ASCITES FORMATION AND REABSORPTION: A STUDY OF FLUID AND PROTEIN EXCHANGE IN THE PERITONEAL CAVITY OF ANESTHETIZED CATS

A thesis presented to

The University of Manitoba

submitted in partial fulfillment

of the requirements for

the Degree of Doctor of Philosophy

by

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TABLE OF CONTENTS

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PREFACE

Sections of this thesis related to the development of the servo-controlled plethysmograph were prepared in collaboration with Ned Mazerall, John Delaive and Gary Scott; each of whom were closely involved in some aspect of the construction of this device. For their most qualified assistance I am very grateful, since it has been with this instrument that much of the data in this thesis was obtained.

As a thesis supervisor, co-worker and friend my liaison with Clive Greenway has been wholly enjoyable. Certainly no apprentice could ask to learn from a more skillful and talented scientist; and no student could expect to be granted more favours, time and personal attention than I have received. It has been a good show, from the back room to the greenhouse.

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enjoyed the company of Deepak and Ratna Bose, Frank LaBella, Marge Turner and my comrades in the G.S.P.A.

Financial support from a University of Manitoba Fellowship is gratefully acknowledged.

This thesis is dedicated to my wife Cathy who has remained patient and loving despite intolerable circumstances; and to my parents who have been thanked so little for giving so much.

INDEX OF FIGURES

LIST OF TABLES

LIST OF PUBLICATIONS

1. Zink, J. and D. Bose. (1974). Cold potentiation of neuromuscular transmission in the avian biventer cervicis muscle. Euro. J. Pharmacol. 28: 149-156.

2. Zink, J., Sasyniuk, B.I. and P.E. Dresel. (1975). Halothane - epinephrine - induced cardiac arrhythmias and the role of heart rate. Anesthesiology 43: 548-555.

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6. Zink, J. and C.V. Greenway. (1977). Function of Intraperitoneal pressure in the formation and reabsorption of ascites in anesthetized cats. submitted to Am. J. Physiol.

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ABSTRACTS

1. Zink, J. and D. Bose. (1973). Cold potentiation of neuromuscular transmission in the avian biventer cervicis muscle. The Pharmacologist 15:222.

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ABSTRACT

Despite intensive research, the phenomenon of ascites in hepatic cirrhosis remains a complex problem with a etiology that is only poorly understood. Controversy related to the isolation of causal factors in ascites has persisted mainly because, in humans, this condition is associated with such a multiplicity of cardiovascular, hormonal and metabolic derangements. While many reviewers have proposed that cirrhotic ascites is the result of the combined effects of portal hypertension and hypoproteinemia which cause fluid transudation from the intestinal vascular bed, there are substantial reasons to believe that in fact ascites is formed primarily from the liver which spills excessive lymph into the peritoneal cavity as a result of intrahepatic vascular obstruction.

It has been the object of this thesis to investigate not only the possible origins of ascites but also to consider the processes responsible for ascites reabsorption from the peritoneal cavity. To this end, studies were conducted on anesthetized cats that were instrumented so that the hepatic venous pressure or portal pressure could be controlled, and the rate of ascites formation or reabsorption could be recorded by the technique of intraperitoneal plethysmography. The level of the intraperitoneal pressure was strictly controlled using a servo-operated plethysmograph.

These studies have revealed that the level of the intraperitoneal pressure is a critical factor controlling the rate of fluid absorption from the peritoneal cavity. However, fluid is absorbed at a constant rate regardless of the concentration of protein in the intraperitoneal fluid; and diuretic agents, which promote the remission of cirrhotic ascites, do not

alter the rate of intraperitoneal fluid absorption under these conditions. The process responsible for fluid absorption appears to remove an iso-oncotic fluid since over a 5 hour period of absorption the protein concentration remained unchanged in the unabsorbed fluid. Together, these findings support the concept that fluid is removed from the peritoneal cavity by absorption into lymphatics which are perhaps located on the peritoneal surface of the diaphragm.

Further studies have shown that the intraperitoneal pressure also affects the rate of ascites formation. Elevation of the combined hepatic venous pressure and portal pressure produced a constant rate of ascites formation which was proportional to the hepatic venous pressure. However, raising only the portal pressure to equivalent levels was not associated with a noticable formation of ascites. From this data, and from previous studies in this laboratory of the in situ liver, it would appear that ascites is more likely to originate from congestion of the hepatic vascular bed than from congestion of the intestine. Additional studies have shown that the rate of ascites formation produced by an elevated hepatic venous pressure is affected by the intraperitoneal pressure in two ways. On the one hand, elevation of the intraperitoneal pressure retards the formation of ascites by reducing the trans hepatic pressure gradient responsible for filtration in the hepatic vascular bed. On the other hand a secondary effect, which may involve obstruction of the hepatic lymph drainage en route through the peritoneal cavity, causes the rate of formation to exceed that which would be expected for a given trans-sinusoidal pressure gradient.

The results obtained in this study suggest that the pathogenesis

of ascites is most properly viewed as a dynamic balance of factors that affect both the rate of formation and the rate of ascites reabsorption from the peritoneal cavity. In this context the intraperitoneal pressure and the rate of intraperitoneal fluid absorption may be much more significant factors in the control of ascites than has been previously recognized. In the early stages of ascites, when the ascitic volume is small, the rate of intraperitoneal fluid absorption is probably somewhat less than the rate of formation. However as ascites progresses and the ascitic volume increases, the intraperitoneal pressure is raised to an extent which is governed by the compliance of the abdominal cavity. This in turn may operate as a feedback mechanism to accelerate the process of ascites reabsorption and perhaps decrease the rate of formation so that the rates of both processes are balanced.

1. The Problem of Ascites

Ascites ("askos", Greek - wineskin or bag) refers to an excessive accumulation of serous fluid within the peritoneal cavity and this condition was labelled as "abdominal dropsy" in nineteenth century medicine. It is now recognized that hepatic circulatory congestion is but one of several conditions that cause ascites.

Certain diagnostic criteria necessary to differentiate this condition from other abdominal disorders were established by Murchison in 1885 (quoted by Summerskill, 1969) who listed progressive enlargement of the abdomen, eversion or hernia of the umbilicus, exertional dyspnea and thoracic breathing. He stressed the importance of percussing for dullness over the flanks with the patient in a supine position and noted that assuming a lateral position was associated with a shift of dullness in response to gravity.

At present, most clinicians can detect ascitic fluid volumes exceeding 1.5 litres, although as little as 120 ml can be identified from roentgenograms (Lawson and Weissbein, 1959). Rarely, but particularly in obese patients, aspiration of the abdomen is considered necessary to establish the diagnosis. Additional findings may include incisional, inguinal or femoral herniae, scrotal edema and the development of pale abdominal striae.

Although ascites may occur in any liver disease, it is the commonest major complication of cirrhosis and suggests a poor prognosis (Ratnoff and Patek, 1942; Keefer and Wilkins, 1970). It is not in itself a critical problem, but many serious complications of cirrhosis are intricately rooted in ascites. In addition, ascites is often indirectly responsible for considerable iatrogenic morbidity since it poses an almost

irresistible challenge to the physician to relieve it. Unfortunately this is often antagonistic to other therapeutic aims and may result in azotemia, hypokalemia and hepatic encephalopathy.

The accumulation of ascitic fluid in excess of 30 litres (Summerskill, 1969) can cause the intraperitoneal pressure to be substantially increased. Although the significance of this has been largely ignored, it is known that anorexia and gaseous distention of the abdomen may result from mechanical interferences with gastrointestinal function. In addition, ascites impairs the function of the cardioesophageal junction by distorting gastroesophageal manometric relationships and permitting gastroesophageal reflux. This may participate in variceal erosion (Simpson and Conn, 1968) which is distinctly uncommon in patients without ascites (Conn, 1972). The development of varices and caval collateral vessels may also be accelerated by elevation of portal and inferior vena cava pressures as a consequence of the increased intraperitoneal pressure in ascites.

Elevation of pressure in the inferior vena cava, which is only partly due to a raised intraperitoneal pressure (Lawrence and Myerson, 1973; Mullane and Gliedman, 1970), is the probable reason for claudication and leg pain in patients with ascites. Together with hypoalbuminemia, the increased venous pressure also contributes to the co-existence of dependent edema which is frequent in these cases.

Although the significance of ascites in the controversial "hepatorenal syndrome" remains somewhat vague, the elevation of intraperitoneal pressure may be detrimental to renal function by increasing ureteral pressure. It has also been suggested that elevation of the caval

pressure imposes an extra load on the renal circulation (Hwang et al., 1950; Conn, 1972). In any case, it is noteworthy that the "hepatorenal syndrome" occurs almost exclusively in cirrhotic patients and rarely, if ever, in the absence of ascites (Summerskill, 1966).

The lymphatic circulation is also disrupted in ascites. It is well established that the diameter of thoracic lymph duct increases several fold to accommodate the marked increase in lymphatic flow (vander Heyde et al., 1964; Zemel and Glutelius, 1965; Dumont, 1975). Possibly this compromises the bacteria-filtering function of the lymphatic system since spontaneous bacterial peritonitis is an additional complication of ascitic patients (Conn and Fessel, 1971).

Elevation of the diaphragm by ascites rotates the heart in the thoracic cavity and raises the intrapleural pressure. This in turn leads to compensatory rise of filling pressure in the right heart with engorgement of the neck veins (Sherlock, 1965) and deleterious effects on cardiac function (Guazzi et al., 1975). Pleural effusions on the right side of the pleural cavity are frequent in ascites (Johnston and Loo, 1964) and may further compromise pulmonary function since hypoxemia, hypocarbia, pneumonia and atelectasis are characteristic of advanced cirrhosis with ascites (Conn, 1972).

Thus it is apparent that the occurrence of ascites is by no means a minor complication of cirrhosis. Rather, it is a condition that should not be ignored, but calls for rational medical management since it is not invariably associated with dire consequences.

I Pre-Galenic History

The respectability of medical knowledge in antiquity is perhaps not surprising when one considers that the earliest recorded description of the splanchnic circulation dates back 30,000 years. Though admittedly crude, there exists a Paleolithic painting in a cave at Lascaux, France which depicts the evisceration of a bison by a spear. Included in the portrayal of the abdominal viscera is a careful representation of the splanchnic vasculature painted in red ochre (Fishman and Richards, 1964). While it is considered that the artist must have engaged several hours in patient dissection, this is a modest effort relative to the undertakings of physicians in ancient Egypt, Assyria and Greece[1].

The papyrus Ebers, dated to the reign of Amenhotep I in 1552 B.C., enumerates in remarkable detail the blood vessels of the human body and contains the earlist known conceptualization of splanchnic vascular dynamics (Joachim, 1890). Essentially an Egyptian medical text, it lists innumerable maladies, surgical procedures and medical treatments -- including the transcription of a prescription written for King Usaphais in 3700 B.C. Of the splanchnic circulation, it is written that:

> "There are four vessels to the liver; it is they which give it humor and air, which afterward cause all disease to arise in it by overfilling with blood"
>
> (Fishman and Richards, 1964)

[1]Mention of African, Indian and Oriental medical practice is omitted but may be obtained from C.C. Mettler's text "History of Medicine" (1947).

2. History of Liver Disease, Ascites and the Splanchnic Circulation

While amiss in some respects it is impressive that Egyptian physicians considered the heart as the seat of the circulation. This belief was contrary to the hepato-centric dogma of other civilizations, yet vanished with the decline of Egypt so that, until the work of William Harvey in 1640, the liver was considered to be the source of blood and the central organ of the cardiovascular system.

While it is scarcely possible to communicate the quality of information in the Papyrus Ebers, it is sufficient to note that the caliber of medical practice in ancient Egypt is surpassed only by the advent of twentieth century medicine. Containing descriptions of most currently known medical ailments, the Papyrus Ebers also dwells on the importance of history taking and physical examinations. For the symptoms of angina pectoris, "pains in his arms, in his breast and in one side of his cardia", squill glycosides are prescribed since "it is death that threatens him" (Sigerist, 1967). Of the numerous pathologies, also listed are "hardening of liver", "water of the liver" as well as various cysts and tumors affecting this organ. It is not unlikely that the first listed condition refers to hepatic cirrhosis since examination of the preserved viscera from Egyptian mummies has revealed the presence of this disease (Mettler, 1947). Considering the caliber of their science and the exceptional rationality of their medical practice, it is not surprising that, for over 2500 years, Egyptian physicians were the most respected practioners in the Mediterranean area. Yet it is rather humbling to see how slowly anatomical and medical knowledge advanced beyond this beginning.

Relative to Egypt, Babylo-Assyrian medicine was quite primitive. Anatomical knowledge was limited and for the most part medical treatment

relied on incantations and divination. The Babylonians made no distinction between arteries and veins and considered the liver to be the source of blood and its circulation. On the other hand, their knowledge of liver anatomy was extremely accurate because they practiced hepatoscopy to receive omens from the gods and to ascertain the nature of most diseases. In earlier times this was performed by laparotomy of the diseased person but later the technique was refined to allow examination of a sheep's liver after the patient had breathed into the animal prior to its sacrifice (Garrison, 1929). Extensive texts were written and utilized to interpret the liver structure and its anomalies. Clay models of the liver have survived from Babylonian times and are better specimens of anatomical illustration than five-lobed configurations that were common in medieval Europe.

In spite of reliance on magic, the medical practice of the Assyrians was quite advanced in other respects. Having knowledge of hundreds of diseases, they utilized a pharmacopeia of several hundred organic and inorganic drugs. In the arena of public hygiene they identified the transmissibility of leprosy and arranged for the strict control of sewage by a system of stone drains (Garrison, 1929). But perhaps of most significance is their control of malpractice by physicians. For centuries this was stricly governed according to the code of Hammurabi (2250 B.C.). In part this decreed that:

> "If a physician has destroyed the eye of a patrician, his own eye shall be destroyed. If he has destroyed the eye of a man's slave, or broken the bone of a man's slave, he shall pay half his value"
>
> (Clendening, 1942)

While the development of Greek medicine led to many advances in the medical sciences, the theories of Erasistratus (327 B.C.) were remarkable for their insight of cardiovascular physiology. Considered to be the first experimental physiologist (Garrison, 1929), Erasistratus described the structure and function of the aortic and pulmonary valves, the cordae tendineae, the aorta and vena cavae. Having conducted detailed analyses of the cardiac cycle he saw the heart clearly as a pump and was close upon the mystery of the circulation, but conceived that the arteries normally contained air to exclude blood. Supposedly, with hemorrhage it was the escape of air that allowed blood to spurt from the arteries. In his study of the circulation he was restricted by hepato-centric dogma and proposed that intestinal peristalsis was a substantial force propelling blood flow into the liver (Mettler, 1947). While he could not identify capillaries he reasoned that they must exist to connect arterial and venous vessels (Garrison, 1929).

In addition to his studies of digestion, metabolism and neural function, Erasistratus described the condition of ascites and postulated that a form of hepatic vascular obstruction was responsible. Thus he explained that the condition was caused by "supposing that blood is prevented from going forward owing to the narrowness of the passages" (Fishman and Richards, 1964). Although he was obviously quite close to establishing a concept of vascular resistance, he paused at the brink of further discovery. Many years later his work inspired the Roman physician Galen (130 - 200 A.D.); though in Greece, his teachings were ignored or held in disrepute. This may have been partly due to his practice of human vivisection (Garrison, 1929).

To say the least, Aristotle and Hippocrates made important contributions to Greek medicine. However, knowledge of circulatory anatomy and physiology was plagued by the belief that "pneuma" or "spirits" were sent forth in the blood vessels to animate the rest of the body. These concepts were further elaborated by Plato who acknowledged the presence of the four basic Elements in the splanchnic vascular bed: air as intestinal gas, fire as body heat, water as the luminal fluids and earth as fecal matter. The animating spirits and the element "air" were vaguely interconnected. Although some believed that air moved into the body through the skin and lungs causing a to and fro tidal movement of the blood, Aristotle disagreed and apparently believed that blood flow irrigated the tissues with "pneumae" (Fishman and Richards, 1964).

In Grecian times physical examination of a patient did not include systematic and exploratory percussion but is interesting that examination of the abdomen was considered more vital than percussion of the chest. Thus, in a thesis on the subject of dropsy, Aretaeus, a disciple of Hippocrates wrote:

> "The symptoms are very great and very easy to see, to touch and to hear; in Ascites for example, to see the tumidity of the abdomen, and the swelling about the feet; the face, the arms and other parts are slender, but the scrotum and prepuce swell and the whole member becomes crooked from the inequality of the swelling. To touch:- by strongly applying the hand and compressing the lower belly; for the fluid will pass to other parts. But when the patient turns to this side or that, the

> the fluid occasions swelling and fluctuation, the sound of which may be heard"
>
> (Mettler, 1947)

Achieving a differential diagnosis of ascites rather than "Tympanites" for example, Aretaeus then prescribed either wormwood or gentian, or both, for treatment of the condition.

Of Roman medicine, the teachings of Galen (130 - 200 A.D.) are most remarkable, and certainly the most voluminous. An exhaustive writer, he is said to be the fountainhead of ready-made theory, having an answer for every problem and a reason for every phenomenon (Garrison, 1929). Educated in Greek medicine, he travelled to Rome at the age of 31 and soon developed an enormous medical practice including service to the Emperor Marcus Aurelius. In spite of his cocksure attitude he was a highly qualified physician and an excellent deductive scientist. Though often disfavoured by his peers in Rome, his influence there was great; and is surpassed only by the awesome importance of Galenic dogma in medieval medicine. For almost 1400 years virtually every facet of European medicine was referred back to Galen as a final authority, from whom there could be no appeal. While his contribution to medicine was highly creditable, it was not infrequent that his imagination extended to extremes, with the result that some of his ideas impeded the progress of medical science in later years. Yet this rigidity would certainly have been distasteful to Galen himself since he rejected some or all of the components of every dogma that existed in his time.

Though it is scarcely possible to list his diverse achievements and studies in medicine, Galen's theories of blood circulation were of considerable significance. His appreciation of the nature of the

arterial pulse was entirely accurate and limited only by the technological development of his day. He described in much detail the significance of arterial dimensions, pulse force and its delay. Also, he recognized arrhythmias of the heart from the irregularity of peripheral arterial pulsations. In one treatise on the subject of the pulse, he wrote:

> "The heart and all arteries pulsate with the same rhythm, so that from one you can judge all .... Now you must remember what a normal pulse is like, and if you find an abnormal pulse of excessive breadth, you should term it 'broad' and if of excessive length 'long' and if of excessive depth 'deep' .... By regularity is meant an even and unbroken series. For example, when the dimensions of a series of pulsations continues the same, the pulse would be termed regular in size. Irregularity means the destruction of even rhythm in whatever varities of pulse it occurs .... For there may be three regular beats, then the fourth irregular, and so on continuously"
>
> (Clendening, 1942)

In similar detail Galen also described the contractility of the heart and the circulation of the blood through the cardiac chambers. However, it is perhaps due to excessive reliance on the observations from primitive species that he assumed that a substantial fraction of the right ventricular output passed directly to the left heart via a sort of persistent foramen ovale (Mettler, 1947).

Although Galen was quite interested in pneumae of all sorts, he correctly disproved the tenet of Erasistratus that the arteries contained air rather than blood (Fishman and Richards, 1964). On the other hand, he erred considerably in his disagreement with Erasistratus on the nature of the hepatic circulation. He identified the hepatic lobular configuration, noting the central vein and its connection to the vena cava. Similarly, at the periphery he observed that "the artery and biliary vessels are clearly seen beside the portal vein". Yet he could not accept the concept that blood flowed through smaller vessels across the parenchyma; and in fact this obstinacy persisted until the work of Glisson, 1400 years later (Fishman and Richards, 1964). Thus while he had located and studied almost all of the splanchnic arteries and veins, he objected to Erasistratus' explanation of ascites as a phenomenon caused by restriction to the free inflow of portal blood. It seemed obvious to Galen that the "attractive faculty" of blood could easily overcome obstacles and, indeed there should be no reason that portal venous flow should always pass from the intestine to the liver, and not on occasion in the opposite direction. Thus he claimed that:

> "when there is an abundance of nutriment contained in the food canal it is carried up to the liver by the veins, and when the canal is empty and in need of nutriment this is again attracted from the liver by the same veins. Thus the stronger draws and the weaker is evacuated"
>
> (Fishman and Richards, 1964)

II Post-Galenic History

From Galen to the time of William Harvey (1578 - 1657), classical knowledge of anatomy and physiology declined in western Europe, although a primitive form lingered on in the Byzantine and Moslem schools. Monastic clerics slavishly copied and recopied Greco-Roman medical texts but such information was utilized only in the anatomical diagrams of monastic leech books.

During the fifteenth and sixteenth centuries, Leonardo da Vinci and Andreas Vesalius emerged as anatomists of distinction but were so greatly influenced by Galenic dogma, that their drawings clearly reflect Galen's concept of the splanchnic circulation rather than their own observations (Fishman and Richards, 1964). Nevertheless, their figures were considerably more accurate and showed an understanding of the circulation much in advance of Galen's. The Renaissance of cardiovascular physiology began in 1640 with the publication of De Motu Cordis by William Harvey. Having studied Galen's physiology, Versalius' anatomy and Galileo's experimental method, Harvey critically examined the entire known history of medical physiology and noted the inadequacies of existing theories. Then, by experimental vivisection, ligation and perfusion, he proceeded to an inductive proof that the heart operates as a muscular pump, propelling blood throughout the body in a continuous circulation. Thus he stated that:

> "Just as the king has the first and highest authority in the state, so the heart governs the whole body"
>
> (Clendening, 1942)

Consequently the liver was dethroned as seat of the cardiovascular system, though it had been considered so for millennia. Yet the significance of Harvey's accomplishments is not so much for proof of the circulation of blood, but rather for the quantitative and mathematical measurement of cardiovascular parameters and the application of these measurements in a deductive proof of this theory.

The crux of his argument was that the actual quantity and velocity of the blood made if physically impossible to do otherwise than return to the heart by the venous route (Harvey; translated 1928). He explains that:

> "The blood is driven round a circuit with an unceasing circular sort of movement. In the mesentery the blood enters through the coeliac and superior and inferior mesenteric arteries and proceeds to the intestines; from these together with the chyle which has been drawn into the veins, it returns through the very numerous branches of those veins into the porta hepatis, and through the liver itself into the vena cava"

The reaction to Harvey's theories was at first a shocked and violent opposition but this soon was quenched by a tide of confirmatory publications. In the year following publication of De Motu Cordis, Gasparo Aselli confirmed and extended Erasistratus' observations on the mesenteric lacteals -- thus providing a means for chyle to be conducted directly to the liver from the intestine. This, however, was soon disproved by Pecquet's description of the thoracic duct and its entry into the subclavian veins (Foster, 1924).

Pecquet's studies of lymph were quickly extended by the research of other lymphologists. These included Francis Glisson who in 1654 published his classic treatise on the liver. This conclusively proved Harvey's claim that blood must flow from the intestine through the liver and to the heart. He demonstrated this by injecting "warm water, slightly coloured with milk" into the portal vein of a human cadaver and found that the liver became pale and no longer sanguinous in appearance. While he believed that the liver might operate as a gland, he considered that it was bypassed by the intestinal lymphatics which, like other such vessels throughout the body, served to return lymph to the blood vessels after it had lubricated the body cavities (Eales, 1974).

To add to this, Thomas Bartholin (1616 - 1681) noted that lymph flowed from the liver rather than into it. This he showed by examination of the structure of the lymphatic valves and by causing engorgement of the hepatic lymph vessels on the hepatic side of a peripherally placed ligature (Eales, 1974). In 1669, Richard Lower of London developed the first experimental model of ascites by partial ligation of the inferior vena cava in a dog. In this animal he observed hepatic congestion, splenomegaly and marked distention of the liver lymph vessels (McDermott and Brown, 1964). The subsequent work of Joyliffe, Munro, Hunter, Hewson and Ludwig provided the final evidence necessary to establish the functional significance of the lymphatic system.

Glisson's research on the distribution of the intrahepatic blood vessels, the nature of bile and the "continuous tunic supporting and protecting the liver" added substantially to Harvey's findings and was in itself a valuable advance in the understanding of the splanchnic circulation. Although neither Harvey nor Glisson were wholly free of the

influence of Galenic tradition, their work initiated a chain reaction amongst their contemporaries bringing explosive progress in the study of virtually all medical sciences.

With the development of microscopy, Malpighi was able to substantiate by direct observation, Glisson's belief that the portal and hepatic veins were connected by a capillary system. This was further advanced in 1830 by Muller and in 1833 by Kiernan, both of whom published detailed monographs on the anatomy of the liver. Slowly the lobular architecture of the liver became established, and with development of the cell theory by Schleider and Schwann, the hepatic parenchymal cells were recognized as lying in columns along the hepatic sinusoids (Garrison, 1929).

The understanding of liver disease progressed substantially slower during this period. While da Vinci had briefly reported on the autopsy findings in a case of hepatic cirrhosis, the first detailed description of this disease is credited to the pathologist Morgagni (1687 - 1771) (Garrison, 1929). From the examination of several autopsy specimens, Morgagni noted "acute yellow atrophy of the liver", rearrangement of the septae within the liver and the coincident symptoms of jaundice, ascites and encephalopathy in the victims (Clendening, 1942).

In 1826, Laennec described a less severe form of hepatic cirrhosis which now either bears his name or is termed anything from "post-necrotic cirrhosis" to "chronic diffuse interstitial hepatitis" (Galambos, 1975). Also, in 1881, the British epidemiologist William Budd contributed to liver pathology by describing a form of atypical cirrhosis (without jaundice) due to auto-intoxication, a syndrome which now bears his name (Garrison, 1929). However, cardiovascular changes in cirrhosis were

largely ignored until the studies of experimental bilary cirrhosis by Hetoen in 1901. His documentation of portal hypertension and porto-caval shunts was supported by the earlier work of Eck (Child, 1953), who in 1877, had shown that ligation of the portal vein would not cause death if a porto-caval shunt was constructed to prevent large volumes of blood from being trapped in the intestinal vascular bed.

Throughout the Middle Ages and Renaissance periods, and indeed until quite recently, the complication of ascites was routinely treated by paracentesis. In accordance with the dubious advice of Dionis (1718), the fluid was best evacuated rapidly with a trocar, provided that a tight abdominal binder was subsequently employed (Mettler, 1947).

For many years after Harvey and Glisson, concepts of splanchnic circulatory physiology were complicated by the proposition (of Glisson) that the "irritabilia" of blood vessels aided the force of cardiac contractility in propelling blood along its course throughout the body. This idea had important proponents including Stephen Hales, who was the first physiologist to study the rate of blood flow in a quantitative sense, and William Cullen, who suggested that "the muscular fibers of the arteries become more irritable as the arteries are more distant from the heart" (Fishman and Richards, 1964).

However, the concept of an arterial pump was eventually disproved, in 1832, by Poiseuille's elegant experiments in which the flow through mesenteric capillaries of frogs, mice and horses was shown to depend exclusively upon arterial inflow and pressure (Fishman and Richards, 1964). Utilizing Newton's laws of motion, Poiseuille went on to formulate the relationships between the volume of flow, viscosity of fluid, dimensions of the conduit, pressure gradient and resistance to blood flow (Garrison,

1929). Yet his work was in part rejected by Claude Bernard who in 1859 claimed that:

> "The hepatic veins in contracting down, squeeze the liver, as it were, like a sponge and the blood, finding an outlet in the direction of the inferior vena cava, is expelled into this vessel which carries it to the heart"
>
> (Holmes, 1974).

Further progression in the study of the splanchnic circulation was made possible by the invention of the "stomuhr" which was elegantly utilized by Burton-Opitz to quantitatively investigate flows within the splanchnic vascular bed. His works, published from 1908 to 1914, examined virtually every aspect of flow through splanchnic vessels. The measurements of resting hepatic arterial and portal venous flows (Burton-Opitz, 1910; 1911a) are considered quite accurate though perhaps slightly underestimated (Fishman and Richards, 1964). In addition, he also examined the effects of raised bile duct pressure, the reciprocity of portal and arterial inflows, hepatic and splanchnic nerve stimulation and infused adrenaline (Burton-Opitz, 1910;1911a,b;1912).

Studies of hepatic blood volume awaited development of the plethysmograph. This was graciously provided in 1873, by the genius of Carl Ludwig after he had tired of his earlier inventions, the kymograph, the stromuhr, and the blood perfusion pump (Garrison, 1929). Ludwig's plethysmograph was utilized by Mosso, one of almost 200 scientists trained by Ludwig, to examine the volume changes of isolated livers perfused with serum. However the preparation yielded rather erratic results and was only marginally im-

proved when used by Lampe and Mehes (1926) and Bauer et al. (1932) to support their proposition that a mechanism existed in the hepatic veins to control venous outflow and liver blood volume. From experiments in dogs, they showed that a sphincter mechanism could be activated by histamine, anaphylaxis and "peptone", but was released by adrenaline or low grade stimulation of the sympathetic nerves.

Further studies of splanchnic blood volume developed from Malpighi's concept of the blood reservoir function of the spleen and liver so that by 1912 August Krogh had outlined the role of the splanchnic veins as an important blood reservoir. Together with Barcroft (1925), Krogh showed that the volume of blood held in the spleen, liver, pancreas and gastrointestinal vessels was greatly increased after denervation but reduced by stimulation of the splanchnic nerves or by the infusion of pressor amines -- effects which they considered relevant to the study of hemorrhage and exercise.

While it is apparent that the development of knowledge of splanchnic cardiovascular function has emerged at a remarkably slow rate over the centuries, it is equally apparent that the greatest obstacle to progress has been the entrenchment of established dogmas. Thus in the twentieth century, the most valuable asset of the investigator should continue to be a critical and curious, but rational attitude in the affairs of science.

3. Literature Survey

I Transcapillary Exchange in the Splanchnic Vascular Bed

The first formulation of a definite capillary filtration theory was put forth by Carl Ludwig in 1861. This proposed that a filtration force, determined by capillary hydrostatic pressure, caused the components of blood to equilibrate with the tissue fluids. However, Ludwig presumed that the fluid filtered into the tissue spaces was returned to the circulation solely by lymphatic transport and it soon became evident that a unidirectional hypothesis for transcapillary movement could not adequately explain either the control of lymph flow or the regulation of the constancy of blood volume (Landis and Pappenheimer, 1963).

In 1896, Starling added absorption to Ludwig's conception of capillary filtration. Measuring the oncotic pressure of the plasma proteins with a crude osmometer, he theorized that a phenomen similar to gel imbibition might participate in the control of fluid transfer. From these calculations he concluded that:

> "although the osmotic pressure of the proteids of the plasma is so insignificant, it is of an order of magnitude comparable to that of the capillary pressures; and whereas capillary pressure determines transudation, the oncotic pressure of the proteids of the serum determines absorption"
>
> (Starling, 1896a)

This interpretation of the forces responsible for transcapillary diffusion and fluid transfer has served as a framework in all subsequent investigations of microcirculatory exchange. The same fundamental principles are now also recognized to control the rate of interstitial fluid transfer into

the terminal lymphatics (Mayerson, 1963; Leak, 1976; Guyton et al. 1976). Thus the regulation of interstitial fluid volume and content emerges as a system controlled by the balance of net fluid and protein transfer at the capillary and lymph interfaces. Expressed in mathematical terms:

where Jv,c is the net volume flow from the capillary into the tissue,

and $Jv,_L$ is the net volume flow filling the initial lymphatic,

then each is controlled by,

$$Jv,c = Kf,c\ (\Delta Pc - \sigma c\ \Delta \pi c)\ ml \cdot min^{-1} \cdot 100\ g^{-1} \text{ of tissue}$$

and $$Jv,_L = Kf,_L\ (\Delta P_L - \sigma_L\ \Delta \pi_L)\ ml \cdot min^{-1} \cdot 100\ g^{-1}$$

Also, where V_T is the interstitial fluid volume (ml/100 g) then,

$$dV_T / dt = Jv,c - Jv,_L \quad ml \cdot min^{-1} \cdot 100\ g^{-1}$$

where Δ Pc is the capillary pressure (Pc) minus the interstitial fluid pressure (P_T) (mmHg)

σc is the reflection coefficient of plasma protein for the capillary membrane such that it is unity if the membrane is impermeable to protein, and zero if there is no restriction to the passage of protein across the membrane

$\Delta \pi c$ is the oncotic pressure of plasma (πp) minus the oncotic pressure of the tissue fluid (π_T) (mmHg)

Kf,c is the filtration coefficient of the capillary membrane ($ml \cdot min^{-1} \cdot mmHg^{-1} \cdot 100\ g^{-1}$) (also termed CFC, see Mellander and Johansson, 1968)

Similarly at the lymphatic interface:

ΔP_L is the interstitial fluid pressure (P_T) minus the lymphatic hydrostatic pressure (P_L)

σ_L is the reflection coefficient of the initial lymphatic wall

$\Delta\pi_L$ is the oncotic pressure of the tissue (π_T) minus the oncotic pressure of the fluid within the initial lymphatic (π_L) (mmHg)

$Kf,_L$ is the filtration coefficient of the initial lymphatics during the filling phase ($ml \cdot min^{-1} \cdot mmHg^{-1} \cdot 100\ g^{-1}$)

To appreciate the whole spectrum of interactive factors which contribute to the pathogenesis of edematous conditions such as ascites, it is necessary to first consider the nature of transcapillary fluid exchange in the splanchnic vascular bed under physiological conditions. The intestinal microcirculation is discussed first simply because more information is available for this tissue.

a) The Microcirculation in the Intestine

i) The Measurement of Capillary Filtration Coefficient

From in vivo measurements in dogs and cats, the capillary filtration coefficient of the intestine has been determined to be approximately 0.110 $ml \cdot min^{-1} \cdot mmHg^{-1} \cdot 100\ g^{-1}$ of tissues. Table 1. shows the range of values that have been reported and includes, for the purposes of comparison, the coefficients obtained in other tissues.

The filtration coefficient is a measure of the "hydrodynamic conductivity" of the microvasculature and is determined by capillary permeability and the perfused area available for diffusion (Folkow and Neil, 1971). It is usually recorded by plethysmographic or gravimetric methods that monitor the net gain of interstitial fluid volume that

occurs with a known (or calculated) increment of capillary pressure. However, formidable difficulties have been encoutered in the attempt to accurately calculate the filtration coefficient of whole organs since often the morphology of the vascular bed as a whole is not sufficiently uniform. It is obvious, for example, that in the intestine the functional design of the mucosal vasculature differs substantially from that of the submucosa or muscularis so that the overall filtration coefficient has a heterogeneous origin (Lundgren, 1967; Folkow, 1967; Jacobson, 1967). While this may not be totally objectionable under resting conditions, it becomes quite difficult to interpret the significance of changes of the filtration coefficient that may occur with conditions that selectively affect the perfusion or permeability of just one part of the vascular bed (Folkow et al., 1963; Wallentin, 1966a; Bassingthwaighte, 1970; Biber, 1973; Svanvik, 1973).

There are other technical problems that complicate the study of filtration coefficients. Lee et al. (1969,1971) and Zweifach and Intaglietta (1969) have shown that, for mammalian capillaries of the omentum and mesentery, the filtration coefficient is 3 to 8 times greater at the venous end of the capillary. If this data is applicable to the intestinal vascular bed it indicates that changing capillary pressure by manipulation of venous pressure would have a greater effect on fluid filtration than if arterial or vasomotor mechanisms were invoked. To some extent this also discredits the isogravimetric technique used by Pappenheimer and Soto-Rivera (1948) and Johnson (1965), but for many reasons the validity of this method has always been questioned (Wallentin, 1966a; Pappenheimer, 1969; Friedman, 1972; Lund et al., 1974).

The possibility has arisen, from in vitro perfusion studies, that the elevation of capillary pressures may alter capillary porosity and increase the capillary permeability to larger molecules (Shirley et al., 1957; Pietra et al., 1969; Taylor et al., 1973). Since it is necessary to raise capillary pressure in order to determine the filtration coefficient, such a phenomenon could limit the usefulness of Kf,c determinations. However there is mounting evidence that this is an artefact induced by in vitro perfusion conditions since in experiments conducted in vivo even large increases of the capillary pressure seem neither to distend the capillary tubes nor increase capillary porosity (Kjellmer, 1965b; Lundgren and Mellander, 1967; Zweifach and Intaglietta, 1969). The "stretched-pore phenomenon" has not been studied in the splanchnic microcirculation but it would be of interest to know whether changes occur in the porosity of the portal capillaries or venules with portal hypertension in hepatic cirrhosis.

The calculation of the filtration coefficient Kf,c (or CFC) for a whole tissue such as the intestine requires that the capillary pressure be increased by a known increment. This is usually accomplished by increasing venous pressure by 5 or 10 mmHg and calculating the transmittance to the capillaries. Unfortunately this is often a source of inaccuracy since there is some disagreement as to what fraction of the pressure increment is experienced by the capillary. In studies of the intestinal vascular bed, Folkow et al. (1963) have assumed that 85% of the venous pressure change is conveyed to the capillaries. In the same organ, Johnson and Hanson (1966) utilize a fractional transmittance of 62% for calculation of the same parameter, however in this case the figure is

derived from experimental computations (Johnson, 1965). Since the increment of intestinal capillary pressure in response to raising venous pressure is affected by autoregulatory adjustments of the pre- post-capillary resistance ratio (Johnson, 1965; Yablonski and Lifson, 1976), direct measurements of the capillary pressure would be beneficial in this organ. In any event, it seems unreasonable to utilize a constant fractional transmittance for the calculation of Kf,c in dissimilar vascular beds (Oberg, 1964). To justify this practice, several workers have estimated that even large errors in the assumption of percent transmittance produce only minor errors in the calculation of the filtration coefficient (Cellander and Marild, 1962; Folkow et al., 1963; Cobbold et al., 1963; Oberg, 1964), however in fact a straight-forward 25% error is introduced if the transmittance is actually 60% but assumed to be 85%.

Elevation of venous pressure for the determination of Kf,c produces a characteristic biphasic volumetric (or gravimetric)response with the intestinal volume increasing rapidly during the first 10 - 15 seconds, then followed by a slower, long-lasting rate of volume change. This response, which occurs in plethysmographic studies of all tissues, is due firstly to a blood volume capacitance effect caused by venous distention; and secondly to an underlying component caused by the increasing tissue volume in response to capillary filtration. These components were initially identified by Lewis and Grant (1925) and have been subsequently confirmed in numerous studies (Pappenheimer and Soto-Rivera, 1948; Mellander, 1960; Johnson and Hanson, 1963; Diana and Shadur, 1973).

In some tissues experimental separation of the two components is not required since the time course of the capacitance effect is so short;

but the somewhat delayed compliance in the intestine (Johnson and Hanson, 1963) may necessitate a check of time course by labelling erythrocytes with ^{51}Cr and monitoring the blood volume over the organ by radioactive collimation (Wallentin, 1966a). In earlier studies the same problem was approached in a different manner and a "pressure plethysmograph" was developed to expell the tissue blood volume at the moment before volume measurements were obtained (Krogh et al., 1932; Landis and Gibbon, 1933).

Since the capacitance vessels in the splanchnic bed do exhibit some delayed dilation when they are distended (Alexander et al., 1953; Johnson and Hanson, 1963), the capillary filtration coefficient could be over-estimated if calculated from part of the volume record that is due to delayed compliance. Considering that the compliance of the intestinal vasculature is 0.34 $ml \cdot mmHg^{-1} \cdot 100\ g^{-1}$ of tissue (Johnson and Hanson, 1963), this could lead to a substantial error. To avoid this possibility, determinations of Kf,c are rarely attempted during the first minute following elevation of the venous pressure. This seems reasonable since investigations of the time course of the initial capacitance effect showed that it had a time constant of 10.9 seconds. On this basis it would be 95% complete within 33 seconds (Wallentin, 1966b; Johnson and Hansen, 1963). However, since the compliance is relatively large, the presence of a delayed component may jeopardize observations of the capillary filtration rate for as long as 2 minutes after the venous pressure is raised.

While measurements of the filtration coefficients of various tissues show considerable variability, and while this may occur even with studies of the same tissue by different investigators (Table 1.), it is important to note that repeated determinations of Kf,c are highly repro-

TABLE 1

Values Obtained for Capillary Filtration Coefficient from Studies of Different Vascular Beds

TISSUE	SPECIES	CAPILLARY FILTRATION COEFFICIENT*	SOURCE
Intestine	dog	0.370	Johnson and Hanson, 1966
	cat	0.120	Oberg, 1964
	cat	0.110	Folkow et al., 1963
	cat	0.070 - 0.100	Johnson and Hanson, 1962
	cat	0.060 - 0.090	Yablonski and Lifson, 1976
	cat	0.064	Wallentin, 1966b
	cat	0.059	Richardson, 1975
Mesentery	frog	0.0056	Landis, 1927
Liver	cat	0.060	Greenway and Lautt, 1970
Hindquarters	cat	0.016	Oberg, 1964
Hindlimb	cat	0.015	Pappenheimer and Soto-Rivera, 1948
	dog	0.014	" " "
	dog	0.012	Kaiser and Diana, 1974
Forearm	man	0.0055	Landis and Gibbon, 1933
	man	0.0057	Krogh et al., 1932
Skeletal muscle	cat	0.040 - 0.050	Eliassen et al., 1974
	cat	0.014	Kjellmer, 1965a
	cat	0.012	Jarhult and Mellander, 1974
Skin	cat	0.035 - 0.038	Oberg, 1964
Heart	rabbit	0.320	Vargas and Johnson, 1964
Lung	dog	0.065	Guyton and Lindsay, 1959
	dog	0.030	Garr et al., 1967
Whole Body	man	0.0061	Brown et al., 1958

* $ml\ min^{-1}\ mmHg^{-1}\ 100g^{-1}$

ducible (Folkow and Mellander, 1969; Taylor et al., 1973). Thus, there is every reason to consider this parameter a valid indicator of capillary perfusion and permeability if measurements are properly obtained. The validity of this experimental approach has recently been certified by the development of conductometric techniques (Friedman, 1968; Fronek, 1971; Menninger and Baker, 1975).

Utilization of the conductometric method for microcirculatory studies is based on Okada's research (Okada and Schwan, 1960) showing that there is an excellent correlation between the hematocrit and electrical conductivity of blood. Whereas measurement of the average whole-organ capillary pressure previously required an in vitro perfusion system with either Pappenheimer and Soto-Rivera's (1948) isogravimetric or Johnson's (1965) zero-flow technique; with an isoconductometric technique Fronek (1971) has obtained identical values without the limitation of earlier methods. This is accomplished by recording the electrical resistance of the effluent blood passing through a conductivity cell and monitoring the balance between filtration and absorption. Friedman (1968) has utilized the same method for the purposes of studying capillary filtration and absorption.

Since the method doesn't involve gravimetric or volumetric measurements, it can be adapted to provide an independent check of the validity of traditional methods used to record capillary filtration. This has been achieved in the recent experiments of Menninger and Baker (1975). To complement their earlier work which used combined erythrocyte and plasma markers to identify the vascular component (Baker, 1970, Moore and Baker, 1971), they have now used the conductometric approach to independently

differentiate the filtration component from the overall biphasic volume increase that occurs when the venous pressure is raised. Their findings indicate that plethysmographic estimates are within 10% of the values obtained by conductometric methods. With any change of venous pressure there were no statistically significant differences between the values obtained by either method (Menninger and Baker, 1975). In addition to validating the usefulness of plethysmographic and gravimetric techniques, with the conductometric recording it is possible to measure the changes of capillary filtration that occur during the initial period after the venous pressure is raised (Fronek, 1971; Menninger and Baker, 1975). This data cannot be accurately obtained with plethysmographic methods since the blood volume is increasing rapidly at this time.

ii) Other measures of Capillary Perfusion and Permeability

There are at least two other important methods which have been used to measure capillary filtration. One of these is the single capillary filtration coefficient (dimensions of $\mu l \cdot sq\ \mu m^{-1} \cdot cmH_2O^{-1}$) which will not be considered in this discussion. An extensive treatment of the theoretical basis and practical use of this parameter can be found in the works of Zweifach et al. (1972; Zweifach and Intaglietta, 1969; Lee et al., 1971).

Another useful index of capillary perfusion and permeability is the PS value developed by Renkin (1959,1968). Briefly, the measurement of PS is obtained by monitoring the radioactivity of a diffusible indicator such as ^{42}K or ^{86}Rb in the arterial and venous blood of a perfused tissue. Since the effluent blood does not re-enter the circulation, the indicator concentration in the arterial blood is constant and the measurement of its concentration in the venous blood can be used to calculate the extraction ratio of the isotope during transit through the capillary bed. Knowing

the blood flow rate, the clearance and PS value can be subsequently calculated. Back diffusion of the isotope during transit of the blood through the capillary is usually negligible but can be corrected for, when necessary. This back-flux of the tracer isotope is limited because the intracellular pool of potassium in tissues such as skeletal muscle or intestine provides an almost infinite sink for the ^{42}K or ^{86}Rb diffusing into the tissue (see Crone, 1969).

This short summary is, of course, a most superficial consideration of the experimental method used to determine PS. For further details of this technique and a discussion of its underlying assumption, the reader is referred to Renkin (1959) and Folkow and Mellander (1969).

The "permeability surface area product" (PS) has two components that in most cases should be considered inseparable. The first is P, permeability; expressed as moles diffusing, per unit time, per unit concentration difference, per unit surface area. The second component, S, represents the total capillary surface area in 100 g. of tissue. Together they form the PS value which is calculated with the dimensions of $ml \cdot min^{-1} \cdot 100\ g^{-1}$ of tissue. With ideal conditions of measurement the PS value is an index of the "diffusion capacity" of a tissue, while the Kf,c (or CFC) represents the "filtration capacity" of the same capillary bed.

Generally speaking, the capillary filtration coefficient Kf,c has achieved a more widespread usage than the PS value. One important advantage of the former value is that it easily lends itself to <u>in vivo</u> experimentation. This not only avoids some of the artificial aspects of tissue perfusion but, more importantly, it permits investigations of the combined capacitance and filtration-absorption effects produced by nervous

stimulation and cardiovascular reflexes (Mellander, 1960; Oberg, 1964; Haglund and Lundgren, 1972). Similarly, the reproducibility and non-invasive nature of Kf,c measurements have allowed it to become popular in a variety of clinical studies. In fact, Brown et al. (1958) have managed to arrive at a Kf,c value applicable to the whole human body by using repeated Valsalva manoevers to raise central venous pressure .... and repeated assumptions to justify their calculation.

Essentially the same pathways in the capillary wall are thought to be used for the transfer of fluid in filtration-absorption and for the diffusion exchange of lipid insoluble substances such as those used to measure PS. This is probably more correct when ^{86}Rb is used in preference to ^{42}K. However, it is more likely that a change of capillary permeability will become evident using Kf,c than when PS is employed — assuming the capillary surface area is known or can be kept constant. This is the case because transcapillary hydrodynamic flow largely conforms to Poiseuille's law so that the rate of fluid transfer changes with the fourth power of the "pore" radius (Garby and Areekol, 1969; Folkow and Mellander, 1969; Taylor et al., 1973). On the other hand, the diffusion transport of small molecules used to measure PS changes only with the second power of the radius. The presence of uncontrolled variables in PS measurements is further shown by the experiments of Lundgren and Mellander (1967) which reveal that the presence of gross transcapillary fluid movements considerably augments the diffusion of solutes across the capillary wall.

