# FURTHER STUDIES ON FACTORS INFLUENCING LIVER INJURY AND LIVER REPAIR*

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THE MORBIDITY AND MORTALITY of surgical lesions involving the biliary tract have decreased progressively during the past quarter of a century. This desirable circumstance is in large part due to the results obtained from animal experiments, which have been applied to similar conditions occurring in man. In few fields has the application of the results of animal experimentation been more fruitful in extending existing knowledge of the patho-physiology of diseased processes than in the liver.

In spite of these advances in our knowledge of hepatic injury much remains unknown. There are not now available methods for accurately detecting even moderate degrees of chronic liver injury. It is often surprising how extensive the histologic change may be, and yet the currently available function tests provide little information that a superimposed acute injury may well induce hepatic incompetency. In few parenchymal organ systems is histologic appearance in chronic disease apt to be so poorly reflected by function tests.

On the other hand, acute hepatic cellular injury, from any cause, is most frequently associated with striking changes in function as determined by these tests, even when histologic evidences of parenchymal injury may be minimal. It is for reasons such as these that widespread biliary cirrhosis resulting from repeated attacks of cholangio-hepatitis may be associated in the chronic state, with little or no evidence of functional incapacity as determined by the turbidity and flocculation tests; and major evidences of functional abnormality may be found in patients with relatively mild attacks of viral hepatitis, in which the histologic evidence of cellular injury is minimal.

We know of no other organ in the body that possesses such an irresistible urge to regenerate after injury even under unfavorable conditions; but it should be just as readily recognized that few organs are more susceptible to a wide variety of noxious agents. Perhaps a wider understanding of certain factors recently shown to cause and to implement widespread hepatic parenchymal injury and the therapy now available which will facilitate repair, will result in a further reduction in the morbidity and mortality of patients with biliary tract disease subjected to operation.

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## ANOXIA AND LIVER INJURY

There are essentially two mechanisms by which anesthesia can effect the hepatic parenchyma—one by direct toxic action of the anesthetic agent and the other by the imposition of anoxia on the parenchymal cells. Every anesthetic agent is not toxic or necrotizing to hepatic cells, although many are. No anesthetization, general or spinal, is without effect on these cells if, during anesthesia and operation, from whatever cause, there occur periods, long or short, where the hepatic cells suffer from oxygen want (Fig. 1).

In the liver which is essentially normal histologically, short periods of oxygen starvation may produce no demonstrable histologic or functional

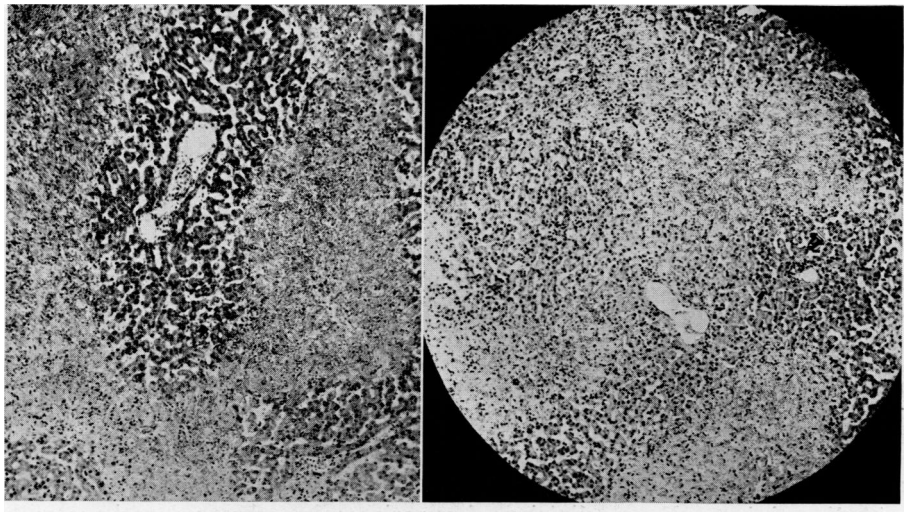

FIG. 1 FIG. 2

FIG. 1.—Necrosis of the liver following ether anesthesia.
FIG. 2.—Necrosis of the liver following nitrous oxide and oxygen anesthesia.

change; but where serious cellular injury, acute or chronic, already exists, even short periods of anoxia may be the motivating factor leading to hepatic incompetency. It must be constantly kept in mind that the hepatic parenchyma is exceedingly susceptible to oxygen want from any cause.

