THE PHYSIOLOGICAL ACTIONS OF PROSTAGLANDIN E_2 ON THE LIVER AND BLOOD-BRAIN BARRIER OF GALACTOSAMINE-INDUCED FULMINANT HEPATIC FAILURE RATS

by

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To You,

For having made this a reality.

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ABSTRACT

The Physiological Actions of Prostaglandin E_2 on the Liver and Blood-Brain Barrier of Galactosamine-Induced Fulminant Hepatic Failure Rats

Basic studies were carried out on the galactosamine-induced fulminant hepatic failure (GalN-FHF) rat model. Physiological, biochemical, and histological changes were followed during progressive development of hepatic coma. Galactosamine (GalN) produced characteristic alterations in the orientation of the hepatocyte's endoplasmic reticulum (ER) such that it completely encircled the nucleus to envelop it. These cellular events led to the onset of hepatocyte karyolysis which resulted in eventual cellular necrosis. The development of brain edema was also studied in the GalN-FHF rat model. It was observed that brain edema followed a biphasic pattern in which cytotoxic brain edema preceeded a vasogenic component. Since prostaglandins have been shown to possess cytoprotective properties, the effect of PGE_2 was investigated once hepatocyte injury had already occurred. PGE_2 prevented GalN-induced hepatocyte necrosis by delaying the cellular events leading to hepatocyte karyolysis and subsequent cellular necrosis. PGE_2 also appeared to prevent the transition of cytoxic brain edema to vasogenic brain edema by preventing perivascular astroglial swelling, thus preventing the total breakdown of the blood-brain barrier.

RESUME

Action Physiologique de la Prostaglandine E_2 Sur le Foie et la Barrière Sang-Cerveau de Rats Atteints de Déficience Hépatique Aiguë Provoquée par la Galactosamine.

Nous avons effectué des études de base sur des rats atteints de déficience hépatique aiguë induite par la galactosamine (GalN-FHF). Nous avons suivi les changements intervenant au cours du développement progressif du coma hépatique, et ce, aux niveaux physiologique, biochimique, et histologique (microscopie optique et électronique, [LM/EM]). Des observations ultrastructurales en microscopie électronique révèlent que, dès le stade I du coma hépatique, la GalN provoque des modifications caractéristiques de l'orientation du réticulum endoplasmique (ER) et des mitochondries de l'hépatocyte, de telle façon qu'ils deviennent étroitement associés à la membrane nucléaire. Durant le stade II du coma hépatique, le ER va jusqu'à encercler complètement le noyau et l'envelopper. Finalement, pendant les stades III et IV du coma hépatique, ces modifications cellulaires aboutissent à la karyolyse de l'hépatocyte résultant en une éventuelle nécrose cellulaire. Le développement d'un oedème cervical étant une complication majeure de la FHF, nous l'avons étudié sur nos rats GalN-FHF par une histologie du tissu (LM/EM) ainsi que des mesures directes du contenu en eau du cerveau et de son gonflement. Nous avons ainsi observé que le développement de l'oedème cervical suit un schéma biphasique dans lequel un oedème cervical cytotoxique précède une composante vasogénique. De plus, le stade II du coma hépatique apparait comme une étape cruciale dans le développement de l'oedème cervical. Pendant les stades terminaux de la GalN-FHF, i.e. les stades III et IV du coma

hépatique, une destruction complète de la barrière sang-cerveau entraine le développement d'un oedème cervical vasogénique. Ceci a été mis en évidence grâce à des colorations au bleu de trypan. D'autre part, comme il a déjà été démontré que les prostaglandines possèdent des propriétés cytoprotectrices, nous avons étudié l'action de la PGE_2 une fois établi le processus de détérioration des hépatocytes. Après avoir injecté la PGE_2 dans la cavité intrapéritonéale d'animaux atteints de GalN-FHF et rendus au stade II du coma hépatique, nous avons déterminé son effet sur le développement de la détérioration des hépatocytes induite par la GalN. La PGE_2 empêche la progression de la détérioration des hépatocytes pendant 8h par comparaison avec des animaux ne recevant qu'une solution saline. La PGE_2 prévient aussi le développement de l'oedème cervical pendant 4h, et, 8h après l'injection, le gonflement du cerveau dû à l'oedème est redevenu normal. Ceci a pu être confirmé à la fois par l'histologie du tissu et par des mesures directes du contenu en eau du cerveau et de son gonflement. De ces études, il apparait que la PGE_2 prévient la nécrose des hépatocytes induite par la GalN en retardant les modifications cellulaires amenant la karyolyse de l'hépatocyte et la nécrose cellulaire qui en découle. La PGE_2 semble aussi prévenir la transition de l'oedème cervical cytotoxique à l'oedème cervical vasogénique en empêchant le gonflement des astrocytes perivasculaires et donc, la destruction complète de la barrière sang-cerveau.

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LIST OF ABBREVIATIONS

ACORC	Artificial Cells and Organs Research Centre
ADP	adenosine diphosphate
ALAT	alanine aminotransferase
Alk. P	alkaline phosphatase
AMP	adenosine monophosphate
ASAT	aspartate aminotransferase
ATP	adenosine triphosphate
BBB	blood-brain barrier
BUN	blood urea nitrogren
b. wt.	body weight
Ca^{++}	Calcium ions
Cl^{-}	chloride ions
CSF	cerebrospinal fluid
^{o}C	degree Celsius or centigrade
dl	deciliter
DOPA	dihydroxyphenylalanine
EM	electron microscopy
FHF	fulminant hepatic failure
FNT	false neurotransmitter
g	gram
GABA	gamma aminobutyric acid
GalN-FHF	Galactosamine-induced fulminant hepatic failure
GMP	guanosine monophosphate
GOT	glutamate-oxalacetate transaminase
GPT	glutamate-pyruvate transaminase
hr	hour

ICP	intracranial pressure
K^+	potassium ions
kg	kilogram
LDH	lactic dehydrogenase
LM	light microscopy
M	molar (moles per liter)
MDH	malate dehydrogenase
mg	milligram
min	minute
mM	millimolar
ml	milliliter
n	sample size (number of observations)
Na^+	sodium ions
NAD	nicotinamide-adenine dinucleotide
NADH	nicotinamide-adenine dinucleotide (in reduced form)
Na^+K^+ATPase	sodium-potassium ATPase
NSAID	non-steroidal anti-inflammatory drugs
PG	prostaglandin
PGE_2	prostaglandin E_2
PUFA	polyunsaturated fatty acid
P_i	inorganic phosphorous
p	probability
pp	pages
SD	standard deviation
SE	standard error
SMAC	sequential multiple autoanalyzer with computer
TX	thromboxane
vs	versus

wt	weight
>	greater than
<	less than
%	percent
μ	micro
μg	microgram
μM	micromolar

Histology

AG	astroglial cell
bv	blood vessel
E	edema; erythrocyte
ER	endoplasmic reticulum
H	hepatocyte
L	capillary lumen
M	mitochondria
N	nucleus
P	portal area of hepatic lobule
V	vacuole
μ	micrometer

PUBLICATIONS

Some of the work which is described in this thesis has been published (or is in preparation for publication) in the following journals;

1. Dixit V, Chang TMS. "Effects of prostaglandin E_2 on the survival time of fulminant hepatic failure rats," Int J Artif Organs 5:588, 1982.

2. Dixit V, Chang TMS. "Preliminary report on the effects of prostaglandin E_2 on brain edema in fulminant hepatic failure rats," Int J Artif Organs 8:55-56, 1985.

3. Dixit V, Chang TMS. "Brain edema and hepatic histopathology in galactosamine induced fulminant hepatic failure rats," Gastroenterology (in preparation for publication), 1985.

4. Dixit V, Chang TMS. "Observations on the effects of prostaglandin E_2 on liver histopathology and brain edema in a glactosamine-induced fulminant hepatic failure rat model," Gastroenterology (in preparation for publication), 1985.

We dance round in a ring and suppose,
But the Secret sits in the middle and knows.

Robert Frost, "The Secret Sits"

CHAPTER I

INTRODUCTION

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INTRODUCTION

1.1 PROSTAGLANDINS (PGs)

Just slightly more than half a century has elapsed since Kurzrok and Lieb (1930) first reported that human seminal plasma had either a contractile or relaxing effect on the human uterus, depending on fertility of the woman from whom the tissue was obtained. However, it was not until the mid 1930s that von Euler (1935) and Goldblatt (1933, 1935) independently demonstrated the presence of a vasodepressor agent and stimulating factor of muscles in human seminal plasma and sheep vesicular gland. At this time von Euler chemically elucidated this "seminal force" as being a lipid soluble material with acidic properties. Subsequently he coined the term Prostaglandin for this biologically active substance, as its source was initially thought to be from the prostate gland. It is now well known that prostaglandins exist ubiquitously throughout the body and are synthesized in practically all mammalian tissues and body fluids (e.g. kidney, lung, thymus, liver, spleen, adipose tissue, uterus, placenta, blood vessel, central nervous system, adrenal glands, gut, menstrual fluid, amniotic fluid, synovial fluid, etc.) (Christ and van Dorp, 1973; Horrobin, 1978). One well documented exception is the mammalian red blood cell. Up until now a

synthesis capability has not been detected in human or subhuman, mature or immature erythrocytes (Shaw et al., 1971; Johnson, 1974).

The occurrence of prostaglandins is not restricted to mammalian tissue only. The presence of large amounts of prostaglandin like material has been identified in Caribbean marine soft coral Plexaura hormomalla as well as several plant species (Schneider et al., 1973; Cao and Cepero, 1976).

1.1.1 Structure and Nomenclature

It is evident from the above statements that the term "prostaglandin" is a misnomer. Despite the ubiquitous distribution of this substance, and for lack of a better terminology, the name prostaglandin has officially taken hold. Since their discovery in the mid 1930s (von Euler, 1935; Goldblatt, 1935), prostaglandin have been subject to much research literature reviews (Horton, 1969; Pike, 1971; Curtis-Prior, 1976; Johansson and Bergstrom, 1982; Vane, 1982).

Prostaglandins are now recognized as a group of naturally occurring cyclical unsaturated fatty acids which possess a wide range of potent and diverse biological activities.

Naturally occurring prostaglandins may be regarded as derivatives of a hypothetical (parent) prostanoic acid, an organic acid containing 20 carbon atoms including a cyclopentane ring and two side chains, one of which contains a terminal carboxyl (-C-OH) group (Figure 1.1). All prostaglandins have in common a trans double bond at C-13 and a hydroxyl group at C-15.

The early classification of these compounds was based simply on their extraction procedure from tissue homogenates. Thus, the letters E and F in prostaglandin nomenclature refer to their extraction by the organic (ethereal) phase (i.e. Prostaglandin E) or in the aqueous phosphate (Swedish fosfat) buffer phase (i.e. Prostaglandin F). Acidic and basic treatment of these prostaglandins yield derivatives designated A and B respectively (Hamberg, 1973). With the advent of modern analytical techniques, prostaglandins are also classified in numerous, often lengthy and overlapping, systems of nomenclature (Nelson, 1974). For example, PGA_2 is also called 5Z, 13E, 15(S)-hydroxy-9-oxoprosta-5, 10, 13-triene-1-oic acid. However, the commonly accepted abbreviation PGA_2 (in this case) is often used.