Another important difference of Kf,c and PS is that the former value is far less dependent on the velocity of capillary flow. With a vascular bed such as the intestine, precapillary sphincters are continually opening and closing (Zweifach, 1973; Gore and Bohlen, 1975). When closure

of a sphincter occurs, with stagnation of flow in the capillary, it is probable that the minute amount of static capillary fluid equilibrates rapidly with the surrounding tissue fluid and both filtration and diffusion processes virtually cease within a few seconds (Lee et al., 1971). However, once blood is again passing through the capillary, even if the flow rate is slow, transcapillary fluid exchange will occur in a normal fashion (Folkow and Mellander, 1969). On the other hand, slowly perfused sections of a capillary network might contribute so little to total net diffusionin the region that the existence of their exchange surface escapes detection in the measurement of capillary diffusion capacity (PS).

iii) Utilization of Kf,c Measurements

As discussed previously, both PS and Kf,c consist of two components; a capillary surface area factor and a permeability factor. Using either parameter it is not usually possible to separate these two components, but with the measurement of Kf,c at least a semi-quantitative differentiation may sometimes be achieved. Kjellmer (1965b) has attempted this in skeletal muscle by first completely relaxing the vascular smooth muscle (e.g. by intense exercise or by vasodilator drugs that do not in themselves affect the capillary membrane). The capillary permeability is then increased by the administration of substances such as histamine or bradykinin which are known to affect vascular permeability. With this approach the change of Kf,c can be taken as an index of the change of permeability for the conditions of the experiment (Kjellmer and Odelram, 1965).

Similar attempts have been made with the study of Kf,c in single capillaries (Zweifach and Intaglietta, 1969). In this case, since only one capillary is involved, any change of Kf,c would indicate a change of

capillary permeability. Using this method, Levick and Michel (1971) have shown that the permeability of selected capillaries in frog mesentery can be varied by changing the protein concentration in the fluid perfusing the blood vessels.

This discussion cannot fully consider the many studies which have employed Kf,c measurements in the investigation of microcirculatory function. Nevertheless a brief survey can illustrate the utility and limitations of this method. In the intestine, which is characterized by a rich vascularization of the mucosa-submucosa, countercurrent exchange of diffusible substances in the villi, and a highly porous capillary endothelium (Grim, 1962; Jacobson, 1967; Svanvik, 1973), Folkow et al. (1963) and Dresel et al. (1966) found Kf,c values of about 0.100 to 0.150 $ml \cdot min^{-1} \cdot mmHg^{-1} \cdot 100\ g^{-1}$, and PS values of approximately 30 $ml \cdot min^{-1} \cdot 100\ g^{-1}$. For the purposes of comparison it is noteworthy that both of these values are almost 10 times are large as the corresponding measurements in skeletal muscle; 0.010 to 0.015 $ml \cdot min^{-1} \cdot mmHg^{-1} \cdot 100\ g^{-1}$ (Cobbold et al., 1963) and 3.0 $ml \cdot min^{-1} \cdot 100\ g^{-1}$ (Renkin and Rosell, 1962) respectively.

Intense vasoconstrictor fiber activation can lower the intestinal Kf,c to 0.040 $ml \cdot min^{-1} \cdot mmHg^{-1} \cdot 100\ g^{-1}$ which is 30% of the control value (Folkow et al., 1964b). Drug induced maximal vasodilation raises Kf,c values into the range of 0.300 to 0.430 $ml \cdot min^{-1} \cdot mmHg^{-1} \cdot 100\ g^{-1}$ (Folkow et al., 1963; Biber, 1973). These values indicate that this tissue has a potential range of Kf,c variation that is approximately ten-fold. A similar span is observed with measurements of PS (Dresel et al., 1966).

However, when these changes of Kf,c are considered relative to the ongoing changes of resistance to blood flow and capacitance, a peculiar pattern emerges. Shortly after the initiation of nerve stimulation the

marked reduction of intestinal flow and the increased pre- to post-capillary resistance ratio return to control levels (Folkow et al., 1964a). Yet the reduction of the functional capillary surface area Kf,c continues unchanged, as does the constriction of capacitance vessels (Folkow et al., 1964b). Thus while flow through the intestine autoregulates, the Kf,c, reflecting capillary perfusion, remains reduced. This pattern is unlike that observed in skeletal muscle and skin (Mellander, 1960).

In studies of this phenomenon Folkow et al. (1964b) have explained that the decreased capillary exchange surface without a maintained increase of resistance could indicate a redistribution of blood flow within the intestine during the autoregulatory phase of constrictor fiber stimulation. This hypothesis is supported by non-quantitative studies with India ink injection which they have interpreted as indicating a continuation of mucosal constriction while the submucosa regions are hyperemic (Folkow et al., 1964b). On the basis of this evidence, they suppose that the diversion of blood flow to subcompartments with an intrinsically lower Kf,c accounts for the maintained reduction of whole organ Kf,c under these conditions (Mellander and Johansson, 1968).

Unfortunately this interpretation, based on circumstantial evidence, is not supported by more recent quantitative studies of blood flow redistribution under these conditions. Rather, since the distribution of blood borne microspheres is the same in all tissue subcompartments during constriction and escape (Greenway et al., 1976), it appears that the autoregulation and hyperemia of intestinal blood flow occurs by relaxation of the same vessels that were originally constricted. In this respect the intestinal vascular bed behaves like the hepatic vascular bed (Greenway and Oshiro, 1972). Thus it is necessary to re-interpret the

reduction of Kf,c observed by Folkow et al. (1964b). One possibility is that, during strong nervous stimulation, the technique of Kf,c determination is inaccurate due to the intense and maintained constriction of the venous capacitance vessels. Because of this the assumed percentage of the increment in venous pressure that is transmitted back to the capillaries could be substantially over-estimated, resulting in the calculation of a lower Kf,c.

Exclusive of this methodological problem, Richardson (1974) has suggested that intestinal blood flow and Kf,c are independent variables, controlled by different smooth muscle elements. On this basis there is no reason to suppose that co-variation of the two parameters is necessary and the capillary resistance ratio which controls Kf,c could be regulated independently of whole organ blood flow.

The changes of capillary perfusion and permeability which occur with the activation of nervous reflexes has been the subject of may studies not discussed here (Oberg, 1964; Wallentin, 1966a). It has also been considered in hemorrhagic hypotension (Haglund and Lundgren, 1973; Haglund 1973) and in relation to mucosal blood supply and passive intestinal absorption (Biber, 1973; Svanvik, 1973).

The foregoing discussion has attempted to consider the theoretical basis and practical applications of both Kf,c and PS as indicators of capillary perfusion and permeability. The possibility of incurring serious inaccuracies with the measurement and interpretation of PS values has limited the routine usefulness of this parameter. Studies of Kf,c also have limitations but these are much less objectionable and more readily defined. As a result the measurement of Kf,c, while not perfect, seems to be the best indicator of capillary filtration coefficient available.

iv) Transcapillary Fluid Exchange with Elevated Venous Pressure in the Intestinal Vascular Bed

Since the intestine is richly vascularized and possesses an enormous surface area for capillary exchange it is conceivable that even moderate increases of portal pressure could cause edema and compromise transcapillary exchange. Considering that life, and even a relatively normal gastrointestinal function, are compatible with marked portal hypertension, this organ must be endowed with fairly efficient mechanisms to limit capillary filtration in these circumstances.

The existence of such a mechanism has been demonstrated by Johnson et al. (Johnson 1965; Johnson and Hanson, 1966; Johnson and Richardson, 1974) and also by Wallentin et al. (1966a,b). Unfortunately these are the only studies of this phenomenon and in both cases the imposed increment of venous pressure is quite modest. Since the absolute levels of venous pressure were not reported it seems likely that these data are only applicable within the normal range of portal pressures. In any case, the studies have shown that elevation of the venous pressure initially causes a marked outward fluid filtration which, within about 5 minutes, declines to within 10-15% of the initial rate (Johnson and Hanson 1966; Wallentin, 1966b). As shown in Figure 1, filtration may sometimes cease entirely, indicating that the previous balance of Starling forces across the capillary wall is altered and re-established in a new equilibrium despite the maintained elevation of capillary pressure.

While there is no doubt that the Kf,c is autoregulated under these conditions, there has been much debate as to the mechanism which may be responsible. There are at least three possibilities:

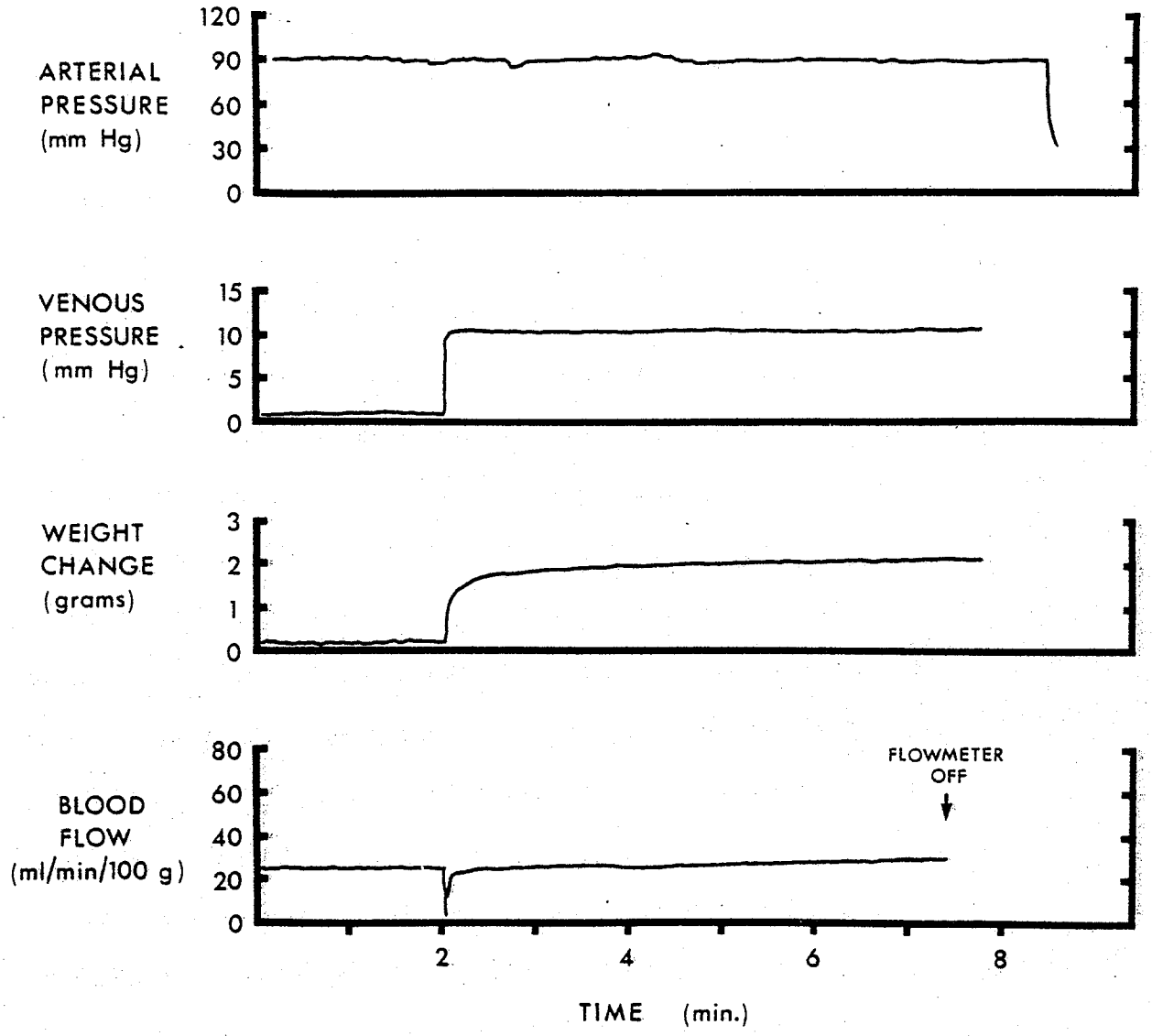


Figure 1. Record of intestinal weight change with sustained elevation of venous pressure. Note that the rate of weight increase gradually decreases until the weight reaches a new equilibrium level. (From Johnson and Hanson, 1966).

1. by myogenic adjustment of the arterioles and pre-capillary sphincters, the pre- to post-capillary resistance ratio could be altered so as to restore the original capillary pressure (Johnson, 1959; 1960),
2. the continued filtration of fluid into the interstitial spaces could raise P_T and counteract the effect of an increased P_c (Wallentin, 1966a),
3. dilution of the interstitial proteins caused by a net gain of tissue water could reduce π_T and counteract the hydrostatic differential by establishing a greater oncotic differential (Johnson and Richardson, 1974).

Whereas myogenic responses (#1.) are certainly important determinants of capillary pressure in the prevention of edema, the autoregulation of filtration (Kf,c) can be demonstrated when the myogenic tone is ablated by the infusion of papaverine or isoproterenol (Wallentin, 1966b). Thus the controversy centers about the latter two possibilities and since there are quite convincing arguments in favour of both mechanisms, it is impossible to make a rational selection of one mechanism over the other at this time. Certainly it is conceivable that both processes could contribute in a co-operative fashion.

The measurement of capillary pressure in the intestine has not met with a great deal of success. Until recently direct measurements in this organ were not technically feasible but Johnson (1965) had calculated a value of 9.7 mmHg using the isogravimetric method. Gore and Bohlen (1975) have managed to obtain direct measurements of P_c in rat intestinal muscle and mucosal villi (Figure 2). Using fiber-optic transillumination and

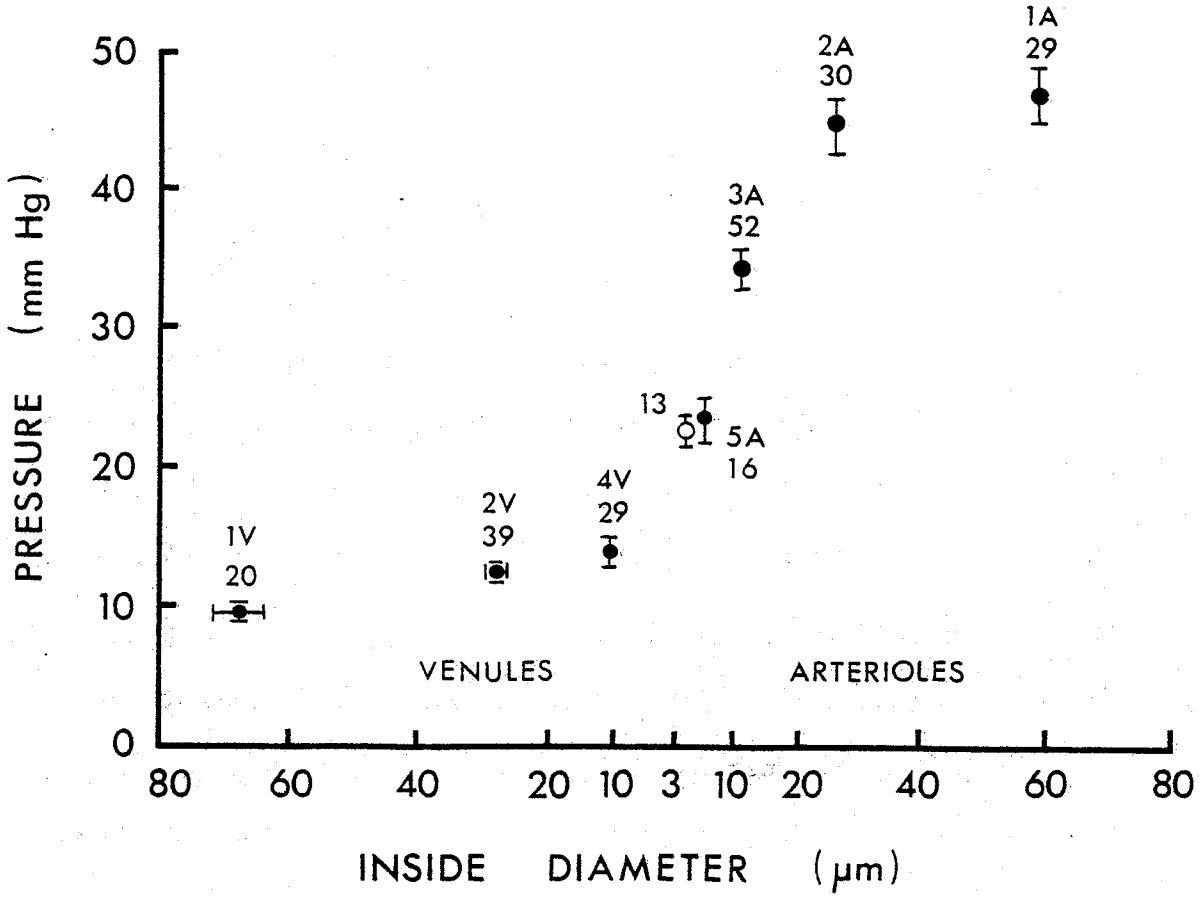


Figure 2. Distribution of mean transmural pressure in the microcirculation of innervated rat intestinal muscle. The notation above each data point refers to the branch order of the vessel type and the number of vessels sampled. A total of 228 vessels in 28 rats were sampled. 1——1 S.E. Open circle is mean capillary pressure. (From Bore and Bohlen, 1975).

recording pressure with a servo-nulling device, they measured P_c to be 24 ± 1 mmHg in capillaries of the longitudinal muscle layer and 14 ± 2 mmHg in mucosal capillaries. In view of recent literature revealing major problems with the isogravimetric method (Lee et al., 1971; Intaglietta and Zweifach, 1974; Lund et al., 1974), it is not surprising that Johnson's calculation falls short of the average P_c estimated by Gore and Bohlen (1975) for the intestinal bed (17 mmHg). Gore and Bohlen's data also reveal that the capillaries perfusing intestinal smooth muscle have a P_c that is approximately equal to π_p whereas those of the mucosa have a much lower value supporting their function as an absorption network.

Tissue pressure has been measured by Johnson et al. (Johnson and Hanson, 1963; Johnson and Richardson, 1974). In accordance with their expectations it averaged 0.1 mmHg ± 0.5 (SD) and showed no significant change with venous pressures ranging from 0 to 20 mmHg. However, these measurements are of doubtful value as they were crudely obtained by the insertion of needle under the serosal surface of the intestine. The pressure monitored in this location would probably be increased only in overt intestinal edema. It remains possible that P_T could be a modulator of filtration within the muscle or mucosal interstitium, though it may be confined to the most minute intercellular spaces. It is beyond the scope of this discussion to further consider the methodology involved with measurements of P_T, however it does seem that this technique has advanced considerably beyond the initial methods which were treated with much skepticism. In recent years the capsular, wick and direct needle measurements have been validated to the extent that it is even possible to detect relatively rapid changes of tissue pressure (Zweifach, 1973; Taylor et al., 1973; Zelis et al., 1974; Brace et al., 1975; Granger and Taylor, 1975).

The control of filtration by the capillary oncotic pressure gradient ($\Delta\pi c$) is again a difficult mechanism to evaluate due to technical problems. Whereas π_p varies from 20 to 30 mmHg in most mammalian species (Landis and Pappenheimer, 1963) this value is not identical with the effective capillary oncotic pressure because the membranes in experimental osmometers do not have the same distribution of pore sizes as do capillary walls. Instead it is necessary to consider the reflection coefficient of the tissue under study and arrive at a value for $\sigma_c \Delta\pi_c$. While the reflection co-efficient is not known for the splanchnic capillaries it is certainly less than 1, the value obtained for capillaries in skeletal muscle.

The tissue oncotic pressure π_T is highly variable from tissue to tissue and its measurement, while not difficult, presents problems of interpretation. Drinker (1945) and Yoffey and Courtice (1970) have maintained that the concentration of protein in the tissue lymphatics represents a mean cross sectional sample of the tissue fluid and is a valid index of π_T. Others have questioned this assumption and presented data to show that the protein concentration of the lymphatic fluid is more concentrated than the calculated or measured tissue protein (Mayerson, 1963; Rusznyak et al.,1967). There is probably no question that the protein in lymph is quite similar to that of the fluid inside implanted capsules (Gibson and Gaar, 1970), but the assumption that it is identical to the protein concentration that determines π_T at the capillary wall seems unlikely (Landis and Pappenheimer, 1963; Taylor et al., 1973). In fact there is evidence that a concentration gradient may exist in the lymphatics themselves. In a recent study of the lymph vessels in cat intestine, Hargens and Zweifach (1976) have sampled lymph from progressively larger

vessels and found that the protein concentration is increased almost three-fold in larger vessels as compared to the terminal lymphatics.

The π_T of the intestine has not been directly measured but has been estimated from the concentration of protein in intestinal lymph (Weiderhielm, 1968; Johnson and Richardson, 1974). Since this is surely more concentrated than the interstitial fluid, the probable value of π_T ranges from 3 to 6 mmHg (Landis and Pappenheimer, 1963). It is somewhat difficult to envisage that interstitial protein dilution could achieve a substantial control of capillary filtration by the reduction of this figure. In addition, studies of tissue water content after periods of raised venous pressure (Davenport and Alzamora, 1962; Yablonski and Lifson, 1976) do not coincide with the amount of fluid known to be filtered (Johnson and Hanson, 1966; Wallentin, 1966b) and the known volume of the interstitial space in the intestine (Bozler, 1961; Barr and Marvin, 1965). Nevertheless if the effective tissue space controlling π_T and Kf,c is a heterogeneous, minute gel-colloid interface close to the capillary wall, it is possible that π_T reduction could be a critical factor controlling the transcapillary filtration and luminal secretion (Yablonski and Lifson, 1976) which occur with elevation of the intestinal venous pressure.

The role of lymph protein (π_L) and hydrostatic pressure (P_L) in the regulation of P_T and π_T is a fascinating but extensive subject that cannot be dealt with here. Further details may be obtained from reviews by Mayerson (1963), Taylor *et al.* (1973), Leak (1976) and Guyton *et al.* (1976). For details of the mechanism by which terminal lymphatics may concentrate lymph protein see Zweifach (1973) and Casley-Smith and Bolton (1973).

To summarize, there appear to be a number of mechanisms involved in the prevention of intestinal edema. The first line of defense may well

be myogenic autoregulation of capillary pressure and tissue protein wash-out. In response to changes of P_T and π_T, lymphatic evacuation of the intestinal interstitial fluid can increase $Jv,_L$ as much as ten-fold (Korner et al., 1954; Yoffey and Courtice, 1970), providing further control of P_T and π_T. Moderate increases of tissue pressure may be involved during the first stages of filtration but greater increases almost certainly occur with prolonged or marked elevation of venous pressure.

The control of intestinal filtration and the possibility that excess intestinal lymph passes into the peritoneal cavity is a key issue in relation to the subject of ascites formation. The long-term control of this process in the face of increased venous pressures will be discussed in Section 3, III of this thesis.

b) The Microcirculation in the Liver

i) Transsinusoidal Exchange

The hepatic circulation is unique not only for its dual blood supply but also because its venous capillaries, or sinusoids, are the most permeable and least pressurized exchange vessels in the body. Yet there have been remarkably few studies into the nature of the hepatic circulation relative to studies of metabolic functions in this organ. Fortunately, information related to hepatic flows, pressures and blood volumes may be obtained from several comprehensive reviews on the liver (Brauer, 1963; Bradley, 1963; Greenway and Stark, 1971; Rappaport, 1973) and thus the current discussion can be restricted to consider the microcirculatory aspects of liver blood flow.

Although liver histology and knowledge of the arrangement of the hepatic microvessels had progressed substantially since Malpighi's

original observations, it was in 1949 that Elias first classified the hepatic architecture according to a lobular, hexagonal pattern based on centripetal drainage into the hepatic venules. Since then, the liver structure has been re-examined by Rappaport (1974; 1975) who maintained that an acinar pattern is more appropriate since it conforms to "basic laws of circulatory physiology". However the distinction of a lobular or acinar pattern seems to be more of an experiment in semantics than circulatory physiology since, in any case, blood flows from the portal venules, through the sinusoids and into the terminal hepatic venules (Bloom and Fawcett, 1968;Elias and Sherrick, 1969), (Figure 3). For the most part, bilary and lymph flows proceed in the opposite direction and there may be some justification for supposing that a form of counter-current exchange may occur between these fluids and the sinusoidal blood (Brauer, 1963).

To this date, sinusoidal pressure has not been measured by direct micropuncture but estimates based on the pressure differential between the portal and hepatic veins have placed it in the range of 1-5 mmHg (Greenway and Stark, 1971; Rappaport, 1973). Wedged hepatic venous pressure has been frequently used as an index of sinusoidal pressure (Friedman and Weiner, 1951; Price et al., 1964) but current opinion is that this technique measures instead portal pressure (Bradley, 1963; Greenway and Stark, 1971; Rappaport, 1973); and even this may be inaccurate in the presence of liver disease (tenHove and Leevy, 1973; Witte and Witte, 1975). Worse still, Sapirstein (1958) has demonstrated that wedged pressures contain a component of the hepatic arterial pressure and accordingly, regular oscillations which are synchronous with the pulse are usually

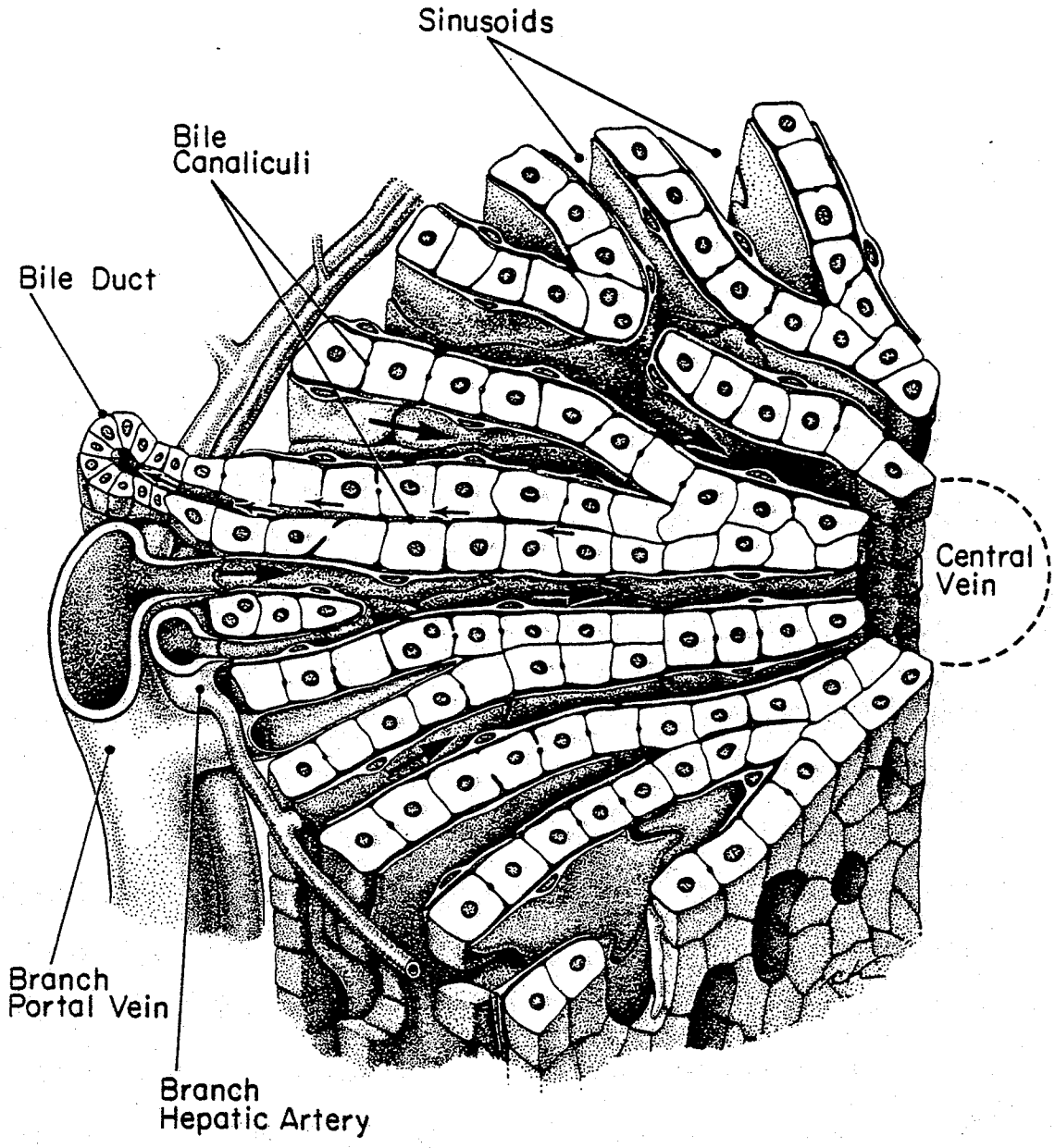


Figure 3. Diagrammatic representation of the radial disposition of the liver cell plates and sinusoids around the terminal hepatic venule or central vein, showing the centripetal flow of blood from branches of the hepatic artery and portal vein, and the centrifugal flow of bile (small arrows) to the small bile duct in the portal space. (From Bloom and Fawcett, 1968).

evident in the tracings of wedged pressure (Sherlock, 1971). Consequently it is not surprising that Friedman and Weiner (1951) have frequently observed wedged hepatic venous pressures that were greater than portal pressure.

The only direct estimate of hepatic sinusoidal pressure comes from the work of Nakata et al. (1960). By insertion of micropipettes into the minute blood vessels of the transilluminated rat liver, pressures of 3.6 $\pm$ 0.14 (S.D.) mmHg were recorded in the terminal portal venules. This pressure fell with transit along the sinusoids to a pressure of 0.7 $\pm$ 0.11 (S.D.) mmHg recorded in the terminal hepatic venules. From this data one may calculate that the sinusoidal pressure must be very low, probably between 0.7 and 1.4 mmHg (Nakata et al., 1960; Rappaport, 1963). Mitzner (1974) has suggested that this value is about 2 or 3 mmHg too low because the margin of the rat liver used for transillumination does not have an arterial input. While this may be the case, it does not necessarily imply that the value is erroneous — given the nature of the arterial perfusion of the sinusoid (Rappaport, 1973). Also, it is possible that the pressures recorded by Nakata in the hepatic venules could have been unnaturally increased by engorgement of the central veins and interference with respiration in the preparation of the liver; so that this effect may offset that due to the lack of an arterial input. Thus, until more direct measurements are obtained, 0.7 - 2.0 mmHg seems a reasonable estimate of sinusoidal pressure. This may decrease further with inspiration (Rappaport, 1973), but the presence of a low closing pressure in the hepatic venous bed probably limits respiratory excursions (Brauer et al., 1956; Mitzner, 1974).

With portal pressures varying from 7 to 10 mmHg (Greenway and Stark, 1971), it is somewhat curious that the sinusoidal pressure is so low.

Nakata's data (1960) indicate that a 40-50% drop in pressure occurs between the terminal portal venules and the sinusoid. Since the portal venules are fairly wide bore vessels (15-20 μm; Rappaport, 1975), some have assumed that the portal blood overcomes a high resistance sphincter at the point where it branches into the sinusoids (Irwin and MacDonald, 1953; Knisely et al., 1957). But since the vessel walls do not contain smooth muscle at this junction others have proposed (Bradley, 1963; Rappaport, 1973) that the large reduction of pressure in the hepatic sinusoids is due to the porosity of their walls which encourages fluid transfer into the space of Disse. This causes the path of plasma to become circuitous and lengthened in traversing the sinusoids. Also, along this route plasma viscosity may be heightened due to the sequestration of erythrocytes inside the sinusoids. Together, the lengthened path and greater viscosity may contributeto an increased sinusoidal resistance according to Poisseuille's formula. In any case, since it is known that there is intermittent flow in the sinusoids (Wakim and Mann, 1952; Seneviratne, 1949; Nakata, et al., 1960), a sphincter mechanism of some sort must be present and could account for the pressure drop, perhaps in combination with Poiseuille's law.

However, given that there is a low sinusoidal pressure, it is difficult to reconcile the continuation of portal perfusion with the entry of high pressure arterial blood into these spaces. Possibly the arterial blood enters sinusoids that are "closed" at the portal end and is subsequently mixed with venous blood. Alternatively, arteriolar sphincters (Elias, 1949; McCuskey, 1967) may control the admixture and in themselves impose a substantial resistance to blood flow. Because there is a fast rate of blood flow in the sinusoids, it is not unlikely that

the energy stored as pressure in the arterioles is rapidly converted to kinetic energy in the sinusoids so that the low pressure portal inflow is not excluded.

With respect to the control of sinusoidal filtration in the hepatic vasculature, it becomes important to consider the permeability of the sinusoidal endothelium. The central regions of the liver sinusoids are composed of particularly thin endothelial cells with large fenestrations. Unlike normal capillaries, these endothelial cells lack cell-to-cell junctions and a basement membrane (Bloom and Fawcett, 1968). Consequently it would seem reasonable to presume that the sinusoidal reflection coefficient, σ_c, and transsinusoidal oncotic pressure gradient, $\Delta\pi c$, must be quite small especially since even large proteins (M.W. 412,000; (Grotte, 1956)) rapidly gain access to the space of Disse. If so, this would indicate that a major force for fluid reabsorption is absent in this organ and fluid exchange is mainly affected by ΔPc and Kf,c.

Data from Greenway et al. (1969; Greenway and Lautt, 1970;1972b) provide the only measurements of hepatic Kf,c that are currently available. Recording the hepatic volume by plethysmography, venous outflow pressure was controlled with an extracorporeal circuit. Elevation of hepatic venous pressure for periods as long as 5 hours showed that the rate of sinusoidal filtration remained strictly constant at 0.060 ± 0.003 ml·min^{-1}·mmHg^{-1}·100 g^{-1} and was not subject to Kf,c autoregulation such as is observed in the intestine under similar conditions (Wallentin, 1966b; Johnson and Hanson, 1966) (Figures 4 and 5). When the plethysmograph was filled with paraffin oil rather than Ringer-Locke solution, fluid was seen to transudate from the liver surface, coalesce and collect at the bottom

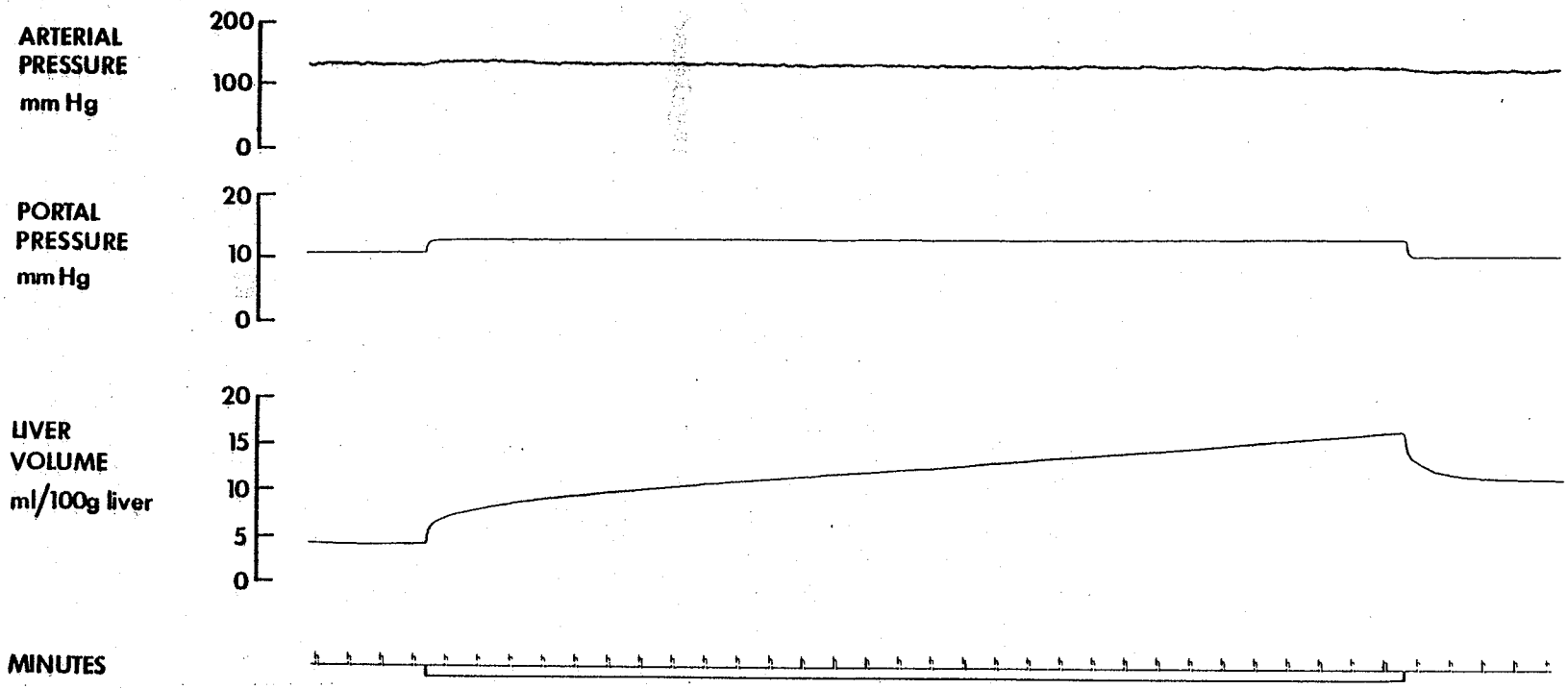


Figure 4. Response of liver volume and portal pressure in one cat when hepatic venous pressure was increased to 4.68 mm Hg for 30 minutes. (From Greenway and Lautt, 1970).

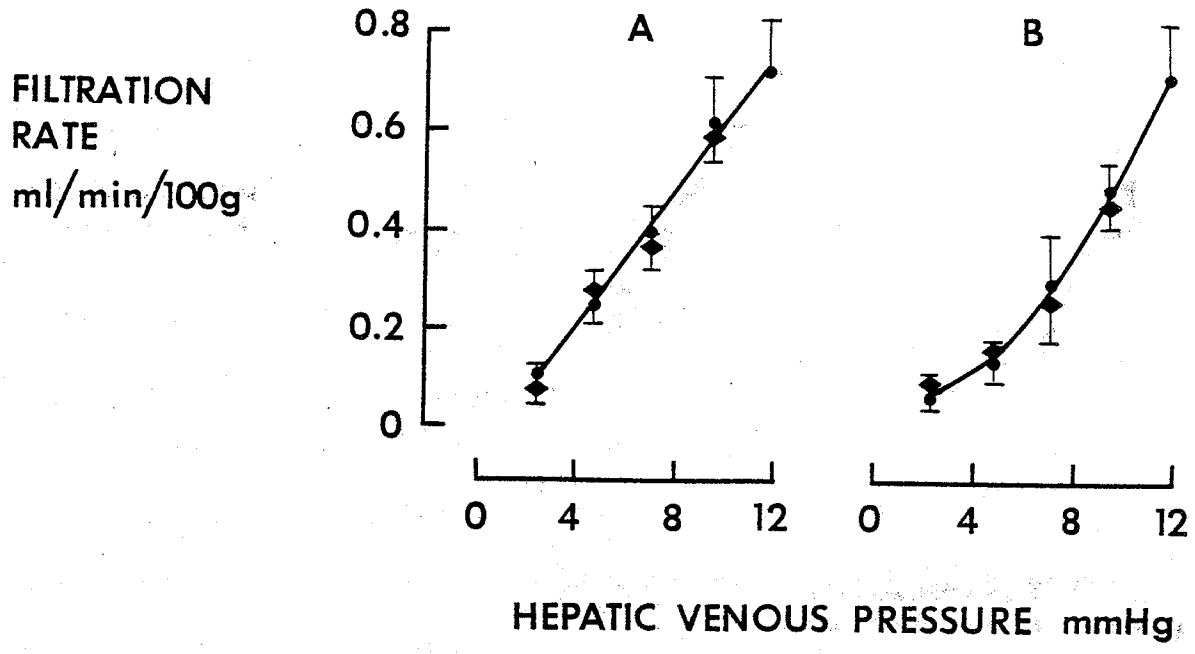


Figure 5. Relation between steady-state filtration rates (mean ± S.E.) and hepatic venous pressures in cats in which the hepatic lymphatic drainage was occluded (A) or intact (B). ● = values during stepwise increases in venous pressure; ◆ = values during stepwise decreases. (From Greenway and Lautt, 1970).

of the plethysmograph. On analysis, its high specific gravity confirmed the presence of protein in concentrations close to that of plasma, as has been reported by Brauer (1959) for the transudate collected from perfused livers which were subjected to passive congestion.

Whereas the Kf,c value recorded for the intestinal vasculature is greatly affected by neural stimuli and blood-borne factors (Mellander and Johansson, 1968), this is not the case in the liver (Greenway, et al., 1969; Greenway and Lautt, 1972b). Stimulation of the hepatic nerves sufficient to cause maximal effects on flow resistance and capacitance had no significant effect on the recorded Kf,c (Greenway et al., 1969) (Figure 6). Also infusions of adrenaline, isoprenaline or histamine had no effect on the rate of transsinusoidal filtration even though hepatic arterial and portal venous flows were markedly altered (Greenway and Lautt, 1972b). Apparently then, the sinusoidal surface area available for perfusion and the permeability of the sinusoidal wall are not readily affected by normal physiological and pharmacological stimuli. This does not necessarily indicate that sinusoidal flow is always constant, or that the sinusoids are incapable of intermittent opening and closing like peripheral capillaries; rather it suggests that, at any given time, the rate of flow and number of open sinusoids are controlled by elements, such as Kupffer cells, that are unresponsive to vasoactive stimuli.

Thus relative to the intestinal vascular bed, the liver microcirculation operates in a distinctly different fashion. Although the filtration coefficients in both organs are similar, the hepatic vasculature seems to lack the oncotic gradient that promotes absorption in the intestinal capillaries. The liver accommodates for this in two ways. Firstly, the sinusoidal pressure (and presumably ΔPc) is very low which

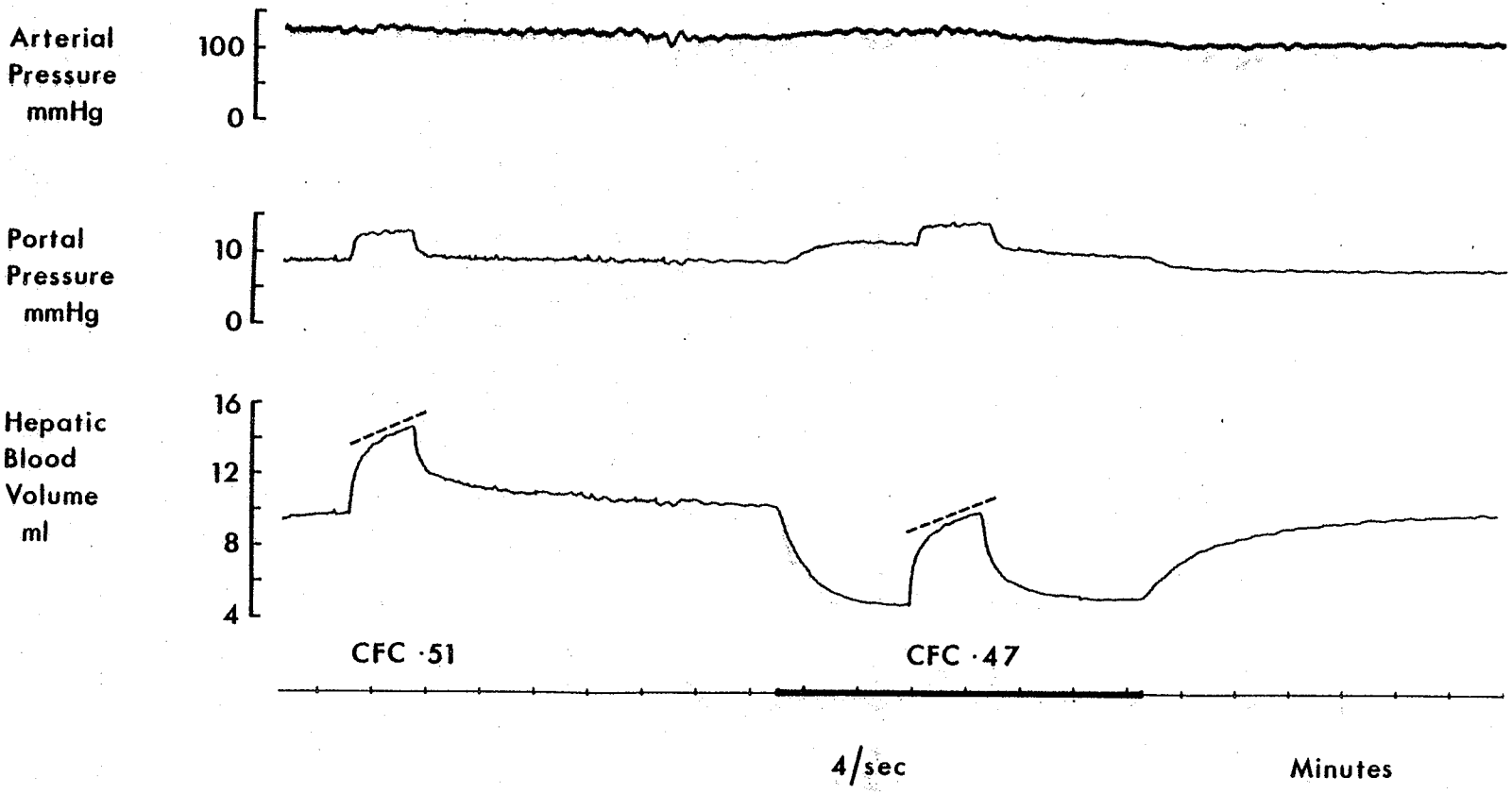


Figure 6. Determination of the capillary filtration coefficient (CFC) by raising hepatic venous pressure 4.7 mm Hg, before and during stimulation of the hepatic nerves at a frequency of 4/sec. The broken lines represent the slopes, taken for the calculation. The hepatic blood volume was determined shortly after these responses to allow calibration of the capacitance response to nerve stimulation in terms of hepatic blood volume. (From Greenway et al. 1969).

tends to balance out the transsinusoidal forces according to Starling's Law. And secondly, the basal rate of liver lymph production is much greater than in other tissues (Brauer, 1963; Greenway and Stark, 1971). With flow rates from 0.4 to 0.6 $ml \cdot min^{-1} \cdot kg^{-1}$ (Brauer, 1963), hepatic lymph production amounts to a substantial component of the thoracic duct flow (1 ml/min; Dumont, 1975). Consequently this provides a unique vent for the removal of tissue fluid not reabsorbed in the sinusoids.

Since the liver, unlike the intestine, is unable to regulate the rate of transsinusoidal filtration in response to a raised venous pressure, it is notably susceptible to venous congestion (Brauer, et al., 1959; Bradley, 1963). In spite of this, marked passive congestion rarely produces irreversible histological changes (Bolton and Barnard, 1931), perhaps because great increasesin the rate of lymph flow (10-20 times) provide some measure of decompression (McKee et al., 1948; Barr et al., 1975). Starling, (1896b) considered that this sensitivity of liver lymph production served as a physiological safety valve to siphon off pressure increasesin the inferior vena cava and right atrium. Whether the hepatic vasculature is in fact designed to serve a more noble function than its own homeostasis is a question of teleology, but certainly it is most prone to edema in cases of pericarditis or right-sided congestive heart failure (Hamilton, 1954; Dunn et al., 1973).

ii) Hepatic Interstitial Fluid and the Formation of Lymph

As mentioned previously, hepatic lymph flow consitutes a major portion (25-50%) of flow through the thoracic lymph duct. Since the lymphatic drainage from the liver contains a high concentration of protein (85% of plasma protein concentration Cain, et al., 1947; Claus, et al., 1954;

Brauer, 1963), the hepatic lymph supplies most of the protein in the thoracic duct lymph (Dumont, 1975).

Since even proteins with large molecular weights readily enter the hepatic interstitial space (Grotte, 1956), it is significant that the protein concentration in the hepatic lymph is not equal to that of plasma. However there is histological evidence that the sinusoid is not highly porous along its whole length but only in its central portion, closer to the central vein or portal triad the fenestrae are less numerous or absent, and a basement membrane may be present in some species (Yoffey and Courtice, 1970; Rappaport, 1973). In these regions a more dilute fluid may be filtered into the extravascular space. In any event, the fact that the liver lymph contains less protein than plasma suggests in turn that this is also the case for the hepatic interstitium --- in spite of the well established sinusoidal permeability.

