THE EFFECTS OF HOMOLOGOUS CROSS CIRCULATION AND IN SITU LIVER PERFUSION ON FULMINANT HEPATIC FAILURE RATS

A thesis submitted for the degree of Master of Science in the Faculty of Medicine, Department of Physiology of McGill University

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CROSS CIRCULATION AND LIVER PERFUSION IN HEPATIC FAILURE

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ABSTRACT

A galactosamine-induced fulminant hepatic failure rat was used to study the effects of homologous cross circulation and in situ liver perfusion. Cross circulation with homologous donor did not significantly improve the survival time or recovery rate of grade II animals. Homologous in situ perfusion significantly improved the survival time and recovery in fulminant hepatic failure only when started in grade II coma; it has no effect in the later stages of coma.

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I N T R O D U C T I O N

Fulminant hepatic failure is defined as sudden severe impairment in hepatic function culminating in encephalopathy, the encephalopathy being the result of hepato-cellular failure and occuring within eight weeks of the onset of the symptoms[1]. It is characterised by jaundice, tremor, fetor hepaticus, and mental changes, starting from slight personality changes and progressing to stupor and coma. The characteristic biochemical abnormality is prolonged prothrombin time and high serum enzymes. The most common cause of fulminant hepatic failure is viral hepatitis A and B[2]. Other causes include hepato-toxicity due to drugs, chemicals and toxins e.g., Paracetamol, mushroom poisoning and phosphorous. Some drugs cause fulminant hepatic failure due to hypersensitivity reactions, e.g., halothane. Metabolic anamalies like acute Wilson's disease and acute fatty liver of pregnancy are a rare cause of fulminant hepatic failure.

The mortality rate in fulminant hepatic failure

is extremely high, being of the order of 80-90%[1]. It is, however, affected by many factors including age, etiology, grade of encephalopathy and presence or absence of complications. With increasing age and grade of coma, the prognosis becomes worse. The mortality rate in fulminant hepatic failure due to hepatitis A is better (17%) than with hepatitis B (19%). Fulminant hepatic failure due to halothane carries a high mortality rate (96%) whereas that due to other drugs has a more favourable prognosis (50%).

The complications most commonly seen in fulminant hepatic failure are gastro-intestinal bleeding, renal failure, respiratory failure, and acute pancreatitis. The presence of any of these seriously affects prognosis and survival.

The treatment of fulminant hepatic failure is not satisfactory[3]. Routine or conservative medical treatment consists of :

1. Protein restriction, antibiotics, and gastro-intestinal cleansing, all aimed at reducing ammonia production.
2. General care of the patient including correction and maintenance of fluid and electrolyte balance and acid base balance.
3. Treatment of comlications.

This type of treatment has failed to improve the grave prognosis and high mortality associated with fulminant hepatic failure. Since the liver possesses the property to regenerate even after enormous insults, a number of temporary measures or hepatic supports have been introduced during the last thirty years[4,5,6]. The aim of such measures is to allow the patient to tide through the crisis of hepatic coma, thereby giving time to the liver to regenerate to a sufficient degree so as to carry out its functions. These include :

1. Corticosteroids.
2. Exchange Transfusion.

3. Cross Circulation.

4. Isolated Liver Perfusion.

5. Hemodialysis.

6. Charcoal Hemoperfusion.

7. Transplantation.

8. Others, including L-Dopa, heparin, hemoperfusion through liver cells in culture, total body wash-out, amino acids, and hemoperfusions through sorbents other than charcoal, e.g., resins.

1. Corticosteroids :

The use of corticosteroids was started in 1951 by Katz and Ducci[7]. A number of reports followed showing benificial effects of such a treatment in uncontrolled trials[8,9,10]. One such study was published by Katz[8] in 1962 which described 23 patients treated with steroids during the period 1951-1961[10]. Besides steroids the treatment regimen consisted of broad spectrum antibiotics, intravenous glucose, maintainence of fluid and electrolyte balance, and in female patients testosterone proprionate.

A survival rate of 39.1% was reported in this series. This group was compared to a series of sixteen patients treated during the period 1940-1950. The treatment consisted of I.V. glucose only. Of these none of the patients had survived. The statistically significant results in this report were attributed to the use of steroids only. This and similar uncontrolled early trials describing the recovery of several patients treated by steroids, provided the rationale for its use.

The first controlled evaluation of corticosteroid therapy was instituted by Ware et al in 1974[11]. In this report no obvious benifit of corticosteroid therapy in fulminant hepatic failure could be demonstrated. But no firm conclusions could be drawn from this study because by chance the number of patients allocated to corticosteroid treatment was small compared to the control group, (four patients treated by steroids as compared to eleven in the control group who received a placebo.), and the mean age was higher.

Another controlled trial was conducted by Gregory by randomly allocating patients to corticosteroid and placebo therapy[12]. In this study 14 patients were assigned to the steroid therapy, and seven of these died. Of the 15 assigned to the placebo group only two died. The difference in the two series was not significant statistically. These results were supported by another controlled study reported by Redeker[13].

Thus it was conclusively shown that cortcosteroids do not improve survival, but whether they have a deterimental effect was not clear from these studies. A prospective co-operative study is being conducted by the Acute hepatic failure study group, and definitive information regarding the value of corticosteroids will then be obvious[6].

2. Exchange Transfusion :

The underlying principle for exchange transfusion was the removal of toxins and supplementing essen-

tial factors. The first patient with fulminant hepatic failure to be treated by Exchange Transfusion was reported by Lee&Tink in 1958[14]. This patient survived. Subsequently some encouraging results were reported by other investigators[3,15,16]. For instance, five out of seven patients in one of Trey's series survived. Unfortunately, other reports that followed were less favourable[17]. There was also the problem of acquiring large quantities of freshly drawn blood since exchange of one blood volume did not result in immediate alleviation of coma[17]. The use of anticoagulants was another unfavourable factor and the risk of subsequent serum hepatitis a real one.

By 1970, 97 patients in hepatic failure due to viral hepatitis had been treated by exchange transfusion and they were reviewed by Rivera et al[18]. Analysis of this seies showed a survival rate of 34%. The survival rate was shown to be higher if the reported study consisted of less than five patients and much lower if five or more patients were involved. Benhamou et al compared

compared the survival rate of 78 patients treated by exchange transfusion with a control of 270 patients treated by various other methods[19]. They found no significant difference in survival between the two groups. McKechnie and Hersh observed only two survivors among 16 patients with acute hepatic failure treated by exchange transfusion[20]. In Ritt's series there were no survivor among eight patients treated by this procedure[21].

In 1973, a controlled evaluation of the efficacy of exchange transfusion in the treatment of fulminant hepatic failure was reported by Redeker and Yamahiro.[22] The patients were randomly allocated to be exchanged or not to be exchanged. The two groups were comparable in age, hepatic function, renal function and depth of coma. Eight patients were exchanged and they all died. Four out of the 13 patients not exchanged, lived. Thus there was no increase in survival among patients with fulminant hepatic failure who were treated by exchange transfusion when compared to patients who were not exchanged. Exchange

transfusion, however, does improve the neurologic status and hemorrhagic abnormality.

3. Haemodialysis :

The use of conventional haemodialysis in the treatment of fulminant hepatic failure was first reported in 1950[23]. In 1956 Kiley et al showed that high blood ammonia levels in dogs with portacaval shunts could be reduced by hemodialysis[24], and then proceeded to treat five hepatic coma patients with this procedure[25]. However, all these patients had chronic liver disease and coma was due to massive gastro-intestinal hemorrhage. They reported lowering of blood ammonia levels after dialysis, but recovery from coma occured 12-24 hours later. A patient with fulminant hepatic failure was treated by hemodialysis in 1961. Although ammonia levels fell considerably, clinical improvement did not ensue[26].

By 1974, there were 19 reported cases of hepatic coma treated by hemodialysis.[27] Ten recovered consciousness

and five survived.Of these only six patients had suffered from acute liver failure. Three recovered consciousness during and after hemodialysis and survived. Another one woke up but died later due to gastro-intestinal hemorrhage. Although not enough patients have been treated by conventional hemodialysis to critically evaluate it's efficacy, it was widely considered to be ineffective.This may be due to the fact that although high ammonia levels are associated with hepatic coma, there is a poor co-relation between the blood ammonia levels and degree of encephalopathy.[28] Further, other methods employed to lower blood ammonia levels, e.g., use of sodium glutamate and L-arginine, failed to provide any benifit.[29,30] Thus, there was a reluctance to accept high ammonia levels as an important cause for hepatic coma.

In recent years considerable evidence has accumulated that the toxins in hepatic coma may belong in the middle molecular range.[31,34] The conventional hemo-dialysis membrane is not permeable to these large molecules. Therefore, membranes with a high clearance for middle

molecules were developed and used. Opolon et al showed that in pigs with experimentally induced hepatic coma the survival time was prolonged when dialysis was carried out using polyacrylonitrile membrane[31] (a membrane permeable to middle molecules). On the other hand, survival was shortened as compared to the controls when cuprophane membrane was used. In subsequent experiments they showed that this detrimental effect of cuprophane membrane may be due to an amplification of imbalance between normal and false neurotransmitters by clearing out low molecular weight substances (e.g. peptides).

