nerves were transected and in the trauma from the forceps and clamps. Manipulation of the liver during surgery, on the dissecting tray, and on the weighing tray could have resulted in trauma to the liver that influenced its responsiveness to the drugs tested.

Swinging a rat by its tail may create nervous impulses that could be important. Rats killed by concussion lost variable amounts of blood; rats treated with Nembutal uniformly lost only a small amount of blood. The variation in blood loss in the rats killed by concussion provides the potential for hypovolemic shock and the nervous system activity associated with it. These responses to hypovolemic shock could
influence drug action and blood flow. The digestive process involves nervous system activity, and this could provide the variation in drug action or control of hepatic blood flow. Nembutal reversibly depresses the activity of all excitable tissues (Harvey, 1975); thus, Nembutal interference with nervous activity could interfere with normal integration of nervous impulses and thereby interfere with drug action or hepatic flow control.

Conclusions can be drawn about presumably important variables and presumably unimportant variables. Digestion, temperature, concussion and manipulation could be important variables. The limitations of the system, Nembutal potentiation of drug action, limitations of the saline for perfusion, anoxia, and temperature do not appear to have been important variable elements of the methods.

The effects of digestion, temperature, concussion, and manipulation appear to have been important, uncontrolled variables in the experiments reported here. A 24-hour fast prior to experimentation is a common element of many experimental methods. Because the digestive process releases many compounds, creates nervous activity, and because the liver is directly downstream from the stomach and intestines to receive the products of the digestive process, great potential exists for a variable influence on drug action and liver flow in all the trials. Temperature may be presumed to have been an important factor in the group A series.
The ten-minute warming of drug solutions at 37° C no doubt inactivated some of the drugs, and isoproterenol is a likely candidate on the basis of its known chemical liability and the results of the perfusion of chilled isoproterenol and propranolol in the last group A trial. The imprecision of concussion killing offers a great potential for interference in the group A and the non-Nembutal-treated group B series. Manipulation of the liver could have resulted in trauma to the liver which influenced its reactivity to the drugs employed. Although it is not certain that such handling would traumatize the liver, the fact that the liver was directly handled raises strong suspicions.

A number of variables do not appear to be important to the methods: limitations of the perfusion apparatus, Nembutal potentiation of drug effects, drawbacks from the composition of the saline solution, anoxia, and the temperature of the perfused liver. Observations throughout the course of the work with group A and group B livers determined that leaks were not a problem; that the pressure and weight transducers were sensitive enough to monitor changes smaller than required; that baseline drift by the Dynograph did not influence results; that the angle of the cannula in the HPV did not mask pressure changes, and it would not have masked weight changes regardless of its action on pressure changes; and that pooling of fluid on the liver or on the tray did not influence weight measurements. Work with both
Nembutal-treated and non-Nembutal-treated livers suggests that Nembutal did not potentiate the action of the drugs by binding with the cytochrome P-450 enzymes and blocking metabolism of the drugs. This idea would be more important if responses had been observed in the Nembutal-treated group B livers, but not in the non-Nembutal-treated group B livers. A perfusion solution was used without allowances for plasma proteins; the work of Pilkins (1969a and b) suggests that this was not a major problem, and the saline solution employed differed little from his. Anoxia from either ischemia or perfusion without erythrocytes did not appear to trouble the livers because the final liver of group A responded dramatically to isoproterenol and propranolol infusions after a surgical period that left the livers ischemic longer than the Nembutal-treated group B livers and a full trial period. Grana et al. (1968) studied the effects of ischemia on dog livers and suggested that anoxia causes the release of compounds which influence hepatic blood flow. Perhaps the last group A liver reacted because that long a time was required to wash out these anoxic metabolites, but this does not seem likely. The temperature of the liver subject to the variations of room temperature and presented with saline and drug solutions cooled by at least a 40-second transit time may have caused some important variation in response to drugs. Again, the observations of the last group A liver denies this. The
10^{-3} \text{ m isoproterenol and propranolol in this case were perfused immediately upon removal from the refrigerator and placement in the water bath. Lower temperatures typically decrease the actions of drugs; in this case, the coldest solutions had the greatest actions. Because of the action of the last group A liver, the decreased temperature of the liver and perfusate do not appear to have been important variables.}