The possibility that a somewhat lower protein concentration exists in the hepatic interstitial space is fortified by the studies of Benson et al. (1955) and Freidman et al. (1956). Their studies on conscious rats with hepatic lymph fistulae suggest that the hepatic lymph undergoes some degree of concentration in a manner similar to that which occurs with the lymphatic drainage of other tissues like the intestine (Mayerson, 1963) (see section 3,I,a,iv). Certainly the extent of this concentration is more limited because of the higher protein concentrations, but since there is a demonstrated inverse correlation of the basal lymph flow rate with its protein concentration, the existence of some form of lymph concentration process should be acknowledged.

While the behaviour of the hepatic lymph provides circumstantial evidence that the interstitial protein concentration in the liver may be

less than that of plasma, it should by no means infer that the levels are as low as in other tissues. However it does present an unusual situation. At least part of the space of Disse contains a sinusoidal filtrate with a protein concentration equal to that of plasma. This fluid borders the hyaluronidate-gel matrix which constitutes the hepatic interstitium proper and contains a somewhat lower concentration of protein. Yet this tissue matrix in turn synthesizes lymph with a protein concentration close to that of plasma.

The selective nature of the hepatic interstitium can be further elaborated by reviewing its permeability to protein relative to substances with lower molecular weights. Brauer's experiments (Brauer et al., 1959) with the perfused rat liver have measured a mean sodium space of 24 ml/100 g, a ^{59}Fe-labelled erythrocyte space of 6 ml/100 g and an extravascular ^{125}I-protein space of 8 ml/100 g. Thus of the total extravascular space of 18 ml/100 g, the interstitium proper (as defined by the space excluding protein entry in the single passage of the bolus injection) is only 10 ml/100 g.

Similar calculations have been made by Rothschild et al. (1966) and Goresky (1963; 1969; Goresky and Silverman, 1965). Goresky's experiments have proved particularly informative through the use of a multiple indicator dilution technique to examine the in vivo distribution of various substances in the liver interstitium. This technique involves a rapid injection into the portal vein of both a reference substance and one or more study substances. Analysis of samples subsequently obtained by rapid serial collection from a catheter in the left main hepatic vein allows a differentiation of the volumes of distribution for various compounds.

Relative to the passage of ^{51}Cr-labelled erythrocytes (intravascular reference standard), labelled water, sucrose, inulin or albumin are delayed in their transit through the liver. The dilution pattern for protein is only slightly prolonged relative to the erythrocyte curve, while tritiated water, because of its entry into the total hepatic water, shows the greatest delay and diminution of magnitude (Goresky, 1969) (Figure 7). Calculation of the extravascular space accessible to the various substances yielded 6.2 $\pm$ 0.8 (S.D.) ml/100 g for albumin, 7.8 $\pm$ 1.3 ml/100 g for inulin and 9.5 $\pm$ 2.1 ml/100 g for sucrose. The smaller monosaccharides, α and β methyl-D-glucoside, were also examined and shown to be distributed in a volume of identical size to that of sucrose (Goresky, 1963; 1969). Thus there seems to exist a characteristic of the hepatic extravascular space that enforces a partitioning effect to restrict the entry of compounds according to their molecular weight. A similar phenomenon can be demonstrated simply by examining the diffusion of substances from a layer of buffer solution into an adjacent layer of hyaluronic acid gel (Ogslon and Phelps, 1961). In addition, since the polyelectrolyte properties of the interstitial ground substance are well established (Inagelietta and Zweifach, 1974), it is noteworthy that Goresky's calculation for the distribution space of ^{24}Na is not significantly different from the distribution of its anion counterpart ^{36}Cl (Goresky, 1969).

While these data provide some glimpse of the hepatic interstitial composition, present techniques cannot accurately resolve the concentration of protein in the intercellular matrix. Surely the selectivity of this compartment cannot be underestimated if only because of its discrimination

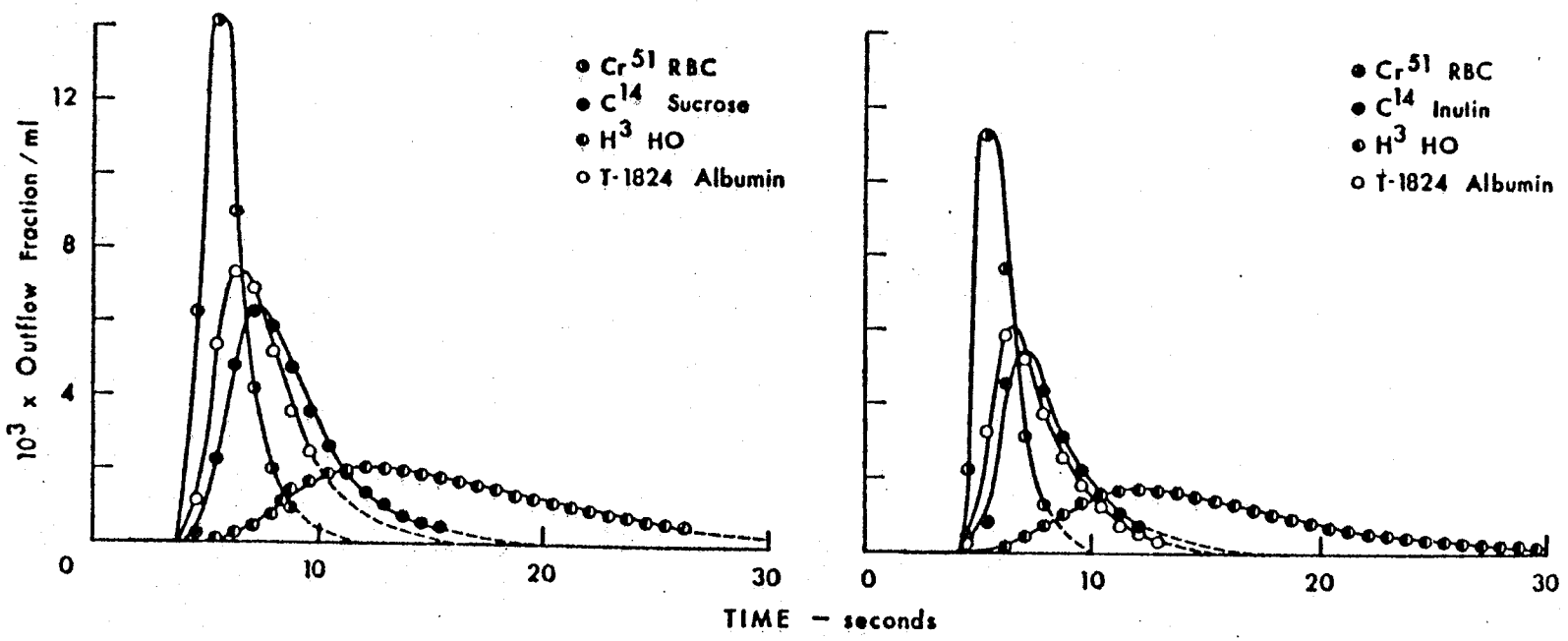


Figure 7. Hepatic venous dilution curves, plotted in a rectilinear fashion. (From Goresky, 1969).

in handling of macromolecules. Protein entry is limited in spite of constant exposure to high levels in the space of Disse. Lymph, which is formed at least partly from its constituent fluid, has an albumin/globulin ratio that is 18% greater than plasma (Nix et al., 1951; Claus et al., 1954). Newly synthesized albumin is specifically routed into the sinusoidal blood rather than into the hepatic lymph (Rothschild et al., 1966;1969a; Yoffey and Courtice, 1970).

Despite the delicate control of the hepatic interstitial contents there is substantial evidence that this is disrupted by the insult of passive venous congestion. Under these conditions the sodium space and colloid distribution space are greatly expanded (Brauer et al., 1959). In addition the selective handling of albumin and globulin disappears, oxygen diffusion is limited and erythrocytes gain entry to the space of Disse (Brauer et al., 1959; Rappaport, 1973).

In accordance with the increase of tissue pressure (presumed), lymph production is greatly increased but the ability to concentrate its protein content is overcome. As a result the sinusoidal filtrate may pass directly into the lymphatics. A similar situation occurs with edematous conditions in tissues such as skeletal muscle or intestine but here results in the production of a protein-dilute lymph (Landis and Pappenheimer, 1963; Rothschild et al., 1969a; Yoffey and Courtice, 1970). However, in the liver, the same phenomenon results in the production of lymph with a protein concentration close to that of the plasma since this is the nature of the sinusoidal filtrate in this organ (Nix et al., 1951; Drapanas et al., 1960; Greenway and Lautt, 1970). Thus the liver is unique in that, with venous congestion and excessive filtration, the concentration of protein in the hepatic lymph is the same as under physiological conditions despite

the fact that flow rates may be increased 35 fold (Nothacker and Brauer, 1950) and the concentrating mechanism bypassed. In fact, the lymph composition differs in only one characteristic feature; the albumin/globulin ratio is reduced to the ratio of plasma (Brauer, et al., 1959; Drapanas, et al., 1960). Yet this most significant clue gives evidence of important changes which have occurred in the mechanism of lymph production by the hepatic interstitium.

Surely it is appropriate to make a similar comparison of the albumin/globulin ratio in ascites versus that found in the residual intraperitoneal fluid under physiological conditions. In ascites, the electrophoretic pattern of the intraperitoneal protein is identical to that of plasma, though lacking the fibrinogen peak due to clot formation (McKee, et al., 1948;1949). In contrast the normal intraperitoneal fluid contains substantially more albumin relative to globulins and is similar in content to hepatic lymph (Courtice and Roberts, 1975).

II The Absorption of Intraperitoneal Fluid

a) Anatomical Nature of the Peritoneal Cavity

Under physiological conditions the peritoneal cavity consists of a potential space confined within the visceral and parietal layers of the peritoneum. Histologically, the peritoneal membrane is composed of a single layer of mesothelial cells overlying a basement membrane and several thin layers of loose connective tissue (Bloom and Fawcett, 1968; Yoffey and Courtice, 1970). The free cells which are found in small numbers throughout the peritoneal cavity are mainly mesothelial cells desquamated from the peritoneal lining but some macrophages, lymphocytes and mast cells are also present.

While most parts of the peritoneum are impermeable to protein (Courtice and Roberts, 1975), this property is absent at the central, inferior surface of the diaphragm (Bolton, 1921; Higgins and Graham, 1929; Casley-Smith, 1964). Thus particles such as glass spheres, india ink, chylomicrons, erythrocytes, and tumor or yeast cells pass from the peritoneal cavity into the lymph vessels underlying the diaphragm, but do not penetrate lymphatics in other parts of the parietal or visceral peritoneum (Yoffey and Courtice, 1970). At the diaphragm, lymph vessels are arranged in two plexuses with intercommunicating vessels; one faces the peritoneum and the other is on the pleural side (French, et al., 1960; Allen, 1967). But it is the structure of the lymphatics on the peritoneal aspect that is of particular interest.

In 1863, von Recklinghausen (quoted by Yoffey and Courtice, 1970) proposed that particles placed in the peritoneal cavity pass into the diaphragmatic lymph vessels through stomata that exist uniquely in this part of the peritoneum. However, since subsequent anatomical examinations could not confirm the presence of such entities (Muscatello, 1895; MacCallum, 1903), this concept has been put aside. Instead, MacCallum (1903) proposed that particles are forced through between the mesothelial cells by respiratory movements. Although Cunningham (1926) subsequently expressed the opinion that absorption could occur directly through the cytoplasm of the mesothelial cells, numerous experimental observations have favoured the form of inter-cellular movement proposed by MacCallum (1903).

More recently, studies with electron microscopy and scanning electron microscopy have shown that the structure of the diaphragmatic lymph vessels in this region of the peritoneum is unique because of

a) the presence of a discontinuous or fenestrated basement membrane, b) the thinness of the layer of mesothelial cells and their absence of cell-to-cell tight junctions, and c) the free passage of erythrocytes through intercellular spaces (Allen and Weatherford, 1959; Smith et al., 1970). Due to differences between species there is some dispute as to the maximum size of particle that can be absorbed through this region of the peritoneum but in most species 20 μm particles are absorbed (Allen, 1956; Allen and Weatherford, 1959).

Application of electron microscopy techniques has also shown that the diaphragmatic lymph vessels lie within lacunae under the peritoneal mesothelium (French, et al., 1960). As illustrated by the reconstruction of a lymph vessel shown in Figure 8, it is evident that overlapping cell-to-cell junctions may fairly readily become separated to allow for the passage of fluid or particles into the lumen of these vessels. Transfer by this process may corroborate earlier studies by Allen (1956) which documented a rhythmic filling of the diaphragmatic lymphatics in synchrony with respiratory movements. Perhaps now we can visualize a regular opening and closing of the junctional flaps between the lymphatic endothelial cells in accord with respiration. Similarly these same overlapping junctions may act as flap-valves to prevent the back-flux of lymph into the peritoneal cavity (Casley-Smith, 1964).

After leaving the diaphragm, lymph mainly enters the large collecting ducts associated with the internal mammary vessels on each side of the sternum. From this point drainage passes through the anterior mediastinal lymph nodes near the thymus and re-enters the circulation via the right lymphatic duct. While in some cases lymph may be diverted to the left side and enter via the thoracic duct, it is important to note

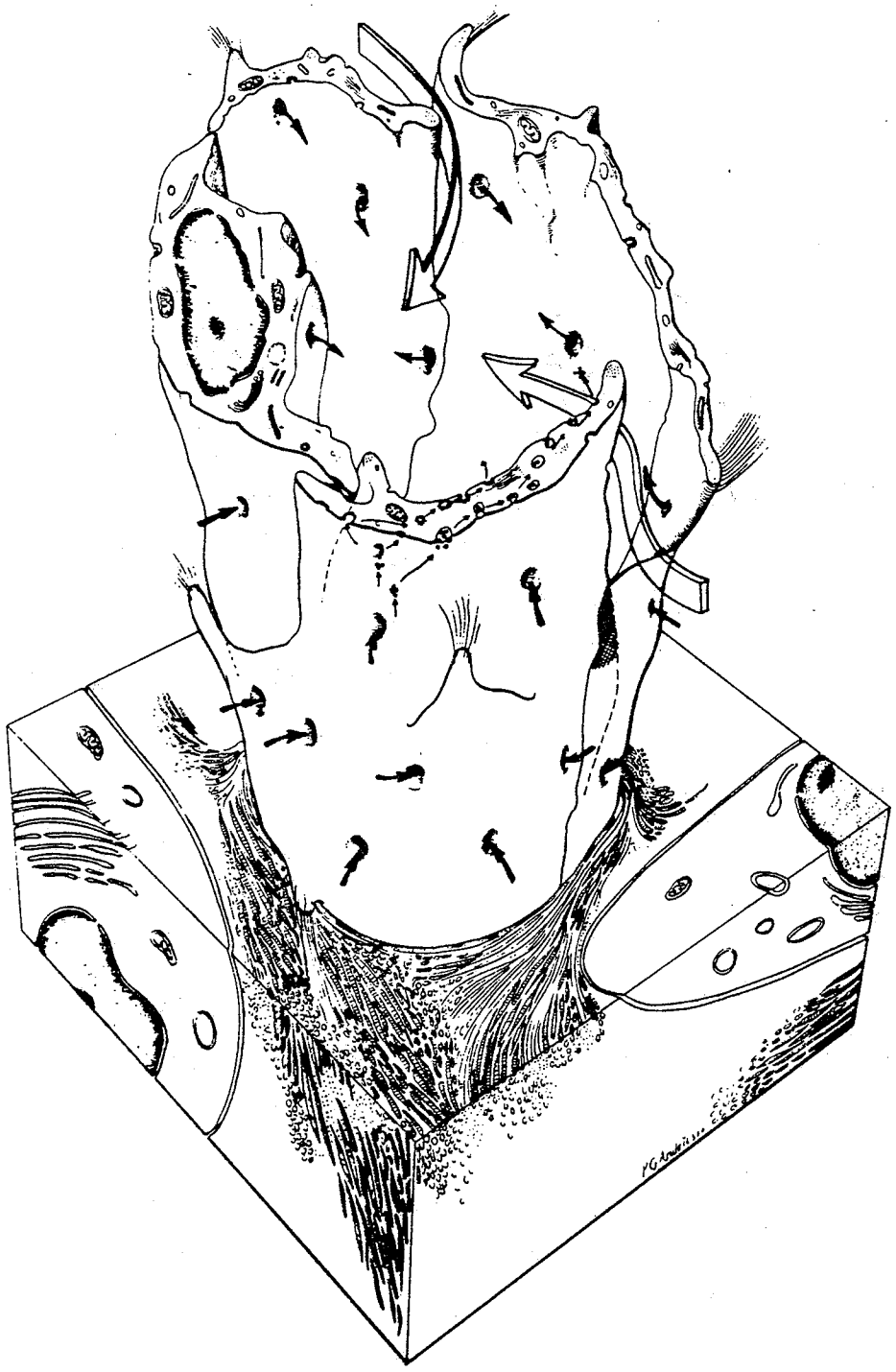


Figure 8, Three dimensional diagram, reconstructed from collated electron micrographs to represent a portion of a lymph vessel in the process of fluid absorption. (From Leak, 1976).

that less than 20% of the diaphragmatic peritoneal lymph drainage is routed through the thoracic duct (Courtice and Steinbeck, 1950a; Olin and Saldeen, 1964; Yoffey and Courtice, 1970).

The peritoneal cavity usually contains a small amount of free fluid (1-7 ml; rabbit) which has a protein concentration of about 2.5 g% (Maurer, et al., 1940; Courtice and Roberts, 1975). The origin of this fluid is not yet established; however certain components of intestinal lymph are absent and the relative concentrations of various macromolecules, are similar to the ratios occurring in hepatic lymph. Thus at least part of this residual fluid may originate from the numerous lymph plexuses that underlie Glisson's capsule (Courtice and Roberts, 1975).

Despite the presence of protein in the intraperitoneal fluid, the impermeable nature of the peritoneal lining (excluding the diaphragmatic region) should not be underestimated. Arturson (1971; Arturson, et al., 1971) has conducted research on this subject by fitting data for the transperitoneal clearance (blood → peritoneal) of albumin and various sizes of Dextran to a standard twin-pore model. The results are compatible with a model membrane system that is quite isoporous with pore radii of 17 Å. Large pores, 17 Å, are quite infrequent, existing in an approximate ratio of one large pore per 10,000 small pores (Arturson, 1971). In this respect the impermeability of the peritoneal membrane is similar to that of the human glomerular capillary membrane (Arturson, et al., 1971).

If this data is considered in relation to Allen's (1956; Allen and Weatherford, 1959) observations of sphere absorption at the diaphragmatic surface of the peritoneum, the increased permeability of the diaphragmatic region is readly apparent. Since albumin has an Einstein-Stokes radius of approximately 55 Å (Arturson, 1971) its passage through

the 17 Å pores of the non-diaphragmatic peritoneal membrane is restricted. On the other hand, at the diaphragmatic surface, where 20 μm spheres are absorbed, there should be no molecular hindrance to the passage of albumin since the pores are over 10,000 times larger.

While on the subject of the physical nature of the peritoneal cavity, a brief comment on the intraperitoneal pressure can be inserted at this point. As recorded in the supine position, the intraperitoneal pressure in humans averages only 1 mmHg relative to the right atrium (Lam, 1939; Drye, 1948; Orerud, 1953; Bjerle and Sandstrom, 1974). Assuming a standing position induces a slightly negative pressure in the upper parts of the peritoneal cavity, with corresponding increases in the pelvic region (Drye, 1948; Orerud, 1953). This pressure is also increased by various physiological and pathological conditions such as coughing, Valsalva manoeuvers, postoperative meteorism and adynamic ileus, and ascites.

b) The Rate of Intraperitoneal Fluid Absorption

Since before the turn of the century, methodological problems have been a major obstacle in the study of fluid absorption from the peritoneal cavity. It was not without good reason that Starling (1896a) put aside his experiments on intraperitoneal absorption with the statement:

> "We must conclude therefore that it is impossible by this method to obtain a definite answer to the question whether blood vessels can absorb isotonic fluids"

Though techniques remained unchanged, subsequent investigators were less discriminating and Starling's method was used in most of the early studies of intraperitoneal absorption (Fleischer and Loeb, 1910c; Clark, 1921;

Schechter, et al., 1933; Kruger, et al., 1962). The sources of error are readily apparent since the technique is not elaborate. A given volume of fluid is placed in the peritoneal cavity, then after a period of time, the animal is killed and the remaining fluid volume is noted.

Aside from yielding only one estimate of the absorption rate for each experiment, the fundamental drawback of this procedure is the inaccuracy caused by the highly variable amounts of physiological "serous exudate" that are present in the peritoneal cavity before the addition of exogenous fluid (Yoffey and Courtice, 1970; Courtice and Roberts, 1975), and the variable amounts of fluid that can be retrieved from the peritoneal cavity at autopsy (Schechter, et al., 1933).

Courtice and associates (Courtice and Steinbeck, 1950a,b; Morris, 1953) developed a slightly more sophisticated approach to the problem. Anesthetized cats, rabbits or guinea-pigs were injected intraperitoneally with small volumes of serum or saline to which Evans-blue dye had been added in standard concentrations. At various time intervals thereafter, the animals were either killed and the peritoneal fluid analyzed, or plasma samples were obtained to assay the dye concentration. This method provides more data, but as for the determination of absorption rates by examination at autopsy, the same objections as listed above again apply. In addition, the analysis of residual dye-protein concentrations in the intraperitoneal fluid may be complicated by non-specific binding of the dye to the membranes within the peritoneal cavity or by separation of the dye-protein complex (Arab, et al., 1973)

More importantly, there is reason to believe that calculation of the rate of intraperitoneal absorption on the basis of the appearance of the dye in plasma introduces a considerable error of underestimation.

Similar studies with the absorption of ^{125}I-labelled albumin have shown that a major fraction of the label reabsorbed from the peritoneal cavity is not found to be present in the plasma (McKay and Greenway, personal communication).

Shear's experiments (Shear, et al., 1965a,b; 1966). conducted with both humans and experimental animals, have utilized a radioisotope technique to record the rate of fluid removal from the peritoneal cavity. This method is significant because it allows the absorption rate to be calculated for various periods during the course of an experiment. The procedure requires addition of a known bolus dose of radio-iodinated serum albumin to the volume of fluid that is placed within the peritoneal cavity. Blood samples and samples of intraperitoneal fluid are subsequently withdrawn at 30 minute intervals for up to six hours, and the rate of absorption is calculated using indicator-dilution principles (Shear, et al., 1965b). For use in human studies the technique is validated in experimental animals by comparing the calculated absorption rate with the actual volume of fluid remaining in the peritoneal cavity at autopsy.

Unfortunately, Shear's method suffers from the inability to control or quantitate the rate of protein absorption from the peritoneal cavity. If no protein was removed and only free fluid absorbed, the accuracy would be limited mainly by non-specific binding of iodinated albumin in the peritoneal cavity. However, since an unknown quantity of protein is absorbed (Courtice and Steinbeck, 1951a) it is necessary to know the remaining amount of radioisotope in order to calculate the intraperitoneal volume by indicator dilution. Shear, et al. (1965b) have attempted to account for loss of the indicator by assuming that it becomes distributed in the plasma volume after absorption. Thus the lost label is

calculated as the concentration in plasma times a constant factor which they presume estimates plasma volume (79 ml/kg body weight x (1 - hematocrit)). This is unlikely to give a reliable figure in both dogs and man (Lawson, 1962); and is further suspect in their studies of the effects of dehydration and diuretics (Shear, et al., 1966). Compounding this problem, the assumption that protein absorbed from the peritoneal cavity remains distributed in the plasma volume grossly underestimates the extent of removal of the intraperitoneal indicator and could be expected to cause a substantial error in their estimates of the rate of intraperitoneal fluid absorption.

Luttwak et al. (1975; Luttwak, 1973) have improved Shear's technique by incorporating several sequential indicator injections (5 mc ^{125}I human serum albumin) and sampling the intraperitoneal concentrations over a 30 minute period after each injection. The intraperitoneal volume is calculated by indicator-dilution with extrapolation to the concentration at the time of each injection and thus the absorption rate can be determined from relative changes of the intraperitoneal volumes. Technical problems are limited to ensuring adequate mixing of the isotope and assuming that there is little intraperitoneal binding and indicator absorption during the 30 minutes after injection. While the accuracy of this method is quite respectable (as shown by test calculations of the volumes before and after paracentesis), its application is limited by the build-up of background radioactivity after three or four injections.

Perhaps due to these limitations of methodology and the lack of an on-line procedure to monitor intraperitoneal absorption, the literature values for intraperitoneal absorption rates are found to vary widely, and in some cases, show overt discrepancies. However it is important first

of all to distinguish iso-osmotic absorption from the absorption of other fluids.

Since Orlow's first experiments in 1894 (quoted by Cunningham, 1926), it has been known that salt solutions are absorbed from the peritoneal cavity at a rate proportional to their osmotic strength. Thus distilled water is absorbed quite rapidly, at least while it remains hypotonic within the peritoneal cavity (Luttwak, et al., 1975). During the initial period subsequent to placement of a hypo- or hyper-osmotic solution in the peritoneal cavity, there is a rapid exchange of water and salts between blood and the intraperitoneal fluid (Clark, 1921; Cunningham, 1926; Schechter, et al., 1933). Within a short period of time (30 - 90 minutes, depending on the osmolarity) this process renders the injected fluid iso-osmotic to plasma, after which it is absorbed at a rate similar to that of physiological saline (Cornstein, 1895; Fleischer and Loeb, 1910c; Clark, 1921; Cunningham, 1926; Luttwak, et al., 1975).

From this it is evident that the protein impermeable nature of the peritoneal membrane is a major determinant of fluid absorption and ionic exchange during the osmotic phase of absorption; and this, of course, is the fundamental principle on which peritoneal dialysis is based. For the most part, proteins remain either in the peritoneal cavity, plasma or tissue fluid while water and electrolytes cross the peritoneal membrane in accordance with the blood-peritoneal osmotic gradient (Fleischer and Loeb, 1910a,b,c). Alterations of this gradient induced by dehydration, adrenaline or intravenous $CaCl_2$ can change the rate of this fluid transfer (Fleischer and Loeb, 1910c; Shear et al., 1965b; 1966), but only in so far as fluid exchange is controlled by osmosis.

In contrast to the absorption of non-isotonic solutions, iso-

osmotic fluids such as physiological saline, Ringer-Locke solution or serum appear to be absorbed, only through a restricted region of the peritoneum at the inferior surface of the diaphragm (Cunningham, 1926; Losowski and Scott, 1973). There is evidence of some removal by drainage into the lymph of the greater omentum but the rate of this removal is small in comparison with the diaphragm and removal of the omentum does not affect the overall rate of absorption (Higgins and Bain, 1930).

There is considerable variation in the actual rates of fluid absorption which have been reported. Many factors are responsible but not the least of these is species differences. The studies of Courtice and Steinbeck (1950b, 1951a) record that 20 ml/kg of homologous plasma is absorbed from the rat peritoneal cavity in 3 - 5 hours, but in the rabbit 8 hours are required and in the guinea-pig complete absorption does not occur for 16 to 24 hours.

An additional factor which affects the rate of absorption of proteinaceous fluids is that in many cases an inflammatory reaction is mounted to the presence of fresh heterologous plasma (Courtice and Steinbeck, 1951a). This does not occur with solutions of crystalline bovine albumin or if foreign plasma is refrigerated for 2 weeks before use. However when it does occur, the intense inflammatory reaction may persist for up to 8 hours, during which time fluid is actually added to rather than removed from the peritoneal cavity.

Presumably in the absence of such reactions, Allen and Vogt (1937) recorded the absorption of plasma from the rabbit peritoneal cavity at 1.7 - 3.5 $ml \cdot kg^{-1} \cdot hr^{-1}$, while Bolton (1921) found that 30 ml of human ascitic fluid with protein added (7.9%) was absorbed from the peritoneal cavity of cats in 7 hours (approx. 1.7 $ml \cdot kg^{-1} \cdot hr^{-1}$). Raybuck *et al.* (1960b) using

anesthetized cats recorded an absorption rate of about 2 $ml \cdot kg^{-1} \cdot hr^{-1}$ for the removal of homologous serum from the peritoneal cavity. Rates in this general range are most commonly reported but the literature on this subject is diverse and includes absorption rates for serum or plasma ranging from 0 to 25 $ml \cdot kg^{-1} \cdot hr^{-1}$. However this is not entirely due to methodological problems since the rate of fluid absorption is also affected by the physiological status of the experimental animal. Anesthetized animals absorb serum or saline at a rate which may be as low as 30% of conscious animals (Courtice and Steinbeck, 1951a; Morris, 1953). Similarly, abdominal massage speeds absorption and a recumbent or pelvis down position slows the rate of removal (Courtice and Steinbeck, 1951b). In addition the rate of absorption is markedly affected by respiration (Courtice and Steinbeck, 1950a; Morris, 1953). Increased diaphragmatic movements associated with 5% CO_2 inhalation increases the absorption rate 2 - 3 fold while section or paralysis of the phrenic nerves retards absorption to a similar extent. Other factors affecting the rate of intraperitoneal absorption have been reviewed by Cunningham (1926) and by Courtice (Yoffey and Courtice, 1970).

The various studies which have compared the absorption rates of physiological saline with plasma, serum or protein-rich fluid have come to quite different conclusions as to which type of fluid might be absorbed more rapidly. But considering the many problems involved with the study of intraperitoneal absorption and the lack of uniform experimental conditions, a lack of consensus is not surprising.

Luttwak, et al. (1975), using conscious dogs with ligated ureters, compared the intraperitoneal absorption rate of saline with solutions of 1.75 and 3.5 g% protein. Their data appear to demonstrate a faster

absorption rate for the higher protein solution relative to saline, but the rate of absorption of the lower protein solution doesn't differ significantly from that of saline or the higher protein solution. Nevertheless, they interpret the results to indicate that the presence of protein slows absorption. Unfortunately the variability of their data compromises this conclusion and since absorption is recorded as percent of an unknown initial volume, it is not possible to correlate the absorption rates to those of other studies. The observation of reduced absorption with protein could in part be caused by a sensitivity reaction to protein since this was derived from homologous plasma; but this cannot be verified. Also, it is disturbing that, during the first two hours of the experiment, the rate of removal of distilled water is equal to that of physiological saline --- an observation clearly at variance with the literature (Cunningham, 1926; Yoffey and Courtice, 1970).

Other studies on this subject have reported the rate of absorption is not measurably affected by the addition of protein to the intraperitoneal fluid (Bolton, 1921; Raybuck, et al., 1960b; Orlow quoted by Yoffey and Courtice 1970). On the other hand Courtice and Steinbeck (1950b,1951a) have observed a stimulatory effect on absorption with intraperitoneal plasma relative to saline. In the absence of statistics it is difficult to assess the variability of their data, however the recorded difference between the two types of solutions is only about 25%.

There are three lines of evidence to support the proposition that the bulk removal of iso-osmotic saline or plasma is by drainage into the lymph vessels at the peritoneal surface of the diaphragm. The first of these stems from direct observations of the peritoneal cavity while such fluids are being absorbed (Allen, 1956; French, et al., 1960; Casley-

Smith, 1964). A great number of studies have shown that when dyes, erythrocytes, radioisotopes, or particles of any type are added to iso-osmotic intraperitoneal fluid, they are found to enter the diaphragmatic lymphatics very rapidly (e.g. intensely stained within 2 - 5 min, Florey, 1927; Higgins, et al., 1930). Furthermore, it appears that passage through this area accounts for virtually all of the absorption of these substances since they are almost never observed within the lymphatics underlying other parts of the peritoneal membrane (Cunningham, 1926; Higgins, et al., 1930; Courtice and Steinbeck, 1951a; Courtice, et al., 1953; Casley-Smith, 1964). These findings have also been corroborated by lymph angiography (Olin and Saldeen, 1964) and scintigraphic studies with ^{198}Au-colloid present in the absorbed fluid (Langhammer, et al., 1973).

Courtice and Steinbeck (1950a; Courtice et al., 1953) have provided further evidence of the importance of this route by studies with cats in which the right and/or left lymphatic ducts were cannulated for collection of the intraperitoneal fluid absorbed through these channels. Marking the intraperitoneal fluid with Evans-blue or a small number of labelled erythrocytes, lymph was collected over a five hour period of absorption. By quantitative comparisons of the absorption rates with the rates of lymphatic recovery, it was demonstrated that over 95% of the absorbed fluid passed through the thoracic and right lymph ducts. The ratio of absorption by the right lymph duct relative to absorption through the thoracic duct averaged 4 to 1 (Courtice and Steinbeck, 1950a) (Figure 9).

The final, and perhaps definitive, body of evidence supporting a diaphragmatic route of absorption stems from studies in which absorption

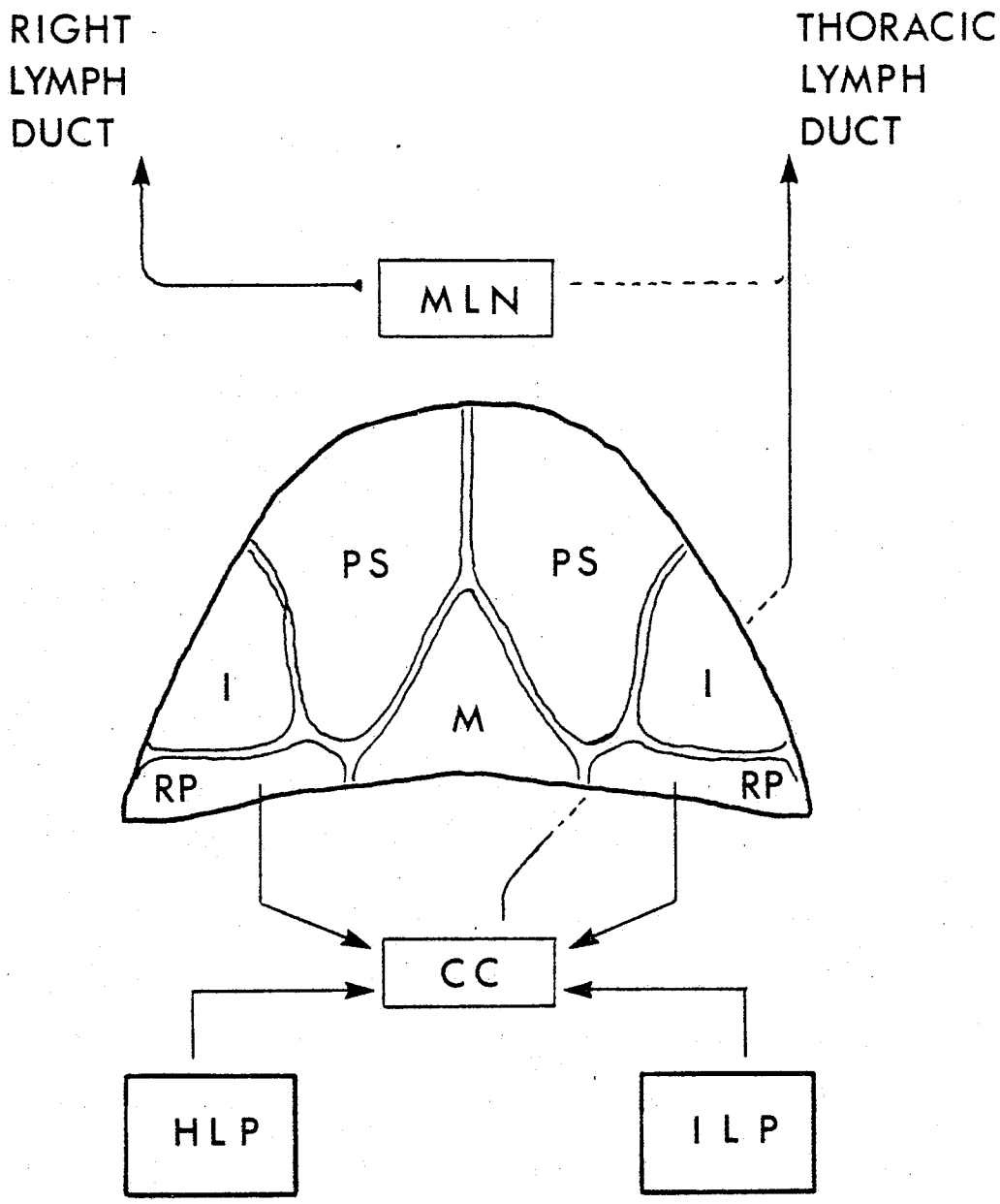


Figure 9. Lymphatic drainage from the peritoneal surface of the diaphragm. Two small retroperitoneal sections of the diaphragm (RP) drain into the cisterna chyli (CC), which also conducts lymph from the intestinal lymph pool (ILP) and hepatic lymph pool (HLP) to the thoracic lymph duct. All of the remaining sections of the diaphragm, including the mediastinal (M), parasternal (PS) and intercostal (I) sections, drain into the mediastinal lymph nodes (MLN) and right lymph duct. (Figure drawn from data presented by Olin and Saldeen 1964, and Courtice and Steinbeck 1950a).

through these lymph vessels was prevented. Whereas Starling (1896a) failed in this attempt because he considered only the thoracic lymph duct, Courtice and Steinbeck (1951b) have succeeded by the combined ligation of the right and left lymphatic ducts. Under these conditions, or when the parasternal lymph channels are ligated, very little (approx. 15%) of the intraperitoneal fluid is absorbed into the circulation of conscious animals. However, large volumes of fluid are recovered from the pleural cavity, presumably due to spillage from the occluded mediastinal lymphatics. Since this pool of fluid is subsequently reabsorbed into the pleural lymphatics, it is reasonable to expect that this would account for the small amount of fluid which gained access to the circulation.

Raybuck et al. (1960b) performed similar experiments in cats which were prepared with fibrotic lesions on the peritoneal surface of the diaphragm. In operations performed two months before the experiment, this was accomplished by abrasion of the inferior surface of the diaphragm and application of talc to the denuded surface. By obliteration of the diaphragmatic lymph drainage with this procedure, the absorption in these animals ranged from 2 to 9% of the rates occurring in animals with intact lymphatics.

III Ascites in Human Disease and In Experimental Animals

Despite intensive research, the phenomenon of ascites remains a complex problem with an etiology that is only poorly understood. Controversy related to the isolation of causal factors in ascites has persisted mainly because, in humans, this condition is associated with such a multiplicity of cardiovascular, hormonal and metabolic derangements. Thus to discriminate the non-causal features which arise secondary to the development of ascites, it is valuable to consider the conditions which can give rise to ascites in experimental animals.

a) Experimental Ascites

i) Models of Exudative Ascites

Ascites produced in experimental animals may be broadly classified as either exudative or transudative. Exudative ascites is generally brought about by an inflammatory or neoplastic process within the abdomen which causes a high protein fluid to weep from the serosal surface. In most mammals inflammatory ascites and peritonitis can be induced by the intraperitoneal injection of either carrageenin (Willis, 1974) or bovine serum albumin with Freund's adjuvant (Baradi and Campbell, 1974; Jacobs and Kaliss, 1975). Sex, hormonal and genetic factors are important determinants of the exudative response (Jacobs and Kaliss, 1975) perhaps by their effects on metabolic processes which appear to transform the peritoneal mesothelial cells into active secretory elements (Baradi and Campbell, 1974).

Exudative ascites can also be produced by the injection of malignant ascitic fluid or transplantable carcinoma cells into the peritoneal cavity (Holm-Nielson, 1953; Straube, 1958; Altman, et al., 1975;

Fastaia and Dumont, 1975). In this case there are at least two processes which contribute to the accumulation of intraperitoneal fluid. Firstly, the presence of viable tumor cells, but not their medium or lyzed contents, initiates an inflammatory reaction involving the mesothelium. The peritoneal cavity subsequently fills with an exudative fluid containing high concentrations of protein, prostaglandins and various autocoids (Sykes, 1970; Feldman, 1975; Greenbaum et al., 1975). A second process obstructs the peritoneal lymph drainage. Both free tumor cells and metastatic clumps of cells from solid tumors are found to migrate to the lymphatics at the inferior surface of the diaphragm and occlude the vessels by proliferation at this site (Holm-Nielson, 1953; Feldman and Knapp, 1974; Feldman, 1975).

The relative importance of these two processes remains a subject of controversy (McDermott and Brown, 1964) but other aspects of exudative ascites are also poorly understood. The inflammatory irritation of the peritoneum seems to occur in two stages. The initial reaction is in many respects similar to that which occurs with the intraperitoneal injection of autocoids but in this case cell-mediated events may trigger the response. Subsequently, and only if the preliminary reaction occurs, a second phase of the reaction allows implantation of the tumor cells in the mesothelium (Straube, 1958; Feldman, 1975). The peritoneal permeability changes which accompany these events have yet to be quantitated and their time course studied. While most researchers believe that protein enters the peritoneal cavity by passive diffusion through the irritated peritoneal membrane (Feldman, 1975; Greenbaum, et al., 1975), Baradi and Campbell (1974) have proposed that the protein is synthesized de novo in the mesothelium and secreted into the peritoneal cavity. In any case, it

is noteworthy that the ascitic protein concentration does not equilibrate with the low protein intestinal lymph and tissue fluids.

ii) Models of Transudative Ascites

Transudative ascites is the form of fluid accumulation that is commonly associated with decompensated hepatic cirrhosis in humans. Compared to exudative ascites, the protein concentration is usually half as great, although in the initial stages of accumulation this may not always be the case (Witte, et al., 1969a,b,c). The first known experimental model of this kind of ascites dates from the observations of Richard Lower in 1669 (McDermott and Brown, 1969). His method, which still remains as the most reliable way to induce ascites, involves the partial ligation of the thoracic inferior vena cava in dogs.

With the refinement of this technique by Bolton (1924) and later by McKee et al. (1948,1949,1950,1952), an aluminum or cellophane band is fitted to the inferior vena cava so as to reduce its diameter by 50 to 75%. This obstruction to hepatic venous outflow does not initially cause pathological changes in the liver but within 2 - 6 days the development of ascites is apparent. However 3 to 12 months after placement of the band the ascitic volume begins to decline and may spontaneously disappear (Bolton, 1914). If the animal is sacrificed at this time, two pathological changes are noted at autopsy. The porto-caval collateral circulation is well developed and often completely established coincident with total occlusion of the inferior vena cava by scar tissue (Drapanas, et al., 1960). In addition there is evidence of hepatic parenchymal degeneration with connective tissue infiltration. Actually, the pathological changes in the liver develop gradually and are only manifest as the characteristic

"hob-nail" liver in the latest stages (Zimmerman and Hillsman, 1930). Liver necrosis and repair can be observed as early as six days after placement of the occluder. This progresses to overt central degeneration with the obliteration of entire lobules in many parts of the liver. Finally, fibrosis of the portal canals and central veins eventually leads to the septal rearrangement that is evident at autopsy (Zimmerman and Hillsman, 1930; Simonds and Callaway, 1932).

The venous obstruction produced by suprahepatic caval constriction leads to numerous manometric and hemodynamic alterations within the splanchnic circulation (Drapanas, et al., 1960; Witte et al., 1971b) but it is pertinent to consider those which are most directly associated with the development of ascites. Using this experimental model in the dog, ascites increases rapidly at a rate of approximately 200 - 400 ml/day (McKee, et al., 1950) until an approximate balance is attained when the ascitic volume averages 2.5 to 5 litres (Drapanas et al., 1960; Orloff, et al., 1964). At this stage, the intraperitoneal fluid is maintained in dynamic equilibrium with the lymph and tissue fluids bordering the peritoneal cavity (Prentice, et al., 1952; Courtice, 1959). If radioactively labelled plasma is injected into the peritoneal cavity, 32% of the original dose is absorbed in 24 hours with only 8% found circulating in the plasma (McKee, et al., 1952). This rapid turnover of the ascitic fluid is also manifest by the rate of appearance of the isotope in the peritoneal cavity when it is injected intravenously (McKee, et al., 1950).

In this form of ascites, flow through the thoracic lymph duct is greatly increased. This was recorded by Starling (1896b) in his earliest studies of edema. At a later time it was also reported by his cohort Bolton (1924) who observed that the increased rate of flow could

not be attributed to the absorption of ascites from the peritoneal cavity.

If the accumulation of ascites is modest, thoracic duct lymph flow may be increased only three-fold to about 90 ml/hr (van der Heyde, et al., 1964; Witte, et al., 1969d); however with greater obstruction of the hepatic venous outflow, the flow rate may be as high as 220 ml/hour (Cain, et al., 1947; Orloff, et al., 1966). Exploratory laparotomy at this stage has revealed a striking distension of the hepatic lymph vessels (Hyatt, et al., 1955; Kato and Tsuchiya, 1964; van der Heyde, et al., 1964) coincident with vigorous transudation of fluid droplets over the entire surface of the liver (Hyatt and Smith, 1954; Hyatt, et al., 1955). Although transudate could be readily collected from the liver surface, this was not possible from the peritoneal or intestinal surfaces which remained only slightly moist after prolonged exposure (Hyatt, et al., 1955).

This data in combination with much additional evidence from both human and animal studies is now taken as presumptive evidence that the ascitic fluid originates from the liver rather than the portal circulation as it was earlier believed (Brauer, 1963; McDermott and Brown, 1964; Greenway and Stark, 1971; Shear, 1973). In addition, this has been confirmed by transferring the liver to a site above the diaphragm a few weeks prior to the experimental production of ascites. With subsequent constriction of the vena cava or administration of carbon tetrachloride, the "ascitic" fluid accumulates almost entirely within the pleural cavity (Freeman, 1953; Aiello, et al., 1960).

In spite of extensive hepatic congestion and fibrosis, the metabolic functions of the liver parenchyma are not grossly compromised

in dogs with experimental ascites. Bromsulphalein clearance is unaltered and the ability to synthesize plasma proteins is quantitatively similar to that of normal animals (McKee, et al., 1948; Hyatt and Smith, 1954). Even so, there exists a unique redistribution of the plasma proteins which is sensitive to the intake of dietary protein (McKee, et al., 1948).

Many studies of protein concentrations in ascites are available but the observations of Hyatt, et al. (1955) are particularly valuable since they include analysis of the transudate from the liver capsule. In this study the ascitic protein concentration averaged 2.8 g% in seven animals. The value for plasma, liver lymph and capsular fluid were all about 40% greater; averaging 4.2 g%, 4.0 g% and 3.95 g% respectively. Similar data have been reported in other studies (Waugh, 1958; Drapanas, et al., 1960; Kato and Touchiya, 1964; Witte, et al., 1971a); with ascitic protein concentrations about 35 - 40% less than the plasma level.

With the spontaneous remission of experimentally induced ascites it is curious that the oncotic and pressure gradients, which initially favoured the transudation of fluid into the peritoneal cavity, are not substantially reduced (Waugh, 1958). Portal pressure often returns to normal levels with the opening of collateral vessels but in many cases this occurs within weeks after placement of the caval band and the build-up of ascites is unaffected (Hyatt, et al., 1955; Drapanas, et al., 1960). Waugh (1958) has speculated that the remission of ascites under these circumstances is due to fibrosis of the liver capsule but there is also reason to suspect that hormonal changes and intrahepatic hemodynamic changes are involved (Drapanas, et al., 1960; Schaffner and Popper, 1963; Papper and Vaamonde, 1971).

Whereas constriction of the thoracic inferior vena cava invariably gives rise to ascites in experimental animals, this is rarely, if ever, the result when the portal vein is constricted (Hyatt and Smith, 1954; McDermott and Brown, 1964). Even if the inferior vena cava is simultaneously constricted above the renal veins (Schilling et al., 1952), portal obstruction fails to produce ascites (Gray, 1951; Douglas, et al., 1951; Raybuck, et al., 1960a) unless the animal is made hypoproteinemic by repeated plasmapheresis (Volwiler, et al., 1950; Wiles, et al., 1952). But even this ascites is transient, and the small volume which is produced contains a very low concentration of protein (Volwiler, et al., 1949).

Witte et al. (1969d; Witte and Witte, 1969;1974) attribute the lack of success with this procedure to the inability to maintain portal hypertension. The extrahepatic portal pressure may rise to 25 or 30 mmHg shortly after the band is in place but over a period of 5 - 15 days the portal pressure gradually returns towards control levels unless the vein is again constricted. Yet in some cases a sustained increase of portal pressure is achieved without the development of ascites. Thus the intrahepatic pre-sinusoidal obstruction caused by experimental silica fibrosis of the liver in dogs (Rousselot and Thompson, 1939) or by infection with Schistosoma japonica in chimpanzees (von Lichtenberg, et al., 1973) is only rarely accompanied by overt ascites.