The most commonly used system of prostaglandin nomenclature is based on the type and position of molecular

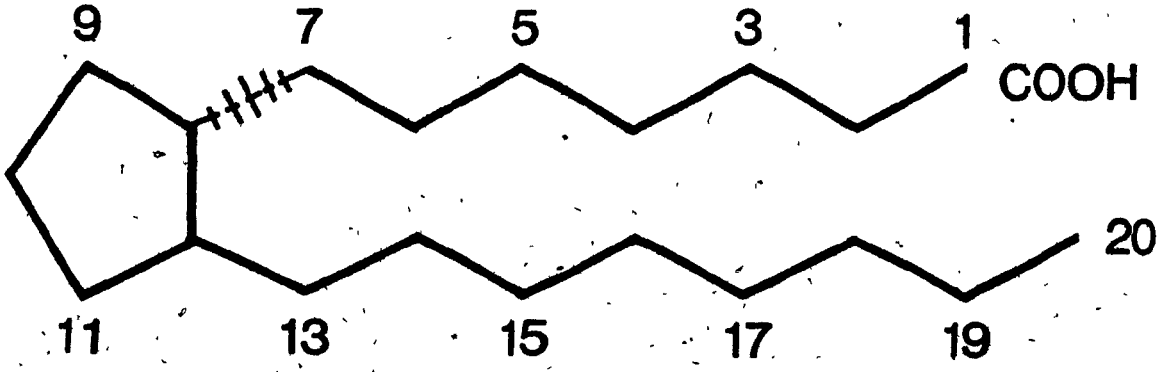


Figure 1.1: Structural formula of the parent (hypothetical) prostanoic acid, from which all naturally occurring prostaglandins may be derived.

groups, and the degree of unsaturation (i.e. carbon, carbon double bonds, C=C) of the side chains on the cyclopentane ring of the basic (parent) prostanoic acid molecule (Figure 1.1) (Crabbé, 1977). Thus, basically, all natural prostaglandins are divided into three groups or "series." Series 1 prostaglandins (e.g. PGE_1, $PGF_{1\alpha}$, PGA_1, or PGB_1) which are derived from dihomogammalinolenic acid (DGLA, 8, 11, 14 eicosatrienonic acid) contains one double bond in the side chains. Similarly, series 2 prostaglandins (e.g. PGE_2, $PGF_{2\alpha}$, PGA_2 or PGB_2) which are derived from arachidonic acid (AA, 5, 8, 11, 14-eicosatetraenoic acid) contain two double bonds in the side chains. Series 3 prostaglandins (e.g. PGE_3 and PGF_3) which are derived from 5, 8, 11, 14, 17 eicosapentanoic acid contain three double bonds in the side chains. These latter prostaglandins are less well documented than their counterparts in series 1 and 2, and are encountered only rarely in nature (Flower, 1977; Needleman et al., 1979).

Because of the rigidity imparted by the cyclopentane ring and the existence of double bonds, various isomers of prostaglandin occur. An alpha/beta (α/β) system is employed to define the stereochemical nature of substituents on the cyclopentane ring. Alpha substituents are situated on the same side of the ring as the aliphatic

side chain (i.e. C-1 to C-7) bearing the carboxyl group at C-1. Beta substituents are located on the side of the ring bearing the alkyl side chain (i.e. C-13 to C-20). The structures of some main series 1, 2, and 3 prostaglandins are shown in Figure 1.2. It will be noticed that these prostaglandins differ from each other in the following characteristic ways:

1) number of double bonds (i.e. basic classification)-C=C
2) number of hydroxyl groups (R-C-OH)
3) presence or absence of keto groups (R-C(R)=O)

Recently the development of specialized analytic techniques had led to the discovery of other important derivatives of arachidonic acid (the fatty acid precursor of most prostaglandins in the body) with potent biological activities. These are: (1) Prostaglandin endoperoxides, PGG_2 and PGH_2 -- an intermediate in the biosynthesis of prostaglandins; (2) Thromboxanes, TXA_2 -- derived from the endoperoxides; and (3) Prostacyclin, PGI_2. All these derivatives share the property of chemical instability, but have potent biological activities (Vane, 1982).

1.1.2 Prostaglandin Biosynthesis

It is now well established that prostaglandins are synthesized de novo in all tissues with the possible

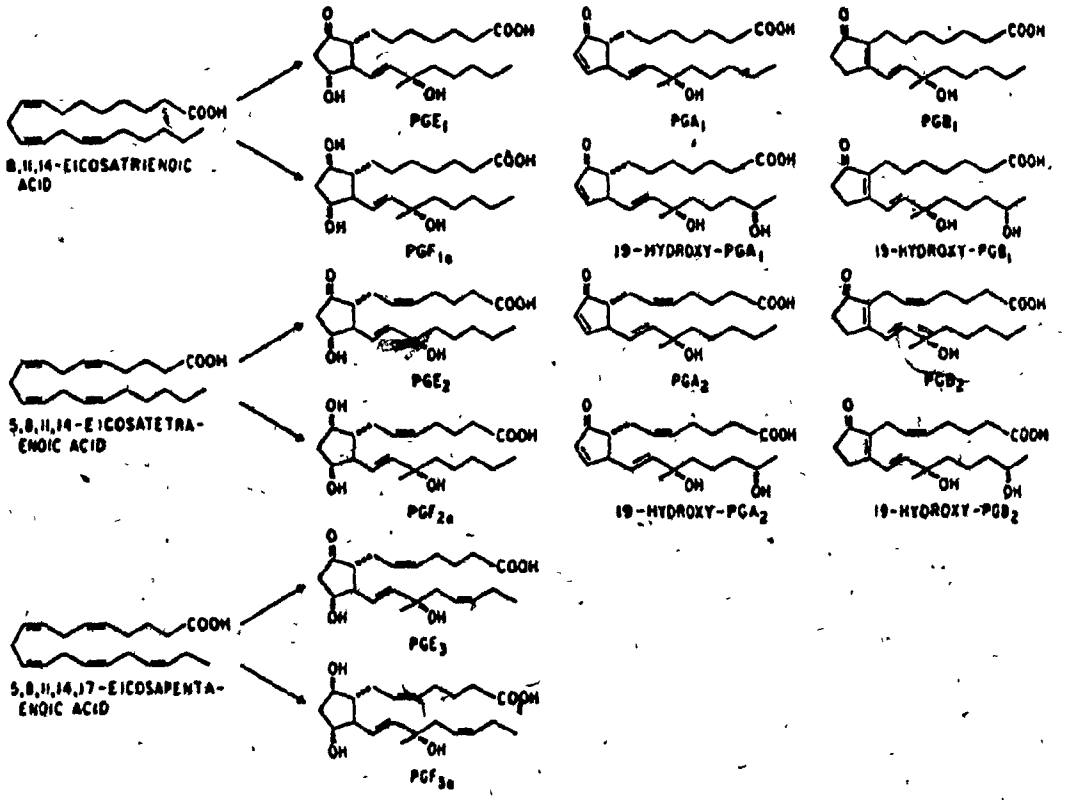


Figure 1.2: Structural formulae of the classical naturally-occuring prostaglandins and their precursors (reproduced from Higgins and Braunwald, 1972).

exception of red blood cells (Christ and van Dorp, 1974; Johnson, 1974). The precise factors governing the synthesis of prostaglandins are not clearly understood. However, it is well documented that prostaglandins are released very easily by pertubation of the cell membrane, induced mechanically or by chemicals (Piper and Vane, 1971; Vane, 1982). During the past two decades voluminous literature has been published on the complex chemical and biological processes involved in the biosynthesis of prostaglandins (Eliasson, 1960; Lands et al., 1971; Samuelson et al., 1977; Mitra, 1977; Lands, 1979; Hammarstrom, 1982). Numerous excellent reviews and symposia proceedings are also available in the literature detailing the various steps in the biosynthesis and inter-conversions of various prostaglandins (Nugteren et al, 1967; Samuelson, 1973; Wolfe, 1976; Hillier, 1978; Vane, 1978). It is intended here only to give a brief overview of these pathways of prostaglandin biosynthesis.

Generally speaking, most prostaglandins (i.e. Series 1 and 2) are synthesized in the body from C-20 straight chain carboxylic acids (i.e. essential fatty acids of the linolenic acid family) which undergo ring closure and the addition of the requisite number of oxygen atoms by complex chemical processes outlined in Figures 1.2 and 1.3. Series 3

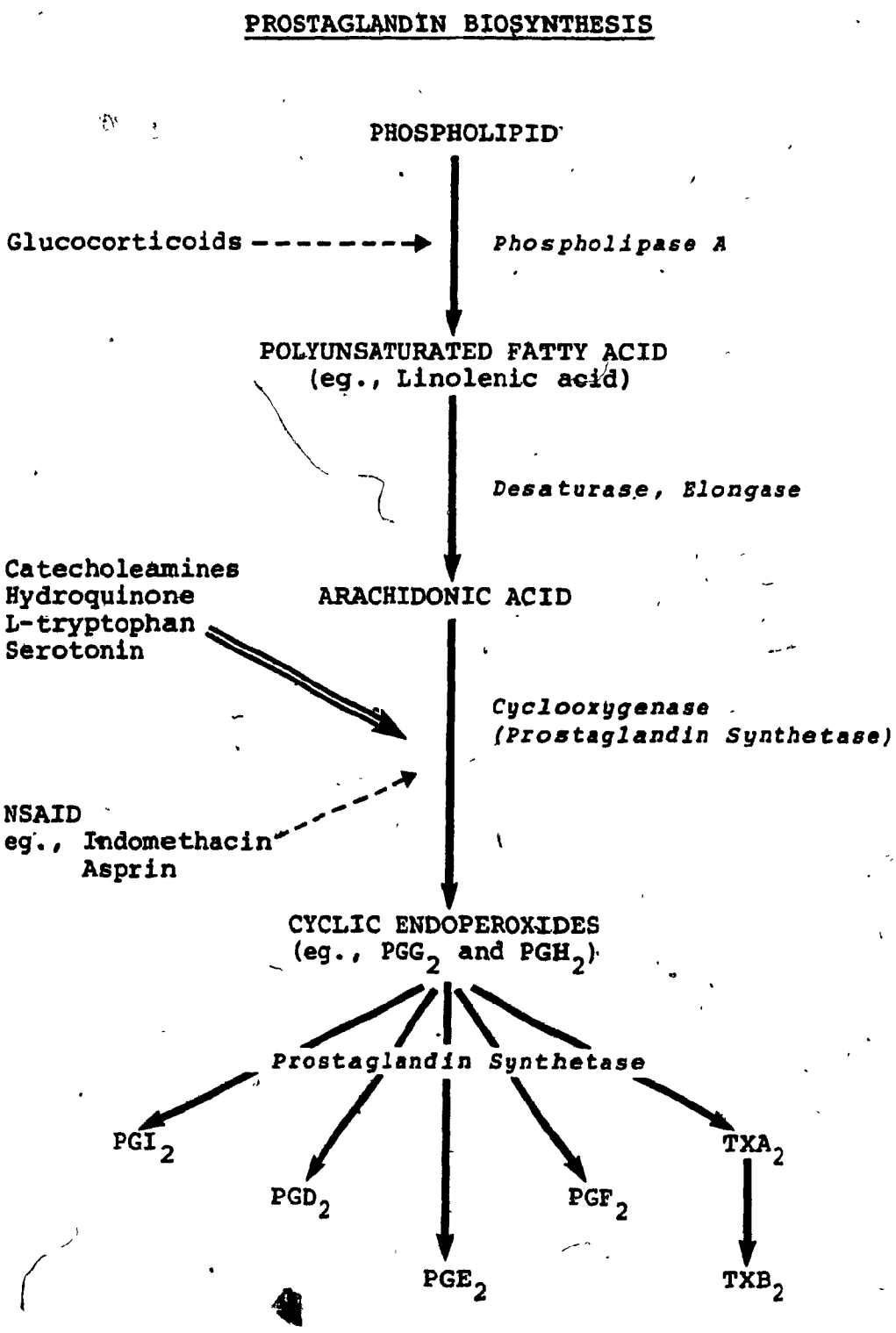


Figure 1.3: Schematic flow chart of prostaglandin biosynthesis. (⟹: stimulation, - - - →: inhibitor). Modified from Crabbe, 1977; Johansson and Bergstrom, 1982).

prostaglandins are synthesized from alpha-linolenic acid which is converted to eicosapentaenoic acid to finally form PGE_3 or PGF_3 (Figure 1.2).