Opolon et al also conducted clinical trials to see the benificial effect of polyacrylonitrile membrane, if any. Twenty-two patients with fulminanat viral hepatitis and coma were treated. Recovery of consciousness was observed in 13 patients (59%) as compared to 23% among 108 patients admitted to the same unit during the period 1965-1974. However, survival rate in both groups was similar (22% with polyacrylonitrile membrane and 18% without).

Polyacrylonitrile hemodialysis treatment was also reported by Silk and Williams.[32] Twenty-four patients were thus treated and eight survived (33.3%). These results are significant when compared to a series of patients treated conservatively in the same unit. But the results of the two groups were not comparable.

4. Charcoal Hemoperfusion :

Charcoal hemoperfusion was carried out for the first time in a patient with grade IV fulminant hepatic coma in 1972 by Chang.[33] Although there was complete recovery of consciousness after the first and third hemoperfusions, and some improvement after the second, the patient did not survive for more than three weeks. Treatment of this patient with EX-01 coil hemodialysis had not affected consciousness.

The second patient with fulminant hepatic failure to be treated by charcoal hemoperfusion was reported by the same group in 1973.[34] In this patient, the onset of hepatic coma and clinical deterioration was ex-

tremely rapid and it was thought that hemoperfusion had been carried out too late to be of any benifit. A third patient[35], after showing no response to conventional hemodialysis for three hours, was treated by charcoal hemoperfusion for 45 minutes. There was no immidiate response, but consciousness started to improve the next day and she survived. All these three patients were treated by ACAC (albumin coated activated charcoal) in which platelet losses do not occur to any significant degree. Three more patients have been treated since, one with viral hepatitis and two with chronic liver disease. None of these recovered.

Charcoal hemoperfusion in a large series of patients (37 patients) was reported from King's College Hospital.[37] The Charcoal used in this series was covered by 4% by weight coating of hydrogel polymer. In this initial series 17 patients (46%) recovered consciousness. The patients were perfused on an average three times and improvement in consciousness took place 12-18 hours following the final perfusion and not during the procedure.

Fourteen patients recovered completely (37.8%). At the same centre survival in 51 patients treated conservatively was 15.3%. In this series small changes in platelet counts were noted during perfusions, considerable drop in platelet count occured from day to day. Four patients had major bleeding episodes which contributed to death. However, the platelet losses in these cases were no greater than in cases where no bleeding occured. The encouraging results led to more clinical trial at this centre,[38] and 34 patients were treated by charcoal hemoperfusion. But in this series only three patients survived (8.8%), so that overall survival in 71 patients treated at this centre was 23.9%. Many of the patients who died in the later series developed severe unresponsive hypotension and the cause of hypotension was thought to be the release of vasoactive substances from platelet aggregates. These substances were demonstrated in blood leaving the charcoal column.

Another centre active in carrying out charcoal hemoperfusion in fulminant hepatic failure patients is George Town University Hospital. A single patient was

successfully treated in 1976,[39] then a series of nine patients treated by charcoal hemoperfusion was reported.[40] Of these nine patients, eight regained full consciousness, but only three survived. Platelet levels decreased dramatically in two patients and so did clotting factor V, VII, X and XI in one patient studied.

Some Japanese investigators have also reported the use of charcoal hemoperfusion in the treatment of fulminant hepatic failures. In one such series 10 patients with fulminant hepatic failure due to viral hepatitis and drug hepatitis were treated by charcoal hemoperfusion.[41] Seven patients had improved consciousness after the procedure and three of these survived. In another series the hepatic assist system consisted of ACAC charcoal hemoperfusion and hemodialysis.[42] Fifteen patients were treated by this procedure, and three survived. In all the cases that survived hemoperfusion had commenced in grade II or III hepatic coma, while in those commencing at IVa or IVb, all died eventually.

The results of all these studies show that the survival rate is not superior to the controls or conservative treatment. However, it has been shown that charcoal hemoperfusion is effective in reversing the encephalopathy of hepatic failure. Recent results obtained using a galactosamine induced fulminant hepatic failure rat model shows that survival can be improved to 70% (from 30%), when charcoal hemoperfusion is instituted in the earlier stages of coma.[102] Also as suggested by Chang, charcoal hemoperfusion may form an important component of a more complex system, rather than constituting alone a complete artificial liver.

5. Liver Transplantation :

In fulminant hepatic failure where the hepatic injury is considered to be potentially reversible, an auxilliary transplant was considered of value. This would allow assistance for long intervals and the sacrifice of the recipient liver is prevented, which can regenerate and function normally. It was also initially assumed that placing an extra liver in the abdomen would be safer and

technically easier than orthotopic transplantation. However, several difficulties associated with this procedure have led investigators to abandon it.[43] It is difficult to find room for an extra organ.[44] It is applicable to non-infectious, non-malignant conditions.[45] But above all the auxilliary organ competes with recipient liver for some metabolite in the portal blood.[44, 46] The liver which first has access to the portal blood retains it's functional and morphological integrity while the other undergoes atrophy. Thus this form of liver trasplants are no longer employed and orthotopic replacement of the liver is the procedure of choice. In this case technical difficulties in removing the diseased liver and replacing it are major challanges.[47] Other problems include coagulation defects, metabolic abnormalities and biliary duct complications.[47] Rejection of the graft is not as severe a problem as with some other kinds of grafts, e.g. kidney.[43,47]

Most patients treated by hepatic transplant have belonged to two major groups, i.e., malignancy of the liver and parenchymatous liver diseases.[48,49] Most investigators feel that fulminant hepatic failure is not an

indication for liver transplantation.[47,50] This is due to the fact that patients in deep hepatic coma have uncertain prognosis, and are bad surgical risks. However, another approach has been suggested by Sutherland et al. This group has shown that hepatocellular transplantation either intraperitoneally or in the portal vein increased the survival of dimethyl nitrosamine treated rats significantly when compared to controls.[51] This approach has not been utilized clinically yet.

6. Isolated Liver Perfusion :-

The perfusion of isolated liver has been used for physiologic studies since the early part of this century, e.g. in the investigation of the dynamics of bile secretion,[52] and the control mechanisms of the hepatic dual blood supply.[53] Advances in the use of pump oxygenators not only provided a great stimulus to these studies, but also opened new avenues for approaching the treatment of hepatic coma.[54] The possibility of substituting a normal liver during the phase of overwhelming hepatic insufficiency seemed attractive. The liver would carry out the

excretory and syntetic functions and risk to the normal donor was not involved, as in the case of cross circulation.

To find out the feasibility of such a procedure some early studies showed the ability of the isolated pig liver to convert ammonia to urea when perfused with homologous blood.[54] Also, pig liver when washed free of its' blood was shown to function satisfactorily.[55] However, in some cases a severe venous outflow block was encountered. Factors leading to this increased vascular resistance were evaluated. Acidosis, adrenaline, histamine, hypothermia and antigenic extracts of some parasites were incriminated in it's causation.[56] Therefore, when in later studies physiologic conditions of temperature, perfusion pressure, PH and strict asepsis were maintained, out flow resistance was not encountered. Perfusing such a liver resulted in good bile flow and effective clearance of ammonia, bilirubin and galactosamine. The liver responded to a choleretic agent by an increased volume of bile excretion.[55]

The first clinical trial using isolated hetrologous liver perfusion in the treatment of fulminant hepatic failure was reported by Eisman in 1965.[55] This series consisted of eight patients. Clinical improvement varied from dramatic return of consciousness to only slight neurological improvement. None of these patients survived on a long term basis.

Subsequent clinical trials were done at various other centres.[57,58,59,60,61] In some of these centres modification and optimization of the method and circuit was also done to mimick physiologic conditions as closely as possible.[62,63] But the results were not encouraging. For example, according to one report 27 patients were treated by isolated liver perfusion by 1971 and only three survived.[59] Of these two were young children. All three survivors had received cortico-steroids and two had exchange transfusions also. The major cause of death in this series was gastro-intestinal hemorrhage.

Sometimes a perfused liver which is functioning

well biochemically will deplete the patient's blood of platelets, worsen prothrombin and clotting time, and produce severe bleeding. This was noted by Hickman in a series of four patients.[60] Winch carried out experimental studies on the mechanism of the coagulation disturbance.[64] It was found that fibrinogen is lost not only to the fluid surrounding the pig liver, but also in the pig's liver itself, as evidenced by the presence of fibrin in the sinusoids at the end of perfusion. This could be responsible for a parallel loss of platelets. These phenomenon could be related to the endothelial damage in the sinusoids due to ischemia during the isolation and preservation of the liver. Winch went on to suggest that to screen out livers that would cause platelet depletion, a test can be done. In this test radioactive fibrinogen is administered to the isolated liver. If there is a fall in its' concentration in the out flow, the liver should be discarded, because such a liver is liable to cause platelet depletion and consumptive coagulopathy.