Chloroform volatilized with air for anesthesia in the experimental animal is twice as necrotizing as when the chloroform is volatilized with oxygen. Even nitrous oxide and oxygen, when insufficient oxygen is used in the mixture, can cause so extensive a necrosis of the liver that the resultant histologic picture may be indistinguishable from that produced by chloroform (Fig. 2).

Any circumstance that unfavorably influences blood flow, or is associated with a decreased oxygen saturation of the arterial blood, will result in injury to the liver cells. Such a condition may initiate injury or may accentuate existing damage.

The evidence now available from many laboratories strengthens the importance of an adequate and optimal oxygen supply to the liver cells during and after operation.

The work of Shorr[1] and Fine[2] and their associates points strongly to the liver as the organ conditioning the development of irreversible shock, while more recent experiments have demonstrated the amazing protection which well oxygenated blood introduced into the portal circulation provides in preventing the development of this serious state.[2a]

There are other factors which may cause local anoxia even when the peripheral evidences of oxygen want may be lacking. The placing of retractors along the right free border of the gastro-hepatic omentum so as to interfere with the normal blood flow to the liver may result in acute hepatic cellular degeneration and necrosis.

Estrada, Simpson and Vars[3] have demonstrated that massive or confluent centrilobular necrosis will occur in the rat following massive gastric distention, and that this is even more marked in the period immediately following partial hepatectomy. The importance of minimizing gastric and intestinal distention in patients with extensive liver injury has received little attention in surgical literature, and yet such a circumstance may so impede hepatic blood flow as to precipitate cellular changes leading to functional incompetency.

To prevent an occasional catastrophe, it may be necessary to maintain gastric intubation for several days after operation in all patients known to have, or suspected of having, pre-existing hepatic parenchymal injury.

Drugs which adversely affect the respiratory exchange, such as morphine and the barbiturates, have long been known to be poorly tolerated by patients with liver injury. Every clinician of experience has observed patients with serious liver injury making a relatively satisfactory recovery from the initial effects of anesthesia and operation, and then, following the injudicious use of such drugs, show evidences of hepatic failure. The anesthetist must be warned of this circumstance, and young house officers must constantly be cautioned that respiratory depressants are to be used in patients with widespread hepatic injury only when all other measures to relieve pain have proved ineffectual. It is well to remember that such a time-honored sedative as chloral hydrate has never been shown to cause hepatic injury.

### THE EFFECT OF PROTEIN STARVATION IN CONDITIONING LIVER INJURY

It is now generally agreed that a diet adequate in composition and in total calories facilitates repair of an injured liver. It is not so generally accepted that under-nutrition frequently conditions the extent of the hepatic injury which results following exposure to an hepatotoxic agent.

Goldschmidt, Vars and Ravdin[4] demonstrated that the most important dietary component in conditioning the liver against injury by noxious anesthetics was protein. They demonstrated that in general the higher the lipid

concentration of the liver, prior to anesthetization with such agents, the greater was the cellular injury; but that an adequate amount of protein in the diet for several days prior to anesthetization provided some protection to the liver even in the presence of large increments of hepatic lipid.