By far the most common experimental model of ascites involves constriction of the suprahepatic vena cava. However, various types of congestive heart failure can be induced in experimental animals with the production of ascites similar to that which develops after caval constriction. This can be demonstrated either with high-output failure after

construction of an aorto-caval fistula (Davis, et al., 1964b; Stumpe, et al., 1973), or with low-output failure after mitral stenosis (Hamilton, 1965; Hamilton, et al., 1954) or combined pulmonary stenosis and tricuspid insufficiency (Hamilton, et al., 1954).

Liver cirrhosis can be produced in laboratory animals by the administration of various hepatoxins, but there has been little attention paid to the formation of ascites in these experiments. Since it is difficult to establish that the experimental lesions are comparable to those in human cirrhosis (Nakata, et al., 1973), there might be little advantage in producing ascites by a toxic injury rather than simply by banding the vena cava. In any case, many months are required for the development of ascites and even then the volume of ascitic fluid is not impressive.

Strict nutritional cirrhosis rarely causes ascites (Gilman and Chaikoff, 1949; Rogers and Newborne, 1973; Wade, et al., 1974) but the administration of carbon tetrachloride may elicit small volumes after 3 to 6 months (Nix, et al., 1951; Mazzacca, et al., 1975). However with the administration of dimethylnitrosamine, large volumes (3-4 litres) of ascites can be produced in dogs; though apparently not in the cat (Zink and Greenway, unpublished observations). This form of cirrhosis is associated with gastrointestinal bleeding, increased cardiac output, portal hypertension and a highly variable protein concentration in the ascitic fluid (0.13 - 4.1 g%; Kreuzer, et al., 1972). Histologically, extensive centrilobular fibrosis seems to account for the increased post-sinusoidal vascular resistance (Butler and Hard, 1971).

b) Ascites in Human Disease

i) Occurrence of Exudative and Transudative Ascites

In human disease it is often difficult to differentiate exudative from transudative ascites. Most clinical studies consider the presence of more than 2.5 g% protein in the ascitic fluid as evidence of an ongoing neoplastic, infectious or inflammatory process; yet about 19% of hospital admissions for uncomplicated cirrhosis and ascites have protein concentrations exceeding this arbitrary figure (Sampliner and Iber, 1974). Also, cases of hepatoma or cholangiocarcinoma differ from the usual abdominal cancers by producing ascites with a much lower protein concentration similar to that which occurs in cirrhosis. Thus it is important to utilize other diagnostic criteria in the identification of exudative ascites.

Ascitic fluid analysis can include smear and culture for microorganisms and tumor cytology as well as erythrocyte and white blood cell counts. Non-infected ascites generally has less than 300 leukocytes/μl, few of which are polymorphonuclear; however prompt ascitic fluid bacteriology is the most reliable measure of infection (Kline, et al., 1976). Ammonia levels above 3 μg/ml are indicative of exudative ascites due to gastrointestinal perforation or urinary extravasation (McDermott and Brown, 1964).

Neoplastic processes responsible for ascites usually involve widespread dissemination of malignant disease throughout the abdominal cavity with multiple nodules growing from the serous surfaces and omentum Common causes are colloid carcinoma of the gastrointestinal tract and ovarian carcinoma (Holm-Nielson, 1953; Hirabayashi and Graham, 1970). Non-cancerous causes of exudative ascites are pancreatitis, peritonitis, myxedema, tuberculosis and various rare granulomatous processes (Donowitz,

et al., 1974). It is not established whether nephrogenic ascites should be considered "exudative" but the ascitic fluid protein concentrations range from 4.3 to 5.7 g% (Craig, et al., 1974).

The literature on transudative ascites in humans is truly voluminous but several reviews on the subject are available (Hyatt and Smith, 1954; Courtice, 1959; Summerskill, 1969; Conn, 1972). While detailed coverage is impossible, the present discussion will attempt to consider the differences of human ascites from experimental ascites in laboratory animals, and examine the most current theories of ascites pathogenesis.

Ascites occurs in human congestive heart failure coincident with generalized systemic edema (McDermott and Brown, 1964). However in humans the incidence of ascites is only 25% (White, et al., 1955; Dunn, et al., 1973), whereas it occurs without peripheral edema in almost 100% of animals with experimentally induced failure. Although central venous pressure is not invariably increased in human heart failure with edema, systemic venous hypertension is even less frequent in congestive failure in animals (Hamilton, 1954; Rapaport, et al., 1958). In the latter case, there may be anatomical and humoral differences which more readily localize edema fluid in the peritoneal cavity (Hamilton, 1954).

In any event both types of heart failure have in common hepatomegaly, engorgement of the hepatic hilar lymphatics, infrequent portal hypertension and widening of the arteriovenous oxygen difference (Witte and Witte, 1975). Most functional disturbances of the liver can be attributed to hypoxia and sometimes centrilobular atrophy (Dunn, et al., 1973). If ascites occurs in human congestive failure, it can be due either to congestion with the retention of sodium and body fluids or to cardiac

cirrhosis. The latter accounts for about 20% of all cases of cirrhosis examined randomly at autopsy (Koletsky and Barnebee, 1944). Still, the development of cardiac cirrhosis may not worsen hepatic function and ascites since often improvement of the cardiac status and relief of venous hypertension leads to the remission of the ascites even though cardiac cirrhotic changes remain (Sherlock, 1957). Also, there is no good evidence that cardiac cirrhosis *per se* increases the incidence of ascites, splenomegaly, portal hypertension or bleeding varices (Dunn, *et al.*, 1973).

The most common cause of ascites in humans is hepatic cirrhosis (either postnecrotic or Laennec's cirrhosis) but severe hepatitis, fatty infiltration of the liver, hepatic vein obstruction (Budd-Chiari syndrome) and numerous other liver diseases also cause transudative ascites. In cirrhosis, the accumulation of ascites may exceed 30 litres (Summerskill, 1969) and raise the intraperitoneal pressure as high as 37 mmHg (Orerud, 1953; Orloff, 1970). The ascitic protein concentration is highly variable and may exceed 4 g% in some cases (Witte, *et al.*, 1969c; Sampliner and Iber, 1974); however the usual concentration is less than 1.5 g% Atkinson and Losowski, 1961; Witte, *et al.*, 1968). Relative to the ascites in animals with caval constriction, protein concentrations are considerably less in human ascites (10 - 15% of plasma protein, c.f. 60-65% in animals; Witte and Witte, 1975).

By the time that cirrhosis progresses to the stage that ascites develops, almost every human physiological function is affected. This complexity has not only impeded progress in our understanding of ascites, but in some cases it has caused a fundamental redirection of experimental approaches to determining the causal factors in ascites. In particular,

two aspects of ascites have interferred with attempts to establish a coherent outline of its pathogenesis. Firstly, there is the whole spectrum of renal and hormonal abnormalities which underlie fluid retention in cirrhotic patients. Secondly, there is the very low concentration of protein in the ascitic fluid.

ii) Renal Involvement in Cirrhotic Ascites

In both humans and experimental animals, the status of cirrhotic ascites is closely correlated to renal dysfunction and sodium retention (Hyatt and Smith, 1954; Losowski, et al., 1963). The ability to excrete urinary sodium is limited even with excess dietary salt, so that the sodium concentration is invariably low in the urine of ascitic patients or experimental animals (Conn, 1972; Blendis, 1975). Oral or intravenous salt loading results in fluid retention and an increased volume of ascites proportional to the exogenous sodium (McKee, et al., 1948; Schilling, et al., Waugh, 1958). Conversely, a low sodium diet diminishes the ascitic volume (McKee, et al., 1948; Courtice, 1959).

Aldosterone concentrations in human plasma are invariably increased with the development of ascites (Luetscher and Johnson, 1954; Wolff, et al., 1958; Coppage, et al., 1965) and this contributes not only to renal sodium retention but also to parallel ionic changes in sweat and saliva (Darnis, 1971). In animals with ascites produced by caval constriction, aldosterone levels are also elevated (Davis, et al., 1957b; Conn, 1972) and the extent of sodium retention is well correlated to the concentration of aldosterone in the plasma (Davis, et al., 1953). While it is generally thought that the stimulus for aldosterone secretion should be hypovolemia due to the loss of intravascular fluid into the peritoneal cavity, the blood volume calculated in both humans and experimental animals

with ascites is consistently elevated above normal (Courtice, 1959; Dykes, 1961; Lieberman and Reynolds, 1967; Lieberman, et al., 1969). Thus the conventional theories of ascites pathogenesis, which account for fluid retention on the basis of pre-renal deviation, are frustrated by the paradox of inappropriate aldosterone secretion (Lieberman and Reynolds, 1967; Epsetin, et al., 1976).

As a result, alternative interpretations have blossomed. Since Eisenmenger's initial proposal in 1952, various investigators have attempted to reconcile the hyperaldosteronism in cirrhotic ascites by viewing the renal disturbances as primary factors which are the cause of ascites rather than secondary adjustments to its occurrence (Halsif, et al., 1953; Papper, 1958; Lieberman, et al., 1970). While at first this seems quite illogical, it is impressive that this theory has received at least tentative acceptance in many reviews on the subject of ascites (Darnis, 1971; Losowski and Scott, 1973; Arroyo and Rodes, 1975). Yet it is the very close interaction between fluid balance and ascites which makes this hypothesis somewhat difficult to refute at the present time.

Basically, the "overflow" theory as developed by Lieberman (Denison, et al., 1971) proposes that cirrhotic changes in the liver cause hormonal and renal hemodynamic aberrations which promote sodium retention and plasma volume expansion. As a consequence of the increased plasma volume, portal hypertension is considered to "mechanically expand the splanchnic bed, creating the conditions for ascites to form as plasma volume expands further" with the progressive renal dysfunction induced by cirrhosis (Lieberman, et al., 1970). The mechanism which activates the kidney in response to hepatic cirrhosis is not elaborated but three possibilities have been put forth: impaired hepatic inactivation of aldosterone

(Barnardo, et al., 1969) or of splanchnic renin (Coppage, et al., 1962); or hepatic production of a humoral stimulator of aldosterone (Orloff, et al., 1965).

In clinical ascites there are few, if any, observations that blatantly disprove the overflow theory. In these patients total body water, total plasma volume, and total body exchangeable sodium are increased (Talso, et al., 1956; Birkenfeld, et al., 1958; Clowdus, et al., 1961). Moderate hyponatremia may be present, but regardless of sodium intake, urinary sodium excretion is markedly reduced ... sometimes to as little as 1 mEq/day (Eisenmenger, 1952; Gabuzda, et al., 1953; Lieberman, et al., 1970). Since water is retained in accordance with the retention of sodium (Courtice, 1959), dietary sodium in excess of 10 - 15 mEq/day directly contributes to fluid retention localized in the ascitic compartment (Gabuzda, et al., 1954; Courtice, 1959; Losowski, et al., 1963). This practically complete retention of sodium is generally associated with severe oliguria that is unrelieved by loading with oral or intravenous water (Darnis, 1971). In the absence of effective medical therapy, this oliguria usually progresses to terminal renal failure (Summerskill, 1969; Conn, 1972).

Both hormonal and renal hemodynamic factors may be responsible for sodium retention in ascites. The glomerular filtration rate (GFR) is found to vary widely in patients just beginning to accumulate ascites but on the average it is reduced only 14% (Jones, et al., 1952; Papper and Vaamonde, 1971). Values for renal plasma flow show a similar trend (Epstein, et al., 1950). Since the GFR may or may not be reduced, it would seem that increased tubular reabsorption is responsible for the retention of sodium. However, a reduction of the filtered sodium may be accessory

in the later stages when the GFR and renal plasma flow are reduced (Summerskill, 1969; Klinger, et al., 1970; Papper and Vaamonde, 1971). Also, when large volumes of ascites are present, the increased intraperitoneal pressure may reduce GFR and renal blood flow either by venous or ureteral compression (Sherlock and Shaldon, 1963; Papper and Vaamonde, 1971); although with paracentesis the GFR is only transiently improved (Gordon, 1960).

Aldosterone is the most obvious hormonal factor that could account for the avid sodium retention in ascites and virtually all studies have recorded increased aldosterone levels in plasma (Luetscher and Johnson, 1954; Coppage, et al., 1962; Summerskill, 1969). Often the levels exceed those found in primary aldosteronism.

The plasma disappearance curves for aldosterone are decreased in cirrhotics with ascites (Coppage, et al., 1962) as is the rate of hepatic clearance (Chart, et al., 1956), but these are accessory factors and only increase aldosterone concentrations by enhancing the primary effect of an increased rate of secretion (Ball, et al., 1957; Coppage, et al., 1962; Summerskill, 1969). On this point the data supplied by Coppage et al. (1962) are perhaps representative. In their study, adrenal aldosterone secretion was investigated in cirrhotic patients with ascites and in normal controls subjected to an equivalent sodium intake. In patients with ascites the secretion rate was increased to 421 - 2080 µg/day (cf 75 - 272 µg/day) and urinary excretion rates were 95 - 249 µg/day (cf 5 - 26 µg/day, conjugated metabolites included). Further evidence of the importance of aldosterone in ascites comes from findings of high potassium concentrations in urine, sweat and saliva, adrenal hyperplasia at autopsy and the therapeutic value of aldosterone

antagonists (Papper and Vaamonde, 1971).

A close correlation of the rate of ascites accumulation and the rate of aldosterone secretion has been demonstrated in both human studies and in experiments with ascites induced by caval constriction in dogs. Davis et al. (1952;1953;1964a) have shown that administration of desoxycorticosterone acetate (DOCA) to dogs with experimental ascites does not further increase the volume of ascites. However, adrenalectomy or metapyrone administration leads to diuresis and the remission of ascites despite the continuation of caval constriction. The animals could be subsequently maintained without ascites on maintenance doses of DOCA; or caused to reaccumulate ascites by increasing the dosage of mineralocorticoid. An equivalent study has been conducted in humans by Denison et al. (1971). In this case ascites was induced in cirrhotic patients without ascites by administration of the mineralocorticoid 9 - α - fluorohydrocortisone.

Other studies of ascites have attempted to demonstrate disturbances of vasopressin and certain natriuretic hormones (Ralli, et al., 1945; Stephan, et al., 1956; Kramer, 1975). Vasopressin may be only a secondary factor in selected cases (Davis, et al., 1957a), but there is mounting evidence that a natriuretic hormone, synthesized in the liver, may inhibit sodium reabsorption in the proximal tubules of the kidney. Since reduced concentrations of this substance are found in the plasma of patients with chronic liver disease and ascites (Kramer, 1975), possibly a deficiency may act synergistically with the excess of aldosterone to produce sodium retention in ascites (Bricker, 1967; Papper and Vaamonde, 1971).

From the preceding discussion it is apparent that most of the

disorders of fluid and electrolyte balance in ascites are compatable with the "overflow" theory of ascites formation. A mechanism to connect hepatic cirrhosis with functional renal changes has not been established but it has been suggested that either a natriuretic hormone or aldosterone could serve as the "missing link" (Lieberman and Reynolds, 1967; Summerskill, 1969; Orloff, 1970). In the latter case, Orloff (1970) has shown that elevation of hepatic venous pressure in experimental animals causes an immediate elevation of the circulating aldosterone concentration without measurable effects on vital signs, behaviour or renal hemodynamics. In any event it should be apparent that all of the data which has been taken as evidence in support of the overflow theory is based purely on the correlation of ascites to renal disorders. As such, this cannot prove that there is any validity to the claim of a primary renal disturbance in ascites.

Yet if the renal and hormonal factors in ascites are secondary to the loss of intravascular fluid by transudation into the peritoneal cavity, it is necessary to establish the stimulus for excessive aldosterone secretion. If such a stimulus were established, the involvement of salt and water retention in ascites could be viewed as a proper homeostatic mechanism to maintain the intravascular volume. The deleterious effects of activating this volume support system would be caused by elevation of portal or hepatic sinusoidal pressures due to the sequestration of splanchnic blood in cirrhosis (Lieberman and Reynolds, 1967; Witte and Witte, 1975) and by possible renal impairment due to overstimulation and oliguria.

To justify this interpretation it would be convenient if the formation of ascites and loss of intravascular volume was associated with

contraction of the plasma volume. Unfortunately the total plasma volume is increased even in the earliest stages of ascites (Eisenmenger, 1952; Darnis, 1971). However in cirrhosis, particularly with accompanying portal hypertension, the measurement of total plasma volume is undoubtedly the most crude index of cardiovascular proprioception, since a major fraction of the blood volume is sequestered in the high-compliance portal vasculature (Boyer, et al., 1966; Zimmon and Kessler, 1974). Thus, by virtue of the redistribution of blood volume it is possible that in fact the thoracic blood volume could be reduced and causing reduced activation of mechanoreceptors in this region —— even though the total blood volume is greater than normal (Courtice, 1959; Epstein, et al., 1976). While at the present time this has not been conclusively demonstrated, certainly it is the most likely explanation of sodium retention in ascites.

Several observations in ascites indicate that a localized contraction of the blood volume activates renal sodium conservation. Coincident with the elevation of cardiac output and development of arteriovenous anastomoses (Krook, 1956; Martini, et al., 1972) the arterial pressure and pulse pressure are often reduced (Darnis, 1971; Presig, et al., 1972), and, as possible evidence of localized hypovolemia, renin levels are consistentlyfound to be much increased in spite of reduced levels of the renin substrate (Biron, et al., 1964; Rosoff, et al., 1975). But perhaps the most significant evidence is that "central hypervolemia" induced in ascitic patients by water immersion to the neck, causes diuresis and natriuresis within a period of two hours (Epstein, et al., 1976). Since this is coincident with the lowering of plasma aldosterone concentrations, this response may be taken to support the thesis that the central blood volume was previously contracted and responsible for sodium retention.

iii) Significance of the Protein Concentration in Ascitic Fluid

The site of the vascular obstruction in human liver disease is highly variable. In hepatic schistosomiasis (von Lichtenberg, et al., 1973; Aboul-Enein, 1973) and in the early phases of Wilson's disease (tenHove and Leevy, 1973), portal hypertension is caused by pre-sinusoidal obstruction (Sherlock, 1974). Although gastrointestinal capillary pressure is increased and blood pools within the portal vascular bed, there is only a transient phase of moderate ascites with perhaps the loss of some fluid into the lumen of the gut (Yoffey and Courtice, 1970; Yablonski and Lifson, 1976). In contrast, veno-occlusive diseases of the liver such as the Caribbean bush-tea poisoning (Bras and Hill, 1956) or hepatic vein thrombosis (Budd-Chiari syndrome, Gibson, 1960), increase the post-sinusoidal resistance and are always associated with massive ascites (Witte and Witte, 1974).

The actual location of the vascular lesion in the various forms of cirrhosis is currently a subject of much debate (Kelty, et al., 1950; Rogers and MacDonald, 1965; Tandon, et al., 1970; Wade, et al., 1974). However from experimental studies and examination of biopsy samples from humans, the consensus seems to favour a "para-sinusoidal" lesion; although the variability is great in any group of patients (MacDonald, 1962; Kato and Tsuchiya, 1964; Nakata, et al., 1973). It is now generally recognized that the ascites which occurs in these patients evolves from fluid transudation from the hepatic vascular bed (Summerskill, 1969; Yoffey and Courtice, 1970; Arroyo and Rodes, 1975), but it is perplexing that the usual ascitic protein concentration is less than 1.5 g% (Hyatt and Smith, 1954; Witte, et al., 1971b). This is unlike experimental ascites in laboratory animals where the protein levels are usually 2.5 -

4.5 g% (McKee, et al., 1952; Waugh, 1958; Witte, et al., 1969d).

Since the protein concentration of ascitic fluid is quite close to that of intestinal lymph (Witte, et al., 1971a), and since portal hypertension and hypoalbuminemia are commonly associated with ascites (Atkinson and Losowski, 1961), for many years it was considered that the intestinal vascular bed was the origin of ascites (Post and Patek, 1942; Higgins, et al., 1947). As a result of this impression, which is still evident in quite recent reviews on ascites (Sherlock and Shaldon, 1963; Losowski and Scott, 1973; Shear, 1973), various theories evolved which attempted to predict the occurrence of ascites on the basis of Starling factors.

At first it was hoped that a critical degree of hypoproteinemia would determine the presence of absence of ascites in cirrhosis (Higgins, et al., 1947; Bjorneboe, et al., 1949; Ricketts, 1951). As it became evident that this was insufficient (Armstrong, 1948; Giges and Kunkel, 1954), Atkinson and Lowsowski (1962; Losowski, et al., 1963) proposed that a threshold for ascites formation could be established by plotting intrasplenic (portal) pressure against the concentration of serum albumin. According to their data, this yielded a "clear-cut" separation between the ascitic and non ascitic groups of patients (Atkinson and Losowski, 1961) (Figure 10). Yet with hindsight, it is apparent that this approach cannot be expected to account for the occurrence of ascites in all cases because it includes only two of the many cardiovascular factors that control fluid exchange in the splanchnic vascular bed (Section 3.I). In addition, a causal relationship of the formative factors in ascites cannot be established by the consideration of these parameters when ascites is fully developed. For example, the concentrations of serum albumin

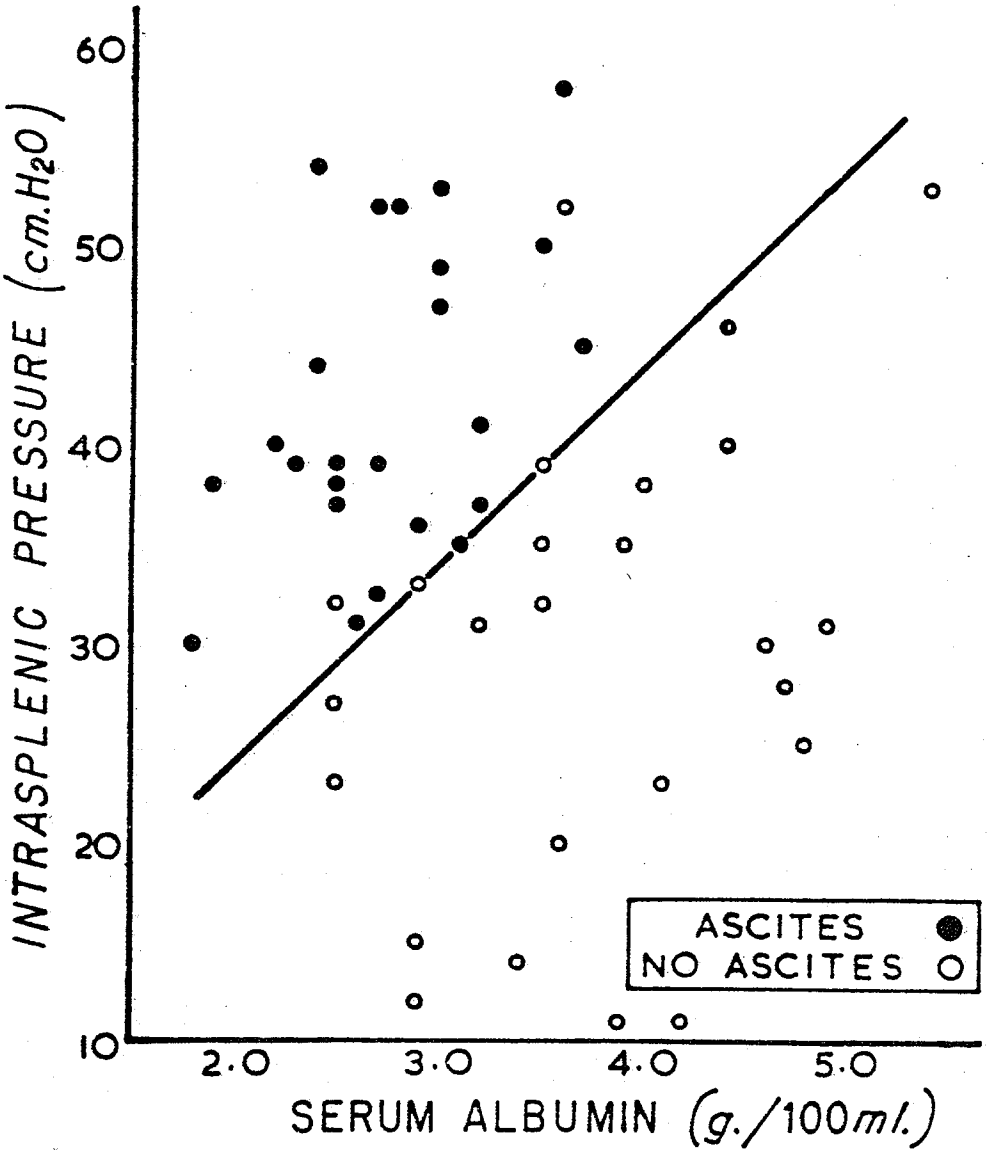


Figure 10. Serum albumin plotted against intrasplenic pressure in patients with chronic liver disease. A straight line can be drawn separating patients with ascites from those without ascites, with a high degree of certainty. (From Atkinson and Losowski, 1961).

are reduced in both human and experimental ascites due to redistribution of the plasma proteins into the ascitic fluid (Boyer, et al., 1969; Zimmon, et al., 1969; Rothschild, et al., 1969b). And, with tense ascites, the portal pressure may be increased by elevation of the intraperitoneal pressure (Volwiler, et al., 1950; Canter, et al., 1959; Iwatsuki and Reynolds, 1973).

Other studies have attempted to further elaborate the transperitoneal gradientswhich contribute to ascites formation by including the components of intraperitoneal pressure and ascitic protein concentration (James, 1949; Waugh, 1958; Cherrick, et al., 1960). Again, these approaches have been unsuccessful due to the neglect of various other factors which are critical in the pathogenesis of ascites (Schaffner and Popper, 1963).

As it came to be recognized that the hepatic vascular bed was an important source of ascites, investigators realized the short-comings of these relatively simple formulae. Yet the low concentration of protein in the ascitic fluid remains a troublesome enigma. For if ascites originates from fluid transudation across the liver capsule and the protein concentration of this fluid is similar to hepatic lymph (3.95 and 4.0 g% respectively, Hyatt, et al., 1954); why then is the concentration reduced 3 to 4 fold in the ascitic fluid? Hyatt and Smith (1954) have suggested that there are two possible explanations. Firstly, it is possible that the process responsible for the reabsorption of ascites could remove more protein than water, resulting in dilution of the fluid remaining in the peritoneal cavity. Secondly, it is possible that the protein in the ascitic fluid could equilibrate with peritoneal and intestinal tissue fluids.

While the first possibility has received little attention, Witte et al. (1969c) have presented somewhat circumstantial evidence to support the latter mechanism. Their studies suggest that in the acute stages of congestive heart failure (Witte, et al., 1969b), or cirrhosis (Witte, et al., 1968), there is a relatively high concentration of protein in the ascitic fluid. However, in patients with chronic congestion or advanced cirrhosis, the ascitic protein concentration is reduced (Dumont and Witte, 1966; Witte, et al., 1969c; Dumont, et al., 1975) (Figure 11).

Witte et al. have interpreted these findings to support their hypothesis of a dual origin of ascites. In the early stages of hepatic cirrhosis, outflow block at the liver predominates and ascites transudates from this organ. Later, in the chronic stages of cirrhosis, or after an ineffective end-to-side portocaval shunt, advanced congestion of the intestinal vascular bed causes ascites to weep from the serosal surfaces of the intestine (Witte, et al., 1969c;1971a)(Figure 12).

Unfortunately this hypothesis is contrary to a considerable body of physiological and medical knowledge. It is well established that the intestinal microcirculation possesses effective defense mechanisms to counteract excessive filtration due to elevation of portal pressure (see Section 3,I,a,iv) and there is some indirect evidence from lymph protein concentrations that this continues even with chronic portal congestion (Figure 13). Whether it operates effectively with chronic congestion is unknown, however graded and progressive constriction of the portal vein in experimental animals and extrahepatic portal hypertension in humans does not cause ascites (Volwiler, et al., 1950; Schilling, et al., 1952; Yoffey and Courtice, 1970).

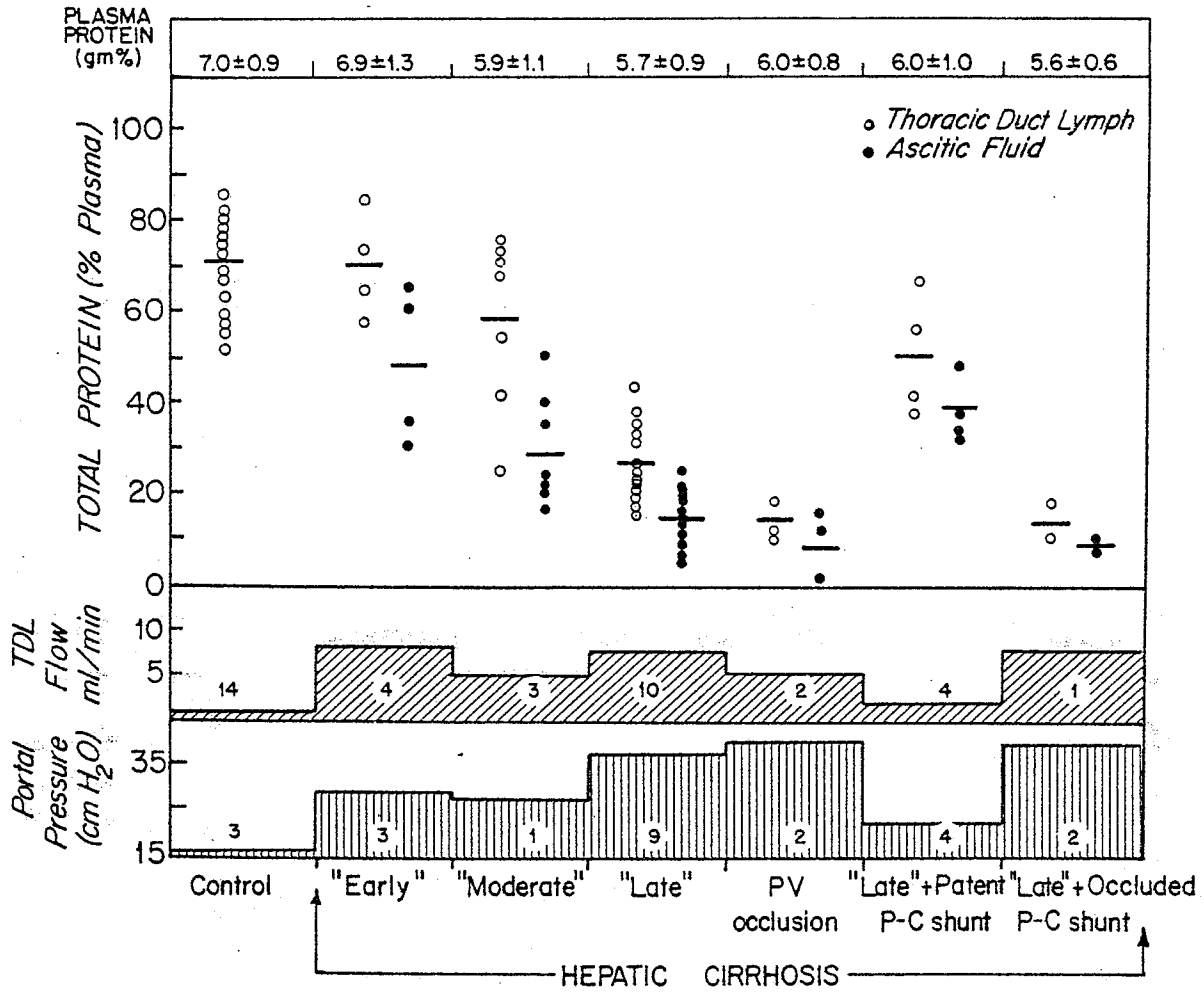
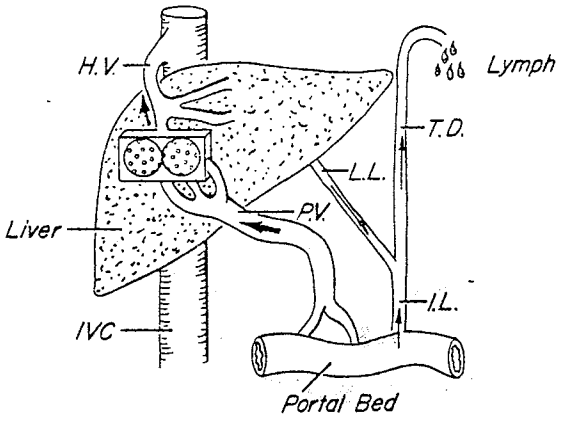
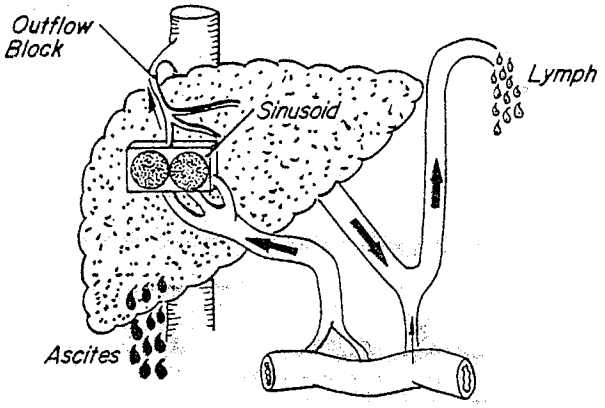


Figure 11. Total protein content in thoracic duct lymph, TDL, and ascitic fluid in 27 patients with hepatic cirrhosis at different stages of the disease. The horizontal bars represent mean values. Alterations in the protein content of each of these fluids are similar and depend on the changing portal circulatory dynamics. Measurements in the thoracic duct lymph of control subjects without liver disease are shown. Mean values of thoracic duct lymph, flow, extrahepatic portal pressure, and plasma protein $\pm$ standard deviation, at comparable stages are indicated. Numerals in the shaded areas represent the number of patients in whom extrahepatic portal pressure and thoracic duct lymph flow were measured. PV, portal vein; P-C, portacaval. (From Witte et al. 1969c).

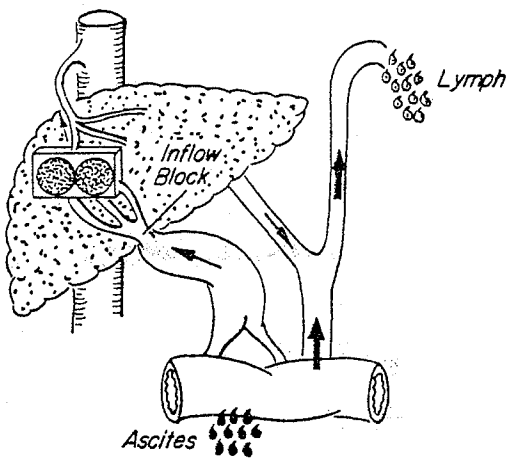
Figure 12. Schematic diagram demonstrating that, depending on portal circulatory dynamics, the origin of ascitic fluid and excess thoracic duct lymph shifts in hepatic cirrhosis. Postsinusoidal obstruction promotes the formation of excess lymph and ascitic fluid primarily from the liver. Presinusoidal obstruction promotes the formation of excess lymph and ascitic fluid primarily from the extrahepatic portal bed. After end-to-side portacaval shunt, ascites probably originates from transected hepatic periportal lymphatics in association with persistent postsinusoidal obstruction, but when the shunt is occluded, ascites originates from unrelieved congestion of the extrahepatic portal bed. TD., thoracic duct; PV., portal vein; H.V., hepatic vein; L.L., hepatic lymph; IVC, inferior vena cava; and I.L., intestinal lymph. (From Witte et al. 1969c).



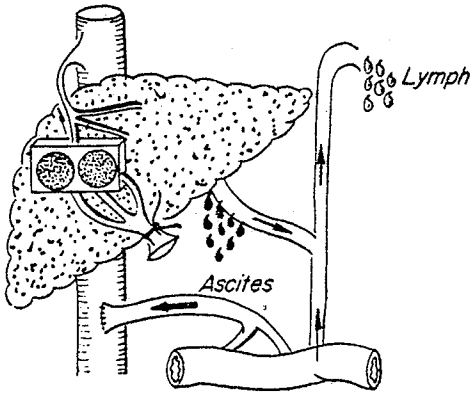
NORMAL



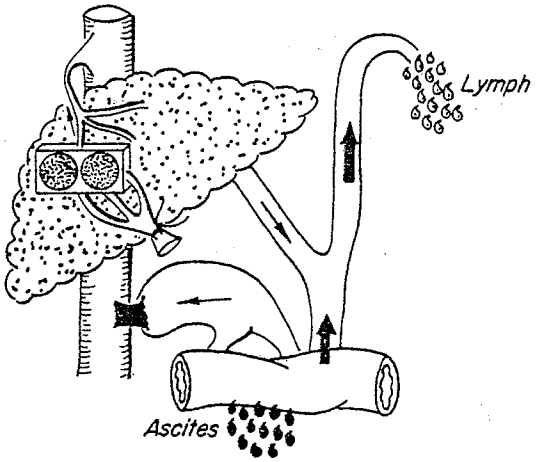
EARLY CIRRHOSIS



LATE CIRRHOSIS

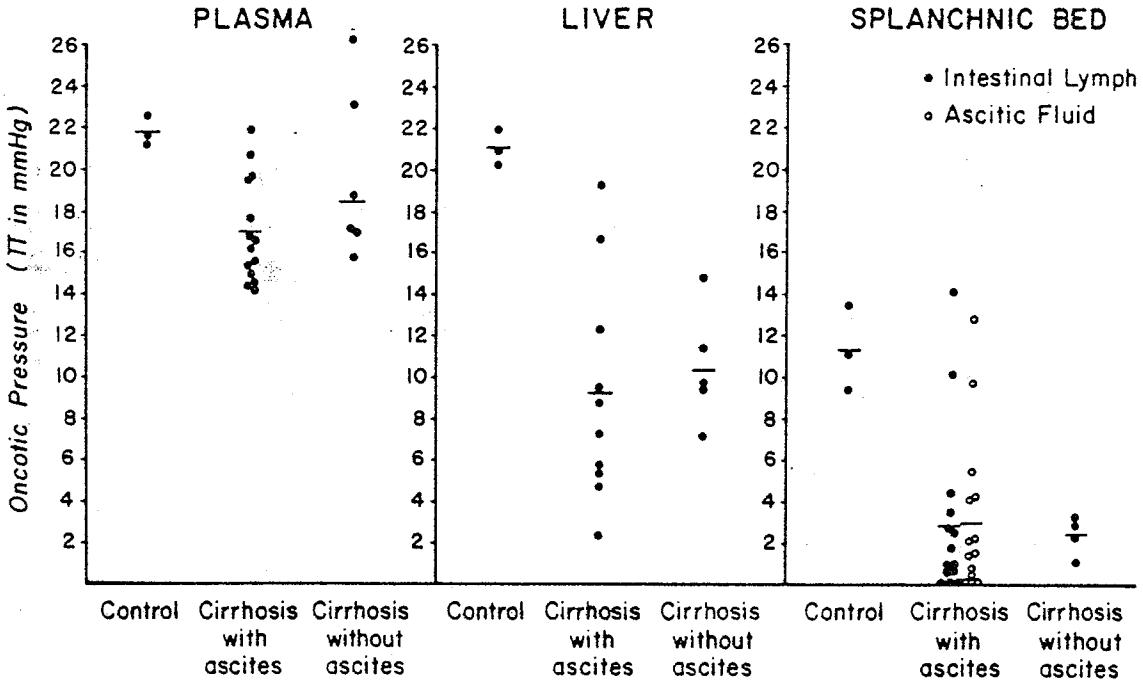


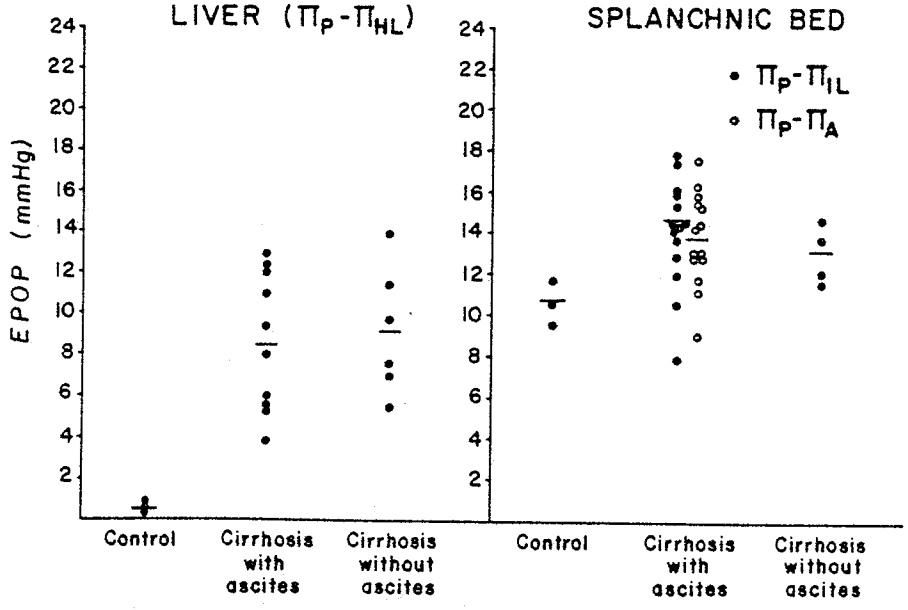
LATE AND EFFECTIVE
END-TO-SIDE
PORTACAVAL SHUNT



LATE AND INEFFECTIVE
END-TO-SIDE
PORTACAVAL SHUNT

Figure 13. A. Calculated plasma oncotic pressure and tissue oncotic pressure in the liver and splanchnic bed in patients with cirrhosis with and without ascites compared with control subjects. B. Effective plasma oncotic pressure (EPOP) in the liver based on the difference between plasma (π_p) and hepatic lymph oncotic pressure ($\pi_{HL}0$) and in the splanchnic bed based on the difference between π_p and intestinal lymph oncotic pressure (π_{IL}) or ascitic fluid oncotic pressure (π_A) in patients with cirrhosis with and without ascites compared with control subjects. (From Witte et al. 1971b).


PLASMA
LIVER
SPLANCHNIC BED
Oncotic Pressure (TT in mmHg)
• Intestinal Lymph
◦ Ascitic Fluid
Control
Cirrhosis with ascites
Cirrhosis without ascites


LIVER (Π_P-Π_{HL})
SPLANCHNIC BED
EPOP (mmHg)
• Π_P-Π_{IL}
◦ Π_P-Π_A
Control
Cirrhosis with ascites
Cirrhosis without ascites

In order for Witte et al. (1969d) to demonstrate even a small amount of fluid transudation from the intestine of dogs, it was necessary to construct an aorto-portal shunt and constrict the portal vein on the hepatic side. This maneuver results in extreme elevation of the portal pressure, bowel edema and chronic diarrhea. Yet in humans with portal hypertension, bowel edema and diarrhea do not occur (Yoffey and Courtice, 1970; Klatskin, 1974) and the portal pressure may be increased only slightly or not at all even with large volumes of ascites (Thompson, 1940; Eisenmenger and Nickel, 1956). This leaves one to doubt the relevance of the dual origin hypothesis since to achieve a sufficient dilution of the ascitic protein, the ascites originating from the liver would need to be diluted by a volume 4 - 5 times as great via transperitoneal diffusion or intestinal transudation.

Probably the most rational approach to the pathogenesis of ascites requires consideration of the many diverse factors which affect the splanchnic circulation and body fluid balance. Thus the rate of hepatic transsinusoidal filtration needs to be considered relative to the outflow capacity of the hepatic lymphatics since what is not removed in the lymph will spill over into the peritoneal cavity (Dumont, 1964;1975). The possibility of ascites formation from extrahepatic tissues needs to be evaluated on the same basis, particularly since the rate of intestinal and thoracic duct lymph flow may bring about some decompression of the portal pressure (Dumont and Mulholland, 1962; Orloff, et al., 1966).

At the same time it is imperative to consider the process responsible for ascites reabsorption from the peritoneal cavity. Ascites can only accumulate if the rate of fluid entry into the peritoneal cavity exceeds the rate of its removal. At the present time the literature on

fluid and protein absorption from the peritoneal cavity is almost completely divorced from the literature which has attempted to interpret the pathogenesis of ascites.

The current management of ascites in chronic liver disease is based on dietary salt restriction and treatment with diuretics (Conn, 1972; Arroyo and Rodes, 1975). While this program provides good control of ascites in most cases, very little is known of the cardiovascular mechanism through which this occurs. Diuretic therapy in ascitic patients is known to reduce portal pressure (Atkinson, 1959; Zimmon and Kessler, 1974), but is it possible that ascites formation or reabsorption are affected in other ways? Probably other measures such as portocaval shunting (McDermont, 1958; Orloff, et al., 1964; Orloff, 1970), thoracic duct drainage or venous shunting (Zotti, et al., 1966; Warren et al., 1968; Donini and Bresadola, 1974), ascites reinfusion (Kaiser, et al., 1962; Vlahcevic, et al., 1967; Parboo, et al., 1974; Levy, et al., 1975) and peritoneo-venous shunting (LeVeen, et al., 1974; Pollock, 1975), could also be used with greater effectiveness if their interactions with the many facets of ascites formation and reabsorption were known.

4. Approach to the Problem

This thesis attempts to integrate certain information related to the absorption of intraperitoneal fluids with studies of the formation of ascites. To this end, experiments were designed to consider the nature of fluid absorption from the peritoneal cavity under various conditions that might be of importance in ascites and to quantitatively examine the interaction between the rates of ascites formation and reabsorption.

Because of the very serious limitations of current methods used to estimate the rate of intraperitoneal fluid absorption (Section 3,II,b), it was necessary to first devise a technique that could provide an accurate on-line record of the rate of fluid transfer into or out of the peritoneal cavity. To meet this need, a plethysmographic method was developed to monitor the rate of change of the intraperitoneal volume. However, in these studies the data obtained by conventional displacement plethysmography proved unsatisfactory due to the unusually large compliance of the peritoneal cavity. Thus the conventional isotonic transducer was replaced with a servo controlled plethysmographic device that was designed so as to record accurately regardless of the intraperitoneal compliance. The function of this apparatus is discussed in Section 5.

Since there is an increased intraperitoneal pressure with the accumulation of ascites (Section 3,III,b,i), the first experiments employed the technique of intraperitoneal plethysmography in anesthetized cats to investigate the rate of fluid absorption from the peritoneal cavity when the intraperitoneal pressure was increased.

Subsequent studies were designed to outline the significance of the intraperitoneal protein concentration. Since the ascitic protein level is particularly elevated in some types of ascites (Section 3,III,b,i), a series of experiments were conducted to determine whether the amount of

protein in the intraperitoneal fluid could speed or retard the rate of iso-osmotic fluid absorption from the peritoneal cavity. Also, to investigate whether the absorption process might affect the protein content of the ascitic fluid by removing a more concentrated or dilute fluid, a separate series of experiments monitored the rate of change of the intraperitoneal protein concentration during a five hour period of absorption.

As mentioned in Section 3,III,b,iii, diuretics are frequently employed to accelerate the mobilization of ascitic fluid. Since this effect occurs quite rapidly in both man and experimental animals (Shear, et al., 1970; Witte et al., 1972) experiments were undertaken to consider the possibility that these drugs may reduce ascites by increasing the rate of fluid absorption from the peritoneal cavity. The results of studies on the intraperitoneal pressure, protein and effects of diuretics are discussed in Section 7,I-IV.

Having considered the factors which might affect the rate of fluid absorption in ascites, subsequent experiments were designed to examine how this process of intraperitoneal absorption would interact with ascites formation to control the net rate of change of the intraperitoneal volume. Using an extracorporeal circuit to control hepatic venous pressure or an hydraulic occluder to increase portal venous pressure, the rate of ascites formation was first established while the intraperitoneal pressure was zero. Afterwards, the intraperitoneal pressure was increased to determine its effect on the rate of ascites formation and to obtain quantitative data for the net rate of intraperitoneal volume changes with various rates of ascites formation and reabsorption. These data are discussed in Section 7,V-VII.