Since prostaglandins are not stored within the cell to any great extent (Piper and Vane, 1971; Lands, 1979), their de novo synthesis and release requires a mobilization of substrate precursor(s) such as free fatty acids. The major fraction of cellular free fatty acids occurs tightly sequestered in the esterified form as cholesteryl esters, triglycerides or phospholipids. Precursor availability is generally considered as the major rate limiting step in the formation of prostaglandins (Lands et al., 1971; Lands, 1979). Polyunsaturated fatty acids (PUFAs), like arachidonic acid, are the principal substrate of prostaglandin and thromboxane formation (Hillier, 1978). PUFA release from membrane phospholipids is facilitated by the action of membrane-bound phospholipases (Figure 1.3). PUFA is in turn acted upon by enzymes (desaturase and elongase) to yield arachidonic acid. The liberation of free arachidonic acid is soon followed by the evolution of prostaglandin endoperoxides (by the action of cyclooxygenase) and the subsequent formation of prostaglandins, thromboxanes, and prostacyclins as illustrated in Figure 1.3 (Lands, 1979; Vane, 1982; Hammarstrom, 1982).

1.1.2.1 Stimulators of Prostaglandin Biosynthesis

A number of compounds such as catecholamines, hydroquinone, L-tryptophan and serotonin are documented as essential co-factors in prostaglandin biosynthesis (Sih et al., 1970). These compounds have been shown to enhance the catalytic conversion of arachidonic acid to the cyclic endoperoxides, PGG_2 and PGH_2 (Miyamoto et al., 1976) (Figure 1.3).

1.1.2.2 Inhibitors of Prostaglandin Biosynthesis

Compounds that can retard or inhibit prostaglandin formation are the non-steroidal anti-inflammatory drugs NSAID (e.g. aspirin and indomethacin), and glucocorticoids (Vane, 1971; Chang et al., 1977). The former attacks the cyclooxygenase moiety of prostaglandin synthetase, essential for the conversion of arachidonic acid to the cyclic endoperoxides PGG_2 and PGH_2. The latter acts by blocking the degradation of membrane phospholipids by phospholipase A_2 (Figure 1.3).

1.1.3 Physiology of Prostaglandins

Despite its concurrent discovery by von Euler and Goldblatt (1935) nearly half a century ago, its precise role in physiology remains to be elucidated. The problem

of the physiological role of prostaglandins is a complex one because there are indications that these ubiquitous substances may play a different role not only in each organ but possibly in each tissue and cell (Horton, 1969). Furthermore, prostaglandins are not stored like histamine or serotonin but are released immediately upon synthesis. Consequently, it is always necessary to initiate synthesis to study release. Thus, release is synonymous with synthesis. In addition, their short half life (1 - 5 minutes) makes precise quantization difficult (Jaffe, 1973).

Much of the physiology of prostaglandins, however, has been elucidated by their pharmacologic effects on various tissues and organs. Numerous excellent reviews on these are available in the literature (Horton, 1969; Hammarstrom, 1982; Curro et al., 1982). Some of these pharmacological actions of prostaglandins are summarized in Table 1.1. Only a brief overview with general examples are given. Prostaglandins have been shown to induce varied physiological effects from species to species. For example, PGE_2 causes bronchodilation in cats but bronchoconstriction in humans and guinea pigs (Table 1.1). In addition, the effects of prostaglandins on the vascular system varies with the anatomical location of the blood vessel. In general, PGEs caused vasodilation whereas PGFs

Table 1.1

Some Pharmacological Actions of Prostaglandins

Species	Prostaglandin	Tissue or Organ System	Effect	Reference
Sheep	PGF_2	Reproductive System	luteal regression	Bygdeman and Gillespie, 1982
Rat	PGE, PGE_2, PGF_2	Reproductive	uterine contraction	Curtis-Prior, 1976
Guinea Pigs	PGE	Respiratory System	Bronchodilation	Piper and Vane, 1971
Cat	PGF_2	Respiratory System	Bronchodilation	Horton, 1969, 1972
Human, Guinea Pig	PGF_2	Respiratory System	Bronchodilation	Jones, 1977
Dog	PGE, PGA	Cardiovascular System	lowers blood pressure, increase cardiac output	Jones, 1977
Rat	PGF	Cardiovascular System	increase blood pressure vaso constrictor	Sammuelson et al., 1977
Dog			increase or decrease cardiac output	Jones, 1977
Dog	PGE, PGA	Gastrointestinal System	inhibit HCl secretion inhibit ulcer formation increase in intestinal motility	Horton, 1969, 1972 Johansson and Bergstrom, 1982

Table 1.1 (continued)

Some Pharmacological Actions of Prostaglandins

Species	Prostaglandin	Tissue or Organ System	Effect	Reference
Rabbit	PGF	Gastrointestinal System	increased intestinal motility	Curtis-Prior, 1976 Johansson and Bergstrom, 1982
Rats	PGE	CNS	depressor effect anti convulsion	Horton, 1969, 1972
Rats	PGF	CNS	no response	Wolfe, 1975
Cat	PGE	Hypothalamus	thermoregulation-fever	Wolfe, 1975
Cat	PGF	Hypothalamus	no response	Wolfe, 1975
Dog	PGF_2	Cerebral blood vessels	vaso constriction decrease blood flow	Yamamoto et al., 1972, 1973 Wolfe, 1975
Dog Cat	PGE	Cerebral blood	vasodilation vasoconstriction	Wolfe, 1975
Rat	PGE, PGE	Neuromuscular Junction	no response	Wolfe, 1975
Rabbit	PGE_2, PGA	Kidney	vasodepressor increase natriuresis.	Horton, 1969, 1972
Dog	PGF_2	Kidney	no effect	Lee, 1973

vasoconstriction in most blood vessels (Jones, 1977). However, in the cerebral blood vessels, both PGE and PGF have vasoconstrictor effects (Wolfe, 1975). Yamamoto et al. (1972, 1973) have further shown that PGE_1 causes vasodilation and PGE_2 vasoconstriction in the cerebral arteries in dogs. Recently, exactly opposite observations were reported by Curro et al. (1982), who demonstrated that PGE_2 was in fact vasodilatory in the cerebral vasculature. Such contradictory reports further suggest to the complex character of prostaglandins in physiology. Thus, because of this and other contradictory observations, the precise physiology of prostaglandins remains controversial. The variable and sometimes diverse biological action of prostaglandins in the same system has thus made it difficult to establish a unified mechanism responsible for the action of prostaglandins in different tissues.

1.1.4 Cytoprotective Property of Prostaglandins

An important adjunct to the diverse physiological properties of prostaglandins is their cytoprotective effect on a variety of organs including the stomach, small and large intestines, pancreas, kidney and liver (Robert, 1981; Johansson and Bergstrom, 1981). The phenomenon of cytoprotection by prostaglandins was first discovered by

Robert (1976) who showed that several natural prostaglandins (PGA_1, PGA_2, PGB_2, PGC_2, PGD_2, PGE_1, PGE_2, $PGF_{2\alpha}$ and $PGF_{2\beta}$) had a unique ability to protect cells of the gastrointestinal epithelium ("direct" cytoprotection) against a variety of potentially noxious agents (e.g. non-steroidal anti-inflamatory drugs (NSAID), steroids, ethanol, hot water, and strong acid or base) which otherwise have the capability of producing cellular damage and necrosis (Robert et al., 1979; Robert, 1981). Prostaglandin was administered orally or sub-cutaneously. In either case, it was observed that "direct" cytoprotection was dose dependent and of the various prostaglandins tested, PGA and PGE were generally the most effective.

It was first suggested that the inhibitory effect of certain prostaglandins (e.g. PGE_2) on gastric acid secretion was the reason for the anti-ulcer activity (i.e. prostaglandins acted as negative feedback inhibition of gastric secretion) (Robert, 1979). However, certain prostaglandins protected the stomach even at non-secretory doses and against necrotizing agents that do not respond to antisecretory drugs such as histamine, H_2 blockers, anticholinergics or antacids (Robert et al., 1979). Furthermore, prostaglandins that do not affect gastric acid secretion (i.e. $PGF_{2\alpha}$ and $PGF_{2\beta}$) also prevented aspirin or

indomethacin induced ulcers (Robert et al., 1967). These observations suggested that prostaglandins could protect the gastrointestinal mucosa by mechanisms other than inhibition of gastric secretion. Since indomethacin is ulcerogenic and its lesions are prevented by prostaglandins, it was postulated that ulcer formation was due to prostaglandin deficiency illicited by aspirin or indomethacin (Robert, 1975; Nezamis et al., 1982). Thus, treatment with prostaglandins would exert a protective effect by replacing the missing endogenous prostaglandins.

Prostaglandin-induced cytoprotection of the gastrointestinal tract was also seen against a variety of physical injury by necrotizing agents such as absolute alcohol, strong acids (e.g. HCl), strong base (e.g. NaOH), hypertonic (25%) saline, and even boiling water (Robert et al., 1979). The mechanism for this is open to debate. Robert's group has postulated that, since prostaglandins are present in most cells, they are necessary for maintaining cellular integrity. Thus, by raising the tissue level of prostaglandins by exogenous administration, the cellular resistance is also enhanced (Robert et al., 1979). Such a mechanism, however, seems unlikely since such necrotizing agents kill the gastric epithelial on contact.

"Adaptive" cytoprotection is another form of cytoprotection which has been observed in the gastric epithelium (Robert et al., 1979). It was observed that mild irritants such as 20% ethanol, or weak acid or base (given 15 to 20 minutes before necrotizing agent) also protected the gastric epithelium from lesions against absolute alcohol, strong acid or base, and even boiling water (Robert et al., 1979). It was speculated that mild irritants might stimulate endogenous formation of cytoprotective prostaglandins by the stomach, implying prostaglandins were the direct cause of cytoprotection. The hypothesis was tested by treating the rats with indomethacin, an inhibitor of prostaglandin synthesis, prior to administering the mild irritants. At an optimal dose of indomethacin, none of the mild irritants were cytoprotective anymore. This result strongly suggests that the mild irritants exert their effect by stimulating the formation of cytoprotective prostaglandin(s) by the stomach (Robert, 1979b). This effect of mild irritants was termed adaptive cytoprotection since it appears to be a local defensive reaction of the body against the introduction of noxious agents.

In the earlier studies in which cytoprotection was accomplished by administration of prostaglandins, one could

argue that the effect was pharmacological, i.e. it did not represent a natural response but was the result of administering a drug. Demonstration of adaptive cytoprotection, on the other hand, suggests that cytoprotection by prostaglandins may in fact be a physiological phenomenon (Robert, 1981).

Since these initial studies (on gastric and intestinal cytoprotection), the cytoprotective property of prostaglandins has been extended to a number of other organs including the pancreas, kidney and liver (Reber et al., 1980; Manabe and Steer, 1980; Stachura et al., 1980; Ruwart et al., 1981a,b).