It has now been demonstrated by other investigators[5-7] that dietary protein will protect the liver from a wide variety of noxious drugs and that protein starvation, or under-nutrition, increases the susceptibility of the liver to these agents. It is not so generally recognized that just as protein starvation accentuates the hepatic necrosis produced by certain drugs, so, too, protein starvation accentuates the injury produced by anoxia. Estrada, Simpson and Vars[3] found more widespread hepatic necrosis in the protein-starved animals following gastric distention than in well-fed animals. The demonstration by

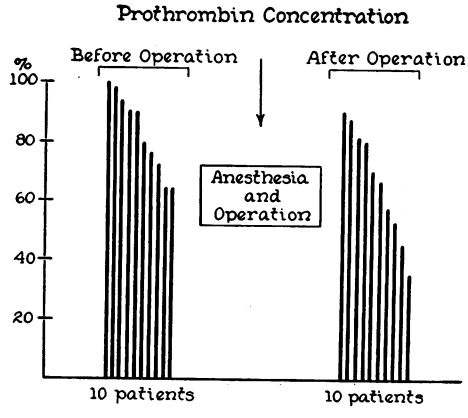


FIG. 3

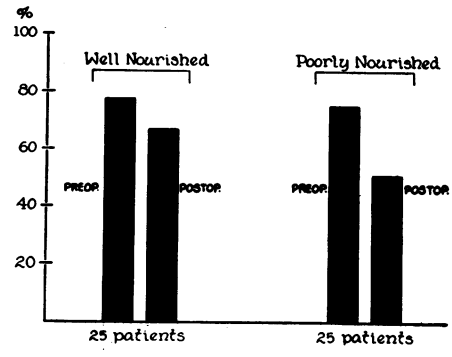


FIG. 4

FIG. 3.—Prothrombin concentration before and after anesthesia.
FIG. 4.—Change in prothrombin concentration at two nutritional levels.

these workers that the more protein-depleted the liver was prior to exposure to gastric distention the more extensive was the resultant injury illustrates the importance of a carefully carried out dietary program prior to operation.

The beneficial effect of protein repletion prior to operation can be demonstrated in man by comparing prothrombin determinations before and after operation in patients with hepatic disease (Fig. 3). An adequate concentration of prothrombin prior to operation may become sharply reduced subsequent to operation by any one or combination of factors which cause parenchymal injury. We have observed, in patients whose dietary intake for some time prior to anesthesia has been adequate, that this sharp decline is minimized in comparison to those whose intake has been inadequate.

It is frequently impossible to determine the protein competency of these patients by any laboratory tests for total proteins, and fractionated proteins may at times give no direct clue to the actual protein intake in the presence

of serious parenchymal change. A careful history prior to hospitalization and an accurate dietary record subsequent to hospitalization are often more revealing than are the laboratory determinations.

Using these criteria, in addition to laboratory tests, to provide the classification of the degree of nutritional repletion or deprivation, we have found the mean fall in the prothrombin concentration on the second postoperative day to have been 10.3 per cent in the well fed group, while in the patients whose mean prothrombin concentrations prior to operation were nearly identical and who had been on a poor dietary intake, the mean decline in concentration on the second postoperative day was 26.2 per cent (Fig. 4).

The evidence now available points strongly to nutritional factors as important etiologic agents in the production of liver injury, either in the form of degeneration or necrosis in acute injury, or as cirrhosis in chronic injury. When the effect of the injurious agent is overwhelming, as occurs in anoxia, the exposure to hepatotoxins and certain bacterial and viral infections, necrosis results. It will also be observed when previous injury provides hepatic parenchyma whose resistance to additional injury has been lowered. On the other hand, repeated exposure to the same injurious agent in lesser amounts will frequently result in some degeneration, associated with a variable degree of cirrhosis.

## INFECTION

We have previously called attention to the fact that the problems associated with acute cholangio-hepatitis are not so serious as they were prior to the availability of the more recently discovered antibiotics. Aureomycin, with its wide antibacterial spectrum, has been used by us with gratifying results. The use of such agents exerts no effect on the underlying cause of the cholangio-hepatitis, but control of the active infection permits operation for the removal of common duct obstruction due to stone or stricture under much more favorable circumstances.

The striking results obtained by Markowitz, Rappaport and Scott[8] following ligation of the hepatic artery of the dog when these animals were protected with penicillin, and the substantiation of these observations by Fitts and Scott[9] and their subsequent observations of the even greater effectiveness of aureomycin in protecting such animals from an otherwise inevitable death, emphasizes again the usefulness of antibiotic therapy.