Another cause of thrombocytopenia brought to

light by Hickman is the immunologic reaction on the part of the pig liver to human blood perfusion. Perfusions of livers from calves, pigs, and lambs with human blood resulted in thrombocytopenia within 30 minutes.[65] In contrast, perfusion of baboon liver with human blood did not provoke significant thrombocytopenia. There was no difference in platelet levels using livers from baboons with blood groups either compatible or not compatible with the human blood being perfused. The baboon liver has also been used for cross circulation and no immunologic reactions have been observed.[90] Therefore Hickman et al decided to use baboon liver only for hepatic assist.

The use of baboon liver for hepatic assist was reported by Abouna in 1972.[66] Two patients in deep hepatic coma due to viral hepatitis were treated by baboon liver perfusion. These patients had failed to respond to exchange transfusion instituted earlier. Both patients made complete recovery. No immunolgic reactions were noted. In these two cases a rise in the level of liver dependent clotting factors accompanied the clinical improvement.

Also, the baboon liver could function upto 24 hours extra-corporeally, whereas in porcine livers it is difficult to maintain perfusion for more than a few hours. This early success with baboon liver perfusion encouraged some investigators to carry out the procedure in a large series of patients. One such report was published in Tuzig, Germany, which showed that baboon liver perfusion did not improve survival in a large series of patients.[67]

7. Cross Circulation :

Cross circulation has been carried out since the later part of the last centuty to study various physiologic processes in animals. The first recorded cross circulation experiment in animals was done in 1890 by Fredericq to study the chemical factors affecting respiration.[68] Since then cross circulation has been utilized to study a variety of physiologic phenomenon. In 1910 Hedon showed that a pancreatectomised dog did not develop diabetes if cross circulated with a normal dog.[69] Nyiri observed that B.U.N. in a nephrectomised dog can be reduced by cross circulation.[70] Cross circulation has also been

used in inducing tolerance to organ transplant[71,72] and in organ failure, especially liver, kidney and bone marrow.[75,76]

In human beings, cross circulation has been utilized in studies of human diseases. In 1936, Rosenthal sought to find pressor substances in hypertension.[77] Bierman, in 1951 used cross circulation in studies of luekemia.[78]

Cross circulation has been an intriguing possibility for supporting patients with organ failure and to sustain life during a potentially reversible illness. It has been utilized in eclampsia, hypertension, and renal disease in 1952.[79] In 1940, Duncan showed clinical improvement in two uremic patients.[80] Further, cross circulation has been used by Warden during cardiac surgery to perform cardio-pulmonary by-pass.[81]

Despite these early experiences, cross circulation was not generally applied to man because of tech-

nical difficulties related to access and heparinization After the development of indwelling arterio-venous silastic-teflon cannulas in 1960,[82] cross circulation was used in the treatment of fulminant hepatic failure, renal failure and bone marrow failure.

In the treatment of fulminant hepatic failure, cross circulation had some advantages when compared to exchange transfusion, a procedure being used extensively in the sixties. Large quantities of freshly drawn blood and heparinization were required for exchange transfusion, but not for cross circulation. With the usual flow of 100 ml/min cross circulation was considered more efficient than plasma or whole blood exchange. Adjustment of electrolyte and acid base balance occured during cross circulation and other metabolic disturbances were also corrected.

Twenty-one patients with fulminant hepatic failure had been treated by cross circulation by 1973.[83,84,85,86,87] Another patient, treated in 1977, makes

the total of twenty-two.[88] Of these four patients survived without continuing evidence of chronic liver disease. Several others regained consciousness for varying lengths of time. There was amelioration of symptoms in 14 patients. Fourteen autopsies were done, and seven showed evidence of hepatic regeneration. Not all patients treated by cross circulation have shown a temporary improvement. There have been two donor deaths due to immunolgic reactions.[86] (One due to anaphylactic shock and the other due to delayed immulogic reaction.)

The published data are inadequate for the evaluation of cross circulation. But it is generally considered that the results are poor. Further, there are obvious dangers to the donor and there have been two donor deaths. In an animal study published in 1969 it was shown that when baboons were cross-circulated for no more than four days, the procedure was relatively safe.[89] Beyond this period, hematologic abnormalities and anaphylactoid reactions potentially fatal for one or both members of the union occured. These authors concluded that cross-

circulation is a safe procedure when carried out upto 4 days and no longer. The results of these studies were applied to clinical trials and cross circulation with the same donor was not carried out for more than 4 days. But in a case reported by Burnell in 1973 the death of a donor resulted from anaphylactic reaction within minutes of starting cross circulation. This was the fourth donor for the patient and the occurence of anaphylactic reaction was presumably due to antigens shared with previous cross circulation donors.

In the early sixties it was proposed that only neurologically dead or fatally ill patients should be used as donors. But this raises social and ethical problems. These mainly centre on the meaning of clinical versus biological death. Therefore, cross circulation with baboon has been proposed. Such a procedure was reported by Saunders in a single patient[90]. In this report there was no immediate or subsequent evidence of harm to the patient. The authors concluded that cross circulation between man and baboon is a safe procedure. However, in a recent report of cross circulation between man and baboon from Germany, the procedure has been shown to be ineffective in the treatment of Fulminant Hepatic Failure[67].

Since the results of these clinical trials are inconclusive, many investigators have given up clinical trials until more experimental work is done in animal models to see if cross circulation increases survival in a large number of test animals when compared to controls. The animal studies conducted so far have have mostly used hepatectomized animals,which differ in the pathogenesis of fulminant hepatic failure in man[91,92]. For example, Sicot et al reported that Systemic-Systemic cross circulation was effective in improving the EEG of hepatectomised rats at a higher exchange flow rate(14.0ml body wt^{-1}), whereas Portal-Systemic cross circulation was effective even at lower exchange flow rates(6.4ml min^{-1}) [91]. (In Portal-Systemic cross circulation,systemic blood from the liver-less rat was infused into the portal vein of the normal rat). Since improvement in EEG does not mean recovery, and we are more interested in finding out whether the animal recovers and lives after the cross circulation procedure, an animal model is needed which could recover and live on a long term basis if the procedure which is being tested is effective. We therefore used glactosamine induced fulminant hepatic failure model in rats. Apart

from the above mentioned criteria, it has other features, which make it superior to other animal models of Fulminant Hepatic Failure known at the present time. This model will be described in detail later.

All the foregoing methods or support systems have generally shown discouraging results. In some cases, e.g. controlled clinical trials of exchange blood transfusion and corticosteroids in fulminant hepatic failure have shown conclusively that they have no beneficial effect on survival. In others, e.g. cross circulation and liver perfusion no controlled clinical trials have been done and investigators are uncertain and indefinite as to the efficacy of these methods in fulminant hepatic failures. Controlled clinical trials are difficult to carry out in patients with fulminant hepatic failure due to many reasons. The number of patients seen at any centre each year is small so that statistical analysis is difficult. Each centre has its own mortality rate, grading of coma and varying extent of intensive care. The prognosis in fulminant hepatic failure depends on a number of factors, e.g. etiology, age, degree of encephalopathy and presence or absence of complications. Further, the course

of disease in each patient is variable and the cause of death multifactorial. Therefore, an appropriate animal model is needed so that the efficacy of different support systems can be tested and compared under constant conditions. A large number of animals can be used in order to do statistical analysis. Requirements for an animal model of fulminant hepatic failure include :[93]

I. Reversibility : The hepatic failure produced should be potencially reversible, to enable animals to respond and recover with suitable treatment.

II. Reproducibility : The mortality should not be 100%, and the pattern of disease should be quite constant, i.e. onset of coma, duration of coma and time of death.

III. Death from liver failure : A selective lesion should be produced which gives rise to death from liver failure, after a suitable time interval which is sufficiently long to allow treatment to be instituted.

IV. Minimal hazard to personnel.

The available animal models of fulminant hepatic failure are :

I. Surgically Induced :

a. Anhepatic Model : They are irreversible and the absence

of damaged liver cells makes them fundamentally different from human fulminant hepatic failure[94].

b. Devascularization : Complete hepatic devascularization produced non-reversible model which lacks reproducibility because survival rates vary enormously[95,96]. Temporary devascularization has been studied in non-anesthetised dogs using balloon occluders on the portal vein and hepatic artery[97]. This gives rise to hepatic dysfunction and death within 12-18 hours. This is a better model of fulminant hepatic failure as compared to the other two mentioned above, but surgical skill is required and not enough time is available to grade coma and apply treatment.

II. Drug Induced Models :

a. Carbon Tetrachloride : It affects not only the liver but also kidney and the lungs. The response is variable and it is dangerous for the staff [93].

b. Paracetamol : The mean survival time is very variable and reproducibility unsatisfactory [93].

c. Dimethylnitrosamine : The model is irreversible [98].

d. Galactosamine : This model was originally used in basic pathological studies [99,100]. It has been developed as a model for treatment of fulminant hepatic

failure[101,102,103], and was shown to be reversible, reproducible and death occured from hepatic failure. This is the model used in this study and will be described in detail later.