5. The Improved Plethysmograph with Servo Control of Hydrostatic Pressure

I Limitations of Conventional Displacement Plethysmography

In spite of some troublesome limitations the conventional fluid displacement plethysmograph has been widely utilized to record changes of blood volume (Roy, 1881; Schafer and Moore, 1896; Greenway and Lautt, 1972a), to study transcapillary fluid exchange (Mellander, 1960; Greenway, et al., 1969), and, under some conditions, to measure blood flow by a non-invasive method (Brodie, 1905; Johnson, 1932; Greenfield, et al., 1963).

As illustrated in Figure 14, the design of this apparatus includes two basic components: a static reservoir containing a physiological buffer solution that is connected to the biological system being studied; and an isotonic transducer which records the rate of volume change by following the movement of a glass bell suspended in a cup over the reservoir. As volume changes are recorded with this system, the amount of fluid within the reservoir either increases or decreases, although the overall volume of the buffer solution may not change but only be redistributed (e.g. in accordance with the volume of the tissue connected to the plethysmograph). If, for example, the tissue volume decreases, a small volume of fluid is displaced from the reservoir so that the glass bell descends to maintain a constant air space above the reservoir fluid. Concurrently, the transducer records the change of reservoir volume by monitoring movement of the bell; and the record is transmitted to a polygraph for display.

With the use of this apparatus to study the rate of change of the intraperitoneal volume, certain problems developed which led to greater inaccuracies than might have been the case in systems with a lower compliance. In addition, the conventional fluid displacement plethysmo-

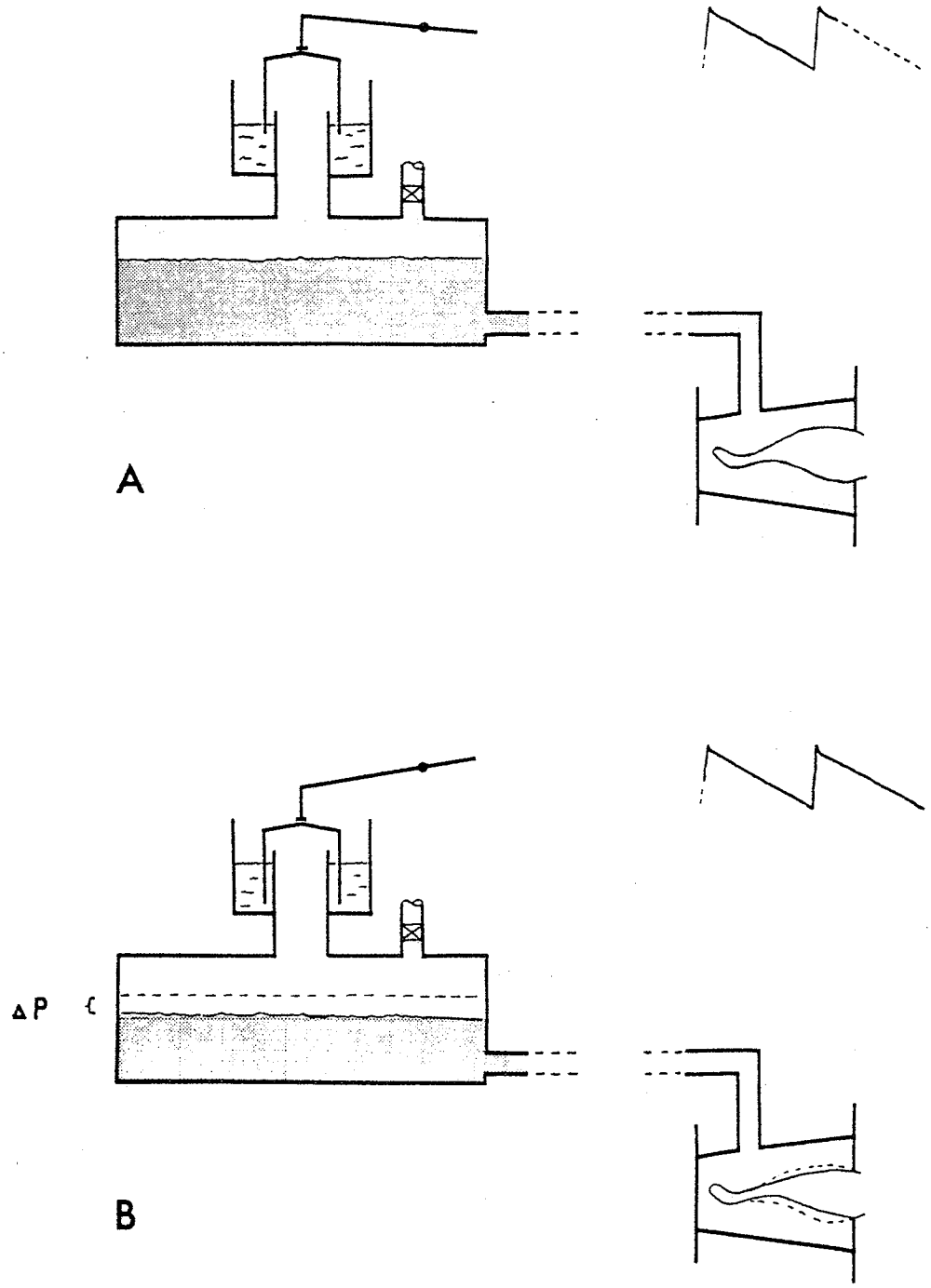


Figure 14. Operation of the conventional plethysmograph. With the reduction of tissue volume from A to B, the fluid level and hydrostatic pressure in the reservoir (Δ P) are reduced as movement of the overlying dome records the change of volume.

graph is limited by the necessity to periodically restore the fluid volume in the reservoir. The addition of this extra fluid may result in temperature, pressure or mixing artifacts at these times. Under circumstances when large blood volume changes occur rapidly, it may be difficult to keep pace by accurate filling of the reservoir. However, the most critical problem with this system is that the hydrostatic pressure is variable with the static reservoir type of plethysmograph.

If, for example, fluid is displaced from the reservoir, the hydrostatic pressure head decreases continuously, until such time as the reservoir volume is restored. Depending on the size of the reservoir, this variation of the pressure imposed on the organ(s) under study can result in a substantial volume artifact caused by blood pooling in the veins or other changes of the system's capacitance.

With subsequent refilling of the reservoir, the hydrostatic pressure is increased, which results in a volume artifact that is proportional to the compliance of the tissue which is being studied. A similar artifact would occur if the volume was changing in the opposite direction, and fluid had to be removed from the reservoir. These problems are particularly accentuated in studies of volume changes in the peritoneal cavity, because in this case, a slow rate of volume change is recorded in a biological system that has a large compliance.

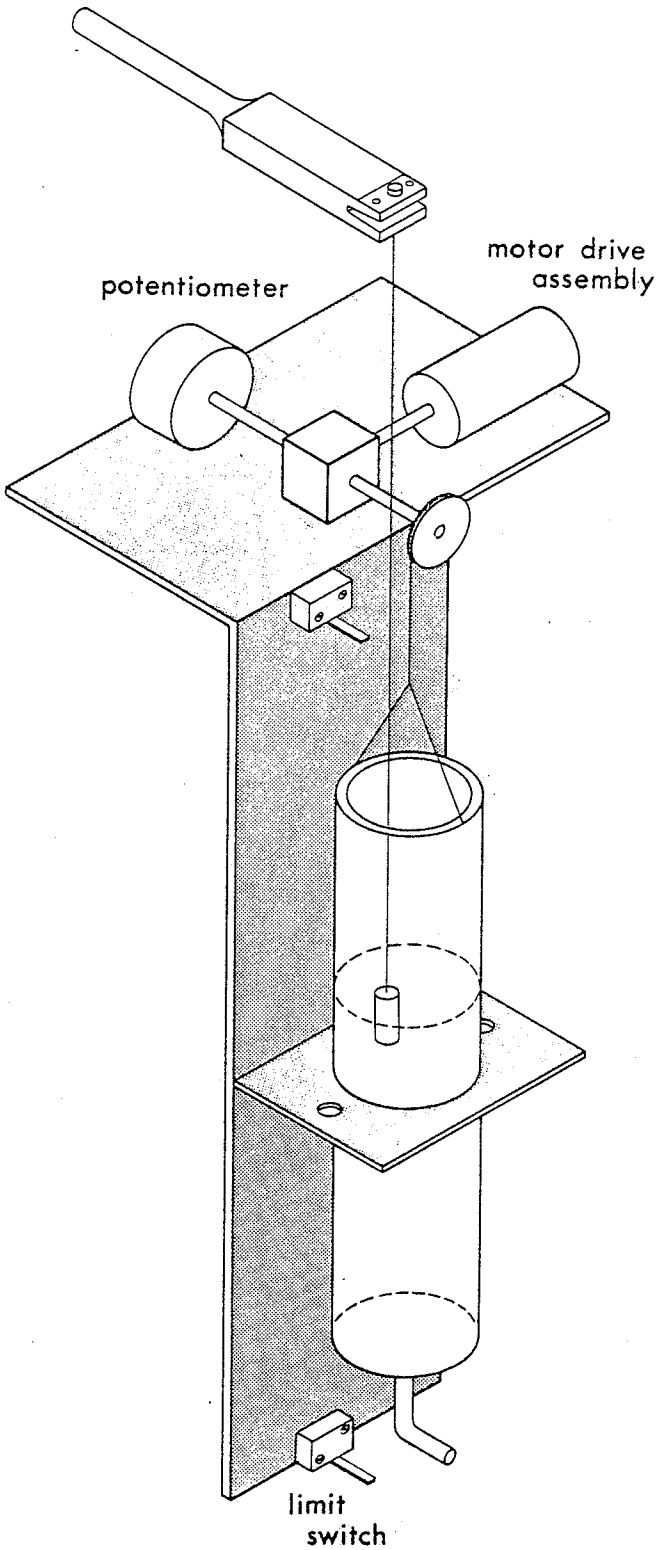
II Description of the Servo Controlled Plethysmograph

To circumvent the problems with conventional displacement plethysmographs, an improved plethysmographic recording device was designed to maintain a constant hydrostatic pressure under all conditions by the use of a servo-controlled vertically mobile reservoir. In addition to pressure stabilization, this also eliminates the necessity of periodically emptying or refilling the reservoir so that mixing problems are limited and there is minimal interruption during the period while volume changes are recorded. When compared to conventional plethysmograph techniques, the use of this apparatus provides increased accuracy and the ability to handle large total changes of volume.

As shown in Figure 15, the present system contrasts the traditional closed-compartment plethysmograph by employing an open reservoir in the form of a vertically mobile plexiglass cylinder. A Silastic tube exits from the lower end of this cylinder so that its fluid is continuous with the fluid in a nearby rigid chamber which encloses the biological system under study - or, in this case, the peritoneal cavity. As fluid exchange occurs, a servo-control system maintains a constant hydrostatic pressure, regardless of the reservoir volume by manipulating the vertical position of the reservoir cylinder.

The circuitry for the servo-control and volume recording mechanisms has been designed to operate in conjunction with two channels of a Beckman Dynograph recorder. As shown in Figures 16 and 17, the servo channel responds in a strictly proportional manner to input signals received from an isometric transducer that is located on a platform overlying the mobile reservoir. From this transducer a small plexiglass

Figure 15. Drawing of servo-controlled plethysmograph showing open, mobile reservoir and the small plexiglass block suspended from an isometric transducer.


potentiometer
motor drive
assembly
limit
switch

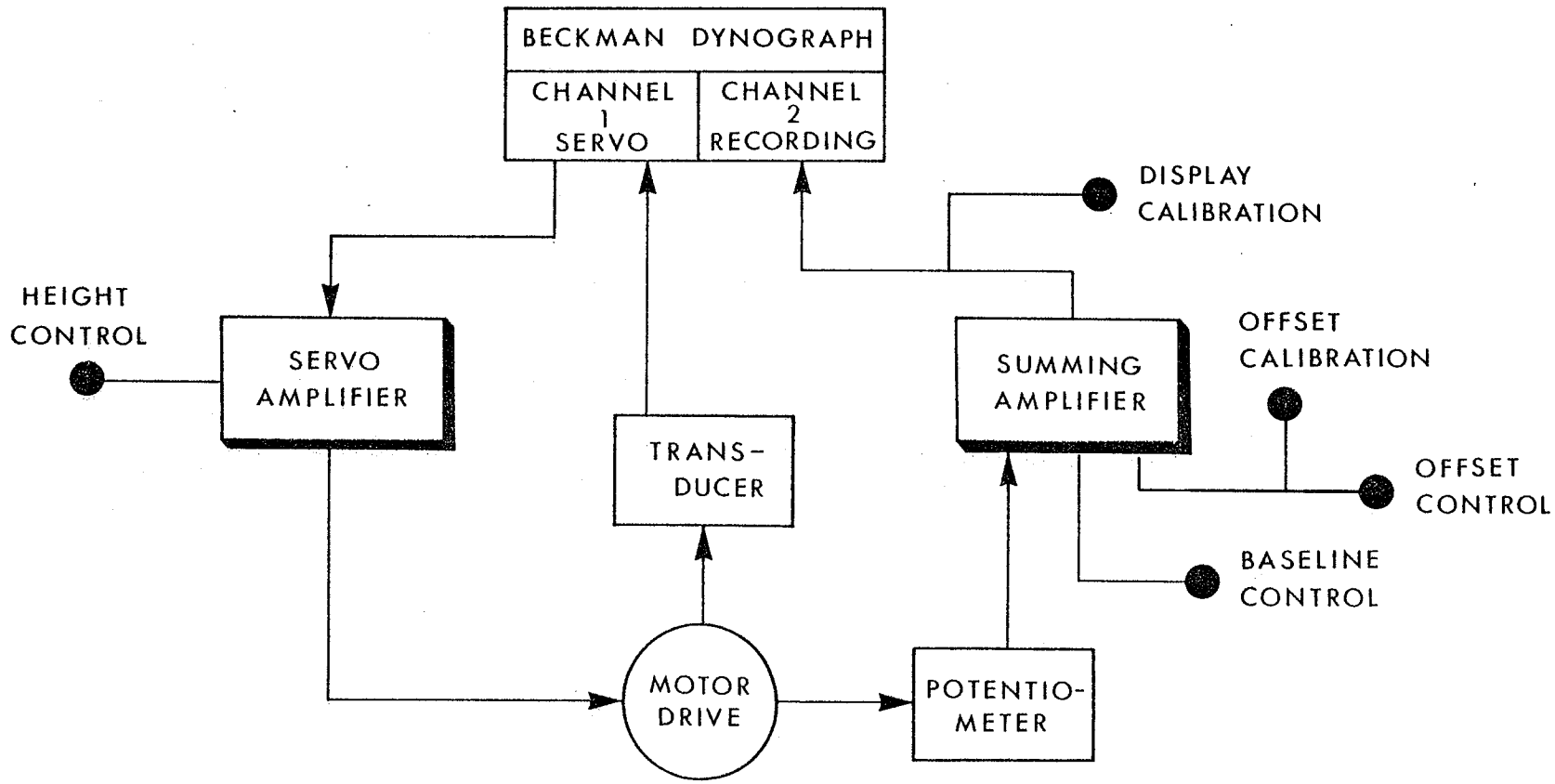


Figure 16. Schematic diagram of servo-controlled mechanism in the improved plethysmograph.

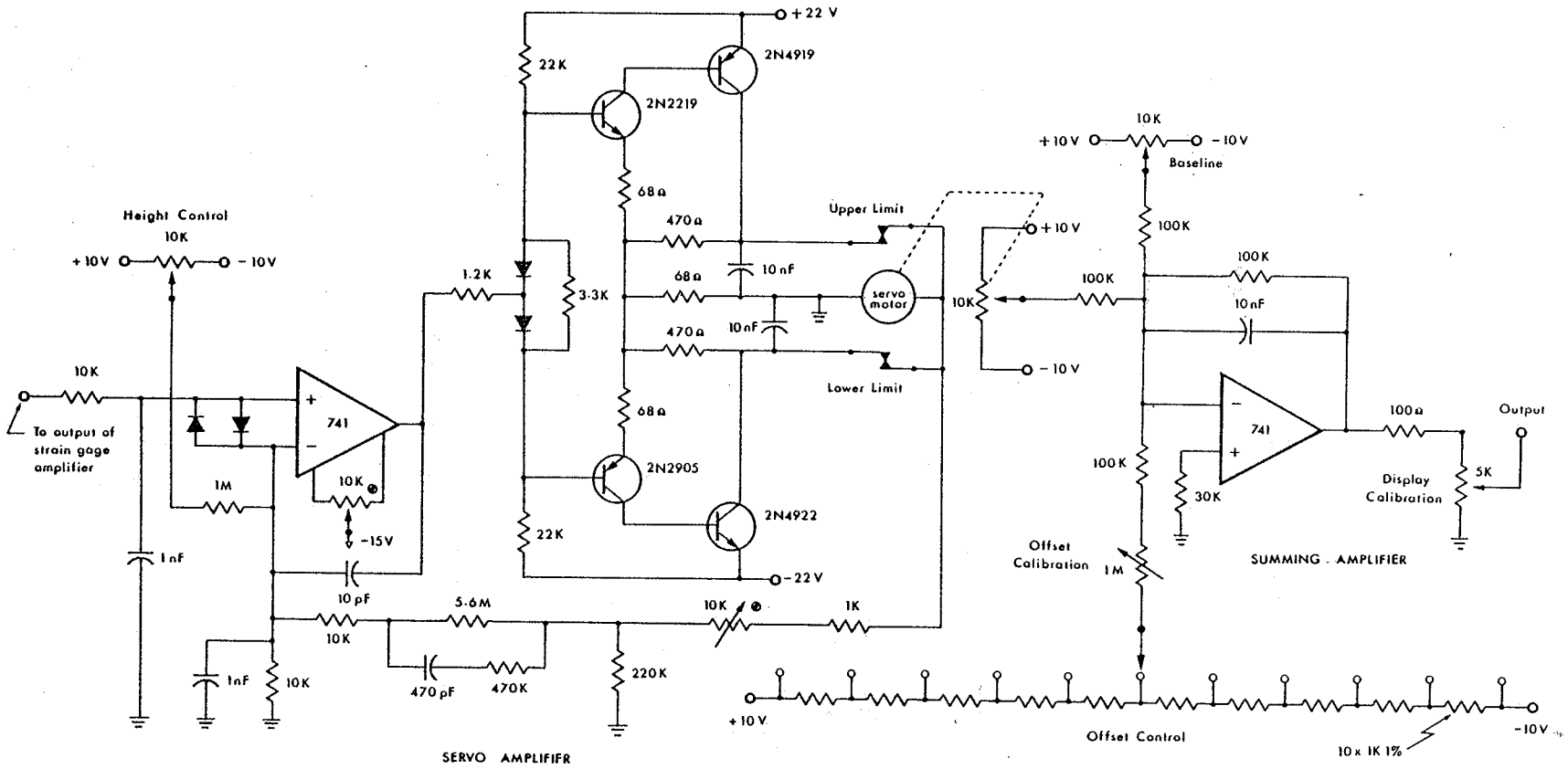


Figure 17. Electronic components responsible for operation of the servo-control mechanism in the improved plethysmograph.

block is suspended through the open reservoir so as to lie approximately half-submerged at the fluid level in the plethysmograph. Thus with fluid exchange occurring so that the level in the reservoir descends, the weight of the block is increased and this results in an increased feedback signal to the servo-control mechanism. Similarly, when fluid is displaced into the reservoir, the feedback signal decreases. This signal is received in the servo-control channel of the Beckman by a strain gauge coupler (Type 9872) operating in the "venous", reversed polarity mode. Alternatively it is possible to operate this servo-control system in response to input from a venous pressure transducer which monitors the hydrostatic pressure either at the bottom of the reservoir cylinder or within the peritoneal cavity.

Input from the transducer passes via the servo channel of the Beckman to a servo amplifier which controls a motor drive assembly (Clifton 27.5-V DC motor) that is located above the reservoir on the same platform as the transducer. By means of reduction gearing the motor operates a pulley to regulate the vertical position of the fluid reservoir which slides up and down in a short plexiglass sleeve. The gear ratio of the motor drive assembly (180:11) is further reduced by a 50:1 worm reduction unit so that a single revolution of the motor produces 0.18 mm vertical movement of the reservoir.

Alternatively the position of the cylinder can be manipulated by a height adjustment control. This allows the operator to set the initial position of the reservoir and its fluid so that the plexiglass block is in an optimal position for feedback control. As a safety precaution, the support frame for the reservoir is equipped with two microswitches that are positioned to impose upper and lower limits to the travel of the cylinder. A control switch (manually operated) is also

provided so that the motor drive assembly can be temporarily disconnected for adjustments of the reservoir, transducer, or other equipment.

Changes of fluid volume are recorded by mechanically coupling the support pulley for the reservoir cylinder to a three-turn Selectrol potentiometer. Thus fluid displacement from the reservoir, which causes the cylinder to be raised, results in an electrical signal from the potentiometer that reflects the change in position of the reservoir and hence the change of its volume. This signal is one of three components delivered to a summing amplifier. A zero control adjustment, which sets the overall base-line position of the recording system, is the second component. The third signal is supplied by a 10-step voltage offset switch thatis used to periodically restore the pen position so that it is within the limits of the recording strip. This function eliminates the need to periodically refill (or empty) the reservoir and provides accurate recordings of volume changes even with very rapid or large total changes of volume. After 10 turns of the offset it can be reset with the zero control adjustment.

Calibration of the recording system is accomplished by adjusting the voltage output of the summing amplifier in response to the addition, by pipette, of a given fluid volume to the reservoir. Subsequently the output of the offset switch can also be adjusted by its calibration control so that each voltage step corresponds to a change in volume that causes full-scale deflection of the recording pen.

The overall sensitivity of the recording system can be set up by adjusting the gain of the preamplifier on the servo channel of the Beckman. Operating with a low input sensitivity improves the signal-to-noise ratio

and eliminates certain undesired volumetric oscillations such as those due to respiration or vibrations transmitted from the operating table. On the other hand this increases the dead-band volume so that, when the direction of fluid movement is reversed, a small part of the actual volume change is not recorded. Thus in practice it is best to increase the sensitivity until the size of the dead-band is reduced to an acceptable level without incurring undesired or extraneous signals.

In our use the dead-band is adjusted to 1-2% of the offset calibration (10 ml.). Under these conditions the plethysmograph records volume changes at rates up to 25 ml/sec without encountering notable lag or overshoot phenomenon. This speed is considerably faster than the highest rates that are encountered in plethysmographic studies (Woodcock, 1974), but it is still less than the theoretical maximum rate of 37.2 ml/sec (27.2 mm/sec. vertical speed) for this device. Since this rate is arbitrarily set by reduction gearing and the diameter of the reservoir cylinder, the apparatus could be modified to further increase its maximum rate of volume change.

Before starting to record, the fluid level in the plethysmograph is preset to a level that is chosen as zero hydrostatic pressure. In the present experiments this was taken as the level of the right atrium, 2.5 cm above the operating table surface. At times when it is necessary to increase the intraperitoneal pressure, the whole plethysmograph assembly can be lifted on its support and locked at a higher level. It will then function as usual to maintain a constant hydrostatic pressure at the new level.

III Advantages of the Servo Controlled Plethysmograph in the Study of Intraperitoneal Volume Changes

The technical problems which occur in the study of intraperitoneal volume changes are mainly related to the large compliance of the peritoneal cavity (8.5 ml/mmHg). Because of this, the volume record is quite sensitive to even minor fluctuations of the ambient hydrostatic pressure, and large capacitance effects occur with purposeful alterations of the hydrostatic pressure.

Several advantages in the design of the servo controlled plethysmograph have minimized these problems so that accurate records of intraperitoneal fluid absorption or formation could be obtained.

By electronically resetting the volume record, accuracy is considerably improved when large or rapid volume changes must be recorded. Furthermore, the conventional fluid displacement plethysmograph cannot record rapid volume changes which cause an off-scale deflection of the recording system because this causes air to either enter or escape from the recording chamber. These problems do not occur with the recording system of the constant pressure plethysmograph since the volume record can always be precisely restored with the offset return switch. However, the fundamental improvement of this design is the elimination of variation in the hydrostatic pressure as volume recordings are obtained.

With the conventional fluid plethysmograph errors due to pressure variation can be reduced, but not eliminated, by increasing the diameter of the reservoir. Thus for a given change of volume there is less vertical fluid displacement. Inevitably this manoever also increases the volume of air within the plethysmograph and increases the

surface area of fluid exposed to this air compartment. The result is an apparatus that is subject to volume artifacts caused by heat exchange between the fluid and air compartments in the reservoir. As described in detail by Vanhuyse and Raman (1972;1974), the air space expands disproportionately, distorting the volume record so that the actual volume changes are underestimated. These errors cannot occur with the new plethysmograph described in this report.

Figure 18 compares the performance of a conventional plethysmograph to that of the new servo controlled device in an artificial system of variable compliance. A constant infusion pump (Harvard Apparatus Co.) was first calibrated and then used to withdraw fluid at a constant rate of 1.27 ml/min while either type of plethysmograph recorded the rate of volume change in the system.

During the time while fluid was being withdrawn, no variation of the hydrostatic pressure was detectable with the constant pressure plethysmograph but with the conventional recording system there was a variation of 0.75 mmHg/10 ml change of volume. The effect of this seemingly small change in hydrostatic pressure is increasingly evident as the compliance of the system is increased. The traces on the left of Figure 18 show that the constant pressure plethysmograph records accurately in all cases. In contrast the traces from the conventional plethysmograph (right of Figure 18) are accurate only when the compliance is zero (uppermost trace). Each lower record on the right side shows the distortion that is present for a given compliance. In the lowermost record, with a compliance of 7.61 ml/mmHg, the constant pressure apparatus indicates that 12.7 ml is removed after 10 min; the other plethysmograph records a volume change of only 7.8 ml. Thus the error component increases

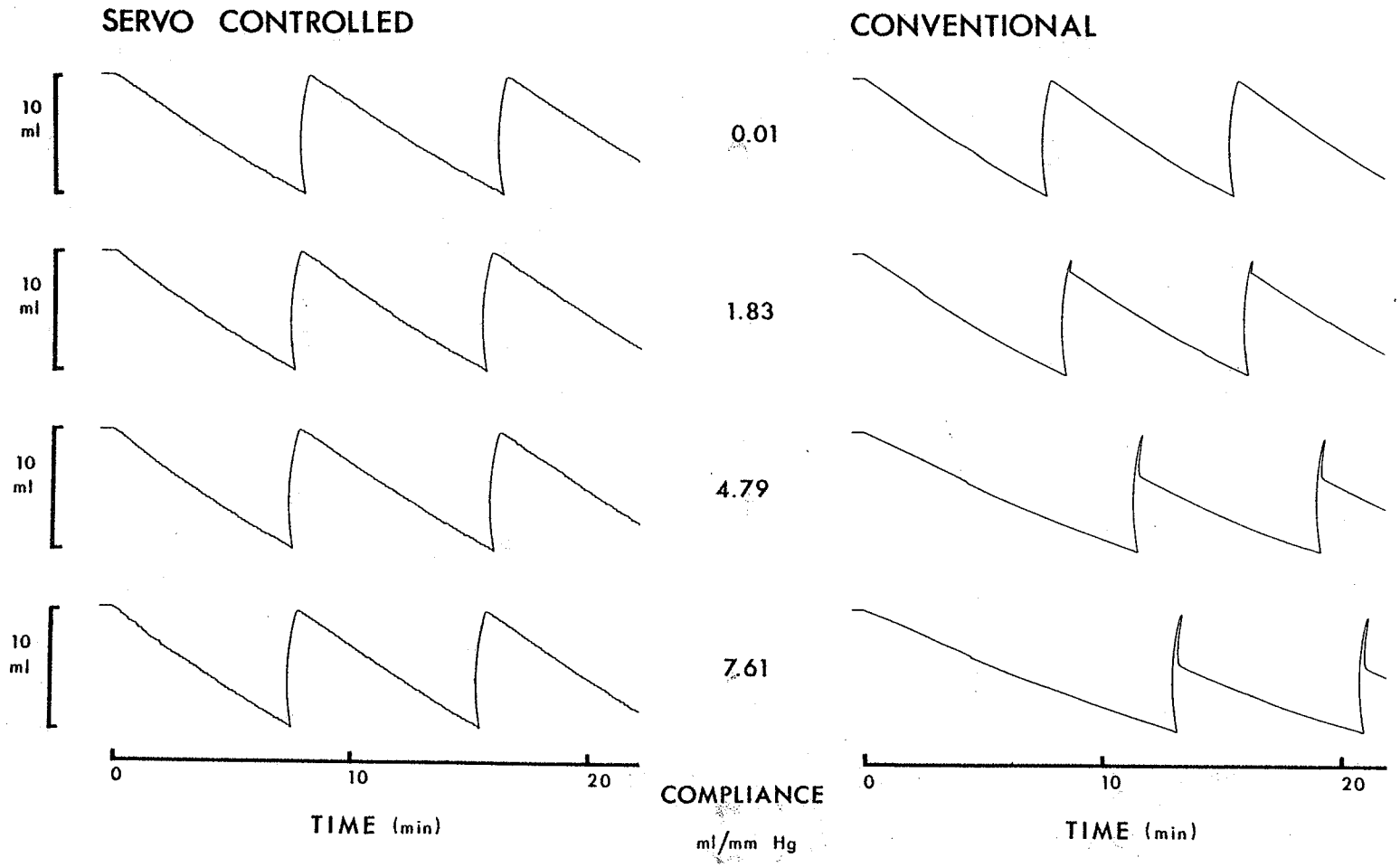


Figure 18. Recordings of volume change obtained by the servo-controlled and conventional plethysmographs in a system of controlled compliance.

as a function of the compliance of the system under study.

While few biological systems have a compliance as great as that of the peritoneal cavity, the vascular compliance in some studies is even greater (Pouleur, et al., 1973; Echt, et al., 1974). In other cases the compliance may be less, but still large enough to be significant. The average compliance of the hepatic vascular bed in cats is 4.5 ml/mmHg per 100 g of liver (Greenway and Lautt, personal communication). In such cases the data obtained by conventional fluid displacement plethysmography could be in considerable error unless the actual compliance is known and can be accounted for. However with studies of intraperitoneal volume changes, the recordings obtained by conventional plethysmographic techniques were of little value due to the high compliance of this system.

Although its use is cumbersome for some studies, the fluid displacement plethysmograph is the most accurate instrument available for the measurement of volume changes (Woodcock, 1974) and with the improved servo-controlled design its usefulness has been further extended to provide accurate volumetric recordings in the present experiments.

6. Methodology

I Surgical Preparation and Intraperitoneal Plethysmography

Experiments were performed on cats of both sexes weighing 1.9 - 3.8 kg (average weight 2.4 kg). The animals were maintained in a controlled animal house environment and fed a standard diet for at least two weeks prior to use. Food, but not water, was removed 24 hours prior to surgery.

The cats were anesthetized by intraperitoneal injection of sodium pentobarbital (30 mg/kg; Abbott) and supplementary doses (2 mg/kg with 1.5 ml saline) were administered intravenously during the course of the experiment whenever reflex ear, limb, and swallowing movements returned. A forelimb cutaneous vein was catheterized, the trachea was cannulated, and mean arterial pressure was recorded from the right femoral artery. Rectal temperature was monitored and controlled by warming the operating table's surface. The bladder was drained by catheterization to prevent abdominal volume artifacts and the subsequent flow of urine was collected in plastic vials which were weighed at ten minute intervals. Urinary flows were calculated assuming a specific gravity of unity.

The surgical procedure for experiments which did not involve preparation of an extracorporeal circuit was as follows. A catheter for sampling venous blood was inserted through the right external jugular vein into the superior vena cava, and the venous pressure in the inferior vena cava at the level of the renal veins was recorded by threading a catheter to this location via the right femoral vein. The abdomen was shaved and opened with a 3 cm incision along the mid-line ligament (Linea alba) below the umbilicus. Any residual fluid present within the

peritoneal cavity was removed with absorbent gauze.

To record intraperitoneal volume changes the reservoir cylinder of the plethysmograph was connected to the peritoneal cavity by Silastic tubing (Dow Corning, 3/16 in I.D.). The fittings at the terminus of this tube are shown in Figure 19. In order to provide a fluid environment, the tube ended in a small plasticized cage (2.5 cm diameter, 8 cm length) which was placed within the peritoneal cavity on the right side of the undisturbed abdominal contents. The cage volume of approximately 25 ml partly prevented the omentum from wrapping around the recording tube but in addition the tubing was perforated along its length within the cage and wrapped loosely with a thin covering of gauze. For recording intraperitoneal pressure and sampling the intraperitoneal fluid, a thin catheter (P.E. 160) was passed through the center of the Silastic tubing and into the peritoneal cavity with its tip positioned over the surface of the liver. The abdominal wall was then closed with sutures to form an air-tight seal. The peritoneal cavity was filled with 80 - 100 ml of 38°C Ringer-Locke solution[1] from the plethysmograph and any residual air was expelled.

To prevent distention of the abdominal wall when the intraperitoneal pressure was increased, the entire abdomen was enclosed in a quick-setting plaster cast (Gypsona bandage). This manoever allowed the peritoneal cavity to function as a plethysmographic chamber since, when dried, the cast was completely rigid and cohesively bound to the abdominal skin. After application of the cast all wounds were closed with sutures and each animal was placed on artificial respiration so as to just suppress

[1]The composition (mM) of the Ringer-Locke solution was: Na^+ 155.8, K^+ 5.6, Ca^{++} 4.3, Cl^- 163.9, HCO_3^- 1.8, Glucose 5.

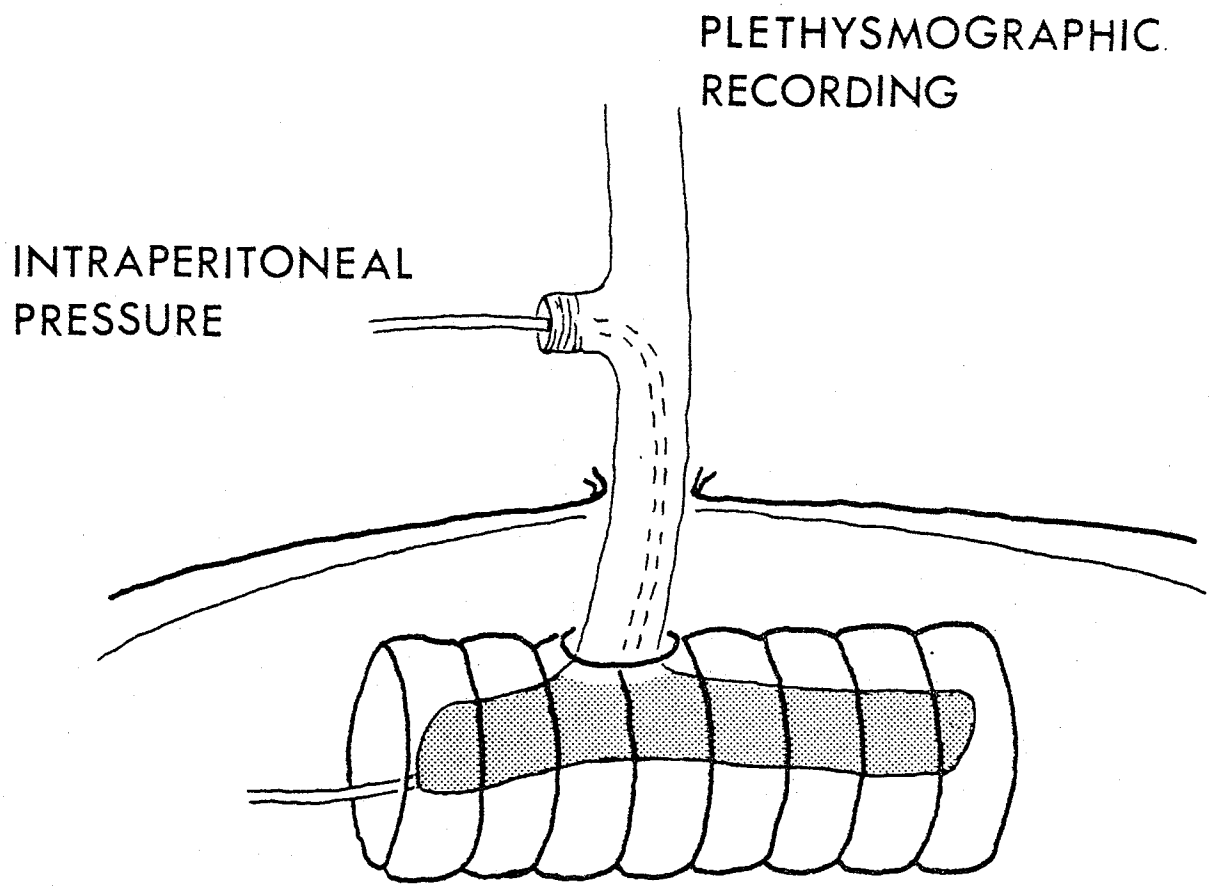


Figure 19. Arrangement of tubing used to connect the peritoneal cavity to the servo-controlled plethysmograph. Shaded area: gauze over perforated Silastic tubing.

spontaneous diaphragmatic movements. To finish, 5 ml of 5% Dextran (Rheochromadex 40, 10% w/v Pharmacia in an equal volume of 0.9 g% saline solution) was administered intravenously.

Arterial pressure was recorded with a Statham P23AC tranducer and venous or intraperitoneal pressures were recorded with Statham P23BC transducers; all were referred to the level of the right atrium and displayed on a Beckman Type RM dynograph.

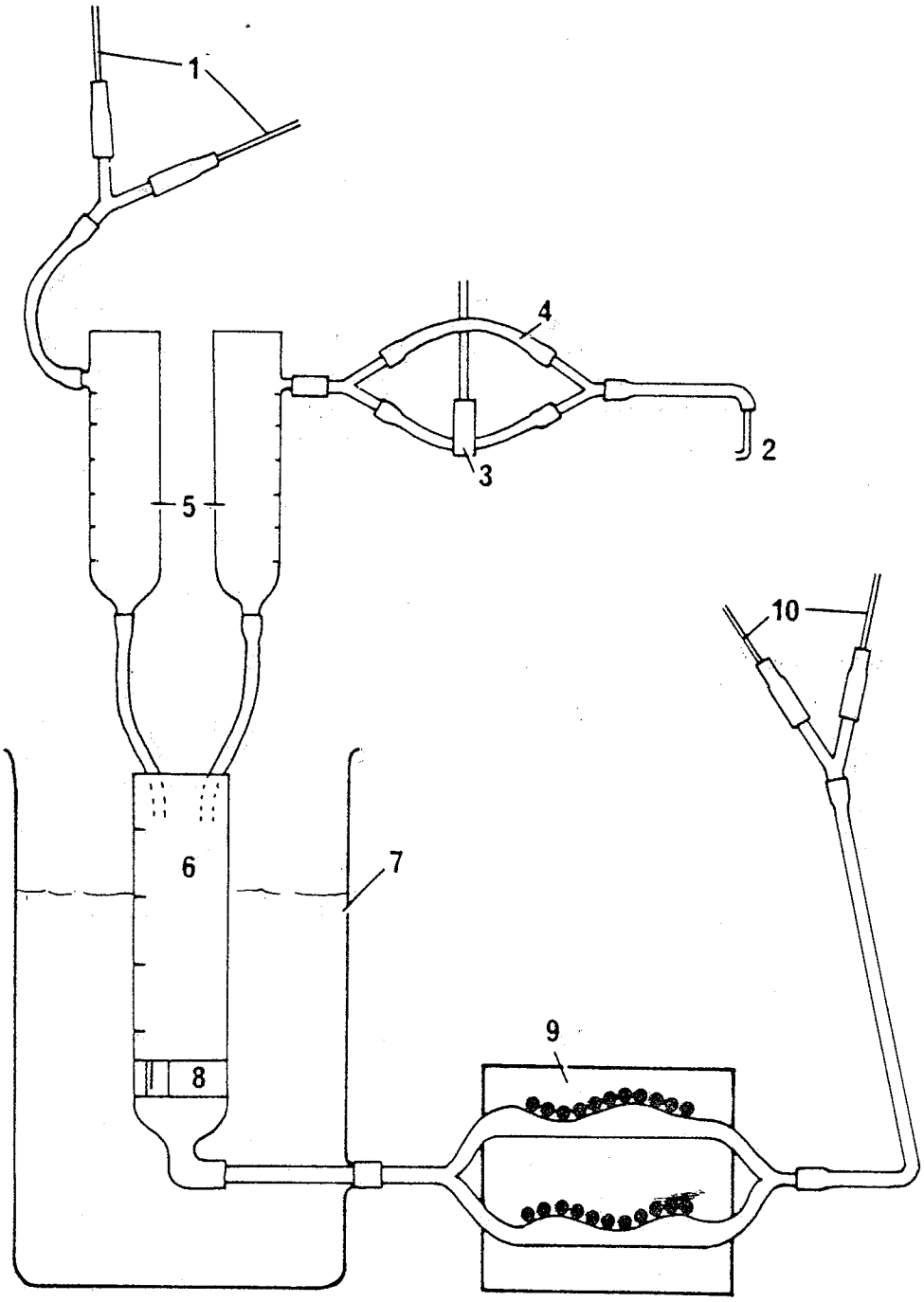
II Control of Hepatic Venous Pressure

In a number of experiments it was necessary to control hepatic venous pressure and the rate of ascites formation. This was accomplished by preparing an extracorporeal circuit to isolate the hepatic venous return so that the outflow pressure could be adjusted.

In these experiments the abdomen was opened with a slightly larger incision and a loose ligature was placed around the abdominal vena cava above the lumbo-adrenal veins. Portal venous pressure was recorded by threading a polyethylene cannula into the portal vein through a small tributary of the superior mesenteric vein draining the appendix. Before closing the abdominal incision, the apparatus required for intraperitoneal plethysmography was placed within the peritoneal cavity as usual and the ligature on the vena cava was passed out through a thin tube so that occlusion of this vessel could be accomplished later in the experiment after the abdomen was resealed.

Figure 20 shows the cannulae and circuitry that was used to reroute the caval blood. The construction of the circuit is modified from a similar preparation which as been previously described (Greenway and Lawson, 1966; Greenway, et al., 1969). Prior to each use the tubing was

Figure 20. Assembled apparatus for establishing hepatic venous long circuit. Blood flows out of the animal via femoral cannulae (1) and hepatic venous cannula (2). Hepatic blood flow is monitored by an extracorporeal flow probe (3). A shunt (4) can be opened to check zero base line, and direct measurement of flow using calibrated reservoir (5) permits accurate calibration of flow. Blood drains into a reservoir (6) where it is warmed by a water bath (7) and filtered through glass wool (8). The warmed blood is pumped (9) back to the cat via jugular cannulae (10). (From Greenway et al. 1969).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sterilized by autoperfusion first with 5% formaldehyde, then 2% ammonia, distilled water and finally Ringer-Locke solution.

The sequence of assembly is designed to reroute blood from the abdominal vena cava to the jugular veins without obstructing the venous outflow from any organ in the process (Figure 21). In preparation, both femoral veins and both external jugular veins were cannulated with teflon tubing (Dupont No. 6416, .066 in I.D.). After the abdominal cast was applied and artificial respiration begun, the chest was opened through the sixth intercostal space and thoracic inferior vena cava was exposed.

At this time 20 - 30 minutes was allowed for hemostasis while a donor cat was exsanguinated under ether anesthesia, and the extracorporeal circuit was primed with approximately 100 ml of blood filtered through fiberglass-gauze and containing 20 mg of heparin (Connaught; 2500 units U.S.P.). A further 10 mg of heparin was administered intravenously to the recipient cat.

A portion of the caval blood was then allowed to circulate from the femoral veins first to a small control cylinder, from there to drain by gravity into the main collecting reservoir from which a peristaltic pump (Harvard Apparatus Co.) returned it to the jugular veins. While in transit through the main collecting reservoir, the blood was passed through a fiberglass-gauze filter and maintained at 38 - 39^{o}C by means of a temperature-controlled water bath around the reservoir.

After the caval blood was rerouted in this circuit for a few minutes, the thoracic inferior vena cava was ligated to temporarily displace the full caval flow from this vessel into the extracorporeal circuit. A glass cannula was then inserted caudal to the tie. After occlusion of the abdominal vena cava below the hepatic veins, this cannula served to

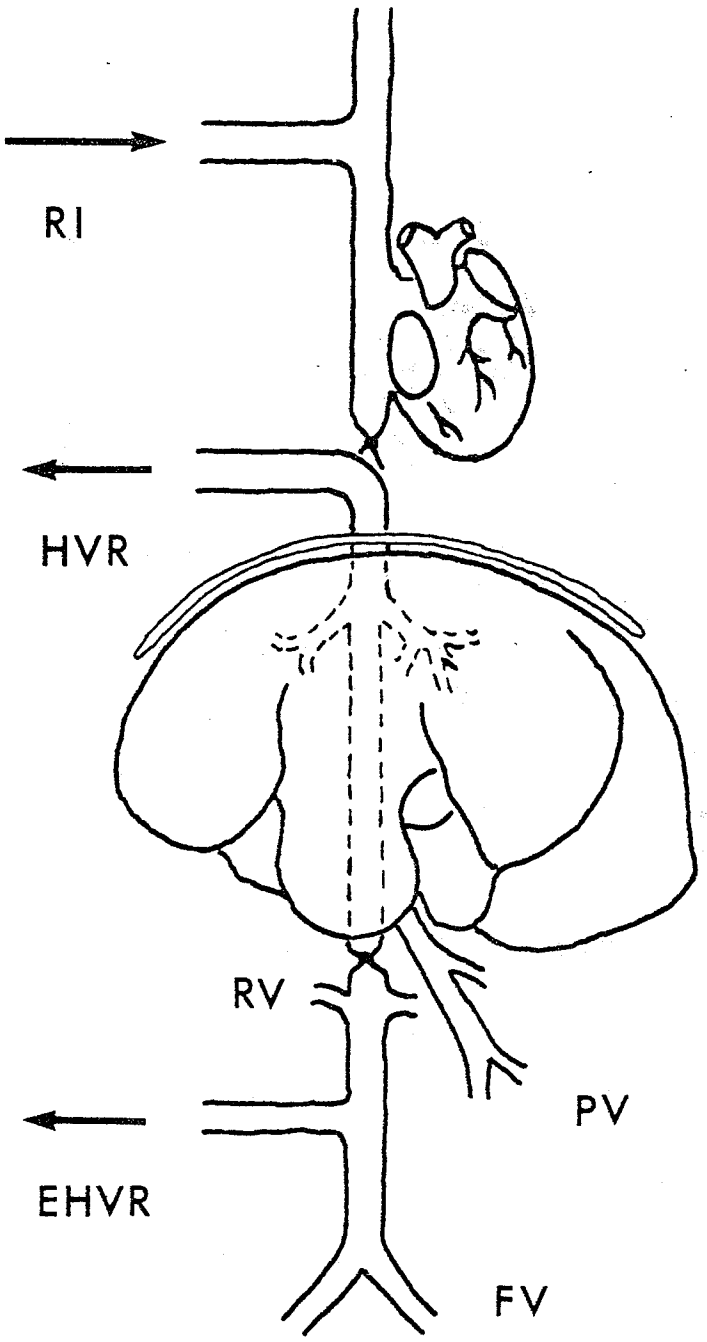


Figure 21. Route of venous blood flow after preparation of the hepatic venous extracorporeal circuit. The hepatic venous return (HVR) drains to one control cylinder and the extrahepatic venous return (EHVR) drains to another; both are reinfused (RI) through the external jugular veins. FV: femoral veins, PV: portal vein, RV: renal veins.

drain the hepatic venous effluent through an extracorporeal, electromagnetic flowmeter (Nycotron, Oslo) and outflow control cylinder into the venous reservoir. A narrow indwelling catheter (PE 60) was threaded from a side branch of the hepatic cannula, past the diaphragm to record hepatic venous pressure.