Recently, numerous studies have demonstrated that prostaglandins (namely PGE_2 and its methyl analog 16, 16 dimethyl PGE_2) were able to protect the liver (i.e. hepatocytoprotection) against acute liver injury produced by a number of hepatotoxins including galactosamine, acetominophen, ethanol, alphatoxin, carbon tetrachloride, alpha-nepthylisothiocyanate (Stachura et al., 1980, 1981; Ruwart et al., 1981a, 1981b, 1982a, 1982b, 1984) and thermal injury (Miyazaki et al., 1983). These studies have demonstrated that RGE_2 was capable of protecting not only liver parenchymal cells but also those of the biliary epithelium from toxic damage. However, for cytoprotection

to occur, it was necessary to administer the PGE_2 just before, simultaneously or immediately following toxin insult to the liver. The mechanism for hepatocyto-protection is unclear. It has been suggested that hepatocyte protection may occur at the membrane level or may be a by-product of alteration of toxin metabolism caused by the prostaglandin (Miyazaki et al., 1983). Since prostaglandins have been shown to protect a variety of cell types (i.e. gastric and intestinal epithelium, kidney, pancreas, and liver cells) there is reason to believe a generalized mechanism for protection of cells from toxins by prostaglandins may exist.

1.1.5 Other Properties of Prostaglandins

In addition to cytoprotection, prostaglandins have also been implicated in DNA synthesis and cell proliferation following partial hepatectomy and ethanol induced suppression of liver regeneration (Miura and Fukui, 1979; Makowka et al, 1982; McNeil and Leevy, 1983). These results strongly suggest that prostaglandins are involved in liver regeneration, although it is not clear what type of prostaglandin is the trigger. An increase in PGE observed during liver regeneration following partial hepatectomy in normal rats suggests that PGE may be of key

importance in DNA synthesis (MacManus and Braceland, 1976). Indomethacin, a potent inhibitor of prostaglandin synthesis, reverses these effects on DNA synthesis (McNeil et al., 1985). The direct mitogenic effect of PGE has also been observed in thymic cells, mouse lymphocytes and cultured rat hepatocytes (MacManus and Whitfield, 1976; Andreis et al., 1981; Otto et al., 1982).

1.1.6 Mechanisms of Prostaglandin Action

The exact mechanisms of prostaglandin action are still to be defined as their exact physiological role remains controversial (Horton, 1969). It is clear, however, that endogenous prostaglandins act locally and rapidly, in small quantities, to produce a variety of cellular responses. It has been suggested that the biological actions of prostaglandins appear to be closely associated with those of various hormones in the body (Horton, 1972). Several workers (Horton, 1969; Lee, 1973; Kuehl, 1973; Wolfe, 1975; Bygdeman and Gillespie, 1982) have postulated that as circulatory hormones and nerve action potential reach the regional cell membrane and exert their biological action, the phospholipidases in the membrane are simultaneously activated to cleave the phospholipids to release polyunsaturated fatty acids, linolenic acid, and arachidonic

acid. These precursors of prostaglandins rapidly follow the "arachidonic cascade" (Figure 1.3) to form the desired prostaglandin(s) (Samuelsson et al., 1977). Thus, it is postulated that on nerve or hormonal stimulation prostaglandins may play a role in the negative feedback mechanism in such peripheral tissues as adipose tissue, stomach, and smooth muscles (Horton, 1969; Wolfe, 1975).

1.1.7 Prostaglandins and the Cyclic AMP System

Cyclic AMP, after its discovery by Rall et al. (1957), has been implicated in various hormone-membrane interactions. It is now well known that cyclic AMP plays a key messenger role to regulate intracellular function as a consequence to external stimuli. A brief schematic representation is shown in Figure 1.4. Briefly, the hormone-membrane interaction excites the membrane-bound adenyl cyclase to convert intracellular ATP to cyclic AMP. The cyclic nucleotide subsequently activates a sequence of protein phosphorylase reactions culminating in the appropriate physiological response.

Steinberg et al. (1964) first postulated that the actions of prostaglandins may also be similarly mediated by cyclic AMP. Much research and extensive reviews in the literature have now confirmed the existence of a cyclic AMP

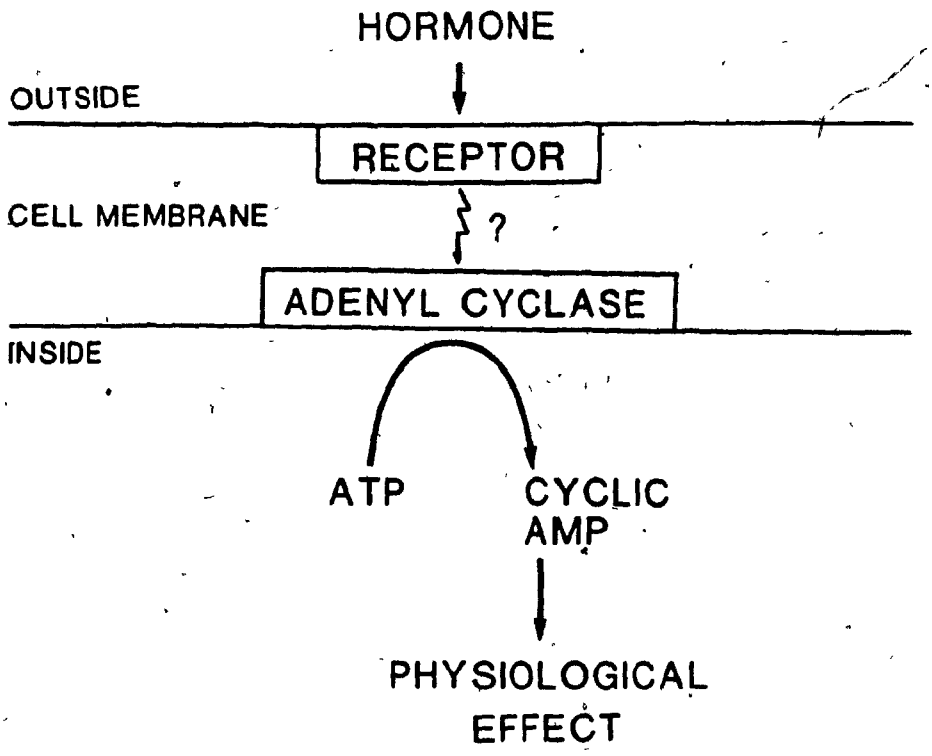


Figure 1.4: A brief schematic representation of hormone-membrane (receptor) interaction which elicits adenyl cyclase to convert intracellular ATP to cyclic AMP. Cyclic AMP subsequently activates a sequence of protein phosphorylase reactions culminating in the appropriate physiological response.

mediated mechanism for prostaglandin action (Hittlemann and Butcher, 1973; Kuehl, 1973; Hammarstrom, 1983). A brief schematic representation of this is depicted in Figure 1.5. The concept that prostaglandins may be formed as a consequence of cyclic AMP action and function as negative feedback regulators is also shown. Despite isolated contrary observations to the negative feedback concept, it is now a generally accepted phenomena (Kuehl, 1973).

1.1.7.1 Ying-Yang Hypothesis of Cyclic AMP and Cyclic GMP

The Ying-Yang hypothesis or the Theory of Dualism was proposed by Goldberg and co-workers (1973). This theory suggests the modulation of intra-cellular (hormonal or prostaglandin related) response due to prostaglandin stimulation is not due to cyclic AMP alone. The theory states that both cyclic AMP and cyclic GMP play a role in regulating cell function with an equal and opposite effect. It has also been suggested that cyclic GMP relates to F prostaglandins in the same way that cyclic AMP relates to E prostaglandins. The presence of receptor sites for F prostaglandins, along with evidence of increased cyclic GMP/cyclic AMP ratio under PGF_2 stimulation, have further strengthened this view. Similar but opposite situation has been observed with PGE_2 (Kuehl, 1973).

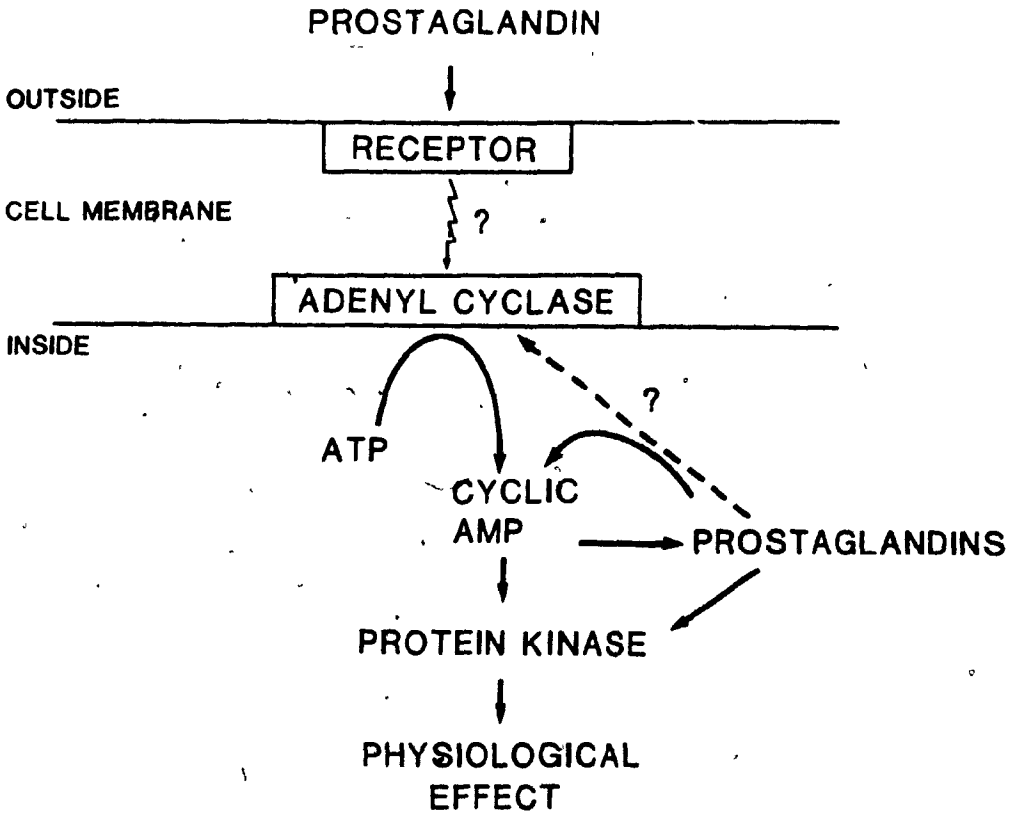


Figure 1.5: A brief schematic representation of prostaglandin-membrane (receptor) interaction which elicits adenyl cyclase to convert intracellular ATP to cyclic AMP. Cyclic AMP subsequently activates a sequence of protein kinase and phosphorylase reactions, culminating in the appropriate physiological response. Prostaglandins, formed as a consequence of cyclic AMP action, may function as negative feedback regulators (- - - - →), or may enhance the protein kinase to augment its action.

1.1.8 Are Prostaglandins Hormones?

The question of prostaglandins being hormones is a complex and controversial one (Horton, 1969). In the classical sense prostaglandins (at least E and F series) cannot be considered as true circulating hormones because they are inactivated during a single passage through the lungs. However, exceptions have been observed in women undergoing labour. In this case PGE_2 and $F_{2\alpha}$ secreted in one area of the body have been shown to circulate past the lungs to cause uterine contractions. In addition, substantial evidence exists to suggest that $PGF_{2\alpha}$ is released in the uterus to cause luteolysis in ovaries of sheep and that PGE_2 is formed in the kidney to regulate hemodynamics of the cortex (Kuehl, 1973).