III. Others : Suitable animal models have not been produced by using fungi, viruses and irradiation.

Galactosamine Induced Fulminant Hepatic Failure in Rats

The amino sugar, galactosamine, when injected intraperitoneally in the rats is taken up by the liver very rapidly. In 3 hours about 20% of the given dose can be recovered from the liver[100]. In the liver the metabolism of galactosame follows in its first steps the pathway of D-galactose and enters the pathway of D-glucosamine. Galactosamine-I-P04 and later UDP galactosamine is formed. UDP Galactosamine is converted to UDP glucosamine. The compound does not serve as a uridylate donor in the uridylyl transferase reaction as does UDP glucose which is generated in galactose metabolism. Therefore, UDP hexosamine acts as a trap mechanism for uridylate, thus interfering with cellular metabolism and causing cellular injury [99,100].

Salient Features of Galactosamine Induced Fulminant Hepatic Failure in Rats.

Galactosamine liver injury has many features in common with human viral hepatitis as judged by histology and biochemistry [99,100,101,103]. Histologically, the hepatic lesion resembles acute viral hepatitis in man. There is intensive inflammatory reaction of periportal areas, proliferation of cholangioles, appearance of uni- and multi-cellular necrosis and Councilman bodies, and lack of fatally infilteration. This reaction is at its' peak at 48 hours after galactosamine injection, when maximal damage to the liver has occured [101,103].

The serum enzymes increase rapidly and reach a peak by 48 hours in the case of SGOT and 72 hours in the case of LDH. Serum alkaline phosphatase does not change significantly [101,103].

The prothrombin time also increases rapidly after galactosamine injection reaching more than 100 seconds after 48 hours, and maintained at this level when followed upto 72 hours. This correlates with a bleeding tendency in the animals [101].

The serum bilirubin increases steadily after

24 hours of galactosamine injection reaching a maximum level at 48 hours. The blood glucose falls steadily so that by 72 hours it is extremly low.

Typical Course of Galactosamine Induced Fulminant Hepatic Failure in Rats.

When 1.1 gm/Kgm of galactosamine was injected intraperitoneally in rats, the course observed was as follows[101,102]. At 48 hours most of the rats were in coma (grade II & III) and histological and biochemical data indicated maximal hepatic damage. By 66 hours majority of rats were either in grade III or had died. Once the animal was in grade III or IV, death occured within a very short period of time ($\frac{1}{2}$-1 hour). Thus most of the animals that died did so at 3±0.5 days. Those that lived this period, survived[101]. When an appropriate dose was given (in this case it was 1.1 gm/kgm) about 30% of the rats lived.

Thus the rat model of galactosamine induced fulminant hepatic failiure shows reversibility, reproducibility, and death occurs from hepatic failure. No other organ is primarily affected except the liver. The histological and biochemical features resembles viral

hepatitis in man. Coma can be graded and enough time is available from the onset of coma to death to institute treatment. Treatment can also be instituted in different grades of coma. No surgery is required to induce the liver failure, and the procedure is not hazardous to personnel. Thus at the present time this model seems to be the most suitable one since it complies to all the criteria mentioned above.

This model has been used successfully in this laboratory for charcoal hemoperfusion studies. In these series of experiments not only the recovery rate of fulminant hepatic failure rats treated by charcoal hemoperfusion was compared to matched controls, but also the effects of hemoperfusion rate and time of initiation of hemoperfusion on the survival of fulminant hepatic failure rats was studied. In the first set of experiments, 21 fulminant hepatic failure rats in grade II coma were treated with one hour hemoperfusion and compared with an untreated group of 23 rats. 71.4% of the treated rats and 30.4% of the untreated ones recovered. Statistical analysis showed a significant increase in recovery for the treated group [101].

In subsequent studies the effect of blood flow rate and time of initiation of hemoperfusion after the onset of fulminant hepatic failure on recovery rate was studied[102]. The effect of blood flow rate was studied by treating 2 groups of animals in grade II coma (48 hours after glactosamine injection, at 1ml/min and 0.5ml/min) and their recovery rates compared to matched controls. The recovery rate for the first group was 71.4% for treated and 30.4% for the controls, the difference being highly significant. However, with a floe rate of 0.5ml/min the recovery rate was 24.2% for treated group and 16.7% for the control; this difference is not significant.

When hemoperfusion was carried out in rats with grade III hepatic coma (66 hours after the galactosamine injection), the recovery rate dropped to 0%, i.e. none of the 14 rats so treated survived. Thus the time of initiation of hemoperfusion has a bearing on the outcome.

It was noted that at 66 hours there is a severe coagulopathy related to bleeding in the rats. In order to rule out the possibility that death of the animals at 66 hours may be due to severe bleeding, exchange transfusion was done before hemoperfusion. In animals with grade II

coma the survival and recovery was significantly increased. For those in grade III coma the survival and recovery was still not significant when compared to the control group.

METHODS

ANIMALS :

Male Wistar rats weighing 270-299 gms were used, the age range being between 47 and 67 days. Standard Purina rat chow and water ad libidum was given to all rats. After glactosamine injection, they were allowed to drink 10% glucose solution instead of plain water, but the diet was the same as before.

GLACTOSAMINE SOLUTION :

D(+) glactosamine hydrochloride (Sigma Chemicals Co.) was prepared just prior to use in the form of 100 mgm/ml sterile normal saline. It was then adjusted to physiological pH with 1N sodium hydroxide solution. The animals were given glactosamine injection intraperitoneally, the dose being 1.1 gm/kgm in groups A,B and C. In group D the dose was reduced to 0.8 gm/kgm.

GRADES OF HEPATIC COMA :

The grading of hepatic coma as used in the rats in this study is as follows :

GRADE I Hepatic Coma : The animal is sedated, but awake.

GRADE II Hepatic Coma : The animal is drowsy.

GRADE III Hepatic Coma : The animal sleeps nearly all the time, but can be aroused.

GRADE IV Hepatic Coma : The animal is comotose, and cannot be aroused.

These grades of hepatic coma have been arbitrarily chosen and do not necess arily correspond to similar grades of coma in man.

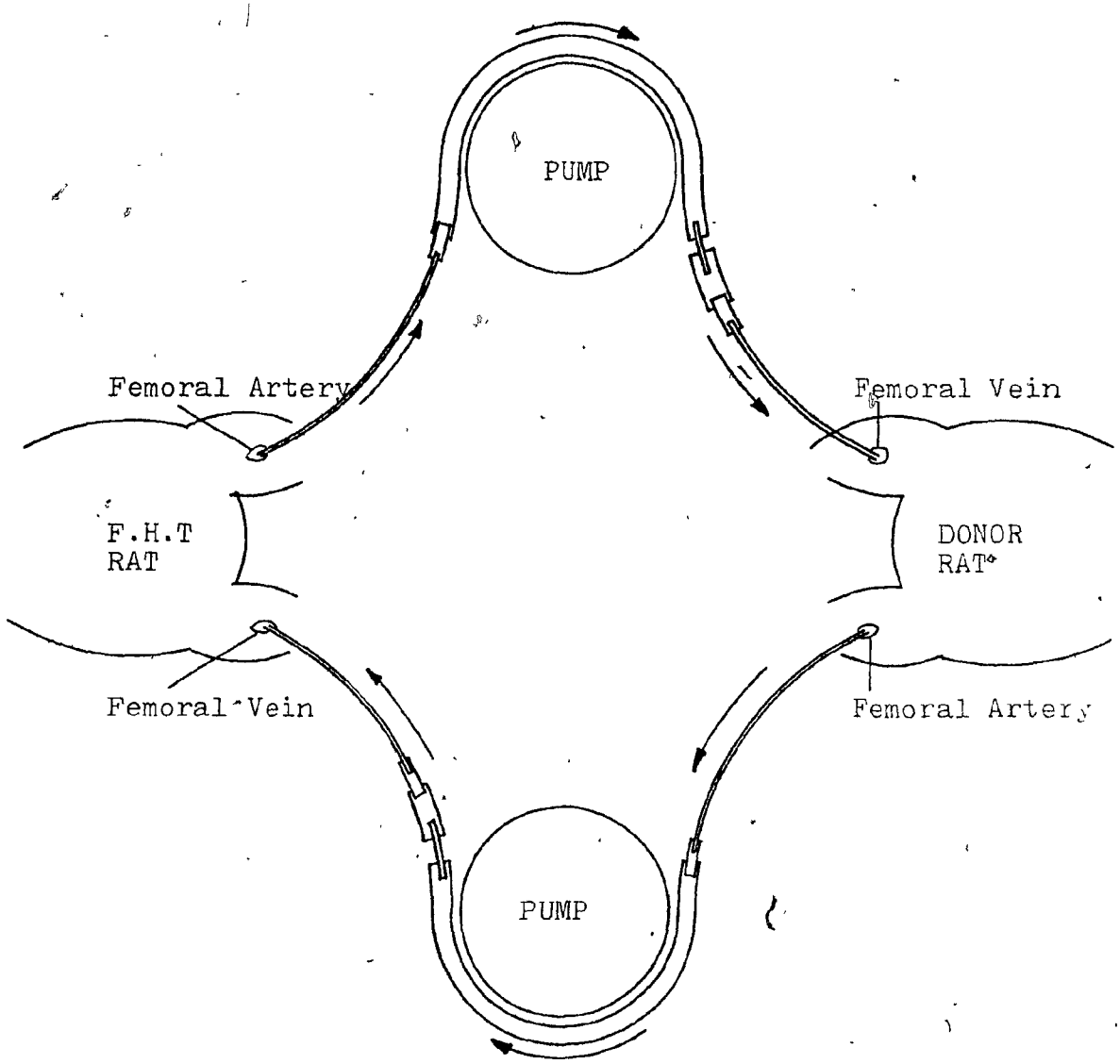
THE CROSS CIRCULATION PROCEDURE :

48 hours after the galactosamine injection, two rats in grade II coma were selected. They were again weighed. It was noted that the rats usually lost 15-20gm in 48 hours. Those rats that did not loose weight were not as sick as the others and almost always survived. So the rats that did not loose weight were not included in the study. One of the rats was picked up at random (usually by flipping a coin) to be treated by cross circulation. (This group will be designated as the treated group or the fulminant hepatic failure rats (F.H.F. rats)). The other was used as a control. Both were given nembutal in a dose of 40 gm/kgm intraperitoneally.