## COINCIDENTAL LIVER AND RENAL INJURY

There has long been serious question as to whether the "hepato-renal syndrome" actually exists. It has now been shown that under certain experimental conditions definite histologic changes may be found in the liver and kidneys of animals exposed to certain noxious agents or to a special dietary program. Either a choline deficient diet or cirrhosis-producing diet will frequently cause simultaneous liver and renal injury, the latter exhibiting itself

chiefly by acute cortical injury. The dietary factors which lead to recovery from the hepatic cell injury will have a beneficial effect on the renal lesions.

### ENZYME ACTIVITY IN THE REGENERATING LIVER

Rosenthal, Vars, Rogers and Fahl[10] have, in view of the biological significance of premitotic cytochemical, studied the enzyme activity of the regenerating rat liver from a half day to eight days after 70 per cent partial hepatectomy. The influence of preoperative protein-depletion and postoperative fasting was also investigated. The following enzymes were assayed: arginase, alkaline phosphatase, adenosine-pyrophosphatase, rhodanase, cholinesterase, and common esterase. With the latter four enzymes, activity per gram of liver protein started to decline just before onset of increased mitotic activity. During the subsequent phase of highest rate of cell multiplication and protein synthesis, activity of these enzymes was reduced by about 20 per cent, and then gradually returned to the original level. The inverse order of changes was found with arginase and alkaline phosphatase, the maximal activity being reached at the onset of increased mitotic activity. The magnitude of the increase of enzyme activity was related to extent and direction of the postoperative change in the animal's metabolism. It thus is evident that during the initial phase of normal regenerative growth pronounced changes in the enzyme pattern are taking place which subsequently disappear in the course of cell division and maturation. It seems possible from their work that stimuli to cell multiplication are transmitted through distortion of enzyme patterns in response to changing environmental conditions.

### DIET AND LIVER REPAIR

Goldschmidt, Vars and Ravdin[4] concluded from their studies that a diet rich in carbohydrate and protein and low in fat was the diet best calculated to prepare the patient with liver injury for anesthesia and operation, and to facilitate repair thereafter.

During the course of these early experiments data were obtained which demonstrated that if the diet contained a sufficient amount of a high quality protein, the dietary fat could be considerable without increasing the liver lipid during the period of special feeding. In such circumstances, the liver lipid decreased quite as rapidly as if the fat were restricted in the diet. In 1945, Hoagland[11] suggested that if the fat in the diet of patients with infectious hepatitis were not restricted to the degree we had suggested, the total caloric intake, which is as important as the composition of the diet, would be greatly increased.

Vars and Gurd[12] have further clarified the relationship of diet to repair in the regenerating liver. They have shown that following partial hepatectomy in the rat, regeneration during a period of two weeks is dependent upon the quality and the amount of protein fed in an otherwise calorically sufficient dietary. Zein as a source of protein was no better than no protein in the diet,

while 27 per cent of casein was considerably better than 18 per cent of casein. The ability of the liver to utilize protein from endogenous or exogenous sources, for rapid repair, was the most important factor in the survival of the animals subjected to preoperation protein depletion and partial hapetectomy.

Vars, Friedgood, Ferguson and Rogers[13] have now studied regeneration of the liver during periods of a high fat dietary. They have studied the effect of high carbohydrate, high protein, low fat and high fat dietaries on regeneration of the liver of the rat following partial hepatectomy. They have