The remaining extra hepatic blood from the abdominal vena cava was bypassed to the central reservoir via the femoral veins. The overall rate of venous return was then adjusted to maintain a constant extracorporeal volume. Thereafter, the venous return remained unchanged for the duration of the experiment.

All wounds were tightly closed and a drainage-suction tube was placed within the chest to maintain a negative intrathoracic pressure of 4 - 5 cm H_2O. Although the animals could have been allowed to breathe spontaneously, artificial respiration was continued throughout the experiment to standardize respiratory movements and diaphragmatic distention; both of which could have affected the recording of intraperitoneal absorption. Hepatic venous pressure was controlled by adjusting the height of the outflow control cylinder but the height of the cylinder for the extrahepatic venous blood was never altered. A flow bypass conduit provided for zero calibration of the hepatic flowmeter. The rate of blood flow from both hepatic and extrahepatic vascular beds could also be measured directly from the output of the control cylinders.

During the course of the experiment 5% Dextran was added as required to maintain an adequate volume in the central collecting reservoir. Positions of all cannulae and ties were verified by post-mortem examination.

III Control of Portal Pressure

In selected experiments, the animals were prepared for the control of portal pressure rather than hepatic venous pressure. In these animals, the portal vein was exposed at the liver and isolated from the surrounding connective tissue and nerve plexus. Portal pressure was recorded as previously described but in this case caution was exercised to ensure that the tip of the portal cannula lay about 2 cm upstream from the exposed site on the portal vein.

A collapsed, hydrostatic occluder was then placed around the portal vein and a control tube was passed out of the peritoneal cavity. After installation of the tubing required for intraperitoneal plethysmography and closure of the abdomen, the portal pressure could be increased by compression of the portal vein with the hydraulic occluder.

A servo mechanism was utilized to control the expansion of the occlusion cuff so that a constant elevation of portal pressure could be maintained in spite of fluctuations in the rate of portal inflow. To this end, a servo-controlled plethysmograph was adapted to serve primarily as a hydrostatic pressure regulator rather than a volume recorder. Thus the constant pressure feature of the plethysmographic device was cancelled with the adaption for pressure control purposes and the recording of portal pressure served as the feedback transducer. Activation of the control mechanism expanded the occluding cuff and increased extrahepatic portal pressure to an extent that was determined by the hydrostatic pressure imposed by the reservoir cylinder. Any tendency to deviate from the preset portal pressure was rectified by servo adjustments of the reservoir height and cuff expansion.

With this approach the portal pressure could be maintained at

a constant elevated level despite ongoing variations of portal blood flow. In all experiments the occluder was carefully installed so as not to raise portal pressure, and, after sustained portal hypertension for periods of 30 minutes or more, control levels returned when the occluding cuff was collapsed. The expansion of the portal cuff introduced a small volume artifact in the record of intraperitoneal volume at the onset and offset of portal hypertension, however, during the intermediate period, pressure was controlled with negligible changes of the cuff volume.

IV Methods Related to Determination of the Intraperitoneal Compliance

In order to validate the accuracy of the plethysmographic technique used in the present study, several experiments were designed to investigate the compliance of the peritoneal cavity and the time course of the initial, rapid capacitance effect that occurred when the intraperitoneal pressure was raised. Two techniques were employed to trace the sources of compliance in the peritoneal cavity.

After preparation of the abdomen for intraperitoneal plethysmography, the animal was placed on artificial respiration as usual. The chest was then opened on the right side at the sixth intercostal margin and the pleural lining of the mediastinum was perforated to connect the right and left pleural spaces. A small tube connected to a second plethysmographic recorder was then placed in the cavity and the incision was closed securely. In this manner the thoracic cavity served as a second plethysmographic chamber and it was possible to monitor the distention of the diaphragm into the thoracic cavity that occurred with elevation of the intraperitoneal pressure.

Also in these experiments, a radioactive technique with ^{51}Cr

labelled erythrocytes, was utilized to assess the component of the compliance that was due to translocation of the splanchnic blood volume. Erythrocytes were obtained from a 5 ml blood sample early in the experiment and labelled with 0.2 mc Na $^{51}Cr\ O_4$ according to the method of Veale and Vetter (1958). When required, the labelled cells were reinfused while suspended in isotonic saline. To block extraneous radioactive emissions, contoured lead shields (1/2 inch) were then placed vertically at the borders of the abdomen, with one in the pelvic region and the other set across the transpyloric plane (Figure 22). The radioactivity of the lead-shielded splanchnic region was then collimated with an open 5 x 5 cm NaI crystal placed 6 - 7 cm above the abdominal cast. Pulses of gamma energies above 100 kev were counted with a scaler timer (Baird Atomic Type 135) and a rate meter (t1/2 - 20 sec).

To correlate splanchnic radioactivity with blood volume, the record was calibrated at the end of the experiment during a nonhypotensive hemorrhage which removed 15% of the total blood volume. Under these conditions, previous data from this laboratory (Greenway and Lister, 1974) have shown that 62% of the blood volume removed is contributed by blood from splanchnic reservoirs (spleen, liver, intestine).

Both splanchnic radioactivity and chest volume data were displayed on the Beckman Dynograph along with other parameters recorded in the study. With the return to control levels of intraperitoneal pressure after periods of elevation as long as 45 minutes, both radioactive and chest volume recordings returned to their initial levels.

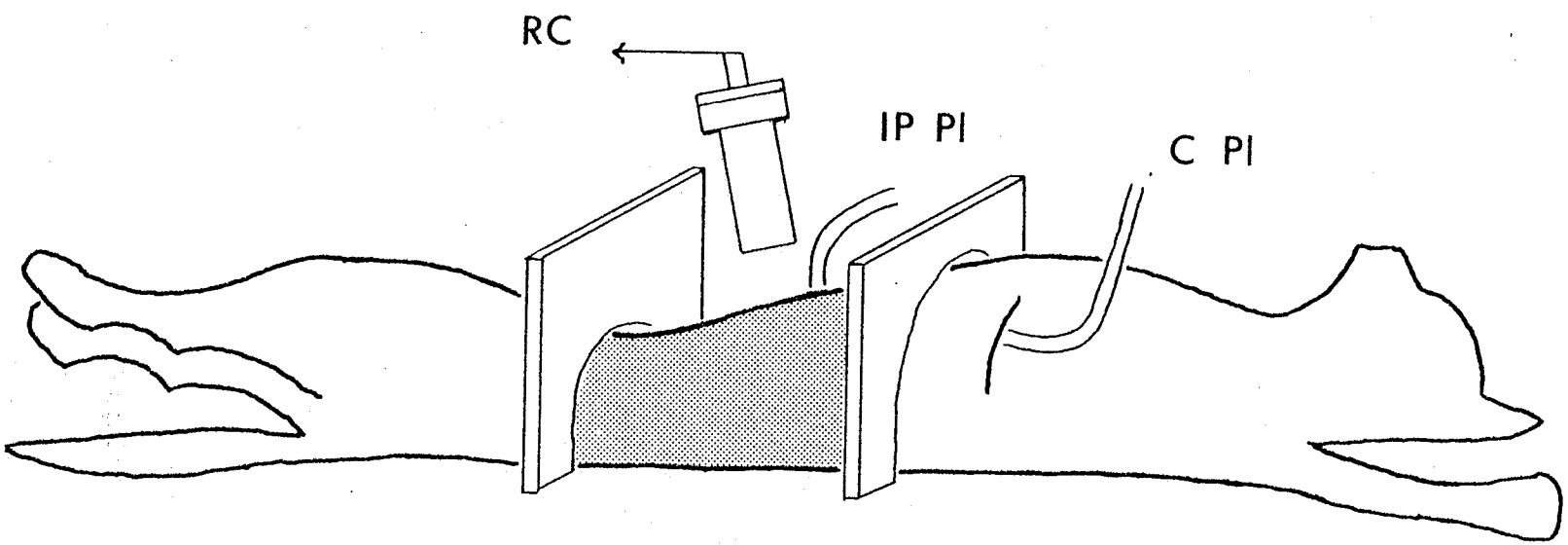


Figure 22. Splanchnic radioactive collimation (RC) combined with chest plethysmography (CPl) and intraperitoneal plethysmography (IPPl) for the study of compliance in the peritoneal cavity.

V Procedures Related to Protein Studies

In the majority of experiments the peritoneal cavity was filled with Ringer-Locke solution that was free of exogenous protein. However, in some experimental series, the Ringer-Locke solution was prepared with a concentration of bovine serum albumin (Sigma grade V) equivalent to that of the animal's plasma protein. This protein level was determined from a citrated blood sample drawn early in the experiment and analyzed by Lowry's assay (Lowry, et al., 1951).

Studies which examined the absorption rate of this fluid at several intraperitoneal pressures were conducted with the same protocol as when the peritoneal cavity contained a protein-free fluid. However other experiments were conducted with the intraperitoneal pressure set to 10 mm Hg throughout, and in these the plethysmographic recording method was slightly modified so that intraperitoneal protein concentrations could be monitored during absorption without the interference of fluid entering from the plethysmograph.

The contents of the peritoneal cavity were divided into two compartments, separated by an impermeable, highly distensible membrane (Figure 23). The "inner" compartment was contained within a loose sack attached to the Silastic tube from the plethysmograph (gauze and cage omitted from recording apparatus). The fluid in this chamber served only to record the rate of change of the intraperitoneal volume and was not in direct contact with the peritoneum. The "outer" compartment contained the Ringer-Locke-Protein solution which flooded the peritoneal cavity and was absorbed during the course of the experiment. The intraperitoneal pressure was monitored in the outer compartment throughout these experiments to ensure the accuracy of the volume record was not compromised

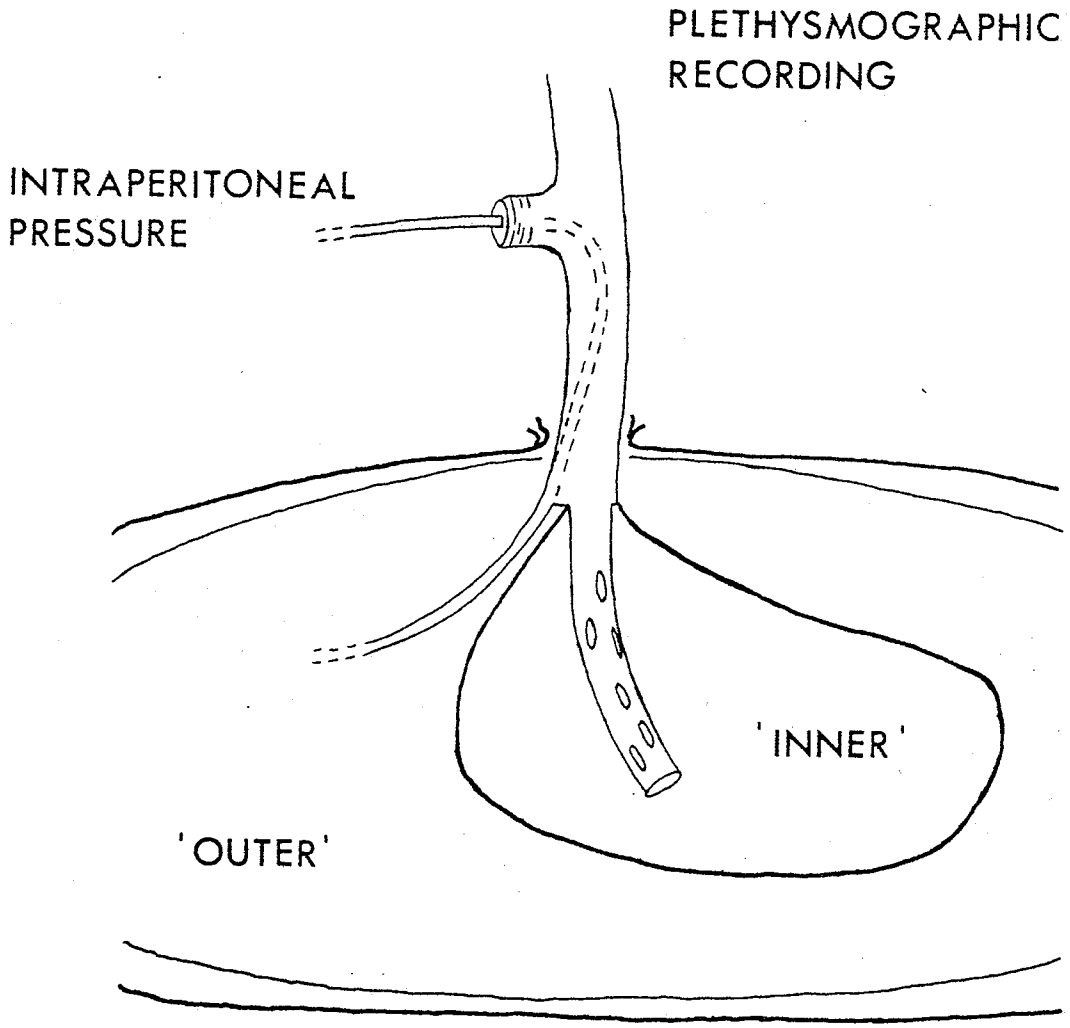


Figure 23. Plethysmographic recording with the dual compartment technique.

by stretching of the interfacial membrane. The same catheter was used to obtain samples of the intraperitoneal fluid for analysis.

Also in these experiments, the rate of entry of endogenous protein into the intraperitoneal fluid was monitored by tracer studies with labelled protein. Bovine serum albumin was labelled electrolytically with ^{125}I using the method of Katz and Bonorris (1968). Dialyzable radioactivity in the protein solution was less than 0.001% of the total radioactivity. An aliquot of the radioactive protein (approx. 1.5×10^6 CPM) was diluted in 1 ml of 0.9 g% saline for intravenous injection at the start of the experiment. Samples of plasma and intraperitoneal fluid were subsequently obtained at regular intervals and the radioactivity was counted in a gamma spectrometer (Intertechnique CG 30).

From determinations of plasma radioactivity and protein concentration, the ^{125}I concentration in the intraperitoneal fluid could thus be used to calculate the addition of endogenous protein during the course of the experiment. The sensitivity of this method is such that it could measure the transfer of as little as 25 mg of protein into the peritoneal cavity.

VI The Determination of Blood Volume

Since a plethysmographic method was utilized to record the rate of intraperitoneal fluid absorption, it was possible that a sustained and gradual reduction of the splanchnic blood volume could result in an overestimation of the rate of fluid absorption. Thus in experiments with Furosemide (Hoechst), it was necessary to determine whether the blood volume was changing during the period when fluid absorption was recorded. Although direct information is lacking, there is reason to suppose that

the accuracy of measuring blood volume using ^{51}Cr labelled erythrocytes alone might be compromised with intravenous furosemide administration. In other types of hypovolemic conditions the time required for cell mixing is prolonged (Lawson, 1962). In addition the marked diuresis induced by furosemide could alter the ratio of systemic hematocrit to whole-body hematocrit. Thus in studies of the effect of furosemide on intraperitoneal absorption, both ^{51}Cr labelled erythrocytes and ^{125}I labelled protein were used in the determination of blood volume.

The labelled erythrocytes and bovine serum albumin were prepared as described in sections IV and V and the levels of unbound isotope were negligible. Three separate measurements of blood volume were made during the course of each experiment with furosemide. Before each measurement a 4 ml blood sample was drawn for the determination of hematocrit, plasma protein, electrolytes, osmolarity and background radioactivity. Subsequently, the combined radioactive markers were injected in a common syringe and flushed with 3 ml of 50% Dextran solution in isotonic saline. Four blood samples (0.5 ml) were withdrawn at 15,30,45 and 60 minutes afterwards. With each measurement of blood volume, the radioactivity of ^{51}Cr and ^{125}I in the initial injection was doubled.

Radioactive blood samples were differentially counted in a gamma spectrometer (Intertechnique CG 30) with the window widths adjusted to separate the peak energies of the two isotopes. The overlap of ^{51}Cr into the ^{125}I channel averaged 2.8% and the overlap of ^{125}I into the ^{51}Cr channel was 0.9%. The data for both markers were corrected for overlap and analyzed separately by standard logarithmic extrapolation methods (Lawson, 1962).

Hematocrit readings (uncorrected for trapped plasma) were ob-

tained in triplicate from blood samples drawn through a short catheter in the left femoral artery. All radioactive determinations were derived from 0.5 ml samples measured by pipette and placed in the bottom of a counting vial. When other analyses were to be performed, the remaining blood was citrated and centrifuged. Plasma was then aspirated for electrolyte determinations by flame photometry (Instrumentation Laboratory #343) and for osmolarity determinations by an osmometer (Fiske).

VII Experimental Procedures

Several series of experiments were performed. In those which were primarily concerned with the absorption of intraperitoneal fluid, the intraperitoneal pressure was either raised to 10 mm Hg and maintained at this level for the duration of the experiment, or, in other studies, set to successively higher levels during the course of the experiment. In all cases the experiments were begun with the intraperitoneal pressure set to zero and a 30 minute period was allowed for equilibration.

Elevation of the intraperitoneal pressure caused a rapid increase of the intraperitoneal volume due to the compliance of the peritoneal cavity. This was followed by a slow and linear rate of volume change which remained steady for the time at a given intraperitoneal pressure. From studies of the time course of the compliance effect (see Results), a 20 minute equilibration time was selected, and in all experiments such a period was allowed after raising the intraperitoneal pressure, before the rate of intraperitoneal fluid absorption was calculated from the subsequent slow rate of volume change. In each case the rate of absorption was determined for a 30 minute period and compared to the observed rates at other pressures, or succsssive periods at the same

pressure, depending on the experimental design.

Other experiments were conducted in which either hepatic or portal venous pressures were controlled in addition to intraperitoneal pressure. The capacitance effects which occurred with increased venous pressures in these studies were also allowed a twenty minute period for equilibration before the rate of filtration or reabsorption was recorded. However, in these studies, time constraints demanded that the period of recording be shortened from 30 to 20 minutes.

With each experiment, data analysis was accomplished by randomized block analysis of variance which incorporated a routine check to ensure linearity. The plethysmographic records were analyzed at 5 minute intervals in recording periods that were 30 minutes and at 2 minute intervals in recording periods that were 20 minutes. Pooled data from several experiments were examined by random design analysis of variance in conjunction with Duncan's test of multiple comparisons as required.

7. Results

I Rate of Intraperitoneal Absorption

When the intraperitoneal pressure was set to zero at the start of the experiment, the ongoing rate of change of the intraperitoneal volume was only - 0.04 ± 0.03 ml/min; indicating only marginal absorption of Ringer-Locke solution from the peritoneal cavity. Elevation of the intraperitoneal pressure to 10 mm Hg caused an initial, rapid increase of volume followed by a slower, linear rate of fluid removal from the peritoneal cavity. Since the rate of intraperitoneal fluid absorption was measured by exclusion of the initial capacitance effect, it was necessary to determine the time course of this capacitance and identify the sources of compliance in the peritoneal cavity.

This was studied in six cats using thoracic plethysmography and a radioactive technique to monitor changes of splanchnic blood volume (see Section 6,IV). The mean compliance was 6.1 ± 0.6 ml/mm Hg when the intraperitoneal pressure was raised from 0 to 10 mm Hg and the duration of the total capacitance effect averaged 12 ± 1.7 minutes[1]. For thirty minutes subsequent to the capacitance response, the rate of change of volume was linear as proven by Armitage's statistical test of linearity (Armitage, 1971), (using 30 single minute volume decrements).

Distention of the diaphragm into the thoracic cavity accounted for 59% of the compliance and showed a time course of 95% completion within 10 minutes. An additional 22% of the compliance could be attributed to

[1] In comparison, when the intraperitoneal pressure was raised to only 5 mm Hg the compliance was slightly greater, 8.5 ± 0.4 ml/mm Hg, but the duration of the total compliance effect was less and averaged 9 ± 1.5 min.

the expulsion of splanchnic blood which showed a similar time course (Table 2). The small proportion of the compliance unaccounted for by these studies was possibly due to compression of air in the intestine and minor distention of the abdominal wall at the edge of the cast.

On the basis of this data an equilibration period of at least 20 minutes was allowed whenever the intraperitoneal pressure was increased during the experiments. The same period of time was allowed for equilibration of the capacitance effects after hepatic or portal venous pressures were increased. In these studies, the time course of the capacitance effects were not studied by radioactive monitoring of the blood volume since this has been previously examined (Greenway and Lautt, 1970).

In addition to these pressure induced blood volume changes the intraperitoneal volume record could also be affected by alterations of the splanchnic blood content caused by the injection of vasoactive drugs. Two instances of this effect are shown (for angiotensin and adrenaline injections) in Figures 24 and 25. In these animals splanchnic blood volume was recorded by radioactive collimation. With both injections, the change of the intraperitoneal volume matched the reduction of splanchnic blood volume ($\pm$ 10%), but more importantly the two parameters returned to control levels with approximately the same time course so that the rate of change of the intraperitoneal volume was the same before and after this transitory reduction of blood volume. From these observations it would seem that the time course of splanchnic blood volume changes is relatively short whether the changes are due to experimental pressure increments or pharmacological effects.

In four separate experiments the uniformity of intraperitoneal fluid absorption was investigated over a period of three hours while the

TABLE 2

A. Intraperitoneal Compliance with Elevation of the Intraperitoneal Pressure from 0 to 10 mmHg

EXPT.#	TOTAL COMPLIANCE	COMPLIANCE EXTINCTION TIME	DIAPHRAGMATIC 95% DECAY TIME	TIME TO DECAY BY 95%	BLOOD 95% DECAY TIME	TIME TO DECAY BY 95%
1.	8.2	7	4.6	6.1	---	---
2.	4.9	16	2.8	10.5	1.2	10.9
3.	6.8	9	3.9	8.1	1.6	7.5
4.	4.6	16	2.6	11.2	1.0	12.8
5.	7.1	9	4.1	6.1	1.8	8.8
6.	5.0	15	---	---	1.1	9.0
MEAN	6.1	12	3.6	8.4	1.34	9.8
± S.E.	0.6	1.7	0.4	1.1	0.2	0.9

B. Observed Intraperitoneal Compliance with Elevation of the Intraperitoneal Pressure from 0 to 5 mmHg

EXPT.#	TOTAL COMPLIANCE	COMPLIANCE EXTINCTION TIME
1.	9.6	7
2.	7.4	13
3.	9.5	6
4.	8.1	8
5.	8.9	6
6.	7.5	14
MEAN	8.5	9
± S.E.	0.4	1.5

STATS: Statistical comparisons to Part A by analysis of variance.

UNITS OF COMPLIANCE: ml/mmHg

UNITS OF TIME: min.

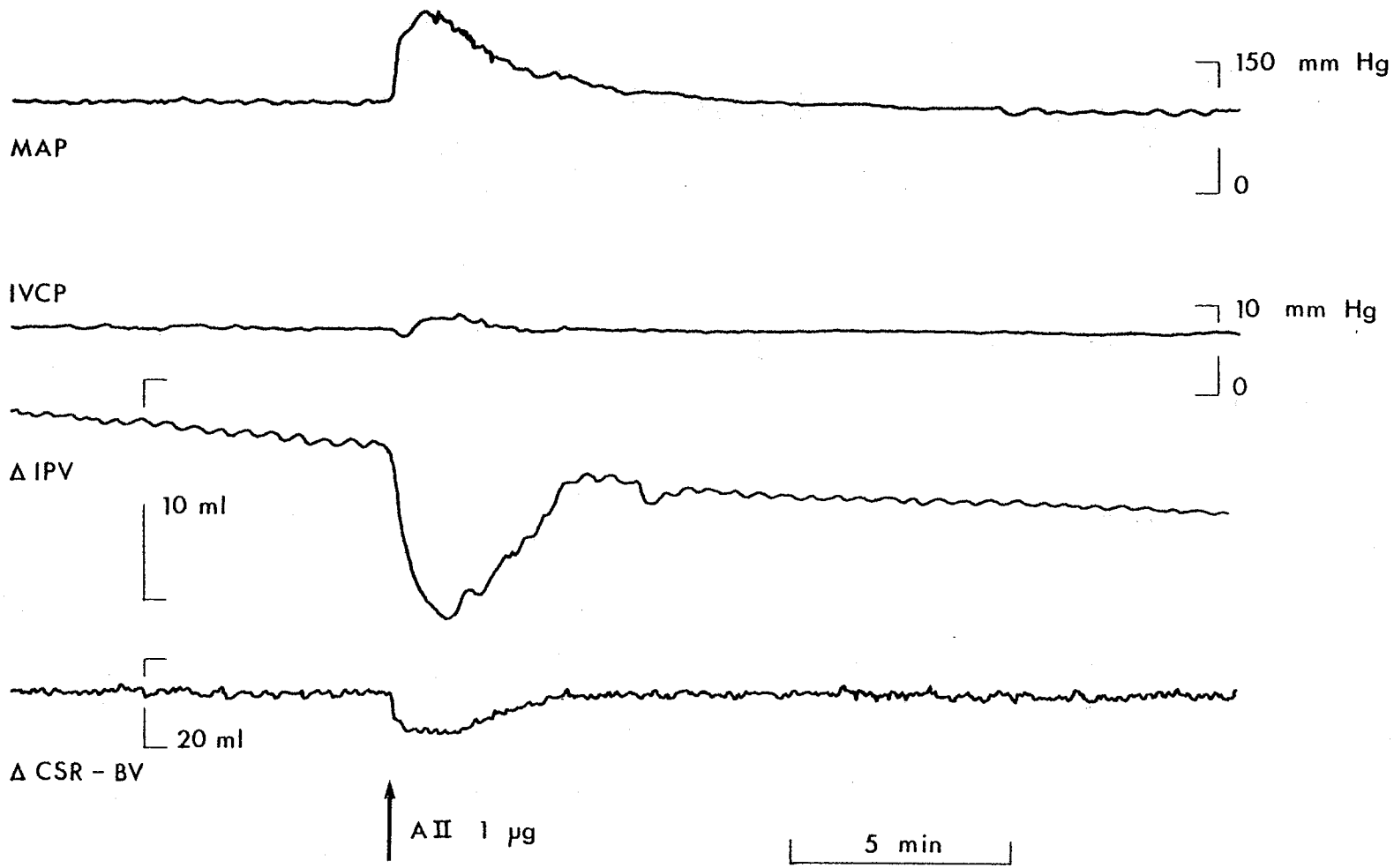


Figure 24. Recording of mean arterial pressure (MAP), inferior vena cava pressure (IVCP), change of intraperitoneal volume (Δ IPV) and change of collimated splanchnic radioactivity (Δ CSR - BV) in one cat (2 kg) given 1 µg of angiotensin II (A II) while the intraperitoneal pressure was set to 5 mm Hg.

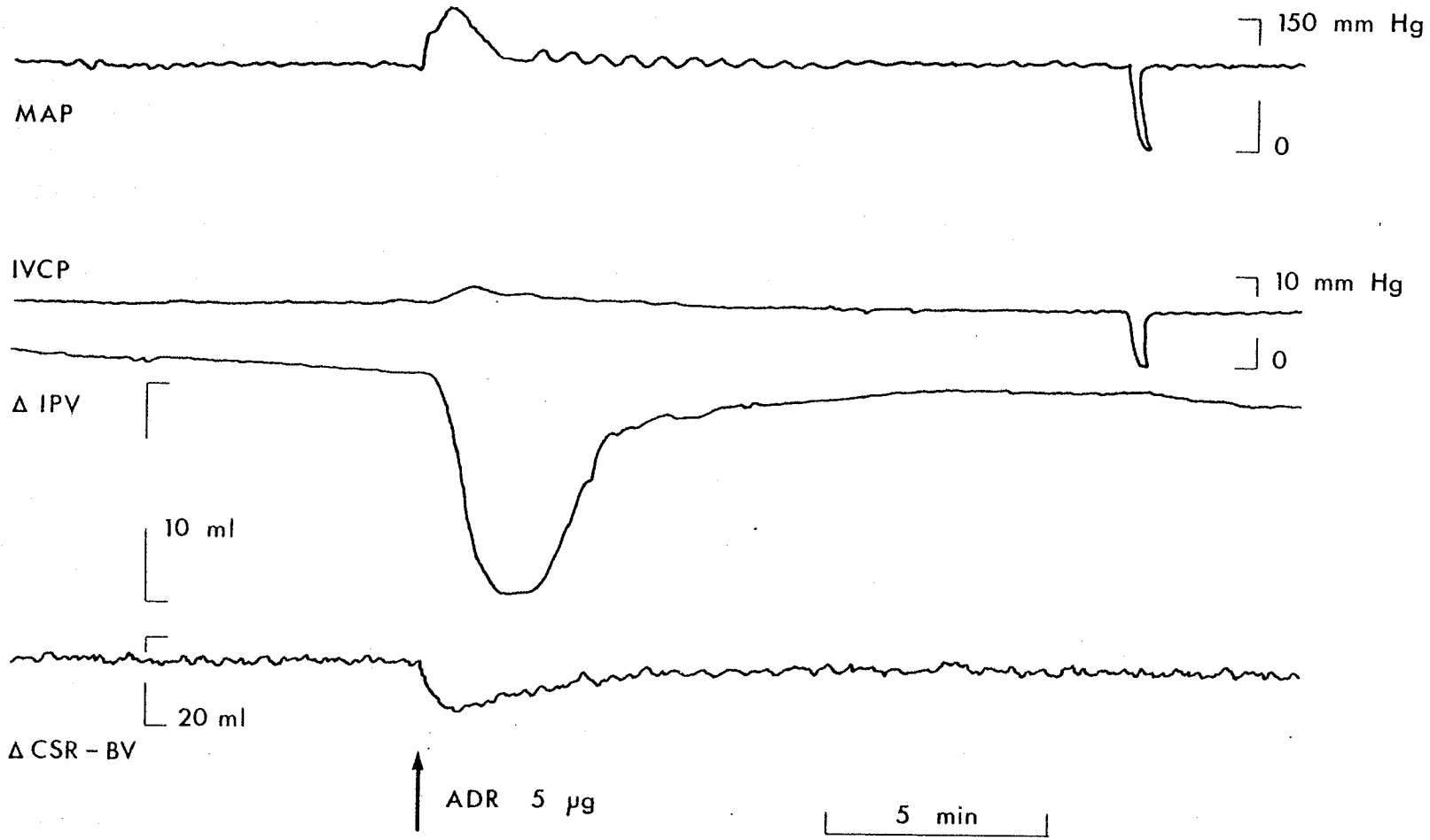


Figure 25. Recording of mean arterial pressure (MAP), inferior vena cava pressure (IVCP), change of intraperitoneal volume (Δ IPV) and change of collimated splanchnic radioactivity (Δ CSR - BV) in one cat (2.4 kg) given 5 µg of adrenaline (ADR) while the intraperitoneal pressure was set to 5 mm Hg.

intraperitoneal pressure was maintained at a constant level of 10 mm Hg. The overall mean rate of absorption of Ringer-Locke solution was 0.22 $\pm$ 0.03 ml/min and a comparative analysis of the rates for 6 sequential 30 minute periods indicated that there was no significant change in the rate of fluid absorption during this time (Table 3).

In three similar experiments the measurement of fluid absorption by intraperitoneal plethysmography was compared to the absorption rate as calculated from the difference of the initial and final intraperitoneal fluid volumes. At the start of each experiment any residual intraperitoneal fluid was removed with absorbent gauze and the volume of fluid added to the peritoneal cavity was carefully measured. After absorption at a constant pressure of 10 mm Hg for three hours, the intraperitoneal pressure was returned to zero for twenty minutes and the animal was sacrificed to allow evacuation of the fluid remaining in the peritoneal cavity. Calculation of absorption by this method yielded values which closely agreed with the data obtained by intraperitoneal plethysmography (2.2, 4.0, 4.5%, respectively in the 3 experiments).

II Effects of Intraperitoneal Pressure and Protein Concentration on Absorption

Ringer-Locke solution was absorbed from the peritoneal cavity at a greater rate when the intraperitoneal pressure was increased. This effect is illustrated in Figure 26 for an experiment that is representative of the findings in 6 animals. After equilibration at each intraperitoneal pressure, the absorption rate was recorded over a 30 minute interval and, in all experiments, there was a significant ($P < .01$) increase

TABLE 3.

Rates of Absorption of Intraperitoneal Fluid over a Period of Three Hours

TIME	0 - 30	30 - 30	60 - 90	90 - 120	120 - 150	150 - 180	MINUTES
EXPT. #							
1.	- 0.11	- 0.19	- 0.14	- 0.13	- 0.14	- 0.15	ml/min
2.	- 0.27	- 0.27	- 0.31	- 0.28	- 0.27	- 0.31	ml/min
3.	- 0.23	- 0.22	- 0.26	- 0.20	- 0.20	- 0.19	ml/min
4.	- 0.24	- 0.21	- 0.18	- 0.18	- 0.17	- 0.17	ml/min
Mean ± S.E.	- 0.21 0.013	- 0.22 0.013	- 0.22 0.013	- 0.20 0.013	- 0.20 0.013	- 0.20 0.013	ml/min

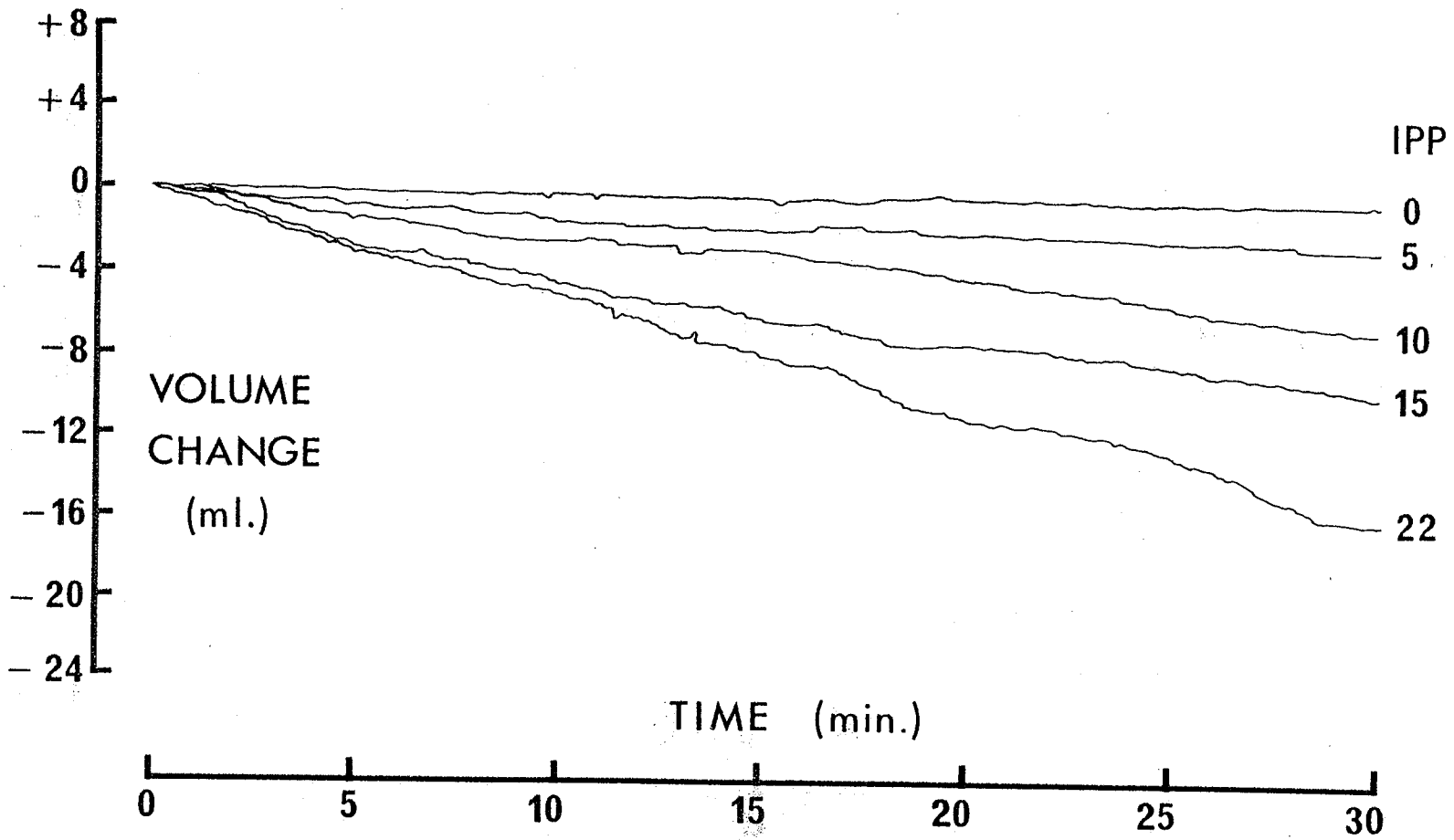


Figure 26. Records of intraperitoneal volume change obtained in one cat for five sequential 30 minute periods when the intraperitoneal pressure (IPP) was set to various levels from 0 to 22 mm Hg. Each recording period is preceded by a 20 min equilibration period (not shown).

in the rate of absorption with greater intraperitoneal pressures.

For the six experiments in which the peritoneal cavity contained Ringer-Locke solution, the combined data for the effects of raising intraperitoneal pressure are shown in Figure 27. It is apparent that there is a nearly linear relationship; with the rate of fluid absorption increasing by 0.021 ml/min for each 1 mm Hg increment of the intraperitoneal pressure. In five similar experiments, the rate of absorption was investigated when the colloid osmotic pressure of the intraperitoneal fluid was elevated by preparation of the Ringer-Locke solution with an added concentration of bovine serum albumin equivalent to the plasma protein concentration in each animal. As shown in Figure 27, the rate of fluid absorption and the effect of increased intraperitoneal pressures were identical to that recorded in experiments when protein-free fluid was present in the peritoneal cavity.

The mean plasma protein concentration was 7.8 $\pm$ 0.3 g% in these animals, and this did not change significantly during the course of the experiment. Protein concentrations in the intraperitoneal fluid at the beginning and end of the experiments averaged 7.9 and 8.0 $\pm$ 0.4 g% respectively. Together this data indicates that the rate of fluid absorption from the peritoneal cavity was accelerated by increasing the intraperitoneal pressure but unaffected by the gradient of colloid osmotic pressure between the intraperitoneal fluid and plasma.

For these eleven experiments the control data for pressures, hematocrit and plasma protein concentrations are shown in Figure 28. When the intraperitoneal pressure was less than 1 mm Hg at the start of the experiments, caval venous pressure in the abdomen averaged 4.5 $\pm$ 0.4 mm Hg. Both pressures increased in parallel as the hydrostatic pressure imposed on the peritoneal cavity was increased. Neither mean arterial pressure

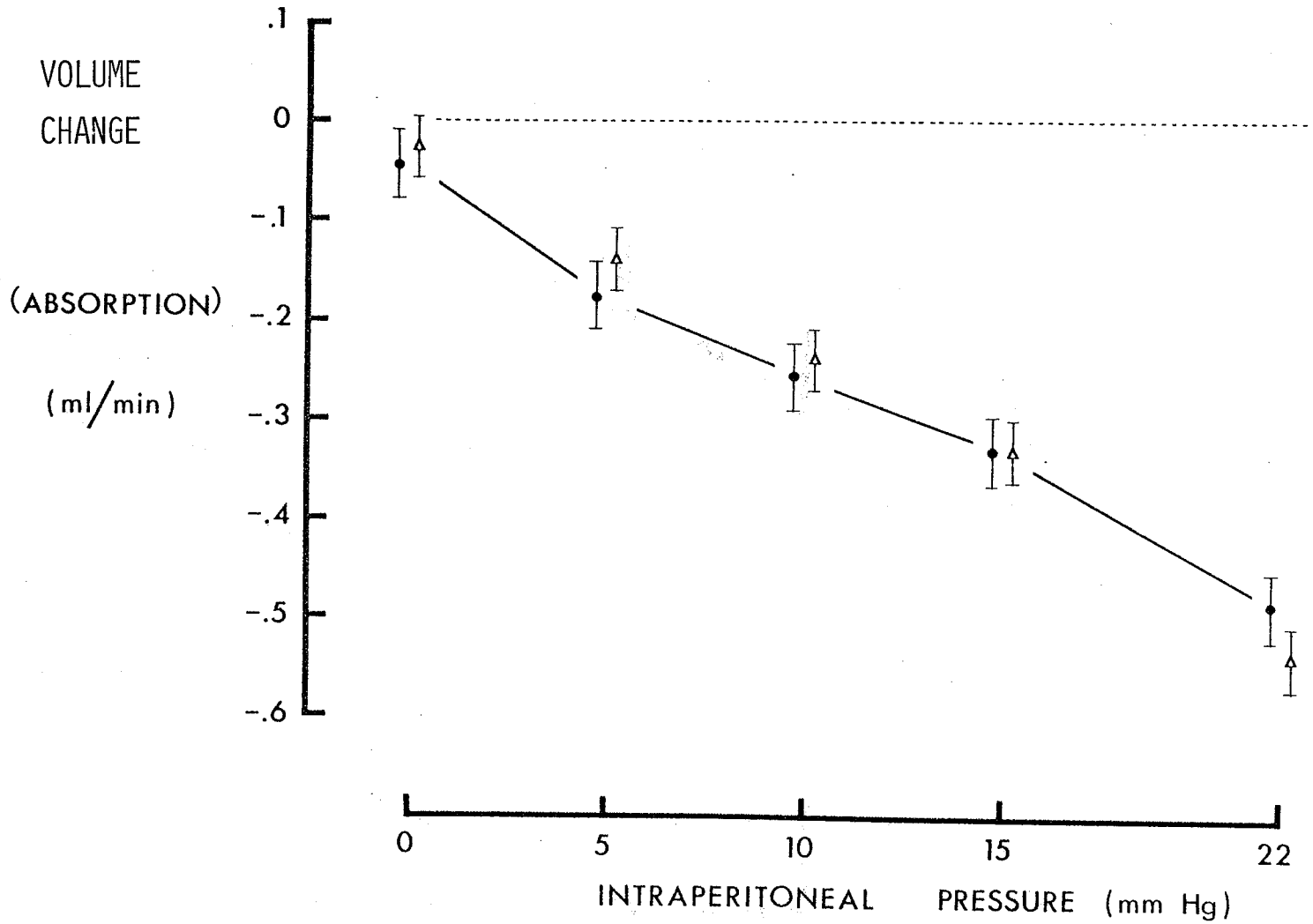
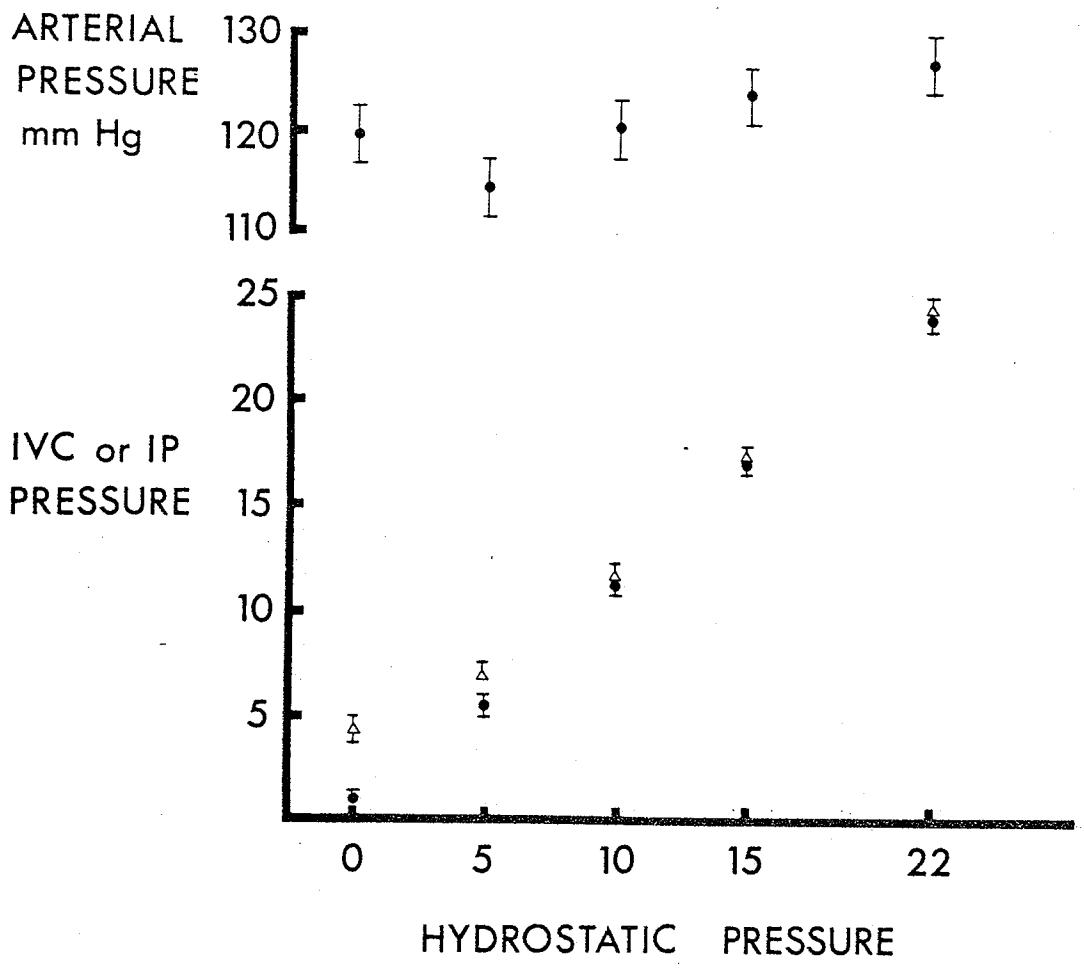


Figure 27. Rate of absorption of Ringer-Locke solution (●) or Ringer-Locke solution with protein (Δ) as a function of the intraperitoneal pressure.



HEMATOCRIT %	32.5 ±1.5	36.3 ±1.5
PLASMA PROTEIN g%	7.8 ±0.3	8.0 ±0.3

Figure 28. Effect of hydrostatic pressure, controlled by the plethysmograph, on arterial pressure, inferior vena caval (IVC) pressure, intraperitoneal (IP) pressure, hematocrit and plasma protein concentration. IVC pressure 1--Δ--1; IP pressure 1--•--1.

nor hematocrit were altered significantly during the experiment ($P < .05$).

III Intraperitoneal Protein Levels During Absorption

The concentration of protein in the intraperitoneal fluid is of interest since it may affect the rate of fluid transfer into the peritoneal cavity from extrahepatic vascular beds. Also it has been suggested (Hyatt and Smith, 1954) that the low protein concentration in ascitic fluid may be due to a hyper-oncotic mechanism of ascites reabsorption. Thus to examine the effect of intraperitoneal fluid absorption on the concentration of protein in the intraperitoneal fluid, a series of six experiments was conducted using the dual compartment technique (see Section 6,V).

Ringer-Locke-Protein solution, with a mean starting protein concentration of 7.7 ± 0.2 g%, was absorbed over a 5 hour period while the intraperitoneal pressure was set to 10 mm Hg. As shown in Figure 29, the assay of hourly plasma and intraperitoneal fluid samples revealed that protein levels in the peritoneal cavity were not significantly changed over this period of time, despite the removal of almost 80% of the fluid initially placed in the "outer" compartment.

Also, in these experiments ^{125}I labelled protein was utilized to record the rate of entry of endogenous protein into the peritoneal cavity. This data showed that only 0.31 ± 0.15 g of endogenous protein was added during 5 hours of fluid absorption. The calculated mean protein concentration of the absorbed fluid was 8.0 ± 0.4 g% which was not statistically different from the mean protein concentration of the intraperitoneal fluid over the absorption period. Thus it would seem that the absorption of intraperitoneal fluid neither concentrates nor dilutes the protein in the fluid remaining in the peritoneal cavity.