In the F.H.F. rats (the treated group), cannulation of the femoral artery and vein was next carried out.

DIAGRAM 1

THE CROSS CIRCULATION PROCEDURE (GROUPS A & B)



For this, the vessels were exposed, the tissues around them cleared and the artery and vein separated by fine curved forceps. Threads were put under either end of the exposed vessels, and using a small stereoscopic dissecting microscope a small cut was made in the vessel. Using a polyethylene tubing (internal diameter 0.011 inch) the vessel was cannulated. This tubing was connected to a wider tubing (internal diameter 0.34 inch) through an intermediate portion of tubing (internal diameter 0.23). In the arterial cannula (the afferent line) the widest polyethylene portion was long so as to circuit around the Minipuls Gilson pump. The same portion of the venous cannula(the efferent line) was small since it would be connected to the afferent line or the arterial cannula of the donor (healthy rat) rat on the other side of the pump. (Diagram No. 1)

All the lines were thoroughly flushed with saline before the cannulations and then primed with heparin, 10 I.U/ml . After the cannulations were completed heparin was given in a dose of 1.333 ml/kg intravenously. 1 ml of a solution made up of 10gm/100ml glucose and 12.5 gm/100ml albumin in normal saline was also given intra-

venously. This solution was given because blood glucose and albumin are very low in the sick rats at 48 hours after galactosamine injection. Also some blood is lost during cannulation, especially from the artery and the volume is thus replaced. Thirdly, it invariably resulted in a better blood flow. The reason may be that since the level of plasma protein was low, fluid shifted to the interstitial space. With the replenishment of some of the protein, this fluid would return to the vascular compartment.

To the donor rat (the healthy cross circulation partner) nembutal was given in a dose of 78 mgm/kgm. The femoral artery and vein were cannulated using the same afferent and efferent tubings as described above for the F.H.F. rat. 1000 I.U. of heparin were given intravenously after completion of the cannulations. A 7gm/100ml solution of Albumin was used to replace any blood losses in the donor rat.

After completion of the cannulations in the F.H.F. and donor rats, cross circulation was carried out between the two. It was done by connecting the afferent line(arterial cannula) of the F.H.F. rat to the efferent

line (the venous cannula) of the donor rat, and vice versa. The connections were made by an 18 gauge needle. One of the pair was placed on a scale to ensure that no changes in the blood volume of either of the rat occur. Each cross circulation procedure was carried out for one hour. Two exchange flow rates were used in two sets of experiments, i.e. 0.5ml/min in Group A and 1.0ml/min in Group B.

After the cross circulation was over, the two rats were disconnected. The blood in its' lines was returned to the F.H.F. rat. Protamine sulphate was given in a dose of 1.5gm/kg intravenously. When all bleeding in the wound stopped, it was closed by metallic clips.

In the control group, all the steps were done in the same sequence as for the F.H.F. rat except no cross circulation was carried out. Thus the femoral artery and vein were exposed. The femoral vein was cannulated. Hapa rin and glucose-albumin solution were given in doses mentioned above. Protamine sulphate was given in the end. The wound was closed by metallic clips after all bleeding had stopped.

The F.H.F. and control rats were closely observed. For the rats that died, the time of death was

noted and an autopsy performed. Rats that survived were observed for one month, and then sacrificed. "Recovery" is defined, in this study, as those animals which continued to live when followed for one month from the time of the galactosamine injection.

IN SITU LIVER PERFUSION :

In-situ liver perfusion was done in 2 sets of experiments : (1) In Grade II hepatic coma (Group IIA)

(2) In Grade III hepatic coma (Group IIB)

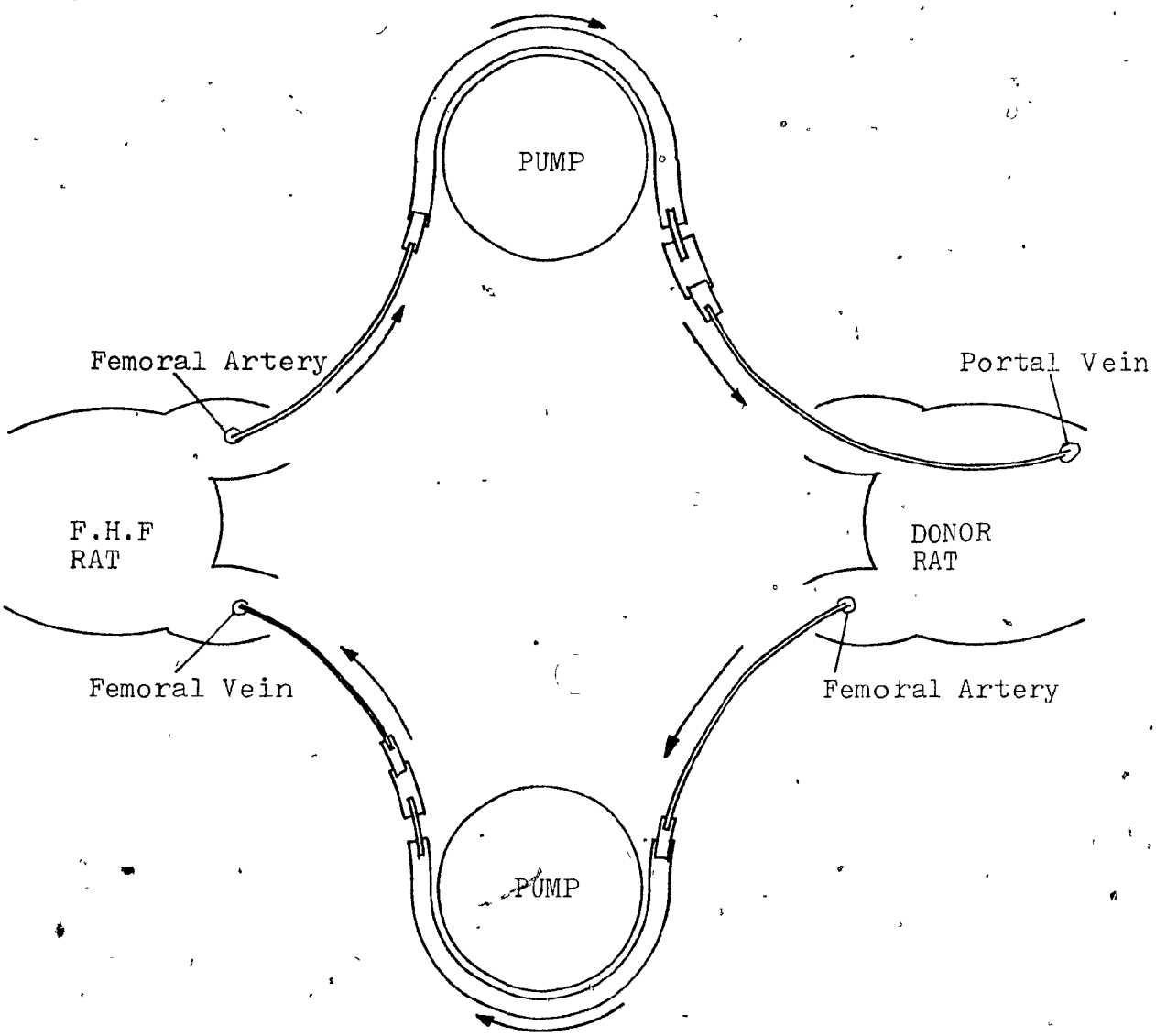
GRADE II Hepatic Coma :

The procedure is essentially similar to the one described above for cross circulation except that in the donor rat for liver perfusion, the portal vein was cannulated instead of the femoral vein. The F.H.F. rat was treated in exactly the same way as for cross circulation.