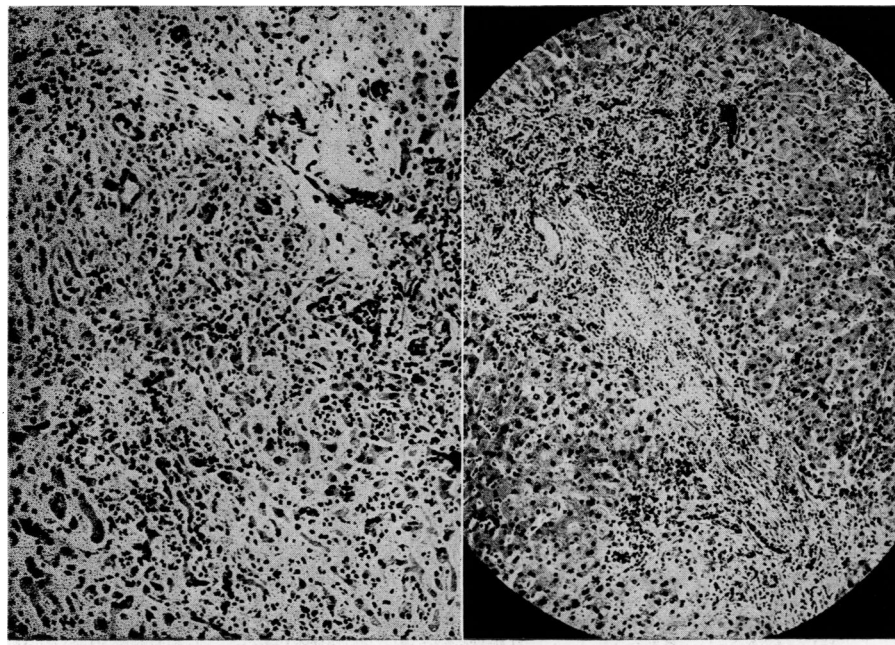

FIG. 5 FIG. 6

FIG. 5.—Widespread cholangiohepatitis with necrosis. Even though the necrosis is extensive mitotic cells are present.

FIG. 6.—Regeneration of hepatic cells in cholangiohepatitis. Twenty days between biopsies.

found that as much as 30 per cent of the total calories can be ingested as fat without unfavorably influencing regeneration, provided a protein of good quality and of sufficient amount is also present in the diet.

It is of importance to determine whether, in addition to ridding the liver of fat during the preoperative period, it is possible to facilitate regeneration of hepatic tissue in the presence of ductal occlusion. It is now well recognized that in infectious hepatitis, as well as in homologous serum jaundice, degeneration and repair progress simultaneously.

Ferguson, Rogers and Vars[14] have studied the regeneration of the partially hepatectomized liver of the rat in the presence of common duct occlu-

sion. In some of the experiments the common duct was ligated some days before partial hepatectomy, and in others at the time of partial hepatectomy. The end-result has been essentially the same. Placed on an adequate dietary, the liver will regenerate, as can be demonstrated by an increase in hepatic cells (the presence of active mitoses persisting for even longer periods than would occur if the obstruction were not present) and an increase in hepatic cellular protein.

The conditions are not exactly similar to those found in man in the presence of common duct stricture or stone, for under the circumstances of their experiments cholangio-hepatitis did not exist. We have, however, now obtained evidence in man that regeneration can take place under conditions of recurrent cholangio-hepatitis (Figs. 5 and 6). Provided an adequate dietary is fed in the preoperative period, cellular repair and regeneration can be demonstrated in man by the presence of mitotic figures in liver specimens taken for study at the time of operation.

The demonstration that liver injury and repair can go on simultaneously in ductal occlusion and cholangio-hepatitis in a manner similar to that previously shown to occur in viral hepatitis gives new impetus to the vigor with which the preoperative dietary program should be pursued. While the influence of certain hormonal factors has been proved to be of definite help in the prevention of liver injury in the experimental animal and in its repair, no such relationship has been proved in man. It is, however, not unlikely that certain hormones may intensify the vigor of the repair which can now be induced by an adequate diet alone.

## SUMMARY

It is more than probable that the reduction in functional capacity of the liver, which at times occurs subsequent to anesthesia and operation is due in large part to anoxia. This takes place during anesthesia from failure of an adequate oxygen supply. It may take place during operation from any condition which impedes blood flow to the liver, even when the circulating blood is well oxygenated, or from a reduced blood flow as occurs in shock. In the patient with suspected acute or chronic liver injury, the prevention of hepatic anoxia during and after operation is of major therapeutic importance to recovery.