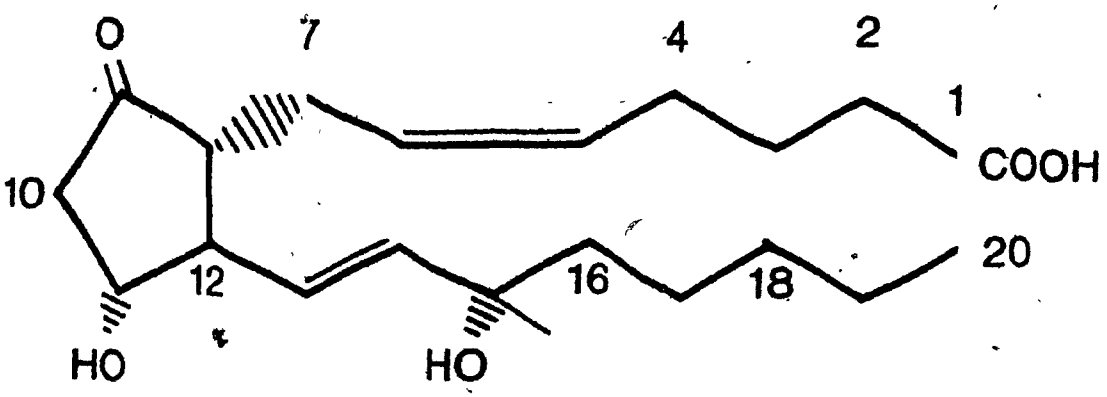
The only prostaglandin that might conceivably function as a true circulating hormone is one of the A series as these prostaglandins are not metabolized by the lungs. Thus, PGA_2 formed in the papilla of the kidney could be elaborated into the venous circulation, following appropriate stimulus (e.g. expansion of blood volume), pass through the lungs to act on peripheral arterioles.

For the majority of prostaglandins (i.e. Series E and F) it has been suggested that they behave like "local hormones" and play an important regulatory role in cellular

function. Recently prostaglandins (specially PGE_2) have also been demonstrated to regulate and trigger red cell formation from hemopoietic stem cells (Dukes, 1982). Thus, the role of prostaglandins as local intracellular regulators (hormones) is established, at least in some instances. In addition, the ability of virtually all cell types to synthesize and respond to added prostaglandins suggests that prostaglandin must also have an important action in the cells which produce them. In accord with the messenger concept of cyclic AMP, prostaglandins would appear to play either an essential intermediate or modulating role on hormone action (Wolfe, 1975; Vane, 1982).

1.1.9 Prostaglandin E_2

Prostaglandin E_2 (PGE_2) is one of the primary end-products of prostaglandin synthesis from arachidonic acid (Figure 1.3). The basic chemical structure of prostaglandin E_2 is shown in Figure 1.6. Early studies with this primary prostaglandin were related to their highly potent effects on smooth muscles, kidney, and the gastrointestinal tract (Eliasson, 1960; Horton, 1969; Pike, 1971; Lee, 1973). In recent years, with the availability of synthetic and purified prostaglandins, these substances have been implicated in the regulation and function of



PGE_2

Figure 1.6: Structural formula for prostaglandin E_2.

practically every tissue and organ system in the body (Kuehl, 1973; Wolfe, 1975; Jones, 1977; Dukes, 1982). Recently they have also been implicated in cell proliferation and DNA synthesis (Andreis et al., 1980; Otto et al., 1982). PGE_2 has been demonstrated to manifest numerous and diverse biological properties which vary from organ to organ. Some representative properties of PGE_2 are listed in Tables 1.1 and 1.2. It is now known that these varied and diverse responses of PGE_2 are mediated by the cyclic AMP system in the body (Hittleman and Butcher, 1973). PGE_2 have been generally shown to be antagonistic to PGF_2 in its action (Curtis-Prior, 1976). For example, PGE_2 relaxes the bronchi, lower esophageal sphincter, arterial smooth muscles, whereas $PGF_{2\alpha}$ constricts these tissues. However, discrepancies have been observed in cerebral capillaries where PGE_2 has been shown to cause both vasodilation and vasoconstriction (Wolfe, 1975) (Table 1.1).

A major and well documented property of PGE_2 is cytoprotection. Cytoprotection by prostaglandins has been extensively reviewed (Miller and Jacobson, 1979; Robert, 1979, 1981; Johanson and Bergstrom, 1981). Of the various prostaglandins, PGE_2 and its methyl analogue 16, 16-dimethyl PGE_2 has been extensively studied in numerous tissues. Strong and convincing evidence now suggests that

Table 1.2

Some Physiological Properties of Prostaglandin E_2

Organ System	Physiological Action
Reproductive System	Relaxes non pregnant uterus Contracts pregnant uterus
Cardiovascular System	Increases cardiac output Increases myocardial contractile force Causes vasodilation Lowers blood pressure
Renal System	Increase natriuresis Causes cortical vasodilation
Respiratory System	Causes bronchodilation
Central Nervous System	Depressive effect Anticonvulsion Increase body temperature
Gastrointestinal System	Inhibits gastric secretion Increases intestinal motility
Endocrine System	Increase cyclic AMP levels Modulates cyclic AMP mediated action of hormones and drugs Promotes glycogenolysis
Immunological System	Inhibit lymphocyte function Inhibitory role in immune response Produces inflamation
Hematological System	Stimulates platelet aggregation

PGE_2 plays a major physiological role in cytoprotection against toxins by causing stabilization of cellular membranes (Robert et al., 1979; Stachura et al., 1981; Nezamis et al., 1982). The mechanism for this is as yet unclear. Increased gastric mucus secretion due to PGE_2 stimulation may play a role, at least in gastric cytoprotection (Nezamis and Robert, 1982). In a unified concept for cytoprotection, it has been proposed that PGE_2-induced cyclic AMP production may play a major role (Robert, 1981). The observation that both PGE_2 and cyclic AMP prevented indomethacin-induced inhibition of sodium active transport in the gastric mucosa suggests that PGE_2 stimulates the sodium pump by activating adenyl cyclase and increasing intracellular cyclic AMP (Chaudhury and Jacobson, 1978). Similar observations have been made in various other types of tissues (Hall et al., 1976). Inhibition of ion active transport may lead to intracellular accumulation of sodium, anions, and water. The resultant osmotic swelling could produce severe cellular damage, altered permeability, and disruption of lysosomes. Prostaglandins (E_2), by activating the gastric sodium pump, may protect the epithelium against such intracellular changes (Miller and Jacobson, 1979).

1.2 PROSTAGLANDINS IN LIVER PHYSIOLOGY

The cytoprotective property of prostaglandins (in particular PGE_2) on liver cells has been well documented. However, there appears to be a paucity of published information regarding the effects of prostaglandins on liver physiology.

It has been demonstrated that the liver (as well as the lungs) efficiently metabolizes as much as 95% of circulating prostaglandins (Dawson et al., 1970). This removal was associated with a rapid and complete decarboxylation of prostaglandin within the liver. Evidence for a prostaglandin receptor was first described in the fat cell (Kuehl and Humes, 1972). However, recently PGE receptors have also been identified on rat hepatocytes (Simigel and Fleicher, 1974). No PGF receptors have yet been identified in rat hepatocytes. Existing evidence suggests that E type prostaglandins exert their action by regulating cyclic AMP levels. Thus, PGE may function to control enzymes associated with cyclic AMP. Recently, contrary evidence was presented indicating that unlike as in most cells, PGE_2 does not appear to be stimulating to cyclic AMP in hepatocytes (Grinde and Ichihara, 1983). Thus, in hepatocytes PGE_2 may in fact depress cyclic AMP

production. This anomalous action of PGE_2 remains controversial.

The effect of prostaglandins on lipid and carbohydrate metabolism has been extensively studied. Its effect on protein metabolism is less studied as it has only marginal effects on the synthesis of protein in bacteria (Ruddon and Johnson, 1967). Recently, however, prostaglandins have been shown to participate in the synthesis and release of different hormones in various endocrine glands (Kuehl, 1973; Wolfe, 1975). The mechanism of action is considered to be mediated by cyclic AMP (Hittleman et al., 1973) (Figure 1.4).

1.2.1 Prostaglandins in Lipid Metabolism

The first observations of the effects of prostaglandin on lipid metabolism were made by Steinberg et al. (1964) who showed that PGE_1 reduces the release of glycerol and free fatty acids from rat epididymal adipose tissue, indicating a direct antilipolytic action of PGE_1. Shaw and Ramwell (1968) extended these findings by showing that when epididymal adipose tissue was stimulated in vitro by lypolytic hormones (e.g. epinephrine, norepinephrine and ACTH), or when the epididymal nerve was stimulated electrically, or when the animals were previously fasted there was

an increased efflux of prostaglandin release. Furthermore, there was a reduced release of prostaglandin (concomitant with reduced free fatty acid release) in the presence of insulin. These observations subsequently lead to the hypothesis that prostaglandins play a physiological role as a negative feedback regulator of hormone stimulated lipolysis in adipose tissue (Figure 1.7) (Shaw and Ramwell, 1968). The nutritional status appears to be an important factor in prostaglandin antagonism of lipolysis; lipolysis in adipose tissue from fasted rats is sensitive to prostaglandin antagonism but not tissue from fed rats (Carlson and Michelli, 1970).

The antilipolytic action of various prostaglandins have been determined and are as follows: $PGE_2 > PGE_1 > PGF_{2\alpha} > PGF_{1\alpha}$. Prostaglandin F_2 was found to be inactive as an antilipolytic agent (Steinberg et al., 1964; Butcher and Baird, 1968).