For cannulation of the portal vein in the donor rat, an upper abdominal incision was used. The intestines were carefully displaced from the right upper abdominal quadrant to expose the portal vein. The edges of the abdominal wound were cauterized to prevent bleeding from these after hepranization. The portal vein was cannulated

DIAGRAM 2

IN-SITU LIVER PERFUSION IN DRADE I HEPATIC COMA (GROUP C)



using the efferent (venous) cannula described above. The wound was kept moist by frequently putting saline at 37°C on it, and heat loss was prevented by covering the wound with a gauze and shinning an ordinary lamp on it. Since some blood loss was anticipated from the abdominal surgery during the liver perfusion procedure, the femoral vein was also cannulated to administer 7gm of albumin solution whenever required. In most experiments, an initial 1-2mls was all that was required. The donor rat was given 1000 I.U. heparin intravenously. In-situ liver perfusion was carried out by connecting the arterial cannula of the F.H.F. rat to the portal venous cannula of the donor rat, and the arterial cannula of the donor rat to the femoral venous cannula of the F.H.F. rat(Diagram No.2). During the in-situ liver perfusion procedure, one of the rats was placed on a scale.

The control rats were cannulated in the same manner as the F.H.F. rat, and treated in same way except that the liver perfusion was not carried out. The average exchange flow rate obtained in this group of experiments was 0.8ml/min. Each procedure was done for 1 hour. As before, the rats were carefully observed after the ex-

cannulated and cannulas passed into the Inferior Vena Cava. The polyethylene tubing was joined to a Silastic tubing and this end was brought out on the back of the neck, subcutaneously. Silastic tubing was used in this part of the cannula because it can be effectively occluded by a strong thread, whereas polyethylene tubing can not be occluded. Thus blood oozing was prevented from the cannula. By sucking at the silastic end of the cannula by a syring, it was made sure that a good blood flow could be obtained. If a good blood flow was not obtained, the position of the cannula in the leg was readjusted. Thus a approximate position for the cannula was obtained and it was glued in this position in the leg and on the back of the neck by crazy glue. The glue also prevented bleeding from these two sites. The leg wounds were closed by metallic clips after all bleeding had stopped. 0.5ml of saline containing 25I.U. of haparin were injected in each tubing on the day of the cannulation. On the following day (48 hours after galactosamine injection) the tubings were flushed with saline to see if they are patent or not and to dislodge any small clots that may have formed. Then 0.5ml of saline containing 12.5 I.U. heparin was injected in each tubing. Glucose

periments. Time of death was noted and autopsies performed on those that died.

GRADE III Hepatic Coma :

Since animals in grade III coma are very sensitive to even extremely small doses of anesthetic, chronic cannulations were done to avoid its' use at the time of liver perfusion.

It was found during the preliminary trials of these experiments, that if nembutal was given a few hours before or after galactosamine injection, the rat is protected from the effects of galactosamine and liver failure does not develop. Therefore, nembutal was given and cannulations done 16 hours after galactosamine injection. It is expected that by this time all the galactosamine has been taken up by the liver, and has exerted its' effect. In these initial studies it was found that the course and development of hepatic failure as well as mortality is not affected when such a regimen is used.

To carry out chronic cannulations in the F.H.F. rats, both the femoral veins were exposed. Using polyethylene tubing(internal diameter 0.011 inch) they were

10 gm/100ml was given subcutaneously in a dose of 1ml/100gm weight of the rat.

Two rats in grade III coma (usually at 66 hours) were selected for each experiment. One was picked up at random for liver perfusion and the other used as a control.

The portal vein and femoral artery of the donor rat was cannulated as described above. The femoral vein was also cannulated for reasons already discussed. In this case, however, a much smaller dose of heparin was used, i.e., 300 I.U. Higher doses caused bleeding in the F.H.F. rats. This dose was adequate for one hour of liver perfusion and did not cause bleeding in the F.H.F. rat.

The F.H.F. rat was placed in a restrainer at the time of the experiment, and 10 I.U. of heparin and 1ml of glucose-albumin solution was injected intravenously through the cannulas on the back of the neck. One of these cannulas was then connected to the portal venous cannula of the donor rat and the other to the arterial line. The donor rat was placed on a scale during the liver perfusion. Exchange flow rate of 1 ml/min was employed. The procedure was done for 1 hour.

The control rat was placed in a restrainer and

through the cannulas on the back of the neck, 10 I.U. heparin and 1ml of albumin-glucose solution was given. The control and F.H.F. rats were followed, as described above.

A number of difficulties were encountered while treating grade III hepatic failure rats. The foremost was due to the fact that animals in grade III hepatic failure are very sensitive to even extremely small doses of anesthetic. So cannulation could not be done immediately before the treatment, as done in the other three groups. Therefore, chronic cannulations had to be done to avoid the use of anesthetic in rats in grade III coma. Chronic cannulations could be carried out either before or after the galactosamine injection. In the initial attempt nembutal was given and cannulation carried out first to a group of rats and later galactosamine was given. In these rats fulminant hepatic failure did not develop and all the rats survived. This was due to the protective effect of nembutal against the action of galactosamine on the liver. Subsequent experiments revealed that if nembutal was given 16 hours after the galactosamine injection, it did not interfere with the actions of galactosamine, which has by this time exerted its full effect on the liver. The time

of onset of coma, the transition from one grade to another and the mortality rate was identical to previously set norms for this model. So this regimen was used for these experiments. As for the chronic cannulations, a number of approaches were tried before a satisfactory method was obtained. The aims for the chronic cannulation were that:

1. The cannulas should not clot for 2-3 days.
2. A minimal amount of heparin should be required, otherwise the animal bleed a lot when in grade III coma.
3. The surgery should be least traumatic, and quick and easy to carry out.
4. The animal should not be able to reach the exteriorized cannulas with its' mouth or hands in order to rupture or pull them out.

The different approaches used are as follows :

I. Use of Silastic tubing (0.3mm x 0.64mm) for cannulation of femoral artery and vein : Silastic tubing was selected in order to minimize clotting and heparin needs. It was found however that cannulation was difficult due to the soft nature of the Silastic tubing and sometimes occlusion or twisting of the tubing occured due to the same reason. Another problem was exteriorizing the tube in

a position where the rat could not reach it. In these experiments they were left in the leg, which obviously did not work.

II. Cannulation of Carolid Artery and Juglar Vein :

Since the back of the neck of the rat is a place where the rat can not reach for the cannulas, these vessels were selected. Silastic tubing was used. Problems related to clotting of the arterial cannula despite moderate doses of heparin led to discontinuation of this method. The surgery was also more traumatic than the femoral cannulations.

III. Circulating Arterio-Venous Shunt : To overcome the problem of clotting in the arterial cannula, a circulating arterio-venous shunt was designed. The connection between Silastic tubings (0.02 x 0.037) in the carotid artery and juglar veins was made of PVC (0.01 x 0.03"). In another case PVC was used in the vein and Silastic in the artery and the two were connected. In still another one, a single piece of Silastic was used to cannulate both the artery and vein, thus eliminating the connections between tubing which could favour clotting. In this last case heparin was given subsequently.

In all these cases, a good flow did not ensue,

and clotting occured on the first or second day. Since silastic tubing in the artery may be partially occluded, causing the failure of this loop, another kind of circulating arterio-venous loop was tried. In this case Polyethylene (PE 50) tubing was used to cannulate the Carotid artery and Juglar vein while the connection was made of Silastic (0.025 x 0.047"). Unfortunately this approach also did not work as most of these cannulas clotted on the 2nd day. In this case the use of Polyethylene tubing could have caused the clotting, so Teflon was used instead of Polyethylene, but the results did not improve.

IV. TDMAC/Heparin Coating of Silastic : To prevent clotting and the use of intravenous heparin, Silastic tubing were coated on the inside by TDMAC/ Heparin complex. The tubes were first heparinized and then cross linked with glutaraldehyde on the next day. The tubes were prepared freshly for each set of experiments. At first a 4% TDMAC solution was used. This proved to be ineffective so that a 7% solution was employed. No subcutaneous or intravenous heparin was used. Initially, in a few experiments clotting was less evident, but in a large series of experiments

the results did not improve.

V. Inferior Vena Cava Cannulations: The failure of the above mentioned methods led us to cannulate the Inferior Vena Cava via the femoral veins, thus eliminating the Cannulation of an artery. In this case the problem was of locating the exteriorized cannula in such a way that the rat could not pull them. Initially, the cannulas were left in the legs, an adhesive tape keeping them in position. Plastic collars were put around the neck of the rat to prevent flexion of the neck. Later a plastic jacket was used to immoblize the back. The rats were somehow able to pull out the cannulas from the legs in both these cases.

Finally, these cannulas were brought out subcutaneously on the back of the neck, an area where the rats cannot reach to pull out the cannulas. Small doses of heparin were used daily, and the cannulas did not clot untill the third day in a majority of experiments done. So this method was used for the in-situ liver perfusion experiments. in grade III hepatic coma (Group IIB). Polyethylene 10 tubing was used to cannulate the Inferion Vena Cava and Silastic was connected to PE10 and brought out on the back of the neck, where it could effectively be occluded by a thread.