A diet which is adequate in total calories and in composition will partially protect the liver from a wide variety of noxious agents and will facilitate repair of the liver damaged by these agents. It will even facilitate repair in the presence of recurrent cholangio-hepatitis in man. The importance of an adequate intake of protein, and of an optimal number of calories in the preoperative period, therefore takes on a new significance, for repair can be begun and functional capacity improved before the patient is subjected to the assault of anesthesia and operation.

## BIBLIOGRAPHY


1 Shorr, E., B. W. Zweifach and R. F. Furchgott: Science, **102**: 489, 1945.
2 Frank, H. A., A. M. Seligman and J. Fine:
(a) J. Clin. Investigation, **25**: 22, 1946.
(b) J. Clin. Investigation, **26**: 530, 1947.
3 Estrada, R. L., Z. A. Simpson and H. M. Vars: To be published.
4 Goldschmidt, S., H. M. Vars and I. S. Ravdin: J. Clin. Investigation, **18**: 277, 1939.
5 (a) Himsworth, H. P., and L. E. Glynn: Clin. Sc., **4**: 421, 1942.
(b) Smith, M. I., B. B. Westfall and E. F. Stohlman: J. Indust. Hyg. & Toxicol., **25**: 391, 1943.
6 (a) Baxter, J. H.: J. Clin. Investigation, **25**: 908, 1946.
(b) ————————: Pharmacol. & Exper. Therap., **91**: 345, 1947.
7 Schifrin, A.: Virchows Arch. f. Path. Anat., **287**: 175, 1932.
8 Markowitz, J., A. Rappaport and A. C. Scott: Proc. Soc. Exp. Biol. & Med., **70**: 305, 1949.
9 Fitts, W. T., Jr., and R. Scott: Unpublished data.
10 Rosenthal, O., H. M. Vars, C. S. Rogers and J. C. Fahl: To be published.
11 Hoagland, C. L.: Bull. New York Acad. Med., **21**: 537, 1945.
12 Vars, H. M., and F. N. Gurd: Am. J. Physiol., **151**: 391, 1947.
13 Vars, H. M., C. Friedgood, C. Ferguson and C. S. Rogers: To be published.
14 Ferguson, C., C. S. Rogers and H. M. Vars: Am. J. Physiol., **159**: 343, 1949.


DISCUSSION.—DR. CALVIN M. SMYTH: There are two or three aspects of this very interesting presentation that I think might bear emphasis. The first of these has to do with the matter of the effect of anesthesia on the liver. If I heard him correctly, Dr. Ravdin said that the damage to the liver, even with some anesthetic agents usually considered innocuous, was dependent upon the length of time during which the liver was subjected to these periods of anoxia. I think that gives us a little pause to think, because it has become popular recently to measure the excellence of an operation by the time it took to perform it. I do not know whether that has impressed other people as it has impressed me, but it seems to me we hear about eight- and nine-hour operations which, because of the time it took them to perform, must be much better than the operation which took two or three hours to perform.

Now, I am not advocating the sacrifice of efficiency to speed. A lot of us were brought up in the "speed" era, and we know that it had many objectionable features. However, I would like to offer the thought to this body that if an operation can be performed efficiently in two hours, there would seem to be but little virtue in prolonging it for five or six, as I am afraid is done sometimes.

I was also very much interested in the point Dr. Ravdin brought out that liver destruction and liver repair could take place simultaneously, and that repair could take place even under conditions which, heretofore, have let us be sort of "horsed" into operating on patients we really didn't think were ready for operation.

It is a comforting thing to know—and have objective evidence such as was presented this morning—that these two things can go on simultaneously, even in severe cholangiohepatitis.

DR. OWEN H. WANGENSTEEN: The persistence and ingenuity with which Dr. Ravdin and his group have followed the problem presented by liver injury and repair has intrigued all surgeons; a debt of gratitude is owing Dr. Ravdin and his associates for the light which their studies have shed on factors which predispose toward, or guard against liver injury. While visiting Dr. Ravdin's clinic last fall, I had the pleasure of seeing the histologic picture of the diffuse necrosis of the liver which accompanies