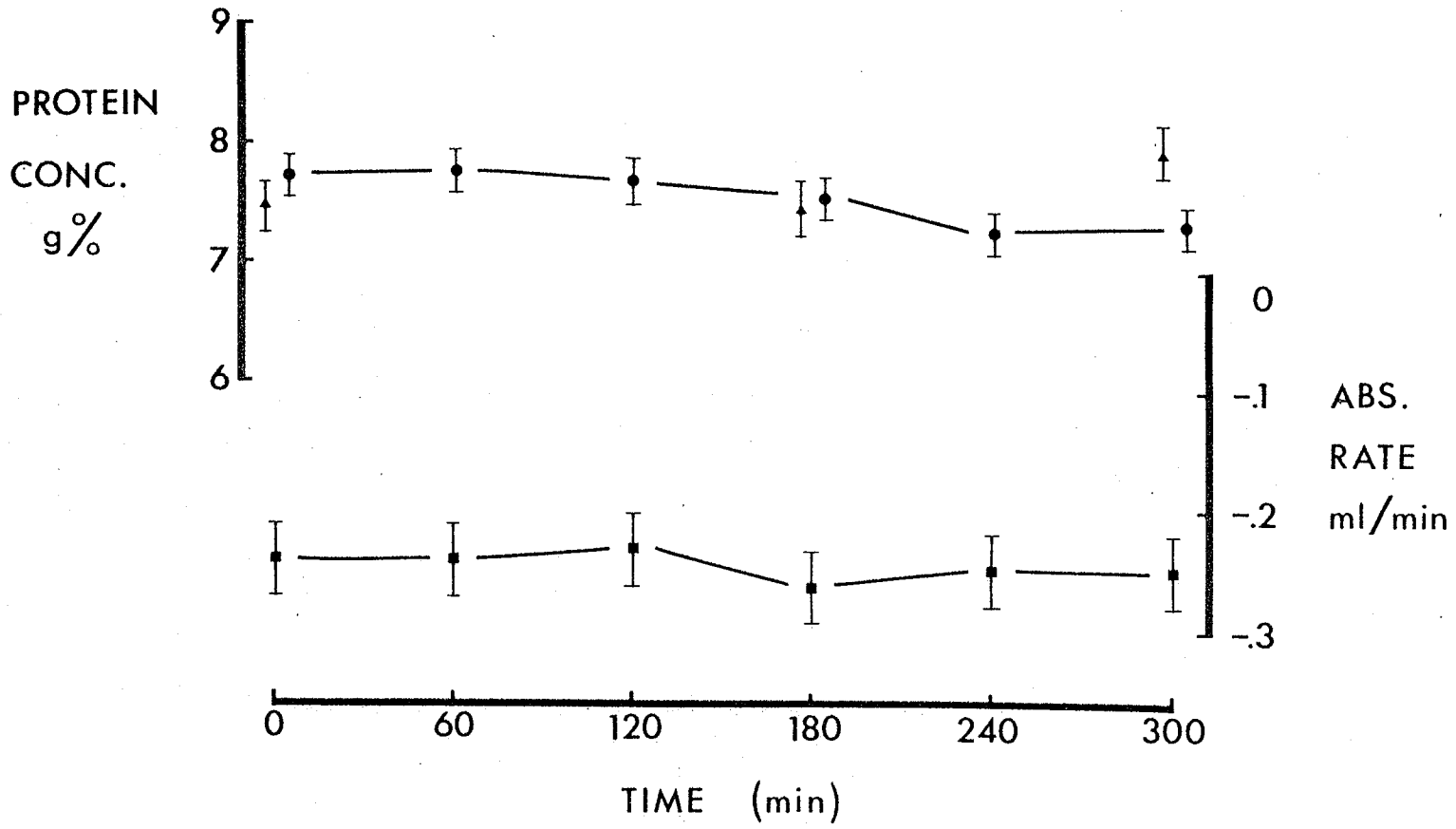


Figure 29. Protein concentrations in plasma (▲) and the intraperitoneal fluid (●) over a period of 5 hours while fluid was absorbed from the peritoneal cavity.

IV Intraperitoneal Absorption after Furosemide Diuresis

Since diuretic administration is a frequent adjunct to the medical management of clinical ascites and in some cases responsible for marked reduction of the ascitic volume, experiments were conducted to determine whether this action was mediated by a direct acceleration of the rate of intraperitoneal fluid absorption. To examine this possibility using the technique of intraperitoneal plethysmography, it was necessary to ensure that the splanchnic blood volume was constant or changing by a measured amount during the time that the absorption rate was determined.

In a series of 6 experiments the blood volume was assayed using both plasma and erythrocyte markers while absorption was recorded with the intraperitoneal pressure set to 10 mm Hg. The rate of absorption of Ringer-Locke solution was compared for two 30 minute periods; one before furosemide administration (3.1 mg/kg), and the other beginning 140 minutes after injection (approximately 2 hours after peak diuresis). Figure 30 outlines the protocol for the measurement of fluid absorption in relation to blood volume monitoring.

The diuretic response to intravenous furosemide became evident about 10 minutes after injection (Figure 31), but if furosemide increased the rate of intraperitoneal absorption, this would have been most apparent about 2 hours after administration. By this time the diuresis of 69 $\pm$ 8 ml resulted in 22% reduction of blood volume from 142 ml before furosemide to 111 ml ($\pm$ 2.9 ml) afterwards (Figure 32). There was marked hemoconcentration and an increase of the plasma protein concentration from 7.7 to 8.8 $\pm$ 0.2 g% but little change of plasma osmolarity or electrolyte concentrations (Table 4). Nevertheless, the rate of fluid absorption was not

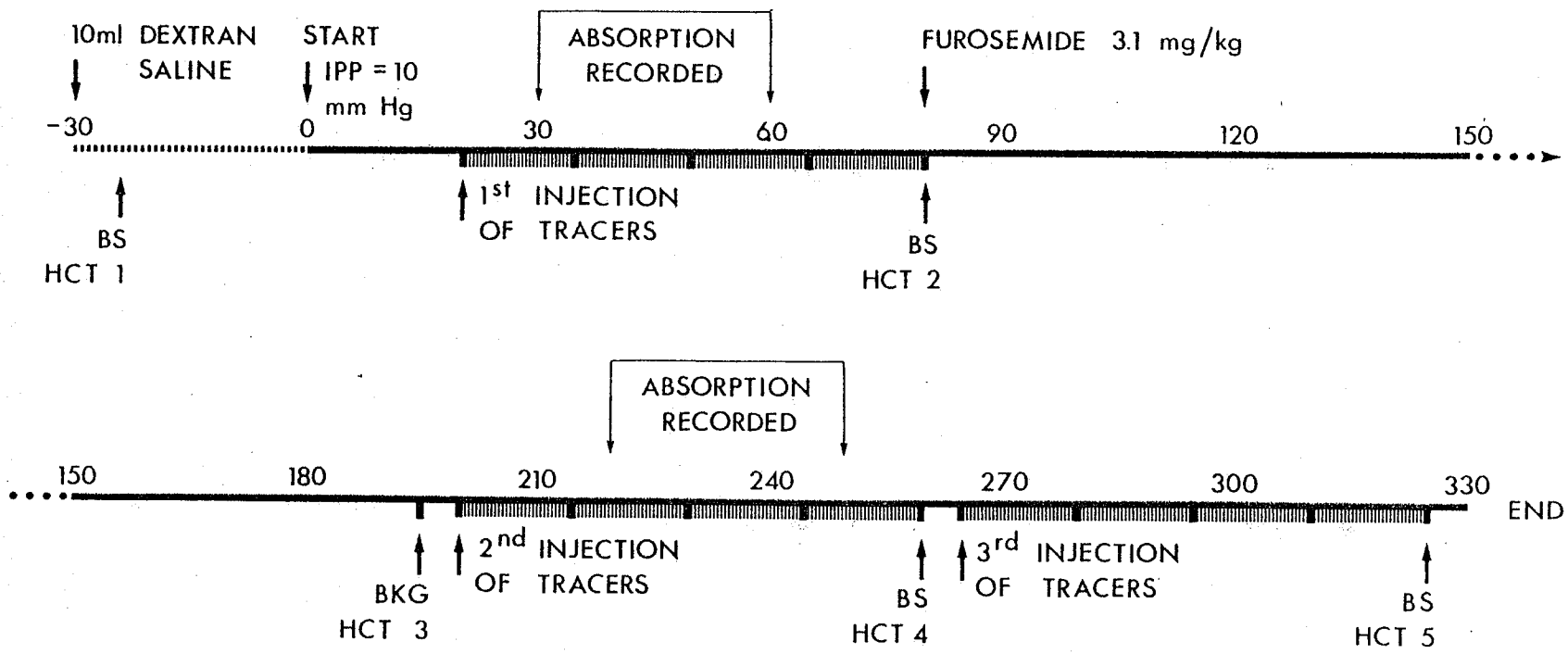


Figure 30. Protocol for measurement of blood volume in experiments with furosemide. Dual isotope tracers injected as shown and 4 blood samples (BS) obtained over a period of 60 minutes after each injection. HCT: hematocrit, IPP: intraperitoneal pressure, BKG: sample for background radioactivity.

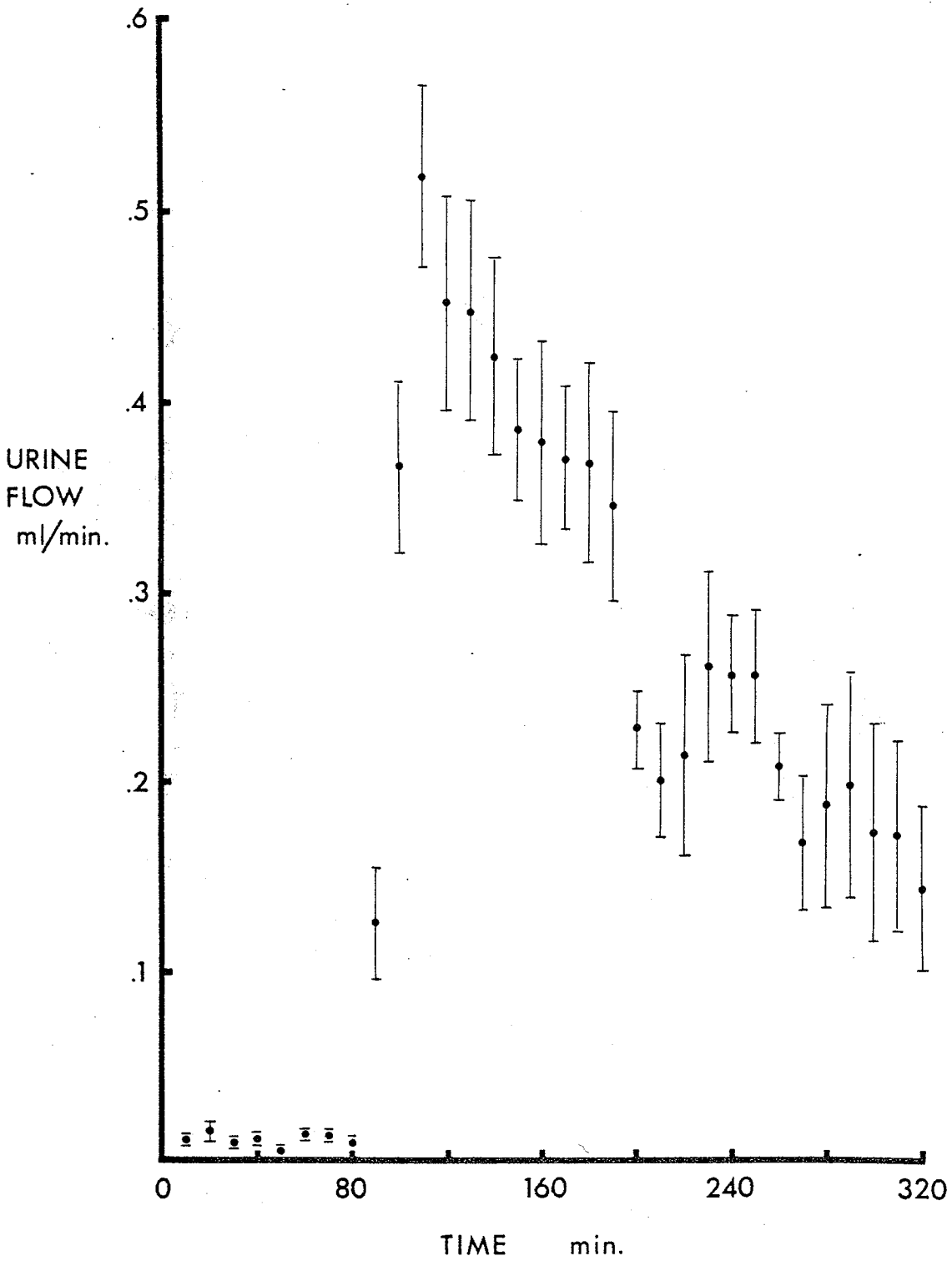


Figure 31. Diuretic response to the injection of furosemide 3.1 mg/kg after 80 minutes of control measurements.

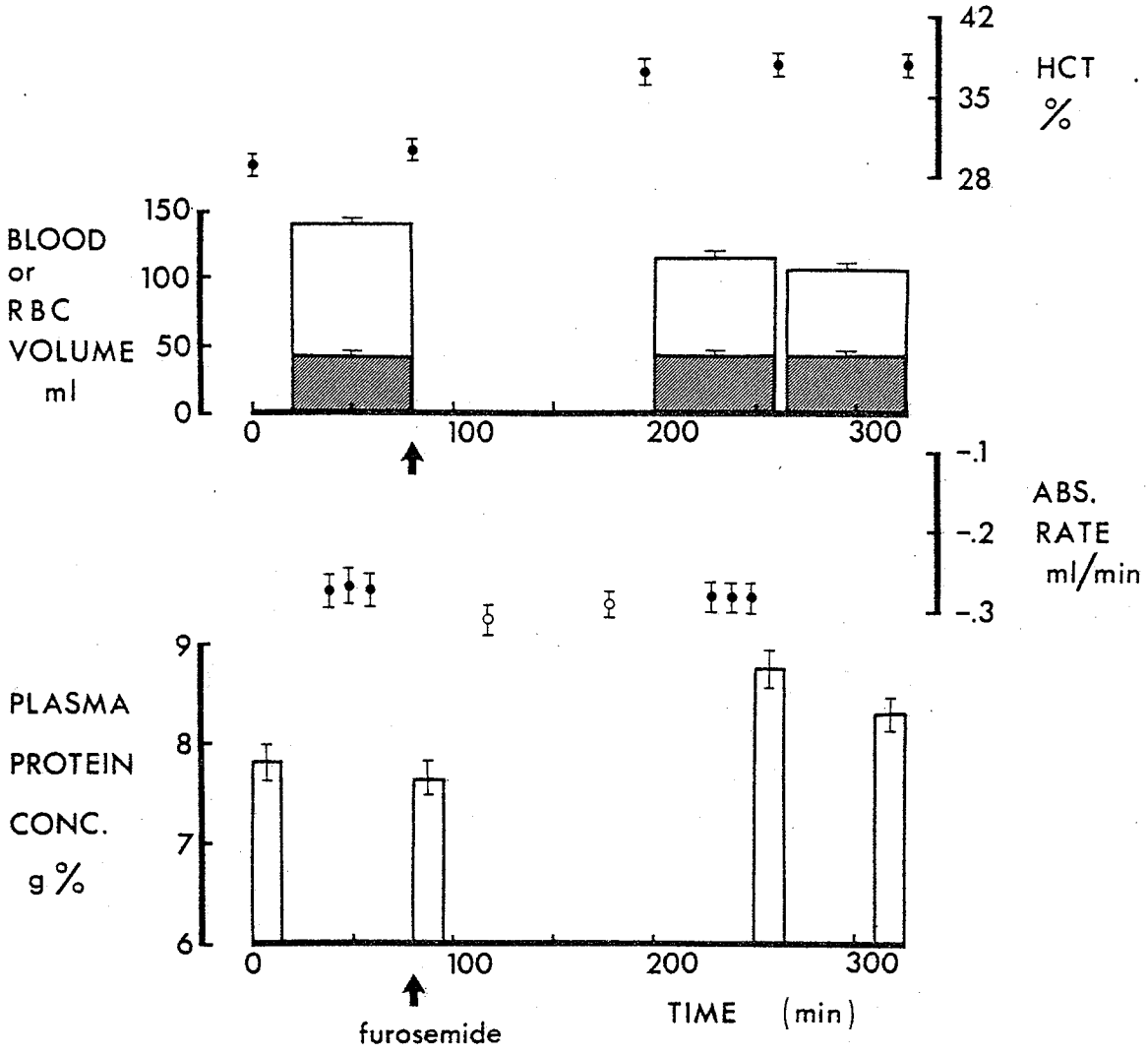


Figure 32. Effect of furosemide injection (3.1 mg/kg) on blood volume, red blood cell volume (shaded area), hematocrit (HCT), intraperitoneal absorption rate and plasma protein concentration.

TABLE 4

Osmolality and Concentrations of Electrolytes in Plasma and Intraperitoneal Fluid Before and After Furosemide Diuresis

		TIME (MIN) 0	80	260	325	± S.E.
Sodium (mEq/l)	plasma	156.7	151.6	158.9	157.8	2.9
	intraperitoneal	152.1	153.4	152.8	155.8	4.1
Potassium (mEq/l)	plasma	4.4	4.2	4.2	4.2	0.1
	intraperitoneal	4.1	4.0	4.2	4.2	0.7
Osmolality ($mOsm/kg\ H_2O$)	plasma	303	306	304	303	4
	intraperitoneal	298	302	302	303	7

Before Furosemide

After Furosemide

significantly increased from the rate before furosemide administration (-0.28 vs -0.29 $\pm$ 0.02 ml/min). If anything, the actual rate may have been slower since the blood volume continued to decrease, though at a slower rate (2 ml during the 30 minute period of recording), during measurement of the absorption rate after furosemide.

Table 5 provides more detailed data for the changes of blood volume after furosemide administration. For each measurement of blood volume in each experiment the data obtained from cellular and protein markers were calculated separately. There is a relatively good agreement for the ^{51}Cr and ^{125}I blood volume data before furosemide administration with the protein marker yielding an estimate about 6.6% greater than the value obtained by the cellular marker.

After furosemide, this difference widens so that the discrepancy becomes 15% and 10% respectively for the two blood volume measurements.

Although the reasons for this discrepancy are unknown, these data justify the use of a dual isotope method for blood volume determinations under these conditions. If one assumes that, in cats, the mixed arterial hematocrit exceeds the whole body hematocrit by a couple of percent, then possibly furosemide could widen this difference. Under control conditions the whole body hematocrit would be only slightly overestimated so that that blood samples would contain a bit more ^{51}Cr and a bit less ^{125}I than the "true" whole body concentrations. This would cause the cellular marker ^{51}Cr to underestimate the blood volume, and similarly the ^{125}I data would overestimate it. After furosemide with the marked rise of the hematocrit of the sampled blood, this discrepancy could be accentuated if the whole body hematocrit did not rise to the same extent.

TABLE 5

Effect of Furosemide on Blood Volume as Determined by Indicator Dilution with Markers Confined to Cellular or Plasma Compartments

EXPERIMENT NO.	A	B	C	D	E	F	MEAN ± S.E.	
^{51}Cr Blood Volume	145	127	142	139	142	129	137 ± 3	Before Furosemide
	114	104	113	110	108	96	108	After Furosemide
	112	96	102	101	102	97	102	After Furosemide
^{125}I Blood Volume	156	135	155	146	149	137	147 ± 3	Before Furosemide
	132	117	127	125	124	118	124	After Furosemide
	121	100	115	116	115	108	112	After Furosemide
Averaged Blood Volume	151	131	149	143	146	133	142 ± 3	Before Furosemide
	123	111	120	118	116	107	115	After Furosemide
	117	98	109	109	109	103	107	After Furosemide
Averaged Red Blood Cell Volume	46	40	46	43	44	40	43 ± 2	Before Furosemide
	46	41	45	44	43	40	43	After Furosemide
	45	38	42	42	42	39	41	After Furosemide

Time	0	80	195	260	325	
Hematocrit ± S.E.	30.5	30.7	36.8	38.1	37.7	± 0.9

V Ascites Formation with Raised Hepatic Venous Pressure

In 14 cats prepared with the extracorporeal circuit, the hepatic venous pressure was initially set to zero by placing the venous outflow control reservoir at a height equal to the level of emergence of the inferior vena cava from the liver. Twenty minutes were allowed for equilibration before beginning the experiment and during this period the mean arterial pressure was 107 $\pm$ 5.1 mm Hg. Portal pressure was 7.6 $\pm$ 0.2 mm Hg. Flow through the hepatic circuit averaged 89 $\pm$ 2.7 ml/min (liver weight 69 $\pm$ 4.8 g) and flow through the extrahepatic circuit averaged 67 $\pm$ 3.0 ml/min. The mean hematocrit was 34 $\pm$ 1.8%.

The effect of increasing hepatic venous pressure was investigated in 10 animals while the intraperitoneal pressure remained constant at either 0 mm Hg or 10 mm Hg. At the beginning of experiments with both the hepatic venous and intraperitoneal pressures set to zero, the intraperitoneal volume remained essentially constant, increasing at a rate of only 0.064 $\pm$ 0.026 ml/min. Subsequent elevation of the hepatic venous pressure produced the usual capacitance response which rapidly equilibrated, revealing a steady rate of fluid filtration into the peritoneal cavity. Since the capacitance responses (3.25 ml/mm Hg) were due to pooling of blood in the splanchnic vasculature, an equivalent blood volume was displaced from the extracorporeal venous reservoir and blood or 5% Dextran in isotonic saline was added to maintain an adequate volume in the main venous reservoir.

With sequential 4 mm Hg increments of hepatic venous pressure, the arterial pressure was unaffected but portal pressure increased progressively with hepatic venous pressure. As shown in Figure 33, this

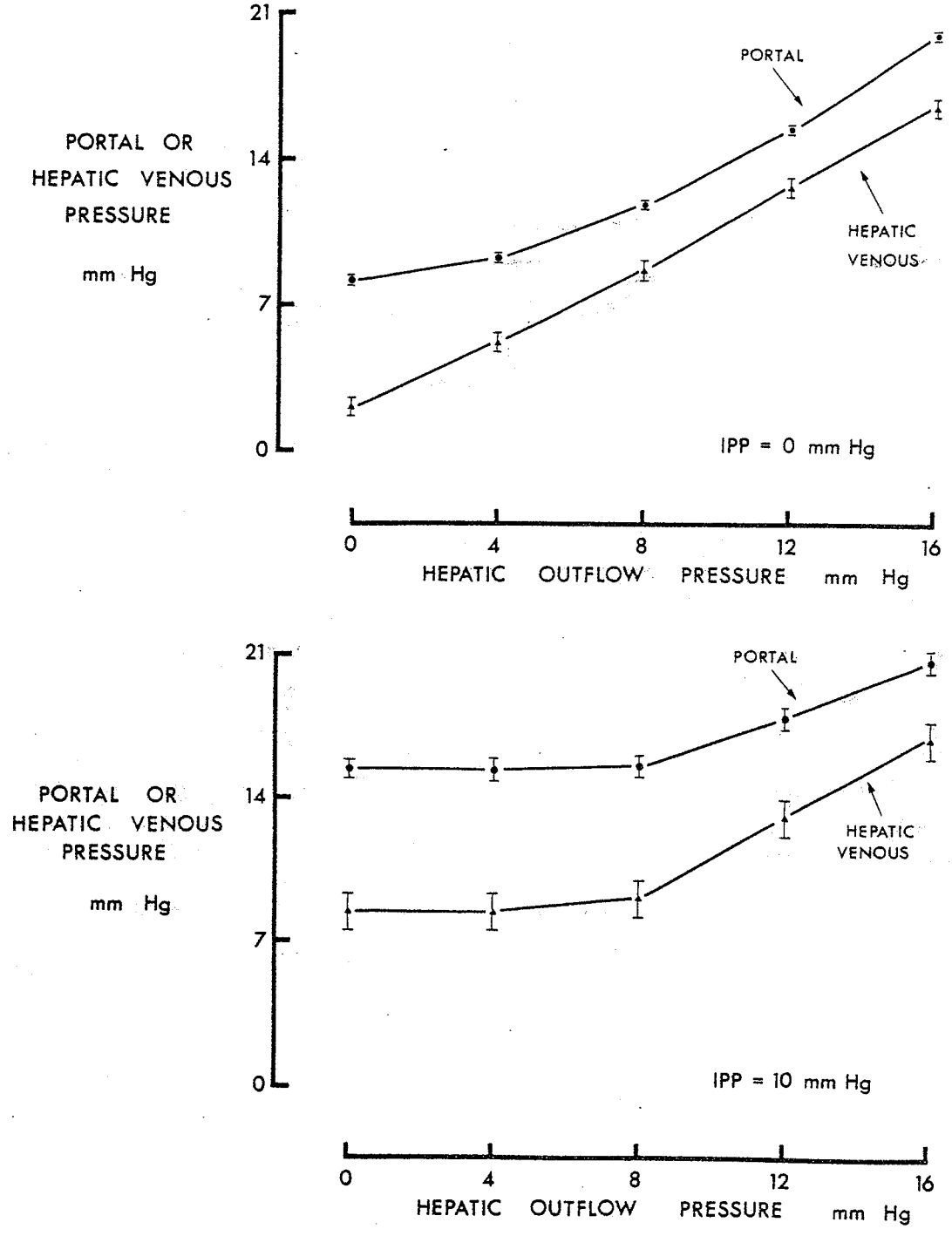


Figure 33. Effect of hepatic outflow pressure, controlled by the venous reservoir, on portal and hepatic venous pressures when the intraperitoneal pressure (IPP) is 0 or 10 mm Hg.

effect on portal pressure was most substantial at the higher levels of hepatic venous pressure when approximately equal increments of portal pressure were obtained. Figure 34 shows that the rate of ascites formation was proportional to the selected level of hepatic venous pressure. However, greater increases in the filtration rate occurred when the hepatic venous pressure was raised to 12 and 16 mm Hg. In each animal a similar pattern was observed, although the absolute rates of fluid filtration could differ substantially between animals.

Since the pathological accumulation of ascites often increases intraperitoneal pressure, the effect of hepatic venous pressure on the rate of ascites formation was subsequently re-examined when the intraperitoneal pressure was raised. The hepatic outflow reservoir was thus restored to the zero level and the intraperitoneal pressure was fixed at 10 mm Hg. This increased portal pressure to 15.3 $\pm$ 0.5 mm Hg and hepatic venous pressure to 8.4 $\pm$ 0.4 mm Hg even though the hepatic outflow reservoir was set to zero (Figure 33).

After 30 minutes was allowed for equilibration and decay of the capacitance effect due to raising intraperitoneal pressure, the rate of intraperitoneal fluid movement was recorded. While the hepatic outflow reservoir was set to zero, fluid was reabsorbed from the peritoneal cavity at a constant rate of -0.242 $\pm$ 0.034 ml/min (cf-0.22 $\pm$ 0.03 ml/min at this intraperitoneal pressure without the extracorporeal circuit).

As the height of the hepatic outflow control reservoir was now raised to 4 or 8 mm Hg the rate of ascites reabsorption was unaffected and remained constant at - 0.255 and - 0.250 $\pm$ 0.034 ml/min respectively (Figure 34). In addition the usual capacitance effects did not occur with these increases of pressure.

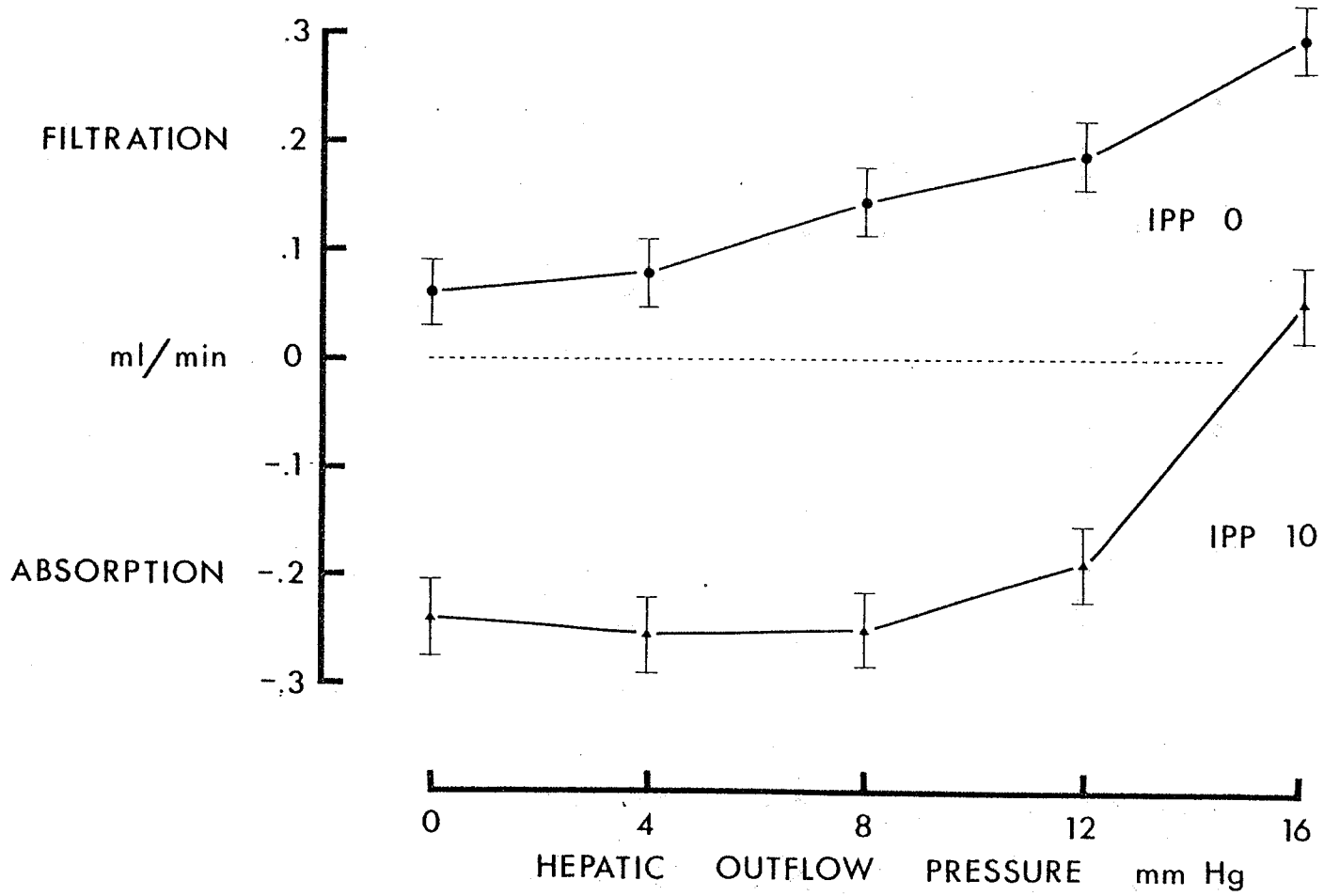


Figure 34. Effect of hepatic outflow pressure, controlled by the venous reservoir, on the net rates of ascites filtration and absorption when the intraperitoneal pressure (IPP) is 0 or 10 mm Hg.

Further elevation of the hepatic outflow reservoir to 12 and 16 mm Hg raised hepatic venous and portal pressures as shown in Figure 33. Thus the hepatic venous pressure was now controlled by the height of the outflow reservoir rather than the intraperitoneal pressure as previously. At the same time ascites formation was stimulated so that the rate of change of the intraperitoneal volume was now in the control of both the rate of fluid absorption and the rate of ascites formation. When the hepatic venous pressure was 12 mm Hg the rate of ascites reabsorption was just slightly reduced to - 0.189 $\pm$ 0.034 ml/min. However when the hepatic venous pressure reached 16 mm Hg, the combined rates of ascites formation and reabsorption were such that there was a net formation of ascites at 0.052 $\pm$ 0.034 ml/min (Figure 34).

VI Ascites Formation with Raised Portal Pressure

Before continuing studies of the interaction between ascites formation and reabsorption it was necessary to ascertain the source of intraperitoneal fluid formation with increased hepatic venous pressures. In a previous study (Greenway and Lautt, 1970), elevation of hepatic venous pressure was shown to cause fluid filtration from the hepatic surface by plethysmographic recording from the *in situ* cat liver. However, since portal pressure is also markedly increased by raising hepatic venous pressure, the intestinal vascular bed could be an additional source of ascites in the present experiments where the entire peritoneal cavity serves as the plethysmographic chamber.

To consider this possibility three separate experiments were conducted to examine the effects of portal hypertension in the absence of

a raised hepatic venous pressure. In each animal this was investigated first while the intraperitoneal pressure was zero and later when it was raised to 5 mm Hg in which case there was an ongoing absorption rate of -0.128 ± 0.034 ml/min. As shown in Figure 35, the rate of ascites formation or reabsorption is not significantly altered when the portal pressure was raised by as much as 16 mm Hg. Thus the intestinal vascular bed was probably not a source of ascitic fluid in the present experiments since the maximum portal pressure attained by raising hepatic venous pressure was 22.5 mm Hg; somewhat less than the pressure attained by compression of the portal vein.

VII Interaction of Ascites Formation and Reabsorption

From the data presented in sections 3,II and 3,V it is apparent that the rate of change of the intraperitoneal volume is reduced by increasing the intraperitoneal pressure and stimulating absorption; conversely, increasing the hepatic venous pressure causes filtration, apparently from the liver, and increases the intraperitoneal volume. However, these two processes are not entirely separate since the intraperitoneal pressure also affects the hepatic venous pressure (Section 7,V).

The interaction of ascites formation and reabsorption was further investigated in a series of four experiments. Initially the intraperitoneal pressure was set to 0 mm Hg and the hepatic venous pressure was 12 mm Hg. After equilibration, ascites was filtered into the peritoneal cavity at a constant rate as shown in Figure 36. For each subsequent recording period the intraperitoneal pressure was increased in a stepwise fashion while hepatic venous pressure remained at 12 mm Hg. The resulting

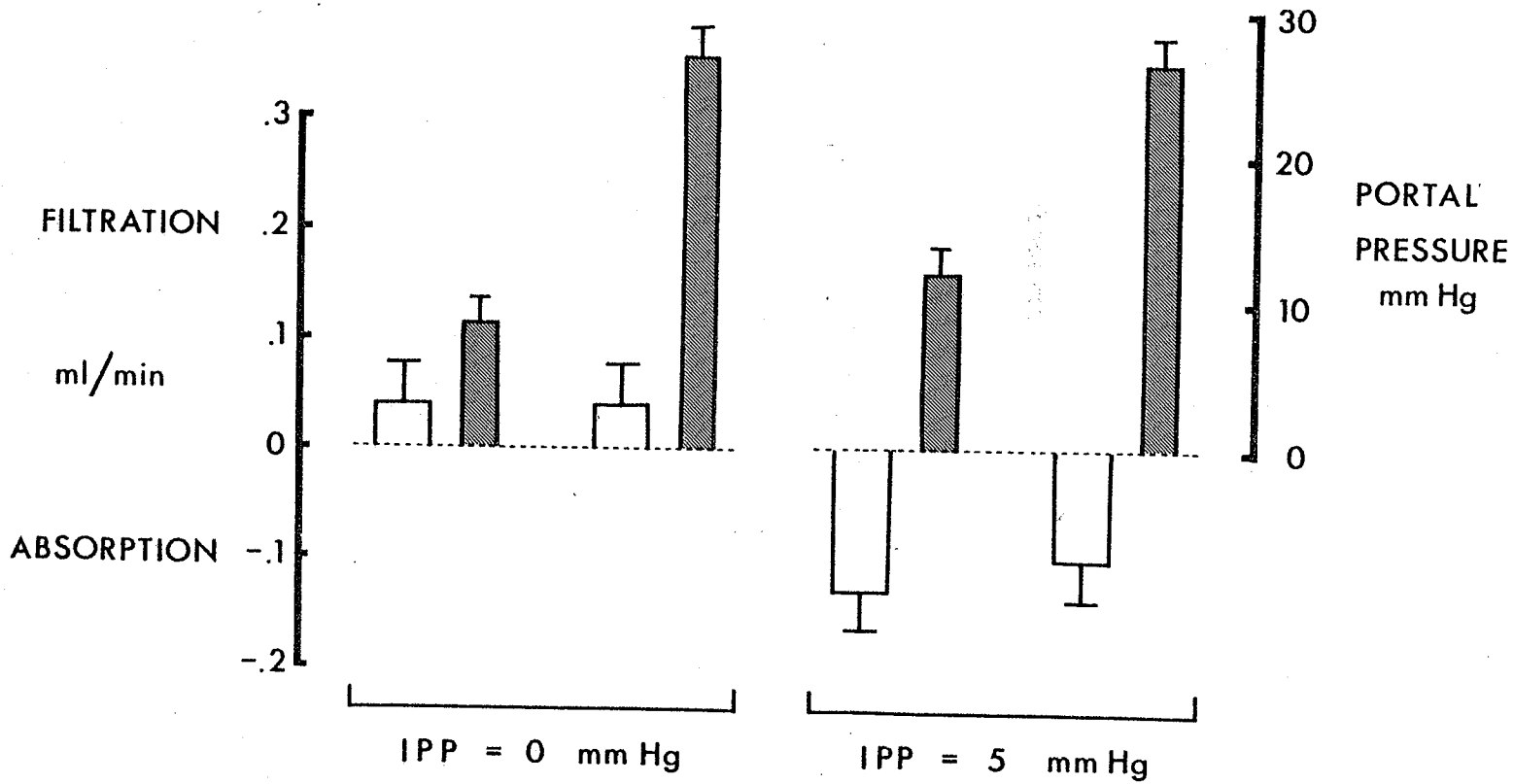


Figure 35. Effect of increased portal pressure (shaded bars) on the rate of ascites filtration or absorption (unshaded bars) when the intraperitoneal pressure is 0 or 5 mm Hg. For each intraperitoneal pressure, control (left) and experimental values (right) are shown.

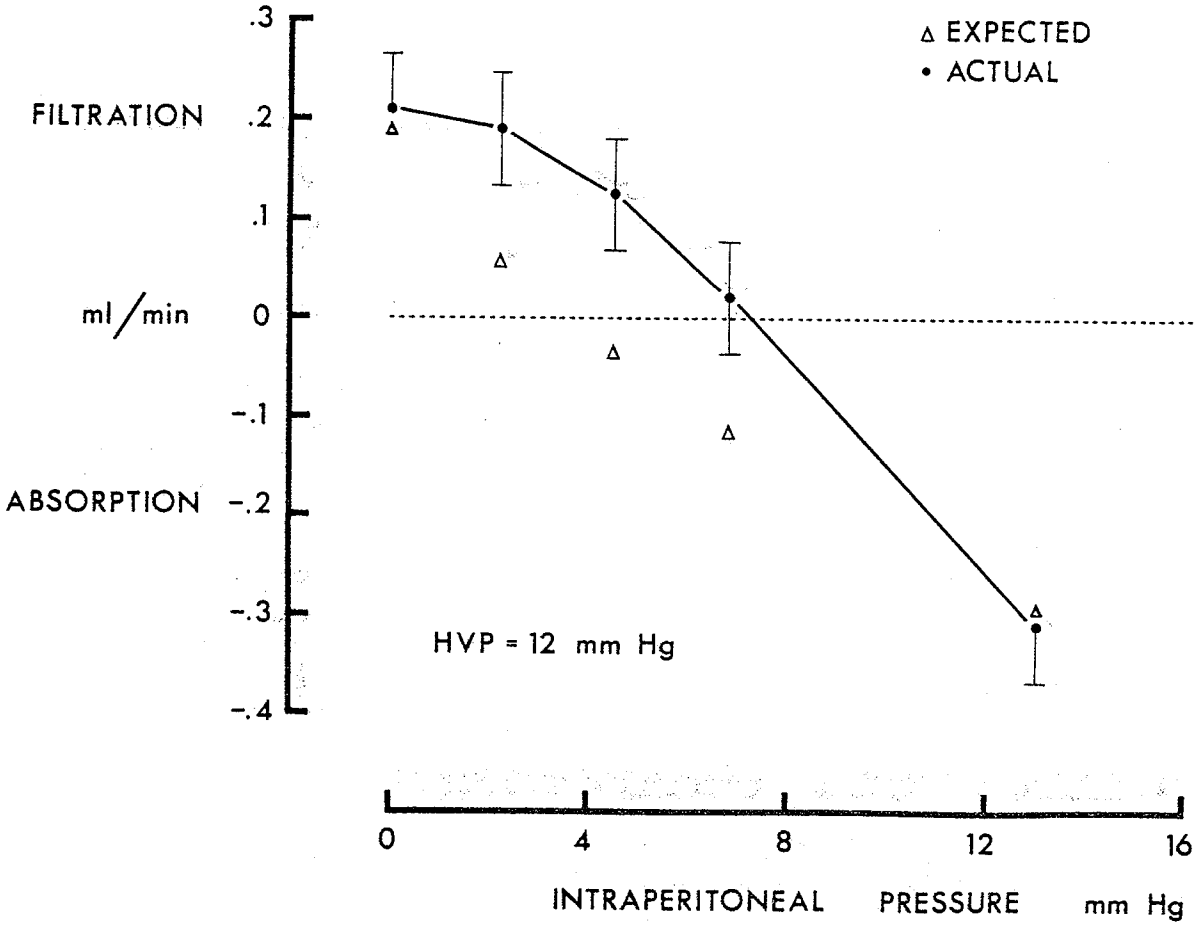


Figure 36. Effect of increasing intraperitoneal pressure on the net rate of ascites filtration or absorption when the hepatic venous pressure (HVP) is set to 12 mm Hg. See text for derivation of expected values.

acceleration in the rate of ascites reabsorption reduced the overall net rate of ascites formation (Figure 36). While the initial rate of ascites formation was 0.210 $\pm$ 0.058 ml/min, increasing the intraperitoneal pressure to 2.2, 4.5 and 6.8 mm Hg reduced the net formation rate so that only 0.020 $\pm$ 0.058 ml/min was formed when the intraperitoneal pressure was 6.8 mm Hg. With further elevation of the intraperitoneal pressure to 13 mm Hg the reabsorption rate overcame filtration and the intraperitoneal fluid showed a net reabsorption at - 0.310 $\pm$ 0.058 ml/min. This rate is equivalent to the rate obtained for this intraperitoneal pressure in earlier experiments when the hepatic venous pressure was not controlled with an extracorporeal circuit (Section 7,II).

With the hepatic venous pressure set to 12 mm Hg, interpolation of the fluid transfer rates from Figure 36 indicates that the rate of ascites formation at this pressure was equally offset by the rate of ascites reabsorption when the intraperitoneal pressure was raised to 7.1 mm Hg. This equilibration pressure varied from 6.8 to 7.4 in four experiments.

8. Discussion

I General Comments

The results of the present study emphasize the need to consider the pathogenesis of ascites as a dynamic process controlled not only by the rate of ascites formation but also by the rate of its removal through intraperitoneal absorption. In this context it would appear that the intraperitoneal pressure is an important factor which governs the rates of both processes in a manner that has not been previously recognized.

Since Erasistratus first attempted to explain the occurrence of ascites on the basis of intrahepatic congestion and resistance to blood flow (Fishman, and Richards, 1964), theories of the pathogenesis of ascites have been almost entirely concerned with the formation aspect of this disorder. Consequent to Starling's demonstration that fluid exchange between vascular and interstitial fluid compartments is regulated by the balance of hydrostatic and oncotic forces across the capillary wall (Starling, 1896a,b), ascites formation in cases of hepatic cirrhosis was at first viewed as the end result of portal hypertension and venous congestion in the intestinal vasculature (Liebowitz, 1959). However, as experimental and clinical data accumulated which disfavoured this source of ascites (Section 3,III,b), scientific attention was diverted to the hepatic vascular bed.

Bolton, et al. (1914,1931) and also Hyatt, et al. (1955) produced ascites in dogs by partial constriction of the suprahepatic, inferior vena cava and fluid was observed to transudate directly from the surface of the liver. Though much additional evidence has since been added to support the hepatic origin of ascites (McDermott and Brown, 1964; Greenway and Stark, 1971; Conn, 1972), quantitative data for the actual rate of ascites transudation from the liver is only obtainable from a single, non-patho-

logical study which examined hepatic filtration due to increased venous pressures (Greenway and Lautt, 1970).

In the absence of firm quantitative data for the rate of ascites formation from the cirrhotic liver, many investigators have been puzzled by the low protein concentration of the ascitic fluid. Because of this, some still maintain that ascites originates from congestion and excessive lymph production in the intestinal vasculature (Losowski and Scott, 1973; Sherlock and Shaldon, 1973). This is perhaps understandable since the concentration of protein in the intestinal lymph is quite similar to that of ascites (Witte et al., 1971a,b), but it neglects the greater body of evidence which favours the hepatic origin of ascites (Section 3,III,b).

The most recent theories of ascites pathogenesis propose that the limited flow capacity of the thoracic duct junction accounts for the development of ascites (Zotti, et al., 1966; Dumont, 1974;1975). This again emphasizes the formation aspect and ignores the importance of absorptive processes which channel lymph mainly through the right lymphatic duct (Courtice and Steinbeck, 1950a). Still, on the basis of this theory, thoracic duct lymph drainage and lymphovenous anastomosis have been enthusiastically adopted by physicians for the treatment of "intractable" ascites (Zotti, et al., 1966; Donini and Bresadola, 1974; Udwadia, 1975). While it is quite apparent that thoracic duct drainage reduces portal hypertension and decreases the volume of ascites, Orloff, et al. (1966) have suggested that this manoeuver is in fact just a difficult way to accomplish abdominal paracentesis --- with all of its hazards and cardiovascular sequelae. Reinfusion of the fluid, or the construction of lymphovenous shunts, seems just to negate any benefits obtained by increasing the rate of lymph drainage. In this case, the fluid reenters the circulation

increasing the blood volume and portal pressure (McDermott and Brown, 1964). Thus it is rather surprising that this theory continues to be promoted with such vigor (Dumont, 1974;1975) when one considers its marginal therapeutic effectiveness and the substantial experimental evidence against it (Orloff, et al., 1966; Warren, et al., 1968; Fomon and Warren, 1969).

In spite of this, the significance of lymph flow through the thoracic duct should not be underestimated since it is this channel which receives most of the hepatic and intestinal lymph flow (Yoffey and Courtice, 1970). Certainly if the outflow capacity of the hepatic lymphatics could accommodate all of the excess fluid filtered across the liver sinusoids, fluid would not be seen to transudate from the liver capsule in ascites (Hyatt, et al., 1955; Van der Heyde, et al., 1964). Yet this should not indict the flow capacity of the thoracic duct junction as a cause of ascites any more than intrahepatic factors which limit the maximal outflow capacity of the hepatic lymph. Similarly, there is little reason to believe that either of these processes are more crucial to the pathogenesis of ascites than is the absorptive capacity of the diaphragmatic lymphatics since this determines the extent to which fluid can be evacuated from the peritoneal cavity.

The present study has considered the pathogenesis of ascites as a composite of several interactive processes which become deranged with the development of chronic liver disease. Thus transsinusoidal filtration, hepatic lymph flow and other factors which contribute to the accumulation of intraperitoneal fluid and protein are integrated with those processes responsible for the removal of ascitic fluid from the peritoneal cavity.