RESULTS

Results will be discussed under the following headings :

I .Cross Circulation

A. Exchange flow rate 0.5 ml/min

B. Exchange flow rate 1.0 ml/min

II.In-Situ Liver Perfusion

A. In Grade II Fulminant hepatic failure rats.

B. In Grade III Fulminant hepatic failure rats.

In all these groups of experiments fulminant hepatic failure rats were treated by either cross circulation or in-situ perfusion and their recovery rate and survival time was compared to matched controls.Before describing the results further, a definition of the terms recovery rate, survival time and matched control is warranted.

Recovery rate : Male Wistar rats 47-67 days old and weighing 250-299 gms, when given an appropriate dose of galactosamine intraperitoneally (which in the case of groups A, B, and C was 1.1 gm/kgm and group D 0.8 gm/kgm) develop maximal hepatic damage at 48 hours and die from

fulminant hepatic failure at 3.5 ± 0.5 days. Those that survive this period live. Recovery rate in this study implies that the fulminant hepatic failure rats continue to live for one month after the galactosamine injection. The rats were observed for this period of time after which they were sacrificed.This arbitrary period of one month is chosen because by this time the rats have completely recovered from F.H.F and continue to live if followed longer.

Survival Time : Rats with galactosamine-induced fulminant hepatic failure and without any treatment usually die at 3.5 ± 0.5 days after galactosamine injection. However, with treatment this survival time can be significantly prolonged.

Random Matched Control : Matched control refers to a rat with fulminant hepatic failure, used as a control for an experiment. The word matched emphasizes the fact that the control rat belongs to the same batch, age and weight group, and grade of coma as the fulminant hepatic coma rat for which it is acting as a control. The matched control and the treated rats were randomly matched on a daily basis to avoid discrepencies arising between rats of different batches. The matched control was treated in the same way

Table 1

EFFECTS OF CROSS CIRCULATION ON THE SURVIVAL OF GRADE II COMA CONTROL AND TREATED GROUPS(DAYS AFTER GALACTOSAMINE INJECTION)

	GROUP A Exchange flow rate = 0.5ml/min		GROUP B Exchange flow rate = 1.0 ml/min	
	CONTROL	TREATED	CONTROL	TREATED
1	3.0 days	survived	survived	4.0 days
2	2.5 days	4.5 days	2.5 days	survived
3	2.5 days	2.5 days	3.0 days	2.5 days
4	survived	3.0 days	2.5 days	4.0 days
5	2.5 days	2.5 days	2.5 days	2.5 days
6	survived	survived	survived	survived
7	survived	3.1 days	3.0 days	2.5 days
8	2.5 days	2.5 days	survived	survived
9	3.3 days	2.5 days	4.0 days	survived
10	survived	survived	2.5 days	4.0 days
11	2.5 days	3.0 days	3.0 days	survived
12	3.0 days		survived	survived
13	survived	-	2.5 days	-
14	2.5 days	-	-	-

as the fulminant hepatic rat except that the specific treatment (cross circulation or in-situ liver perfusion) was not carried out. Both the recovery rate and the survival time of the treated and matched control rats were compared.

I. Cross Circulation :

A. Group IA : In group IA cross circulation between a fulminant hepatic failure rat and a donor rat was carried out at an exchange flow rate of 0.5 ml/min for a period of one hour. Eleven sets of experiments were done. In each set fulminant hepatic failure rats in grade II coma (48 hours after galactosamine injection) were treated by cross-circulation with a normal donor rat at rate and period of time specified above, and the recovery rate and survival time was compared to matched controls. The recovery rate in the treated group was 36.36% (3 rats out of 11 survived). In the matched control group the recovery rate was 35.71% (5 out of 14 rats survived). Thus there was no significant increase in recovery rate in the treated group when compared to the matched control group (Table II). The average survival time in the treated group was 2.95 days as compared to 2.70 days in the matched control group

Table 2

RECOVERY RATE AFTER CROSS CIRCULATION IN CONTROL AND TREATED GROUPS

Group	Degree of Coma	Exchange Flow Rate ml/min	Control		Treated		Significance Chi Square
			Total	Survival %	Total	Survival %	
A	II	0.5	14	35.71%	11	27.27%	N.S.
B	II	1.0	13	30.70%	12	50.00%	N.S.

N.S. = Not significant

(Table I &III). The increase in survival time in the treated group is not significant when compared to the matched control group. Thus the increase in recovery rate or survival time in the treated group is not significant as compared to the matched control group when grade II F.H.F. rats were treated by cross circulation at an exchange flow rate of 0.5 ml/min.

B. Group IB : In group IB cross circulation was carried out for 1 hour at a flow rate of 1.0 ml/min. Grade II fulminant hepatic failure rats (48 hours after galactosamine injection) were treated by cross circulation with a normal donor rat. 12 set of experiments were done. The recovery rate in the treated group was 50% (6 out of 12 rats survived). In the matched control group the recovery rate was 30.70% (5 out of 13 rats survived). Statistical analysis showed that the increase in recovery rate in the treated group is not significant when compared to the matched control group (Table II). Similarly, the increase in survival time (3.25 days in the treated and 2.83 days in the controls) was not significantly increased in the treated group (Tables I & II). Thus cross circulation did not significantly increase the survival time or recovery rate.

Table 3

COMPARISON OF SURVIVAL TIME (IN RATS THAT DIED) AFTER CROSS CIRCULATION(DAYS AFTER GALACTOSAMINE INJECTION)

Group	Degree of Coma	Exchange Flow Rate ml/min	Average Survival Time		Significance Chi Square
			Control	Treated	
A	II	0.5	2.70 days	2.95 days	N.S. ($P > 0.3$)
B	II	1.0	2.83 days	3.25 days	N.S. ($P > 0.1$)

N.S. = Not significant

In both these groups of experiments there were no major technical problems or problems with bleeding. The mortality rate (65% and 70% respectively in groups IA and IB) in the untreated rats or control rats, and the time of death (2.70 days and 2.83 days for Group IA & IB respectively) was within the normal limits for this F.H.F rat model as tested and reported previously in hemo-perfusion studies done in this laboratory [101,102,103].

II. In-Situ Liver Perfusion :

Group IIA - In Grade II Fulminant Hepatic Failure Rats :

In group IIA in-situ liver perfusion was carried out at 0.8 ml/min for 1 hour between a grade II fulminant hepatic failure rat (48 hours after galactosamine injection) and a normal rat. 11 sets of experiments were done. The recovery rate in the treated group was 54.5% (6 out of 11 survived) as compared to 0% (none of the control rats survived) in the matched control group. The difference in the recovery rate between the two groups was significant (Table V). The average survival time in the treated group was 3.9 days as compared to 2.5 days in the matched control group(Table IV & VI). This increase

Table 4

SURVIVAL OF TREATED AND CONTROL RATS AFTER IN SITU LIVER PERFUSION(DAYS AFTER GALACTOSAMINE INJECTION)

	GROUP C In situ liver perfusion Grade II Hepatic Coma		GROUP D In situ liver perfusion Grade III Hepatic Coma	
	Control	Treated	Control	Treated
1	2.5 days	survived	3.3 days	3.3 days
2	2.5 days	survived	3.3 days	3.3 days
3	3.0 days	2.5 days	3.3 days	survived
4	2.5 days	survived	3.0 days	3.3 days
5	2.5 days	survived	3.0 days	survived
6	2.5 days	survived	3.3 days	3.3 days
7	2.0 days	2.5 days	3.3 days	3.0 days
8	2.5 days	survived	3.3 days	3.3 days
9	2.5 days	4.5 days	3.0 days	3.3 days
10	2.5 days	4.5 days	3.0 days	4.3 days
11	2.5 days	5.5 days	3.3 days	3.3 days
12	-	-	3.3 days	3.3 days

in survival time was also significant (Student 't' test <0.05). Thus in-situ liver perfusion in grade II fulminant hepatic failure rats significantly increased the recovery rate and survival time as compared to the controls.

The exchange flow rate used in these experiments was 0.8 ml/min. This was due to technical difficulties in obtaining an exchange flow rate of 1.0 ml/min as done in all other groups of experiments. However, the results are still significant with the lower exchange flow rate.

Another point which requires attention in this set of experiments is that the mortality rate in the control or untreated group is 100% which is unusual for this model because about 30% animals survive when untreated. This discrepency was ascribed to higher sensitivity of this batch of rats to galactosamine. Therefore a study was undertaken to determine whether the sesitivity of the rats had in fact changed, and if so to find out a new dose of galactosamine which would give 30% survival in the untreated rats. 10 rats were given galactosamine in a dose of 1.1 gm/kgm, and they all died by the third day. Decreasing doses were given to batches of ten rats. At a dose of 0.8 gm/kgm a general mortality rate of 30% was

Table 5

RECOVERY RATE IN CONTROL AND TREATED GROUPS AFTER IN SITU LIVER PERFUSION

GROUP	Grade of Coma	Exchange Flow Rate ml/min	Control		Treated		Significance Chi Squar
			Total	% Survival	Total	% Survival	
C	II	0.8	11	0%	11	54.5%	S
D	III	1.0	12	0%	12	16.7%	N.S.