The mode of the antilipolytic action of prostaglandins is considered to be mediated via adenyl cyclase system (Horton, 1969). Several hormones activate adenyl cyclase to form cyclic AMP which is converted to 5'AMP by phosphodiesterase in the adipose tissue. Hormone sensitive lipase, which hydrolyzes triglycerides in adipose tissue, is activated by cyclic AMP-dependent proteinkinase and

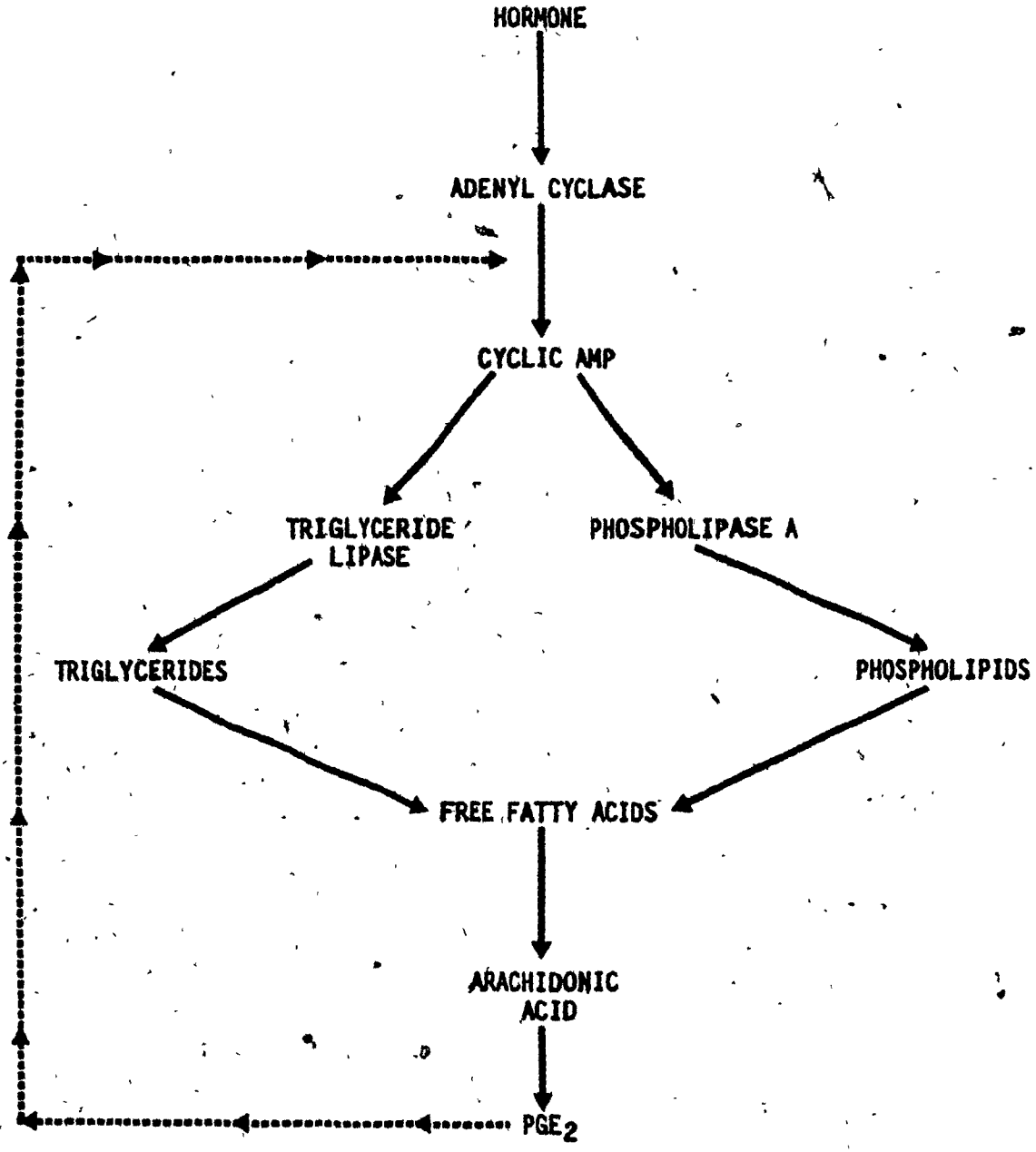


Figure 1.7: A schematic flow chart showing the physiological role of PGE_2 as a negative feedback regulator of hormone-stimulated lipolysis in adipose tissue (⋯⋯▸ negative feedback inhibition).

appears to be the rate limiting step (Butcher and Baird, 1968). Hence, a second messenger, cyclic 3',5',-AMP seems to play a key role in mediating the lipolytic actions of catecholamines and other hormones by activating this lipase (Butcher and Sutherland, 1967).

1.2.2 Prostaglandins in Carbohydrate Metabolism

Relatively few studies have been made on the effect of prostaglandins on carbohydrate metabolism. Intravenous injection of PGE_1 and PGE_2 causes significant hyperglycemia in a variety of animals (Bohle and May, 1968; Miller et al., 1983). On the other hand, PGE_1 fails to increase blood glucose in adrenalectomized rats (Bergstrom et al., 1968). Furthermore, unrelated hypotensive agents can also induce hyperglycemia (Bergstrom et al., 1968). These observations indicate that two separate mechanisms may be responsible for the genesis of PGE_1-induced hyperglycemia: (1) a direct glycogenolytic action of PGE_1 on the liver and (2) reflexly increased secretion of catecholamines secondary to the hypotensive action of PGE_1 (Bergstrom et al., 1968; Bohle and May, 1968). From in vivo experiments, it appears that the former is rather feeble compared to the latter mechanism for the production of hyperglycemia.

In other studies, Crawford and Haessler (1968) noted the similar action of PGE_1 and insulin in reducing lipolysis in adipose tissue. Subsequently, they showed that PGE_1 may promote fatty acid re-esterification and lipid synthesis from glucose and acetate. Prostaglandin E_1 was also "insulin-like" in stimulating ^{14}C incorporation into fatty acid and glycogen. Recently, prostaglandin inhibitors, such as indomethacin, significantly blunt plasma glucose and hepatic glucose output in response to glucagon and or epinephrine (Miller et al., 1983). Thus, both carbohydrate and lipid metabolism appear to be intimately interrelated at least with respect to prostaglandin modulation.

1.3 PROSTAGLANDINS IN LIVER DISEASE

Definitive studies implicating a role for prostaglandins in liver disease are lacking. It is well documented, however, that prostaglandins play important regulatory roles in lipid and carbohydrate metabolism (Shaw and Ramwell, 1968; Bergstrom et al., 1968). E type prostaglandins have been found to be strong inhibitors of lipolysis and may play a critical therapeutic role in preventing fat deposition in liver disease. Hepatocytes have prostaglandin binding sites which appear to be highly

specific for E type prostaglandins. The binding site, however, does not differentiate PGE_1 from PGE_2 (Simigel and Fleicher, 1974). Furthermore, the binding site which appears to be mediated by cyclic AMP can be down regulated in a manner similar to a receptor (Robertson et al., 1980).

Recently, it was shown that isolated liver tissue have a small, but finite, rate of prostaglandin production which decreases with increasing age (Murota and Morita, 1980). Whether these observations imply an in vivo steady state role for hepatic prostaglandin production is presently unclear. An increased concentration in the blood might imply an increased role for prostaglandins, whereas the opposite may imply a diminished role. Since it is believed that prostaglandins act mainly close to or at their site of synthesis, changes in blood levels of prostaglandins might well reflect an alteration in its clearance or metabolism rather than of synthesis or release (Lifschitz, 1983).

Plasma measurements of prostaglandins in subjects with liver disease have been reported by only a few investigators (Trewby et al., 1975; Zusman et al., 1977; Loginov and Markova, 1979). Trewby et al. (1975) have reported extremely low levels of prostaglandin E in patients with acute fulminant hepatic failure. In patients with chronic liver failure, the prostaglandin E levels were also

significantly reduced with respect to control values (Zusman et al., 1977; Loginov and Markova, 1979). Loginov and Markova (1979) suggest that serum concentrations of prostaglandins depend on the severity of the disease. Thus, in chronic hepatitis the levels of prostaglandin A and E were unchanged in relation to the control group and in liver cirrhosis it was decreased. Since a close relationship exists between free unsaturated fatty acids and prostaglandins synthesis, it was suggested that decreased prostaglandin levels were caused by deficiency of their precursors in liver cirrhosis (Loginov and Markova, 1979). This was confirmed when an oral or intravenous infusion of a solution containing 70% linolenic acid significantly raised the prostaglandin level in the blood at the end of treatment. At this time, a rise in concentration of endogenous prostaglandins accompanied normalization of liver functions (Loginov and Markova, 1979). It has been speculated that patients with severe liver disease might be nutritionally deficient in certain essential fatty acids (Loginov and Markova, 1979; Lifschitz, 1983). In experimental settings, animals on severe dietary restrictions have lower levels of prostaglandin production (Hurd et al., 1981). Conceivably, patients in severe liver disease could also have their rate

of prostaglandin production limited by the severe dietary restrictions (Loginov and Markova, 1979; Lifschitz, 1983). Whether this phenomenon is in fact clinically important is not known.

It has been suggested that exogenous prostaglandin administration may also have a beneficial effect in liver disease (Loginov and Markova, 1979; Dixit and Chang, 1982; Chang, 1982). A recent controlled study showed that PGE_2 injected into galactosamine-induced acute fulminant hepatic failure rats significantly increases survival time but not the survival rate in these rats (Dixit and Chang, 1982). Furthermore, PGE_2 also significantly prevented the development of brain edema (a major complication in acute liver failure) in these animals (Dixit and Chang, 1985). In the few uncontrolled clinical studies (on patients with cirrhosis and ascites) involving exogenous prostaglandin administration, no significant improvements were seen in those patients (Lifschitz, 1983).

1.4 LIVER-BRAIN INTERRELATIONSHIP

The liver-brain interrelationship, i.e. the notion that the liver is associated with the brain and the mind, enjoys a mysteriousness that has puzzled physicians since the beginnings of recorded medical literature. It is not intended here to give a detailed treatise of the progress made through the centuries towards elucidating hepato-cerebral relationships. Numerous excellent books and literature reviews are available in the medical literature (Frerichs, 1860; Adams and Foley, 1953; Brown, 1957; Garrison and Fielding, 1961; Brown, 1970; Conn and Lieberthal, 1979).

Much has been written in recent years on the matter of liver-brain relationships (Fischer and Baldessarini, 1971; Hoyumpa et al., 1979; Zieve, 1979; Schafer and Jones, 1982; Laursen, 1982; Crossley et al., 1983). The topic is highly complex and controversial, regardless of the type of liver disease. Since this thesis deals primarily with fulminant hepatic failure (FHF), the liver-brain relationship will be discussed in this context, unless otherwise indicated. A detailed description of FHF is given in a later section of this thesis. For the present, it will suffice to say that FHF is an acute disorder of the liver involving massive hepatocyte necrosis (Trey and Davidson, 1970). The disease

is characterized by a rapid onset with the development of distinct neurological abnormalities, commencing with confusion and stupor, which rapidly progress to coma and subsequent death. The various grades of hepatic coma have been described in Table 1.3. Thus, fulminant hepatic failure can result in disturbances in all parameters of liver and brain function.

The postulate in the liver-brain relationship of acute liver disease is that the liver is the initial seat of the disease and that the involvement of the nervous system (i.e. the development of hepatic coma) is secondary.

The liver can be considered as one of the most important organs in the body by the sheer multitude and diversity of its function. A brief summary of its numerous functions is presented in Table 1.4. It is evident and now generally accepted that the liver plays a key role in: 1) storage (glycogen), 2) synthesis (albumin, etc.), and 3) detoxification (metabolic) functions in the body. Any compromise in these functions, as is seen in FHF, can present a potentially life threatening situation. In FHF the functioning liver biomass can no longer provide these essential liver functions. Consequently, various toxic metabolites or by-products can build up in the blood. The exact nature of these toxins is unknown at this time.

Table 1.3

Description of the various grades of hepatic coma during galactosamine-induced fulminant hepatic failure in rats.

GRADES OF COMA	
GRADE I	SLOWNESS IN MOVEMENT (LETHARGIC) DIFFICULT TO DIAGNOSE IN RATS SOME PILOERECTION
GRADE II	SLOWNESS IN MOVEMENT DROWSY CONFUSION
GRADE III	SLEEPS MOST OF THE TIME BUT IS AROUSABLE CONFUSION VERY LITTLE MOVEMENT MAY OR MAY NOT RESPOND TO STIMULUS MAY BE HYPERACTIVE AT TIMES CONVULSIONS
GRADE IV	UNAROUSABLE NO MOVEMENT UNRESPONSIVE TO STIMULUS CONVULSION

Table 1.4
Functions of the Liver

1. The formation and excretion of bile acids.
2. The formation and excretion of bilirubin.
3. The metabolism of nutrient substances.
4. The conversion of glucose to glycogen and the reconversion of glycogen to glucose.
5. The storage of glycogen.
6. The synthesis of urea.
7. The formation and maintenance of serum proteins.
8. The metabolism of lipoproteins.
9. The metabolism and esterification of cholesterol.
10. The metalization of proteins.
11. The amination, deamination, transamination of proteins, amino acids and peptides.
12. The metabolism of hormones.
13. The metabolism of phosphatases, oxidases, dehydrogenases and other enzymes.
14. The degradation and resynthesis of hemoglobin.
15. The metabolism of prothrombin, thrombin and other blood coagulation factors.
16. The metabolism of copper-albumin fractions.
17. The metabolism of ceruloplasmin.
18. The metabolism of iron.
19. The metabolism of zinc.
20. The neutralization of foreign metals.
21. The formation of lymph.
22. The metabolism of porphyrins.
23. The detoxication and inactivation of metabolic waste and foreign materials.
24. Reservoir of blood.
25. Water and electrocyte metabolism.
26. The metabolism of ammonia.
27. The metabolism of mucoproteins.
28. The formation of antibodies.
29. The metabolism of vitamins A, B, C, D, E, K.