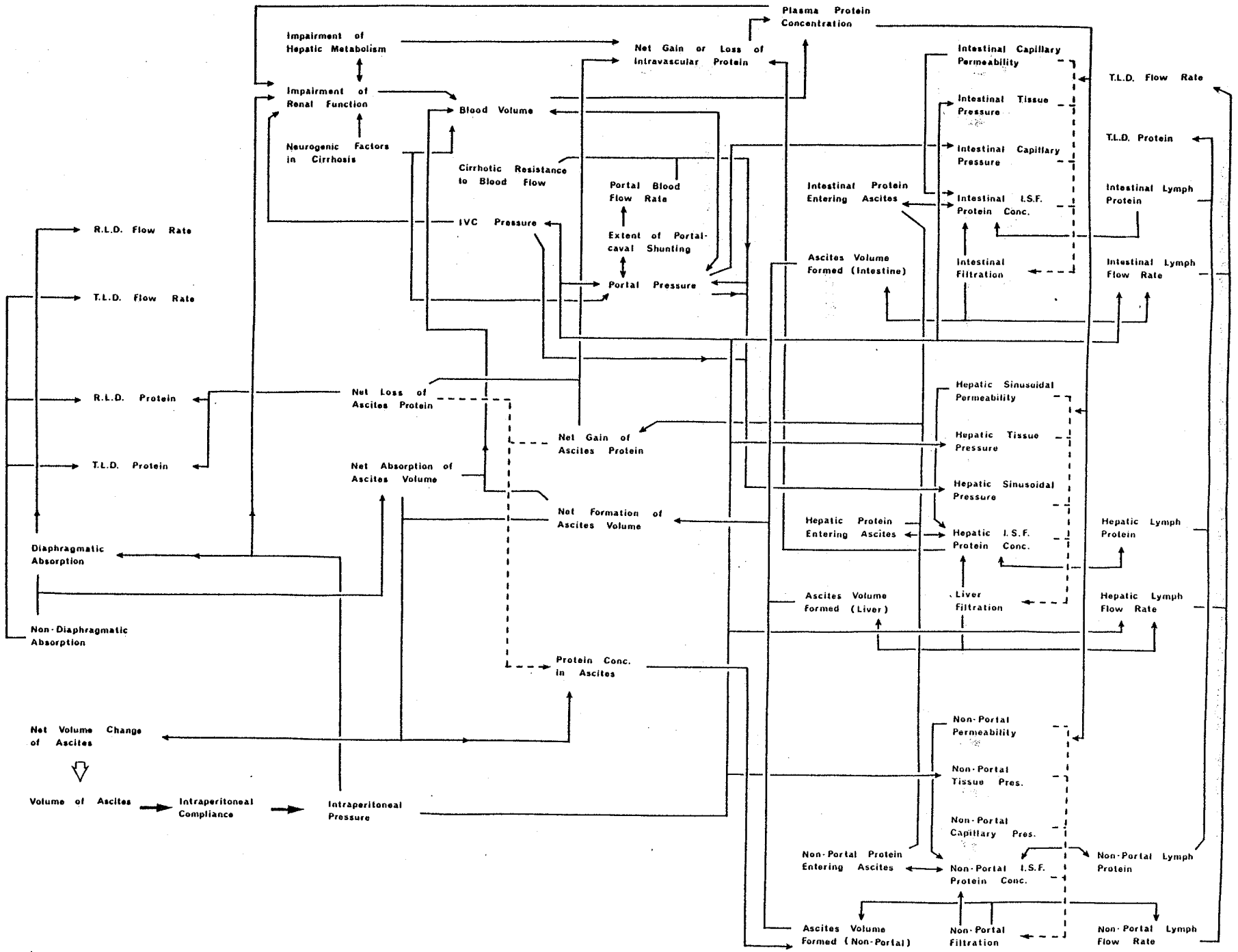
To this end the experiments have attempted to provide quantitative in vivo data for the rate of ascites formation as this relates to

the ongoing process of ascites absorption. Similarly, the absorption of fluid and protein from the peritoneal cavity has been examined with a view to establish the rates of removal as they might be affected by conditions which exist in ascites.

If the results of the present investigations are considered in relation to what is currently known of the cardiovascular and metabolic disorders occurring in cirrhotic liver disease, it becomes apparent that there are a multiplicity of factors which may contribute to the pathogenesis of ascites. Figure 37 attempts to outline these factors, showing their interaction with processes that either form or remove ascites fluid and protein. While somewhat complex, this diagram can be viewed as an alternative to current theories (Sherlock and Shaldon, 1963; Shear, 1973; Dumont, 1975), which are perhaps overly simplistic and are unable to account for the entire spectrum of changes occurring in clinical and experimental ascites.

With this "all-inclusive" interpretation, the tissues responsible for ascites formation and the entry of protein into the peritoneal cavity are listed on the right. Elevation of the hepatic sinusoidal pressure is considered to be the primary disorder which initiates excessive trans-sinusoidal filtration in most cases of hepatic cirrhosis. This fluid is in part removed by lymph drainage since the rate of hepatic lymph flow is increased (Baggenstoss and Cain, 1967; Witte, et al., 1971b) but the remainder transudes from the liver capsule to form ascites. The intestine, and also non-portal tissues bordering the peritoneal cavity, may also contribute fluid or protein as shown in the diagram. Together these tissues determine the net gain of ascites protein and the net formation of the ascitic volume.

Figure 37. Schematic diagram of some processes that control the rate of accumulation of ascites. RLD: right lymph duct, TLD: thoracic lymph duct, ISF: interstitial fluid, IVC: inferior vena cava.


Plasma Protein Concentration
Impairment of Hepatic Metabolism
Net Gain or Loss of Intravascular Protein
Intestinal Capillary Permeability
T.L.D. Flow Rate
Impairment of Renal Function
Blood Volume
Intestinal Tissue Pressure
T.L.D. Protein
Neurogenic Factors in Cirrhosis
Intestinal Capillary Pressure
Cirrhotic Resistance to Blood Flow
Portal Blood Flow Rate
Intestinal Protein Entering Ascites
Intestinal Lymph Protein
R.L.D. Flow Rate
IVC Pressure
Intestinal I.S.F. Protein Conc.
Extent of Portal-caval Shunting
Ascites Volume Formed (Intestine)
Intestinal Lymph Flow Rate
T.L.D. Flow Rate
Intestinal Filtration
Portal Pressure
R.L.D. Protein
Net Loss of Ascites Protein
Hepatic Sinusoidal Permeability
Net Gain of Ascites Protein
Hepatic Tissue Pressure
T.L.D. Protein
Net Absorption of Ascites Volume
Hepatic Sinusoidal Pressure
Net Formation of Ascites Volume
Hepatic Protein Entering Ascites
Hepatic I.S.F. Protein Conc.
Hepatic Lymph Protein
Diaphragmatic Absorption
Ascites Volume Formed (Liver)
Liver Filtration
Hepatic Lymph Flow Rate
Non-Diaphragmatic Absorption
Protein Conc. in Ascites
Net Volume Change of Ascites
Non-Portal Permeability
Non-Portal Tissue Pres.
Volume of Ascites
Intraperitoneal Compliance
Intraperitoneal Pressure
Non-Portal Capillary Pres.
Non-Portal Protein Entering Ascites
Non-Portal I.S.F. Protein Conc.
Non-Portal Lymph Protein
Ascites Volume Formed (Non-Portal)
Non-Portal Filtration
Non-Portal Lymph Flow Rate

On the left side of this figure, diaphragmatic and possible non-diaphragmatic absorptive processes are shown. These determine the net removal of ascites protein and the net absorption of ascites volume as shown. Thus in conjunction with formative processes, the ascitic volume is determined by the balance of the rates of formation and reabsorption. Similarly, the protein concentration of the ascitic fluid is controlled by the rates of protein entry and removal as well as the rate of change of the ascitic volume (by dilution or concentration).

The results of the present study show the importance of intraperitoneal absorption as a process which governs the volume of filtered fluid remaining within the peritoneal cavity to form ascites. The use of a servo-controlled plethysmograph ensured that the intraperitoneal pressure remained constant while fluid absorption was recorded from the rate of change of the intraperitoneal volume. Since the experimental animals were physiologically normal, the rate of change of the intraperitoneal volume was assumed to represent the true rate of fluid absorption without a significant filtration component. If such studies were attempted in animals with experimentally induced ascites, this would not be justified and the rate of fluid absorption could not be determined. Similarly, the absorption rate was assumed to be negligible in those experiments which recorded ascites formation as a function of hepatic venous pressure while the intraperitoneal pressure was zero. To differentiate the rates of absorption and filtration from the net rate of volume change in cases in which both the intraperitoneal pressure and hepatic venous pressure were varied, the rate of absorption was considered equivalent to the rate observed for the corresponding intraperitoneal pressure when the hepatic venous pressure was unaltered.

Using this plethysmographic method, Ringer-Locke solution was shown to be absorbed from the peritoneal cavity at a steady rate which was unchanged while the intraperitoneal pressure was maintained constant for periods as long as three hours (Table 3). This concurs with the observations of Shear, et al. (1965b) that the absorption of saline placed within the peritoneal cavity of dogs becomes linear after a 10 - 15 minute period of rapid osmotic equilibration. In all experiments the rate of fluid absorption was directly proportional to the intraperitoneal pressure and this relationship was unchanged whether the peritoneal cavity contained a protein-free fluid or the same fluid with a protein concentration equivalent to that of the animal's plasma (Figure 27).

The observation that intraperitoneal protein levels do not affect the rate of fluid absorption from the peritoneal cavity is noteworthy since considerable significance has been attached to the intraperitoneal protein concentration as a determinant of the ascitic volume (Sherlock and Shaldon, 1963; Witte, et al., 1971b). Thus many investigators believe that the ascitic volume is reduced if higher protein concentrations occur in the ascitic fluid (Shear, 1973; Losowski and Scott, 1973), but actually this has never been demonstrated in either clinical or experimental studies. Presumably this impression arises from the common but superficial view which considers ascites to be controlled by the overall balance of Starling factors across the peritoneal wall. Speculation naturally leads to the suggestion that the accumulation of intraperitoneal fluid should be governed by the oncotic differential between plasma and ascites. Yet this ignores the hepatic origin of ascites and the limited importance of oncotic factors in the control of transsinusoidal filtration. In fact, Mankin and Lowell showed in 1948 that the volume of ascites in

cirrhotic patients was unaffected by either dilution of the intraperitoneal protein concentration or elevation of the plasma protein levels produced by intravenous infusions of concentrated human serum albumin. The results of the present study corroborate these findings for the absorption aspect of ascites and indicate that the rate of removal is also unaffected by the intraperitoneal protein concentration.

Other studies (Courtice and Steinbeck, 1950b; Luttwak, et al., 1975) have used different techniques to compare the absorption of serum and saline from the peritoneal cavity. In some cases the proteinacious solutions were absorbed more rapidly whereas in others the reverse was observed. The methodological barriers to obtaining accurate data in these studies have been discussed in Section II,b of the Literature Survey (3).

Although elevation of the intraperitoneal pressure has been observed in experimental ascites (Waugh, 1958) and is frequently viewed as a secondary complication of abdominal distention in human ascites (Mankin and Lowell, 1948; Losowski, et al., 1963; Iwatsuki and Reynolds, 1973), the significance of this factor has been largely overlooked. The results of the present study, as shown in Figure 27, indicate that this pressure might not be an inconsequential side effect of fluid accumulation, but instead the increased intraperitoneal pressure may [illegible] increase the rate of ascites reabsorption from the peritoneal cavity.

Other studies in experimental animals and in man (Courtice and Steinbeck, 1950a; Shear, et al., 1965a) have recorded intraperitoneal absorption rates that are similar to those recorded in the present study. Species differences are significant (Courtice and Steinbeck, 1950b;1951a); but the most serious obstacle to valid comparisons with these rates is that the intraperitoneal pressure was not recorded, let alone controlled at a constant

level, in these investigations. Some studies (Courtice and Steinbeck, 1950b; 1951b; Kruger, et al., 1962) have compared the rates of absorption of small versus large volumes of fluid placed in the peritoneal cavity but the probable elevation of intraperitoneal pressure with the larger volumes is ignored. Luttwak, et al. (1975) have selected fluid volumes which "maximally expand" the peritoneal cavity and monitored absorption over a 24 hour period by serial assays of the intraperitoneal volume. During this time a decline of the intraperitoneal pressure would certainly have been observed if this parameter were recorded but in the absence of this data the progressive slowing of absorption was inexplicable.

Isotonic saline is absorbed from the peritoneal cavity of man at a rate which ranges from - 0.495 to - 0.621 ml/min (Shear, et al., 1965a). This is about 3 times the rate of fluid removal recorded in cats when the intraperitoneal pressure is set to 5 mm Hg (i.e. - 0.178, Figure 27). In dogs, saline is absorbed from the peritoneal cavity at a rate of about - 0.28 ml/min (Shear, et al., 1965b). Differences of body weight, technical limitations and the unknown intraperitoneal pressure could be expected to influence the rates recorded in studies of other species but in addition it should be noted that anesthesia and controlled respiration tend to reduce the rates recorded by intraperitoneal plethysmography.

II Protein Concentration in the Intraperitoneal Fluid

It is difficult to explain the low protein concentration of ascites that occurs due to advanced hepatic cirrhosis since the primary source of intraperitoneal fluid is believed to be excess hepatic lymph containing high levels of protein (Freeman, 1953; McDermott and Brown, 1964; Yoffey and Courtice, 1970). Witte, et al. (1971a,b) and Losowsky and Scott

(1973) are among those believing that these protein levels are strong evidence that the ascitic fluid is formed from intestinal lymph. However, as discussed in Section 3,III,b, there are many reasons why this conclusion is unacceptable. Also, even in the early stages of hepatic cirrhosis or in experimental ascites due to caval constriction, when it is almost certain that fluid transudates from the liver to form ascites (Drapanas, et al., 1960; Witte, et al., 1968), the ascitic protein concentration is still somewhat lower than that of the hepatic lymph (Hyatt, et al., 1955). This has led to the suggestion that the protein content of the intraperitoneal fluid is diluted by fluid from intestinal or non-portal tissues which diffuses along a concentration gradient into the peritoneal cavity (Yoffey and Courtice, 1970).

In the midst of this controversy the process of ascites absorption has been neglected but Hyatt, et al. (1955) have speculated that the dilution of intraperitoneal protein could be accomplished by a selective reabsorption process which removes more protein than fluid. The absorption of concentrated protein solutions was investigated in the present study to consider this possibility.

As shown in Figure 29, the results indicate that fluid absorbed from the peritoneal cavity contains a protein concentration equivalent to that of the intraperitoneal fluid. This at least excludes one possibility that could account for the dilution of ascitic protein. But in addition, since the intraperitoneal protein concentration remained constant over a period of 5 hours, it would appear that the diffusion rate of protein-dilute fluid into the peritoneal cavity must be exceedingly small. Although one might be tempted to suggest that the effects of a slow rate of entry of dilute fluid would be more pronounced over a longer period of time, this is

not the case if isooncotic fluid is absorbed from the peritoneal cavity. The proof of this can be demonstrated with a computer model of the rates of intraperitoneal fluid and protein exchange.

The details of this procedure are included in Appendix A, but in summary the data show that the ultimate concentration of the ascitic protein is determined by the ratio of the rate of entry of protein-concentrated fluid to the rate of entry of protein-dilute fluid. Thus if ascites is formed from hepatic lymph containing 5 g% protein which enters the peritoneal cavity at a rate of 0.3 ml/min, and this is diluted by a slow rate of diffusion from extrahepatic tissues at perhaps 0.015 ml/min, then the concentration of ascitic protein will not be less than 4.75 g%. Similarly, to achieve the 4 - 5 fold dilution that would be required to dilute hepatic lymph to the concentration which occurs in the ascitic fluid (Witte, et al., 1969c), the rate of diffusional entry would need to be about 0.24 ml/min. A dilution factor of this magnitude was certainly not evident in the present experiments and in studies of experimental ascites in animals, fluid is not seen entering the peritoneal cavity from any surfaces but that of the liver (Volwiler, et al., 1950; Hyatt, et al., 1955; Drapanas, et al., 1960).

This paradox cannot be adequately resolved from the data that are presently available but the possibility that some dilution of the ascitic protein occurs by transperitoneal diffusion or the congestive entry of intestinal lymph should not be excluded. Perhaps with peritoneal inflammation or in cases when portal hypertension is accompanied by marked hypoproteinemia the significance of dilution processes from these tissues could be increased (Volwiler, et al., 1950; Witte, et al., 1969d). However it seems that greater attention should be paid to the protein concentration of

the fluid which transudates from the liver since this forms the bulk of the ascitic fluid.

While the concentration of protein in the hepatic lymph is substantially greater than the concentration in ascites, this difference is less marked in the later, chronic stages of ascites formation (Witte, et al., 1969c; Dumont, et al., 1975). In addition it is possible that in chronic human ascites the transudate from the liver capsule contains substantially less protein than the hepatic lymph (Section 3,I,b,ii). This could occur due to reduction of the hepatic interstitial protein concentration with the development of increased sinusoidal permeability in cirrhosis (Schaffner and Popper, 1963) or due to thickening of Glisson's capsule around the liver which also occurs (Waugh, 1958), and may restrict the passage of protein in the liver transudate. The role of these factors in relation to processes which may further dilute the ascitic protein concentration are shown in Figure 37.

III Diuretics and Intraperitoneal Absorption

Diuretic agents are frequently employed in the management of human ascites. The administration of these drugs mobilizes ascitic fluid at a rate which is about 3 times greater than the rates observed in patients undergoing spontaneous diuresis (Shear, et al., 1966).

Although the site and mode of action of various diuretic preparations on the kidney are well delineated (Bank, 1968), the mechanism by which they accelerate the removal of ascites is still speculative (Lieberman and Reynolds, 1966; Arroyo and Rodes, 1975). In studies of dogs with experimental ascites, Witte, et al. (1972) have shown that intravenous ethacrynic acid causes marked reduction of the abdominal girth in a period of just two

hours. In this case the decline of ascites could be due to either reduced formation or an increased rate of reabsorption. However, the rapidity of this effect and other studies in normal animals which have shown that ethacrynic acid increases the rate of lymphatic absorption from some tissues by a direct, non-renal effect (Szwed, et al., 1971; Maxwell, et al., 1974), suggested that diuretic agents might reduce ascites by increasing the rate of intraperitoneal fluid absorption.

This possibility was examined in the present study by inducing diuresis with furosemide. The selected dosage (3.1 mg/kg) was about twice as great as has been used in other animal studies (Witte, et al., 1972; McNeill, 1974) to ensure that maximal diuresis occurred in each experiment (Figure 31). Even under these conditions the rate of fluid absorption from the peritoneal cavity was not increased despite marked changes of plasma volume and protein concentration (Figure 32). With diuretics that alter plasma osmolarity there may be a transient period of transperitoneal fluid reabsorption (Shear, et al., 1966), but this does not occur with drugs such as furosemide which do not alter the transperitoneal osmotic balance (Table 4).

It is commonly known that intravenous furosemide can produce marked hypovolemia (Summerskill, 1969; Conn, 1972) but there has been an absence of accurate data to quantitatively substantiate the clinical signs of this condition. In the present study, the use of radioisotopes labelling both cellular and plasma constituents gave evidence of an altered ratio of the systemic to whole body hematocrit which occurred with the 33% reduction of the plasma volume induced by furosemide (Table 5). Hemoconcentration increased the plasma protein level from 7.8 to 8.8 $\pm$ 0.2 g% but due to the peculiar osmotic characteristics of plasma proteins this would be expected

to disproportionately increase the plasma colloid osmotic pressure by almost 20 mm Hg (Landis and Pappenheimer, 1963).

Since diuretic agents undeniably reduce the volume of both clinical and experimental ascites (Shear, et al., 1970; Witte, et al., 1972) but do not affect the rate of intraperitoneal absorption, their mechanism of action is probably to reduce the rate of ascites formation. Atkinson and Losowski (1961) have suggested that this occurs because ascites formation from excess intestinal lymph is curtailed due to elevation of the plasma colloid osmotic pressure and the reduction of portal pressure with hypovolemia (Atkinson, 1959; Zimmon and Kessler, 1974). While this interpretation may be valid in some patients, it should not be overlooked that the colloid osmotic pressure of the ascitic fluid rises in proportion to that of plasma (Mankin and Lowell, 1948).

A reduction of intrahepatic vascular pressures is a more plausible explanation of the reduced rate of ascites formation, particularly since furosemide is known to markedly reduce hepatic portal inflow by mesenteric vasoconstriction (McNeill, 1974). Transsinusoidal filtration and hepatic lymph production would be reduced with the lower hepatic sinusoidal pressure. At the same time the fraction of the liver transudate which continues to filter into the peritoneal cavity might contain increased protein concentrations in accordance with the increased plasma protein levels. The effect of diuretic agents on hepatic lymph flow in ascites is unknown. However it may be surmised that the flow of hepatic lymph is reduced, since this source accounts for most of the thoracic duct lymph flow which has been shown to be reduced by 60% under these conditions (Witte, et al., 1972).

All of the features of intraperitoneal fluid absorption observed in the present study are compatable with the conventional view that isotonic

fluids are removed from the peritoneal cavity by lymphatic absorption rather than by uptake into blood vessels. Lymphatic absorption, presumably at the inferior surface of the diaphragm (Section 3,II,a), seems to be the only mechanism which can account for: a) the isotonic absorption of intraperitoneal fluid, b) the unchanged rate of absorption over a wide range of intraperitoneal protein concentrations, and c) the absence of an effect of furosemide on the intraperitoneal absorption rate in spite of the substantial elevation of the plasma colloid osmotic pressure.

On this basis, fluid within the peritoneal cavity differs from peripheral subcutaneous fluid which for the most part can be directly reabsorbed by the blood vessels (Landis and Pappenheimer, 1963). A similar comparison may be appropriate for edema and ascites since fluid from the former compartment is more readily mobilized with spontaneous or drug induced diuresis (Shear, et al., 1970).

The acceleration of absorption rates due to increased intraperitoneal pressures is also compatible with the lymphatic mode of removal. Although the present study cannot determine the actual mechanism of this effect, from a consideration of the anatomical nature of the diaphragmatic lymphatics (Section 3,II,a) the most reasonable hypothesis seems to be that elevation of the intraperitoneal pressure, which results in distention of the diaphragm into the thoracic cavity (Table 2), causes the diaphragmatic lymphatics to become stretched so that their absorptive capacity is increased. This could happen either by a direct effect on the vessels themselves or perhaps by causing the lymphatics to protrude from lacunae on the diaphragmatic surface so that their exposure to the intraperitoneal fluid is improved (Yoffey and Courtice, 1970). If a stretch-related mechaniam is responsible for the effects of an increased intraperitoneal

pressure, this might operate in a manner similar to the potentiation of intraperitoneal absorption that occurs when respiratory movements are increased due to the inhalation of 5% CO_2 (Courtice and Steinbeck, 1950a).

IV The Control of Ascites by Formation and Reabsorption

In a previous study by Greenway and Lautt (1970) liver volume changes were investigated *in situ* using direct hepatic plethysmography. With this technique the steady-state rate of filtration was found to be a linear function of hepatic venous pressure with a slope of 0.060 ± 0.003 ml·min^{-1}·mmHg^{-1}·100g^{-1} when the hepatic lymphatics were ligated. However, if the lymphatics were not ligated, the relationship was not linear and less fluid was filtered into the plethysmograph at lower hepatic venous pressures. Similar filtration rates were obtained at the highest hepatic venous pressure (11.7 mm Hg) whether or not the lymphatics were ligated.

In the present study, ascites formation was also induced by raising hepatic venous pressure but the rate of formation was monitored by intraperitoneal plethysmography so that the interaction with absorption processes and the effects of increased intraperitoneal pressures could be examined. With the intraperitoneal pressure set to zero, there was a non-linear relationship between the filtration rate and hepatic venous pressure since in this case the hepatic lymphatics were not ligated (Figure 34). Relative to the previous data the curve has the same shape although the rates of filtration are slightly less. In part this may be caused by some elevation of the rates that were recorded previously from the *in situ* liver due to compression of the external hepatic lymph vessels by the plethysmograph.

Hepatic venous pressures up to 16 mm Hg produced a constant rate

of ascites formation for periods in excess of 30 minutes after equilibration. While pressures this high have not been previously investigated for the hepatic vascular bed, the rate of filtration recorded by in situ liver plethysmography has been shown to be constant for over 4 hours while the hepatic venous pressure was set to 9.35 mm Hg (Greenway and Lautt, 1970).

In the present study, elevation of hepatic venous pressure also raised portal pressure as shown in Figure 33. This was particularly noticeable with elevation of the hepatic venous pressure to higher levels; in which case the increment of portal pressure was equal to that of hepatic venous pressure. Since the technique of intraperitoneal plethysmography recorded the accumulation of fluid from any splanchnic source it was necessary to consider that filtration from the intestinal vascular bed could be significant when the portal pressure was increased to levels as high as 22.5 mm Hg. This possibility was explored with the use of a servo-operated portal venous occluder which allowed the portal pressure to be fixed at an elevated level for prolonged periods without extensive surgery, intestinal resection, arterial perfusion, or the use of vasoactive drugs as in previous studies of intestinal filtration (Wallentin, 1966a; Johnson and Richardson, 1974).

As shown in Figure 35, the results of these experiments indicate that the elevation of the portal pressure from 8.4 to 26.4 $\pm$ 0.5 mm Hg for a period of 40 minutes does not cause any detectable rate of filtration from the intestinal vasculature. Similar results were obtained when the intraperitoneal pressure was maintained at 5 mm Hg during the period of portal hypertension.

These findings support and extend the observations of Johnson and Hanson, 1966; Johnson and Richardson, 1974) and Wallentin (1966b).

Studies by these authors have shown that after an initial period of blood volume expansion and filtration the intestinal vascular bed accommodates to the raised venous pressure and further filtration ceases (see Section 3,I,a, iv). In the present study this was also observed when the portal pressure was elevated to pathological levels for prolonged periods of time. This was not possible in the studies by Johnson and Hanson (1966) or Wallentin (1966b) because extensive surgery entailed a considerable risk of bleeding with increased venous pressures.

In any case it now appears that the mechanism which protects the intestinal vasculature from excessive filtration after a modest increment of venous pressure is also operative when the portal pressure is increased to levels which would be considered hypertensive. Thus with the elevation of hepatic venous and portal pressures in the present study, this data indicates that the accumulation of intraperitoneal fluid is due to a primary excess of fluid filtration from the hepatic vascular bed. Whether this is also the case in humans or animals with chronic ascites has been discussed in Section III,b,iii of the Literature Survey (3).

The filtration due to a raised hepatic venous pressure models to some extent the pathological accumulation of ascites in hepatic cirrhosis. However, in cirrhosis, the progressive build-up of ascites causes the intraperitoneal pressure to increase to an extent which depends on the compliance of the peritoneal cavity. As shown in Figure 27, this elevation of the intraperitoneal pressure in turn accelerates the rate of ascites absorption from the peritoneal cavity.

Thus ascites can be viewed as a dynamic condition where the rate of accumulation is determined by a quantitative balance of the rate of fluid formation and the rate of its removal from the peritoneal cavity. These

rates in turn are determined by hepatic sinusoidal pressure and the intraperitoneal pressure. However, since the intraperitoneal pressure increases as more fluid is filtered into the peritoneal cavity, this could operate as a feedback mechanism to limit the accumulation of ascites and thus actually determine the intraperitoneal volume in a patient with stable ascites. These interactions are graphically shown on the left of Figure 37.

This reasoning suggests that the intraperitoneal pressure and the rate of intraperitoneal fluid absorption may be much more significant factors in the control of ascites than has been previously recognized. However, in addition to its stimulatory effect on ascites reabsorption, elevation of the intraperitoneal pressure could also affect the rate of ascites formation by reducing the transsinusoidal pressure gradient responsible for filtration in the liver. This possibility was investigated by comparing the rate of ascites formation in response to increased venous pressures either when the intraperitoneal pressure was zero or when it was increased to 10 mm Hg.

The results indicate that elevation of the intraperitoneal pressure does affect the rate of ascites filtration from the liver but not just by reducing the transsinusoidal pressure gradient. Figure 33 shows that increasing the intraperitoneal pressure also increases the hepatic venous pressure, perhaps by compression of the hepatic veins within the peritoneal cavity. Thus when the hepatic venous pressure is initially set to be zero, elevation of the intraperitoneal pressure does not affect the transsinusoidal pressure gradient since the hepatic venous pressure is increased in proportion to the expected increase in tissue pressure. On the other hand, if the hepatic venous pressure is initially greater than zero, elevation of the intraperitoneal pressure would partly reduce the pressure

gradient responsible for fluid filtration. Figure 34 shows that when the intraperitoneal pressure is fixed at 10 mm Hg there is a constant rate of fluid absorption from the peritoneal cavity as long as the hepatic outflow pressure (i.e. the height of the venous control reservoir) remains less than 10 mm Hg. Under these conditions hepatic venous pressure is fixed by the level of the intraperitoneal pressure. However, when the hepatic venous pressure is raised to levels in excess of the intraperitoneal pressure, filtration resumes and opposes the ongoing rate of absorption so that the net effect is a reduction in the rate of removal of intraperitoneal fluid.

When the hepatic venous pressure is 16 mm Hg and the intraperitoneal pressure is 10 mm Hg, the transsinusoidal pressure gradient responsible for fluid filtration is comparable to that which is present when the hepatic venous pressure is 6 mm Hg with the intraperitoneal pressure at zero. This suggests that the rate of ascites filtration should be approximately 0.11 ml/min (Figure 34) in association with a reabsorption rate of - 0.25 ml/min (Figure 27); yielding an expected net rate of fluid transfer of - 0.14 ml/min. However the observed rate is 0.05 ml/min indicating that the actual filtration rate is approximately 0.30 ml/min or almost 3 times greater than the rate of filtration when the intraperitoneal pressure was zero.

Thus in addition to its effect on the rate of ascites reabsorption, the intraperitoneal pressure also influences the rate of ascites filtration for a given hepatic venous pressure. When the intraperitoneal pressure is fixed to a level less than that of the hepatic venous pressure two factors affect the rate of ascites formation. On the one hand the

pressure gradient responsible for filtration is reduced as previously described; but in addition a second factor causes the rate of filtration to be greater than what would be expected for the re-established pressure gradient.

While the mechanism of this secondary effect has not been established in the present study, one possible interpretation is that elevation of the intraperitoneal pressure could obstruct lymphatic drainage by compressing the hepatic lymph vessels in the peritoneal cavity. Thus for a given venous pressure less of the filtered fluid could be siphoned off by the hepatic lymphatics, causing a greater rate of transudation into the peritoneal cavity (Figure 37). This situation would be similar to the formation of ascites as recorded from the in situ liver after ligation of the hepatic lymph vessels (Greenway and Lautt, 1970).

The significance of a raised intraperitoneal pressure in ascites was further examined in a series of experiments designed to mimic, in a stepwise fashion, the elevation of intraperitoneal pressure which might occur as the peritoneal cavity fills with ascitic fluid. The hepatic venous pressure was fixed to a level that caused a reasonable rate of ascites formation (i.e. 12 mm Hg); and for each increment of the intraperitoneal pressure it was possible to calculate the net rate of change of the intraperitoneal volume that would be expected if the only effects of the increased intraperitoneal pressure were a) stimulation of the rate of ascites absorption, and b) slowing of the rate of ascites formation caused by a reduction of the transsinusoidal pressure gradient. This "expected" value for the net rate of volume change is marked by the triangle symbols in Figure 36. The deviation of the observed data from these estimates thus occurs due to secondary effects of the intraperitoneal pressure on

the filtration rate in addition to the straight-forward reduction of the transsinusoidal pressure gradient.

The expected rates of ascites accumulation indicate that the rates of formation and reabsorption should become equal when the intraperitoneal pressure is raised to just 3.5 mm Hg. At this pressure the rate of ascites reabsorption is - 0.145 ml/min (Figure 27) and the pressure gradient responsible for filtration is 8.5 mm Hg (12 - 3.5). This corresponds to a filtration rate of 0.145 ml/min (Figure 34) when the intraperitoneal pressure is zero. However, as shown in Figure 36, raising intraperitoneal pressure causes the rate of filtration to be greater than what might otherwise be expected.

The overall net rate of ascites accumulation is increased at each point where the intraperitoneal pressure is raised until it comes to exceed the set height of the hepatic outflow reservoir. Consequently the actual intraperitoneal pressure which equalizes the rates of formation and absorption is 7.1 mm Hg; considerably greater than the value expected if the intraperitoneal pressure only affected filtration by reducing this transsinusoidal pressure gradient. Similarly the calculated filtration rates at the intraperitoneal pressures of 2.2, 4.5 and 6.8 mm Hg are greater than expected on the basis of a simple gradient effect by 82%, 125% and 140% respectively. In most cases, this additional effect to increase filtration balances the reduction caused by the lower transsinusoidal gradient so that the primary result of a raised intraperitoneal pressure is achieved by accelerating ascites reabsorption. However, with greater increments of the intraperitoneal pressure, the pressure gradient is sufficiently reduced that the rate of filtration also slows, and so contributes to the net reduction in the rate of ascites accumulation.

These experiments show that it is possible, at least under experimental conditions, to consider ascites as an interaction of separate formative and absorptive processes and to arrive at a quantitative estimation of these rates. In this context the intraperitoneal pressure seems to play a fundamental role by controlling the ongoing rates of both processes.

V Summary

The results obtained in the present study suggest that the pathogenesis of ascites is most properly viewed as a dynamic balance of factors that affect both the rate of formation and the rate of ascites reabsorption from the peritoneal cavity. Figure 37 attempts to illustrate the interactions which might occur between various processes which participate in the control of ascites.

This "all inclusive" approach proposes that in most cases of cirrhotic liver disease in humans the initial formation of ascites occurs due to an increased resistance to blood flow through the liver which elevates the hepatic sinusoidal pressure (Kelty, et al., 1950; Kato and Tsuchiya, 1964; Nakata, et al., 1973; Wade, et al., 1974). This leads to an increased rate of transsinusoidal filtration which is only partly compensated by increased drainage through the hepatic lymphatics; leaving the remainder of the fluid to be transudated across the liver capsule into the peritoneum where it forms the bulk of the ascitic volume and protein content. Additional fluid, and perhaps protein, may enter from intestinal or non-portal tissues but these rates are slow in relation to transudation from the liver.

When the ascitic volume is small in the early stages of ascites

formation, the rate of intraperitoneal fluid absorption is considerably less than the rate of formation. However, as ascites progresses and the intraperitoneal volume increases, the intraperitoneal pressure is raised to an extent which is governed by the compliance of the peritoneal cavity. This in turn accelerates the diaphragmatic reabsorption of ascites so that the rate of fluid removal approaches the rate of its entry into the peritoneal cavity. At the same time the increased intraperitoneal pressure may reduce the rate of ascites formation if the reduction of the transsinusoidal pressure gradient is sufficiently great to offset the interference with hepatic lymph drainage. Thus the equilibrium volume of ascites, if attained, is dependent on a progressive increase of the intraperitoneal pressure until the rates of fluid transfer are balanced.

As shown in Figure 37, other factors may have important effects to alter the course of ascites. Due to the variation which occurs in the development of cirrhosis and in its medical management, the ultimate rates of ascites formation and reabsorption could be affected by quite different processes. In some patients the progressive impairment of renal function or hepatic metabolism could promote a greater rate of transinusoidal filtration. In others, diuretic therapy or a portal-caval shunt may achieve some decompression of the hepatic sinusoids. In the latter case it is interesting that paracentesis may be beneficial if ascites returns shortly after the operation to establish the shunt. Here the removal of ascites reduces the intraperitoneal pressure which had previously increased the pressure in the abdominal vena cava both by direct vascular compression and by causing the diaphragm to obstruct venous outflow by distortion of the vein at the abdominothoracic junction (Vix and Payne, 1972). After paracentesis, reduction of the caval pressure may reduce sinusoidal pressure

and increase portal-caval shunting so that ascites does not reoccur.

The benficial results from paracentesis under these conditions is an exception to its general lack of effectiveness in the routine management of ascites. In most cases the intraperitoneal volume increases rapidly after paracentesis so that the previous ascitic condition is quickly re-established. From the data obtained in the present studies this is not unexpected because paracentesis would sharply reduce the intraperitoneal pressure and the rate of ascites reabsorption, while formation continued at a rate that was unchanged or possibly even increased.

In other cases of ascites a reduced rate of intraperitoneal fluid absorption may be primarily responsible for an increased rate of ascites accumulation. For example, in clinical and experimental carcinomatous ascites, migrating tumor cells occlude the diaphragmatic lymphatics (Feldman and Knapp, 1974; Feldman, 1975) and reduce the intraperitoneal capacity for absorption (Fastaia and Dumont, 1976). Also, in cases of cirrhotic ascites, Popper and Schaffner (1957) have reported fibrinous adhesions on the peritoneal surface of the diaphragm which could compromise lymphatic absorption at this site.

This consideration of the factors which may affect the pathogenesis of ascites attempts to account for most of the troublesome "enigmas" that are characteristic of this disorder (Witte, et al., 1971b; Conn, 1972). The authors of previous studies (Mankin and Lowell, 1948; Waugh, 1958) have been perplexed by the essentially constant "transperitoneal hydrostatic and oncotic gradients" in cirrhotic patients during both the remission of ascites and during its rapid reformation after paracentesis. The results of the present study suggest that the portal pressure and the transperitoneal oncotic pressure gradient may be relatively unimportant in the control of

ascites. Instead, the net rate of accumulation should be viewed as a balance of the underlying rates of formation and reabsorption —— with the principle controlling factors being the hepatic sinusoidal pressure and the intraperitoneal pressure.

ADDENDUM

It is possible that the absorption rate which is recorded with an elevated intraperitoneal pressure includes an unidentified filtration component. This could occur if the intraperitoneal pressure did not raise hepatic interstitial pressure to the same extent as hepatic venous pressure. However in this case fluid filtered from the liver sinusoids would not enter the peritoneal cavity due to the greater intraperitoneal pressure. With this fluid remaining in the hepatic interstitium the tissue pressure would increase to the level of the intraperitoneal pressure.

If this sequence of events does occur with elevation of the intraperitonea pressure one might expect that, in the present experiments, this process would be completed during the period of equilibration so that the absorption rates wo be accurately recorded. In any case there was no evidence of liver edema.

The experiments discussed in series 7,III provide additional evidence that the background rate of fluid filtration is minimal when the intraperitoneal pressure is elevated. Since the protein content of fluid which is filtered from the hepatic surface resembles that of liver lymph and plasma (hyatt and Smith, 1955), the absence of a significant rate of protein transfer from plasma into the peritoneal cavity indicates that there is also a negligible rate of fluid filtration ender these conditions.

9. Appendix A

Computer Model of Intraperitoneal Protein Concentrations

The concentration of protein within the peritoneal cavity is determined by the rate of protein addition or removal and the rate of entry or exit of protein-dilute fluid. If, in ascites, the main source of fluid is a high-protein transudate derived from the hepatic vascular bed, then it is the concentration of protein in this fluid which should control the protein concentration in the intraperitoneal fluid. However the question arises as to whether the addition of protein-dilute fluid from extra-hepatic vascular sources might achieve a substantial reduction of the ascitic protein levels if the dilution continued over a prolonged period of time.

In the consideration of this possibility a mathematical model can be constructed to simulate the processes of fluid and protein exchange under conditions when the ascitic volume is in a state of dynamic balance; that is the overall rate of fluid entry is equal to the rate of its reabsorption. Figure 38 illustrates the amount of dilution that could be expected over a period of 300 minutes for various rates of addition of protein-dilute fluid. For these data the initial intraperitoneal volumes and protein concentrations were 100 ml and 7.7 g%, respectively. Ascites absorption is considered to be iso-oncotic at a rate of 0.26 ml/min, and the overall rate of formation is also equal to 0.26 ml/min.

In Figure 38 each successively lower line shows the dilution which would occur with the formation process including a greater proportion of protein-dilute fluid. Thus ascites may be formed from either a pool containing 7.7 g% protein or from a pool containing no protein. In the uppermost trace there is no dilution since ascites is formed only from the protein-rich source. In each of the lower lines the formation rates (ml/min) from

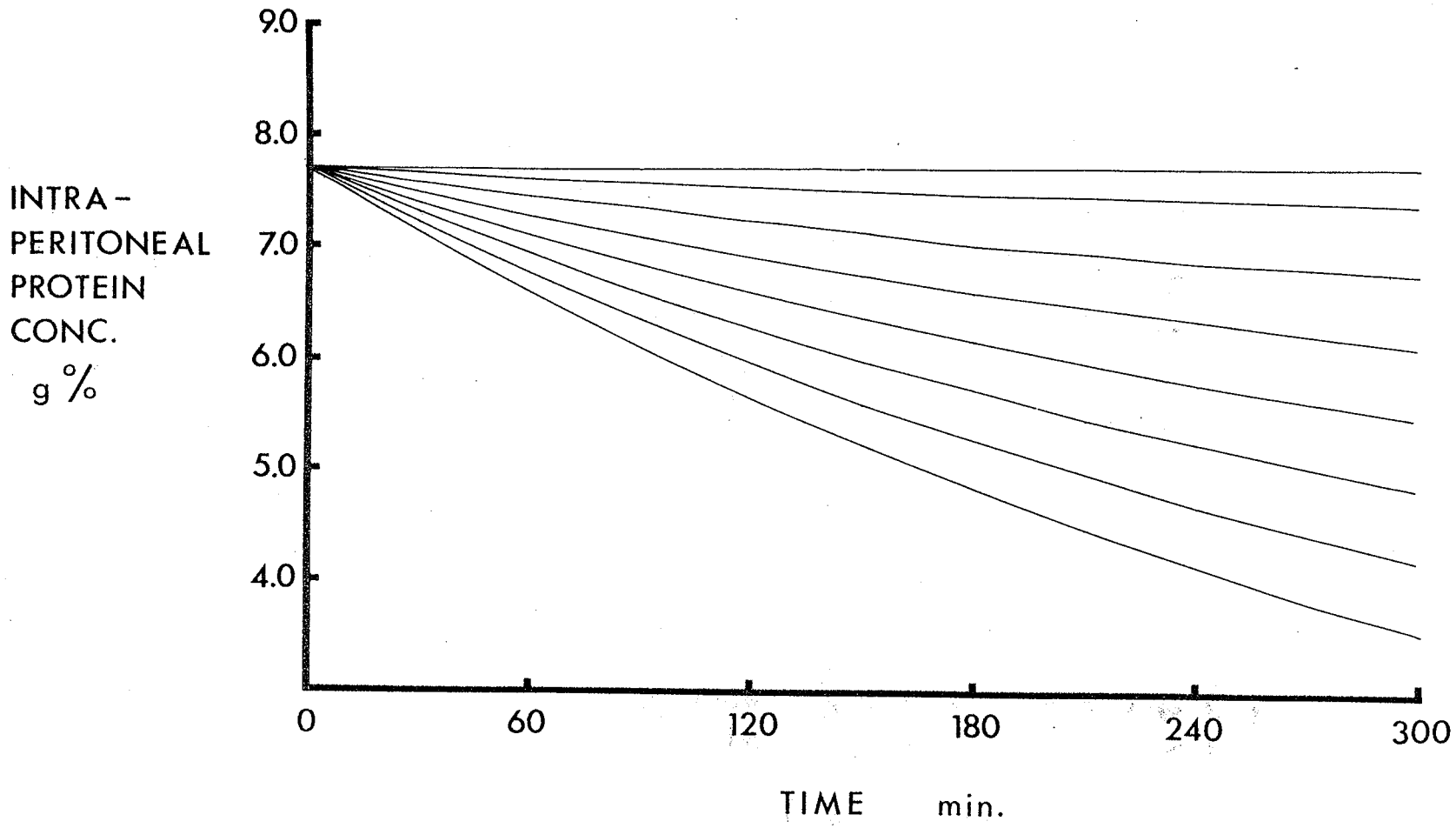


Figure 38. Expected changes of intraperitoneal protein concentration with a constant rate of ascites formation and reabsorption. See text for details.

the protein-rich and protein-dilute sources are: 0.24, 0.02; 0.20, 0.06; 0.16, 0.10; 0.14, 0.12; 0.08, 0.18; 0.04, 0.22; 0.00, 0.26.

Figure 39 illustrates the progress of dilution of the ascitic protein over a longer period of time. In this instance the four curves represent dilution ratios of 0.257, 0.003; 0.25, 0.01; 0.20, 0.06 and 0.13, 0.13 for the formation rates (ml/min) from the protein-rich and protein-dilute sources. In each case the equilibrium concentration of protein in the intraperitoneal fluid (i.e. 7.6, 7.4, 5.9 and 3.9 g%) is determined by the ratio of the rates of fluid entry from the two sources and their respective protein content.

This mathematical extrapolation thus shows that a slow but prolonged input of protein-dilute fluid cannot achieve substantial dilution of the ascitic protein concentration if reabsorption is iso-oncotic. Instead, a relatively high rate of dilution (i.e. from extrahepatic sources) would be required to reduce the concentration of protein to levels much lower than those which occur in the fluid that transudates copiously from the hepatic surface.

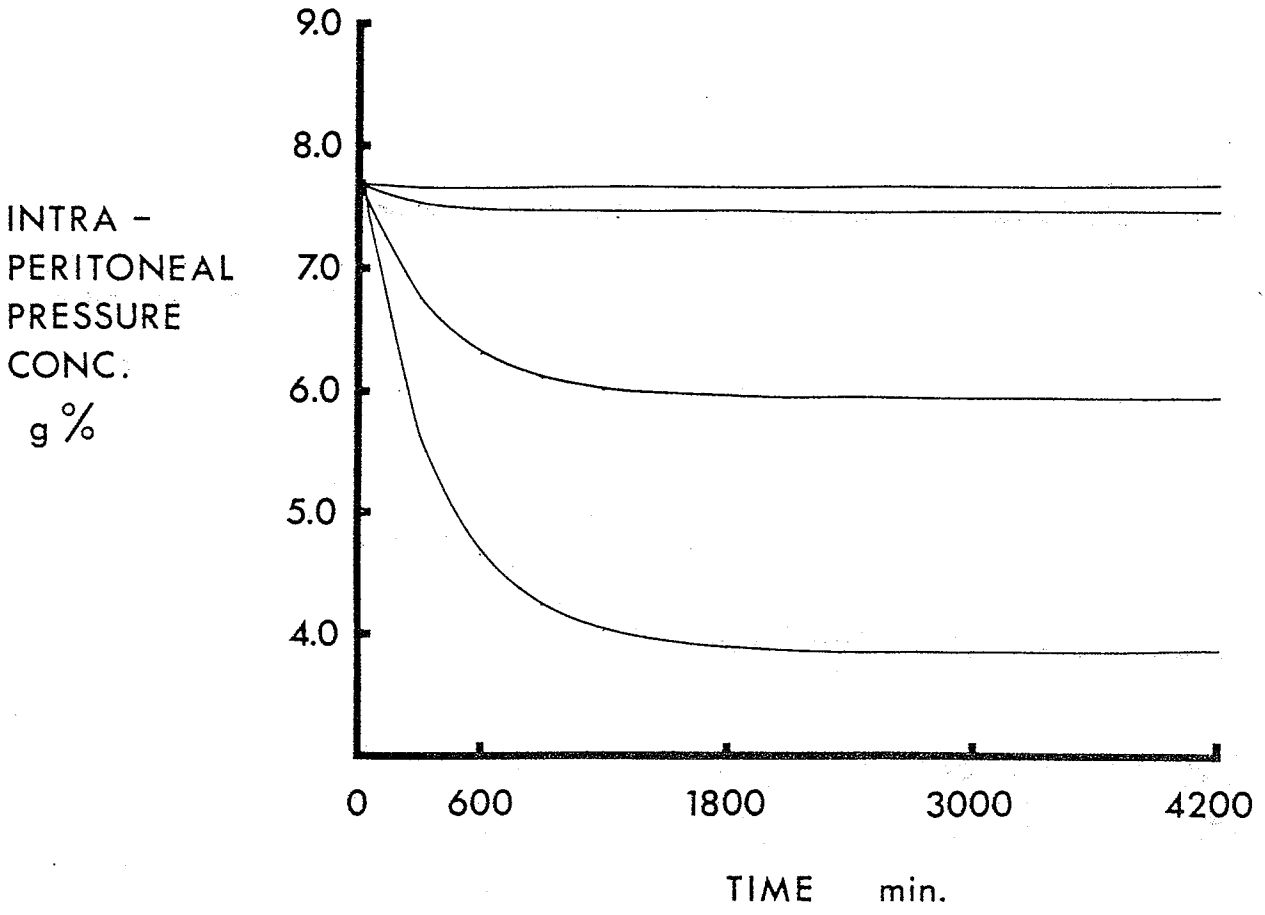


Figure 39. Long term, expected changes of intraperitoneal protein concentration with a constant rate of ascites formation and reabsorption. See text for details.

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