S = Significant

N.S. = Not significant

obtained. In the subsequent experiments (Group IIB) in which this dose was employed a similar 30% survival rate was obtained. This applies only to general survival rate of all the animals because those rats which have progressed to grade III coma, all died. The 30% survival rate obtained for the general survival rate was due to the rats which either did not become comotose or which lapsed into grade II coma and recovered without progressing to grade III. Therefore, the mortality rate of grade III coma would be expected to be 100% (Table V).

In-Situ Liver Perfusion :

Group IIB - In Grade III Fulminant Hepatic Failure Rats :

In this group 12 fulminant hepatic failure rats in grade III hepatic coma (66 hours after galactosamine injection)were treated by in-situ liver perfusion for a period of 1 hour at flow rate of 1 ml/min. Two out of 12 rats recovered in the treated group (16.6%),and to 0% in the control group(none of the 12 rats recovered). The difference is not significant statistically (Table V). The average survival time was 3.37 days in the treated group and 3.2 days in the control group (Table VI) which is also not significant. Thus the increase in recovery rate and survival

Table 6

SURVIVAL TIME OF TREATED AND CONTROL RATS(THOSE THAT DIED) AFTER IN SITU LIVER PERFUSION(DAYS AFTER GALACTOSAMINE INJECTION)

Group	Degree of Coma	Exchange Flow Rate ml/min	Average Survival Time		Significance Student's t-test
			Control	Treated	
C	II	0.8	2.5 days	3.90 days	$P < 0.05$
D	III	1.0	3.2 days	3.37 days	N.S.

N.S. = Not significant

time in animals with grade III hepatic coma when treated by in-situ liver perfusion is not significant when compared to matched controls.

D I S C U S S I O N

The pathogenesis of hepatic coma in fulminant hepatic failure is not well understood. Many factors seem to be involved. Accumulation of toxic metabolites especially nitrogenous waste products, fatty acids, mercaptans, false neurotransmitters and amino acid imbalance have been incriminated.[104] Another possibility, not much investigated is the absence of a substance or substances produced by the liver and vital for the normal brain function. A knowledge of the pathogenesis of hepatic coma is essential to formulate rational therapy. However, the survival in a patient with hepatic coma due to fulminant hepatic failure depends on many factors, for example, the brain abnormality should be reversible and the liver should be able to regenerate. Obviously, a patient will not survive if the liver, for some reason cannot regenerate, or if the brain dysfunction has become irreversible. But unfortunately, at the present time, the factors regulating hepatic regeneration are not well known, and the transition of reversible brain dysfunction to the irreversible state cannot be judged in hepatic coma.

Cross circulation and isolated liver perfusion have been used clinically to a limited extent as hepatic assists. Controlled clinical trials have never been done and their efficacy in hepatic coma remained unclear. The rationale for their use is that the liver of the normal

donor in cross circulation and the isolated liver in liver perfusion will remove toxic metabolites and supply deficient substances to the sick partner.

The cross circulation and in-situ liver perfusion procedures done in the present study are expected to work on the same principles mentioned above, i.e., removal of toxin and provision of essential but deficient substances by the healthy rat's liver to the sick rat.

The essential difference between the cross circulation and in-situ perfusion is that in in-situ liver perfusion more efficient detoxification of the blood can take place as whole of the sick partner's blood passes initially through the liver. In cross circulation effective removal of the toxin may not occur since blood circulates through other tissues of the body before reaching the liver. This would be particularly true if the hypothetical 'toxins' have high affinity for the tissues. As far as supply of essential substances is concerned, there should be no difference between the two procedures, as whatever is produced by the liver will be present equally throughout the vascular compartment.

The results of the cross circulation experiments show that it is not effective in increasing survival time or recovery rate in fulminant hepatic failure at lower or higher exchange flow rates. Two exchange flow rates were used in the cross circulation experiments because it has been postulated that the failure of cross circulation in patients may be due to lower flow rates used in these trials.[91]

The exchange flow rates in most clinical trials have been 50-200 ml/min. According to these investigators lower exchange flow rate would not be able to remove the toxin effectively if the toxin turn-over rate is high.

The maximum exchange flow rate that can be obtained in a patient is 200-300 ml/min. In our experiments, we used 2 exchange flow rates in 2 sets of experiments. The flow rate in group A was 0.5 ml/min (equivalent to 125 ml/min in a 70 kgm man) and in group B, 1.0 ml/min (equivalent to 250 ml/min in a 70 kgm man). In both the groups the difference in recovery is not significant between the treated and control groups. Thus cross circulation is not effective within the practical limits of blood exchange flow rates. But, in-situ liver perfusion in grade I hepatic coma at an exchange flow rate of 0.8 ml/min gave significant results in the treated group when compared to the controls. Also, charcoal hemoperfusion, carried out in this laboratory at 1 ml/min in the same animal model in similarly controlled trials resulted in a recovery rate of 71.4% in the treated group as compared to 30.4% in the matched control group.[101] The difference in recovery between the two groups is highly significant. However, when an exchange flow rate of 0.5 ml/min was employed during the hemoperfusion procedure, the recovery rate in the treated group dropped to 24.2% as compared to 16.7% in the control group, the difference being insignificant.[102] Thus, the rate of exchange flow has important implications

on the outcome and adequate exchange flow rate should be employed in any of these procedures. However, the exchange flow rates effective in charcoal hemoperfusion and in-situ liver perfusion are not effective in cross circulation. Higher exchange flow rates are advocated for cross circulation but they are not practicable. In a recent review, exchange flow rates for cross circulation likely to be effective as inferred from experimental work are of the order of 800 ml/min, which are clearly not obtainable.[6]

The effectiveness of in-situ liver perfusion as compared to cross circulation may indicate that toxin removal may be more important than the replacement of some factor in the treatment of fulminant hepatic failure. This concept is supported by the results of the charcoal hemoperfusion studies done in this laboratory and mentioned above. The exact nature of the toxin involved in the genesis of hepatic coma in fulminant hepatic failure is not known. Disappointing results obtained by hemodialysis using the conventional membrane suggest that they may be non-dialyzable.[26] Since this type of membrane has a high clearance for small molecules but a very poor clearance for middle molecules, the toxins may belong to the later group. This concept is supported by the fact that charcoal hemoperfusion in patients with grade IV hepatic coma due to fulminant hepatic failure has resulted in the recovery of consciousness of a high percentage of

patients.[33,34,35,37] This has been suggested to be due to the higher clearance of middle molecules in the charcoal hemoperfusion. The subsequent use of polyacrylonitrile membrane (PAN) for the treatment of fulminant hepatic failure strengthened this proposal.[27] This membrane has a high clearance for the longer molecules, and its use resulted in a significant increase in the recovery of consciousness in animals as well as in clinical trials.[31]

The inherent ability of the liver to regenerate plays a vital role in the final outcome of the syndrome. It has been shown that plasma from patients with hepatic coma is cylotoxic to isolated liver cells in vivo, when compared with plasma from subjects without liver disease. This effect has been shown to be reduced by charcoal column hemoperfusion.[105] It is possible that the higher recovery rate obtained by liver perfusion is due to more efficient removal of the 'toxin', which in turn favours hepatic regenaration.

This study shows that liver perfusion is effective in the treatment of rats in grade II hepatic coma, but not in grade III hepatic coma. It is important to note here that the grades of coma as used in this rat model do not correspond to the same grades of coma in man. In man, survival rate is quite higher in grades II & III hepatic coma (66% and 48% respectively)and recovery is still possible in patients in grade IV hepatc coma.This is not the case in the rat model that

we used. At the dose of galactosamine being used, about 30% rats survive without any treatment. Of these, about half recover sfter going into grade I hepatic coma, whereas the other half do not become comatose at all.So spontaneous recovery is possible in grade I coma. But once the animals pass into grade II hepatic coma,their condition deteriorate more rapidly, and they almost always die. Thus, grade I hepatic coma in rats can be regarded equivalen to early stages of hepatic coma in man where chances of spontaneous recovery are present, and grade II equivalent to very late stages of hepatic coma in man, since the chances of spontaneous recovery are almost nil.

The poor results obtained in grade II hepatic coma rats, as compared to the results in grade I, maens either that liver perfusion is not effective when the abnormality becomes irreversible once the rats are in grade II coma. If the former is the case, some other more effective type of treatment may improve the survival rate in grade II hepatic coma. It is interesting to note that the liver perfusion in this experiment has been carried out under ideal conditions for this hepatic support,i.e., in the absence of immunological reaction and ischemic and anoxic injury that would be expected to occur during the isolation and preservation of the organ.

The sensitivity of the different batches of rats to galactosamine may vary. Increased sensitivity

may be the cause for zero percent survival rate seen in the control group IIA, where about 30% of the rats would expected to live. The dose of galactosamine in group IIB rats was therefore reduced and adjusted to get 30% survival in the untreated rats (this dose was 0.8 gm/kgm as opposed to 1.1 gm/kgm being used in other batches). Since control and test animals from the same batch were used for this study, and matching them was done on a daily basis, such variations are not expected to onfluence the results.

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