1.4.1 Postulated Mechanisms Implicated in the Liver-Brain Interrelationship During Liver Failure

When considering the enigma of hepato-cerebral relationships during acute liver failure there appears to be no general agreement as to the mechanism of action (Conn and Lieberthal, 1979; Zieve, 1981; Fisher, 1982; Schafer and Jones, 1982; Jones et al., 1984). Conn and Lieberthal aptly concluded, following a review of this topic, that "when one is finished reviewing all the possible mechanisms for the biochemical pathogenesis (of hepatic encephalopathy) one is left with no completely satisfying answer and a number of quite unsatisfactory ones."

What is clear, however, is that following acute liver damage, characteristic and progressive neurological abnormalities and encephalopathy develop. While it is clear that a normally functioning liver is necessary to maintain normal brain functions, the mechanism by which the liver fulfills these vital functions is unknown. It was once believed that the liver may produce a substance (other than glucose) which is necessary for the maintenance of normal brain functions. It was found that inclusion of the liver into a cat brain perfusion circuit increased the survival time of the brain preparation (Geiger and Yamasaki, 1956). More recently, however, carefully

controlled cross-circulation experiments in rats indicated that brain function of liverless rats improved more rapidly when their aortic blood was infused into the portal vein (systemic-portal cross circulation) rather than into the jugular vein (systemic-systemic cross circulation) of a normal donor rat (Roche-Sicot et al., 1974). Thus, if hepatic coma was due to a lack of substances produced by the liver the two would be equally effective. Hepatic coma seems more likely to be related to failure of the liver to remove some toxic substances that are endogenously produced. For some time, ammonia had been considered the prime candidate. However, despite high levels of ammonia in the blood of patients with liver failure, there is only an approximate correlation between this and the depth of coma (Conn and Lieberthal, 1979). Although it may be an important factor, ammonia does not seem to induce coma directly (Cole et al., 1972). Furthermore, the electro-encephalographic changes introduced by hyper-ammonia in rats does not resemble those seen in the galactosamine induced FHF rat model (Pappas et al., 1984).

There is at present no concensus as to which "toxin" or metabolite mediates the neurological alterations in liver failure. Various theories have been proposed as follows:

1.4.1.1 Theory of Synergism

This theory has recently been reviewed in great depth by its founder (Zieve, 1979, 1981). Briefly, it states that in hepatic failure various "toxic" (i.e. those molecules with coma producing potential) substances (e.g. ammonia, mercaptans, fatty acids, and phenols -- all of which are elevated in liver failure) can accumulate and interact synergistically to produce neurological alterations and coma. Accumulation of ammonia and its effects on the central nervous system are central to the synergism hypothesis. Such a hypothesis is difficult to prove or disprove. Recently, however, an electroencephalographic analysis of comatose rats in liver failure found no support for this hypothesis (Pappas et al., 1984).

1.4.1.2 The False Neurotransmitter Hypothesis

The false neurotransmitter hypothesis of hepatic encephalopathy was introduced in 1971 by Fischer and Baldessarini. The primary abnormality is thought to be an alteration in amino acid metabolism which results in alterations in brain neurotransmitters. According to this theory, substances such as octopamine, formed by bacterial action in the colon, functioned as weak or false neurotransmitters replacing the true transmitters, norepinephrine

and dopamine. Blood levels of octopamine are indeed increased in liver failure and correlate approximately with the degree of coma (Manghani et al., 1975; Chase et al., 1977). In liver failure the plasma concentration of aromatic amino acids (tyrosine, phenylalanine, and tryptophan) increases in relation to that of the branched chain amino acids (valine, isoleucine, and leucine) and this would augment the inhibitory effect by increasing the cerebral synthesis of false neurotransmitters, e.g. octopamine, with a deficiency of the true neurotransmitters norepinephrine and dopamine (Fischer, 1975; Bloch et al., 1978; James et al., 1979) (Figure 1.8). The arousal effect of levodopa and the beneficial effect of the dopamine against bromocriptine in patients with hepatic coma would support this hypothesis (Parkes et al., 1970). However, there is strong negative evidence indicating that intraventricular injection of octopamine, in quantities sufficient to cause considerable depletion of brain norepinephrine and dopamine resulted in no change of consciousness (Zieve and Olsen, 1977). Furthermore, a recently completed controlled study on the infusion of branched-chained amino acid to correct the amino acid imbalance (alterations) in liver failure indicated that this treatment neither improves cerebral function or

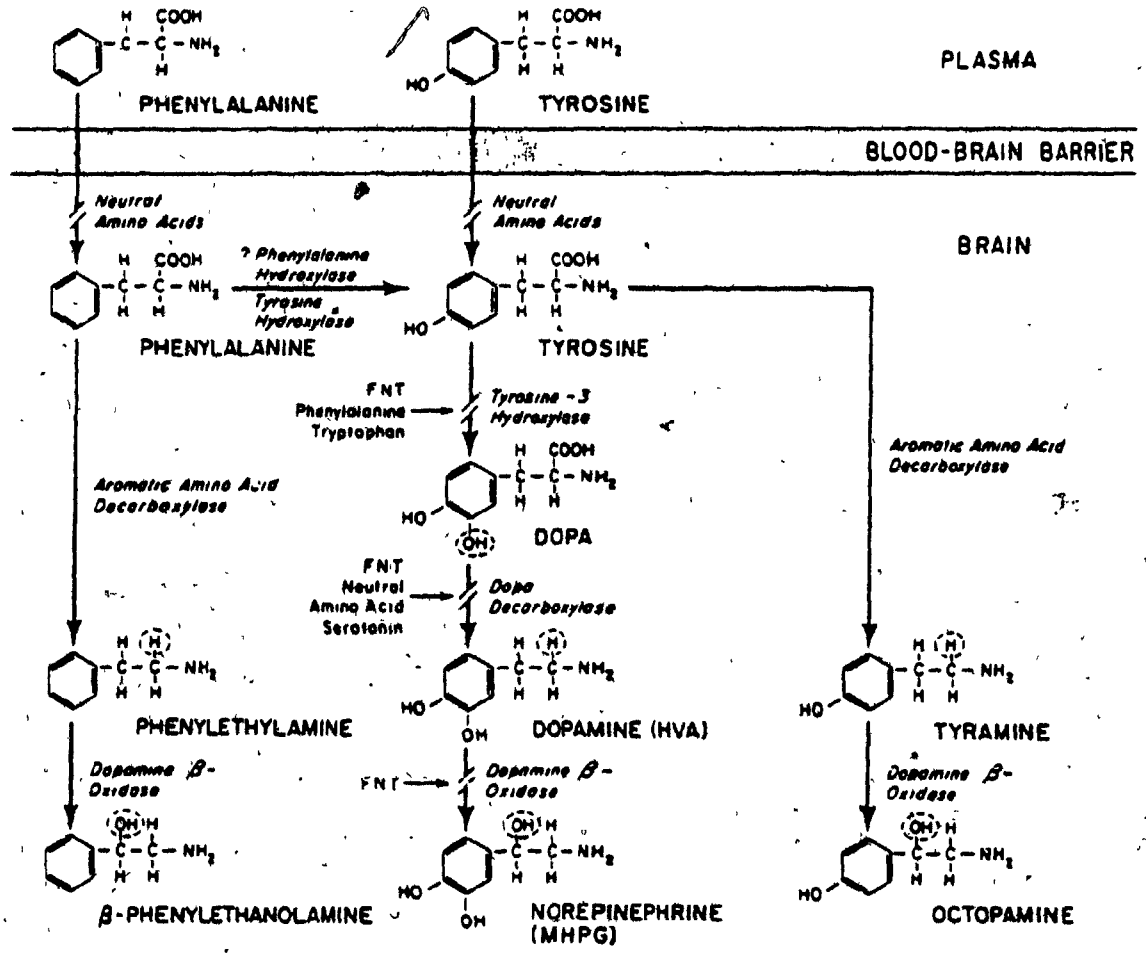


Figure 1.8: A schematic representation of the synthesis of catecholamines and phenylethylamines. Blocks in the synthetic function are indicated by lines and suggest the numerous sites at which catecholamines may be blocked. If tyrosine, for example, cannot go to dopa, it is primarily decarboxylated to tyramine and then to the false neurotransmitter (FNT), octopamine (adapted from Fischer, 1975).

decreases the mortality of patients with hepatic encephalopathy (Wahren et al., 1983), thus raising further doubt over this hypothesis.

1.4.1.3 Middle Molecule Hypothesis

Many investigators have thought that "middle molecules" or substances having a molecular weight between 500-5000 Daltons may be responsible for the neurological alterations observed during FHF (Chang and Migchelsen, 1973; Opolon et al., 1975; Denis et al., 1978; Leber et al., 1981; Contreras et al., 1982; Crossley et al., 1983). It has been postulated that build up of these and other toxic molecules may be the underlying reasons for the development of hepatic coma (Chang and Migchelsen, 1973; Holloway et al., 1979; Zieve, 1979; Chang and Lister, 1980, 1981; Gaguer et al., 1980, Hoyumpa and Schenker, 1982; Crossley et al., 1983; Denis et al., 1983; Shu and Chang, 1983). Justification for this lies in Chang's initial observation (1972) that charcoal hemoperfusion (i.e. the perfusion of the patient's blood through an extracorporeal column containing activated charcoal) resulted in the complete recovery of consciousness in patients with grade IV hepatic coma (Table 1.3). These first observations by Chang (1972) have since been confirmed at various centres

world wide (Gazzard et al., 1974; Blume et al., 1976; Chang, 1976; Schreiner, 1977; Amano et al., 1978; Bartels, 1978; Gelfand et al., 1978; Krumlowski et al., 1978; Odaka et al., 1980; Agishi et al., 1980). It has been suggested that the success of treatment by hemoperfusion may be due to the removal of middle molecules and other loosely protein-bound molecules to result in the reversal of coma (Chang and Migchelsen, 1973). Recently, microencapsulated activated charcoal has been demonstrated to significantly remove middle molecules and other toxins implicated in liver failure (Chang and Lister, 1980, 1981; Shi and Chang, 1982; Nishiki et al., 1984). Thus, it is possible that middle molecules and/or other molecules may play a role in the development of coma in liver failure.