Observations of the wash-out of blood from group A and group B livers raise the possibility that sphincter constriction and/or vasoconstriction resulted from both nerve stimulation and the release of autacoidal factors. That Nembutal-treated livers were better cleared of blood and their viscera appeared redder suggest vasoconstriction induced by concussion. To be sure, this effect could be caused by Nembutal-induced vasodilation. Also, one cannot rule out the possibilities that all the patches were the result of constriction mediated by nerve stimulation that gradually relaxed due to washout; however, nerve synapses are thought to be well-isolated and not easily subject to wash-out. Concussion would be expected to create similar conditions in any part of the liver affected; in the absence of local influences, one would expect a minimum of differently-behaving patches of tissue. The immediate clearing of the livers was, doubtless, the result of flow through open channels. Those patches which persisted until the end of
perfusion probably were caused by long-enduring, concussion-induced constriction. Patches which cleared during the course of perfusion probably were results of the action of autacoidal factors. The idea of Grana et al. (1968), that liver damage may result from stasis of flow caused by vaso-active compounds released during ischemia and anoxia, offers an explanation for the release of the autacoids that are hypothesized to produce patches that disappear during perfusion. The lightening of dark patches could result from washout of autacoidal effects produced by local release of factors and, possibly, from the beginning of the washout of the effects of nervous stimulation. It may take some time for flow through blocked sinusoids to washout autacoidal factors released originally and during the period of time until substantial flow is reestablished. A positive-feedback cycle is probably at work: Flow must be re-established to washout autacoidal factors, which in turn increase flow, which washes out more factors. Eventually some of the effects of nervous stimulation may be washed out too. The conjecture that the lightening of patches is the result of the wash-out of the effects of autacoidal factors and not the effects of nerve stimulation is supported by two ideas. First, lightening occurs over the same period of time as the disappearance of some other patches presumably the result of the action of autacoidal factors. Autacoids are blood-borne; thus, they are very
susceptible to wash-out. Second, neurotransmitters, which mediate the effects of nervous stimulation, are not blood-borne; therefore, they are not as susceptible to wash-out as autacoids. Furthermore, neurotransmitters and the entire synapse area are thought to be well-sequestered. The purpose of this sequestration is to minimize the amount of neurotransmitter lost with each firing of the nerve. With markedly decreased flow, due to the effects of nerve stimulation, the sequestration is even more pronounced. As a result, autacoidal factors should be washed out much more easily than the effects of nerve stimulation.

Autacoids are most likely to restrict drainage by the mechanism suggested by Elias and Popper (1955). Constriction of segments of the HV in response to autacoids would be expected to produce patches similar to the ones observed in perfused livers, and constriction of HV-IVC sphincters would be expected to darken entire lobes (darkened entire lobes were observed occasionally). In human livers, sinusoids only drain into the central veins, which drain into several layers of the HV; in rat livers, sinusoids drain into central veins and into several levels of the HV through channels similar to the ones described by Elias and Popper (1955). Because of this organization, HV constriction causes closure of both individual sinusoids and groups of sinusoids. The flow from other, open sinusoids through the constricted sublobular HV segments
constricts autacoidal-induced HV constriction by washing out autacoids. Eventually, autacoids would be washed away, the HV musculature would relax, and flow through the blocked sinusoids would be reestablished.

CONCLUSIONS

The methods employed by these experiments were not adequate to demonstrate hepatic actions of the drugs tested. The inadequacy of the methods employed very possibly may relate to the lack of a 24-hour fast for the rats prior to the use of their livers, warming of drug solutions in the water bath during the group A series, the imprecision of concussion killing, and manipulation of the liver. Limitations of the system, Nembutal potentiation of drug actions, limitations of the saline solution, hepatic anoxia, and the effects of variations of room temperature do not appear to have been important to the inadequacy of the methods.


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