1.4.1.4 The Gamma Aminobutyric Acid Hypothesis

The gamma-aminobutyric acid (GABA) hypothesis is currently the front runner among the various explanations for cerebral alterations during liver failure. According to this hypothesis, GABA, which is produced by bacterial action in the gut, contributes to neuroinhibition (after crossing the blood-brain barrier) by a process of post synaptic inhibition (Schafer and Jones, 1982; Jones et al., 1984). GABA, a potent inhibitor of single neurons (Krnjevic

and Phillis, 1963), is the principal inhibitory neurotransmitter of the mammalian brain; 25 - 45% of all nerve endings are GABA-ergic (Roberts et al., 1981). Recent evidence obtained from the galactosamine-induced FHF model in rabbits strongly supports this hypothesis (Pappas et al., 1984). It was seen that the pattern of post synaptic neuronal activity in liver failure and coma was similar to that in barbiturate and benzodiazepine induced coma (Schafer et al., 1984). These drugs induced their inhibitory effects by interacting with binding sites on the GABA receptor complex on post synaptic neuromembranes. The number of binding sites for GABA and benzodiazepines on postsynaptic neurons is greatly increased in liver failure (Schafer and Jones, 1982). Furthermore, those with overt hepatic encephalopathy tend to have very high GABA-like activity in their serum (Frenci et al., 1983). These observations lend further support to the GABA hypothesis and may explain the increased sensitivity of patients with cirrhosis to barbiturates and benzodizapines.

1.4.1.5 Other Theories

Other theories, such as the one currently being developed at King's College by William's group (Crossley et al, 1983), implicates that a multitude of as yet undefined

potentially toxic molecules (which are elevated in liver failure) mediate permeability changes in blood-brain barrier to enhance the neurological abnormalities occuring in liver failure (Zaki et al., 1983). In some ways it resembles the synergism hypothesis of Zieve (1981) but it also implicates the evolution of cerebral edema as a major factor for the neurological alterations. Cerebral edema is a major and frequent complication in hepatic coma (Ware et al., 1971). It has been suggested that accumulation in the blood of toxic substances, normally metabolized by the liver, may interfere with the maintenance of transmembrane ion gradients necessary for normal neuronal activity. Ammonia, along with other "toxins" and "middle molecules" which may be involved in the cerebral disturbances during liver failure, have been shown to inhibit Na^+K^+-ATPase activity (Foster et al., 1974; Seda et al., 1984). These toxins have recently been shown to also affect the permeability of the blood-brain barrier in experimental acute liver failure (Zaki et al., 1983, 1984). Thus, the inhibitory mechanism in the brain may be attributed to the increased permeability of the blood-brain barrier to "toxins" normally excluded from the brain by an intact blood-brain barrier. Additionally, a similar inhibitory

(i.e. mechanism Na^+K^+-ATPase inhibition) could also lead to concurrent development of brain edema to further complicate hepatic coma in FHF (Crossley et al., 1983a).

1.5 THE BLOOD-BRAIN BARRIER IN LIVER DISEASE

The title of this section is rather broad, at least in terms of the area it encompasses. It is not intended here to present an indepth treatise on the physiology of the blood-brain barrier (BBB), rather a brief description relevant to this thesis will be presented. Some excellent recent reviews on this topic have been published (Rapoport, 1976; Goldstein et al., 1976; Pardridge and Oldendorf, 1977; Livingstone et al., 1977; Horowitz et al., 1982; Laursen, 1982; Bradbury, 1984).

1.5.1 The Blood-Brain Barrier -- Structure and Function

The concept of the blood brain barrier (BBB) developed from the initial observations by Ehrlich (1885) and Goldmann (1909) that intravenous injections of certain analine dyes and trypan blue resulted in the vital staining of various organs except the brain. Now, nearly a century after, the physical entity of the blood-brain barrier has been defined and is described as a regulatory interface between the blood and the nervous system. The barrier

exists in the choroid plexus and essentially all areas of the brain parenchyma except the hypothalamus. The blood-brain barrier comprises of capillary endothelia (or epithelia of the choroid plexus) joined together by tight junctions that restrict intercellular diffusion. In cerebral blood vessels the astrocyte endfeet circumvent the entire vascular surface. Thus the astrocytes are also an important component to the structure as well as function of the blood-brain barrier (Zulch, 1967; Laursen, 1982). Lipid-soluble solutes easily penetrate plasma membranes and also equilibrate rapidly between blood and brain. Lipid-insoluble nonelectrolytes and proteins enter the brain (by carrier mediation) from the blood much more slowly than they enter other tissues (Rapoport, 1976). Thus far, eight independent carrier systems which mediate the influx of essential substrates into the brain have been identified as follows (Pardridge and Oldendorf, 1977):

1) Hexoses: Glucose transport through the blood-brain barrier is saturable, stereo-specific, and sodium independent. It is neither active nor energy dependent. Other hexoses competitively inhibit glucose transport. Since the transport capacity (V_{max}) for all hexoses is constant, the movement of the carrier

through the membrane, not the sugar binding, is the rate limiting step. The glucose carrier is half saturated at a serum glucose concentration of 7mM. A fall below this value will lead to a proportionate fall in therate of glucose entry into the brain and may lead to hypoglycemic brain damage.

2) **Amino Acids** (3 systems): Amino acid transport across the blood-brain barrier is mediated by facilitated diffusion, a process that is equilibratory (i.e. not concentrative), sodium and energy-independent, and stereo specific. Because the amino acid transport activities of nerve and glial cell membranes are much greater, it has been inferred that transport across the blood-brain barrier is the rate limiting step in the supply of amino acids to the brain. Three independent amino acid carriers have been identified for: a) **Acidic Amino Acids** -- glutamate and aspartate; b) **Basic Amino Acids** -- arginine, ornithine, and lysine; c) **Neutral Amino Acids** -- phenylalanine, tyrosine, tryptophan, valine, leucine, isoleucine, histidine, and threonine. The rate of entry of any particular amino acid is strongly influenced by the presence of the other competing amino acids (Rapoport, 1976).

3) Monocarboxylic Acids (e.g. lactate, pyruvate or ketone bodies): A carrier mediated, saturable, and stereo specific transport mechanism system. Transport of lactate is inversely proportional to the pH.

4) Purine Compounds (e.g. adenine)
5) Amines (e.g. choline)
6) Nucleosides (e.g. adenosine)

These have only recently been discovered and also appear to be saturable and energy-independent processes (Pardridge and Oldendorf, 1977)

1.5.2 Blood-Brain Barrier Abnormalities in Liver Failure

Because of the extremely sensitive regulatory nature of the blood-brain barrier, it has been suggested that it can be a vulnerable site in various disease states (Fishman, 1975; Rapoport, 1976). This is especially so in liver disease (Finlayson, 1982; Laursen, 1982; Hawkins et al., 1983; Goldstein, 1984).

It is now well recognized that the blood-brain barrier is damaged, or at least its permeability is significantly altered, during acute liver failure (Livingstone et al., 1977; Horowitz et al., 1983; Zaki et al., 1984). No single "toxin" is likely to account for these features. Elevation of water soluble toxins such as ammonia and amino acids, as

well as lipophilic toxins such as phenols, fatty acids, and mercaptans have all been reported in liver failure (James et al., 1979; Chang and Lister, 1980; Zieve, 1981; Denis et al., 1983). Further, these toxins can also alter the permeability of the blood-brain barrier (Zaki et al., 1983).

Using a partial hepatectomy model of liver failure, Livingstone et al. (1977) demonstrated that the blood-brain barrier had become permeable to D-sucrose, insulin, and, L-glucose (substances normally prevented from crossing the blood-brain barrier) in comatose animals. These results were recently confirmed using the galactosamine-induced fulminant hepatic failure model in rats (Zaki et al., 1984). Furthermore, these studies also demonstrated an increased blood-brain barrier permeability to amino acids in liver failure. In this same model, but in rabbits, Horowitz et al. (1983) used radio labelled isotopes to demonstrate a general or non-specific increase in permeability of the blood-brain barrier (in the gray matter) which preceeded the development of overt encephalopathy.

In other studies, Hawkins et al. (1983), using elegant quantitative autoradiographic techniques, have demonstrated increased brain uptake of amino acids in practically all regions of the brain during liver failure. It was suggested that increased uptake of amino acids may have

been due to an increase in the transport carrier density in the cerebral capillaries.

Thus far this review has been limited to permeability changes of the blood-brain barrier during liver failure. The question of total barrier breakdown during liver failure is controversial (Ousafor, 1983; Crossley et al., 1983b). Livingstone et al. (1977) have reported that intravenous trypan blue (normally excluded from the brain due to an intact blood-brain barrier) resulted in total staining of the brain in late stages of liver failure and coma. Others have not observed such changes (Hawkins et al., 1983). Differences in experimental models and time of sampling (i.e. at a particular stage of liver disease) may account for such differences. In the experimental as well as clinical situation, osmotherapy (using mannitol) for cerebral edema has shown benefit in some but not in all cases (Hoyumpa et al, 1979; Zimmerli et al., 1981; Canalese et al., 1982). This implies that osmotherapy may not reduce brain edema in those cases with damaged blood-brain barrier. Time of treatment (i.e. in early or terminal stage of liver failure and coma) may also account for these observations (Ede et al., 1982).

1.5.2.1 Brain Edema in Liver Failure

Brain edema is best defined as an increase in brain volume owing to an increase in its water content (Fishman, 1975; Rapoport, 1976). Thus, the integrity of the blood-brain barrier is the major factor in the mediation of brain edema. Chemical or physical injury to the blood-brain barrier can result in its permeability changes or even total barrier breakdown (Rapoport, 1976).

Brain edema may be classified as being either cytotoxic or vasogenic in origin (Klatzo, 1967). The former implicates a metabolic origin (possibly due to metabolic endotoxins etc.) involving cytotoxic damage to the membranous components of the blood-brain barrier (i.e. capillary endothelium and its supporting astroglial cells) to cause intracellular edema. The latter suggests physical or traumatic injury to the cerebral blood vessels, causing leakage of plasma proteins into the extracellular region of the brain, to result in extracellular edema (Figure 1.9).

The development of brain edema is perhaps the major and most frequent complication in fulminant hepatic failure (FHF) (Ware et al., 1971; Murray-Lyon et al., 1975; Berk and Popper, 1978; Ede et al., 1982). In patients it has been implicated as an immediate cause of death, possibly due to uncal and cerebellar herniation (Pirola et al.,

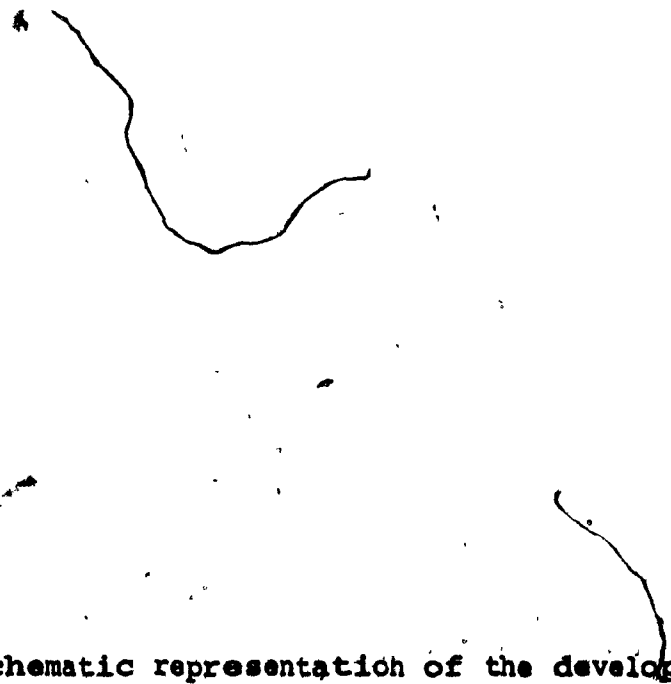
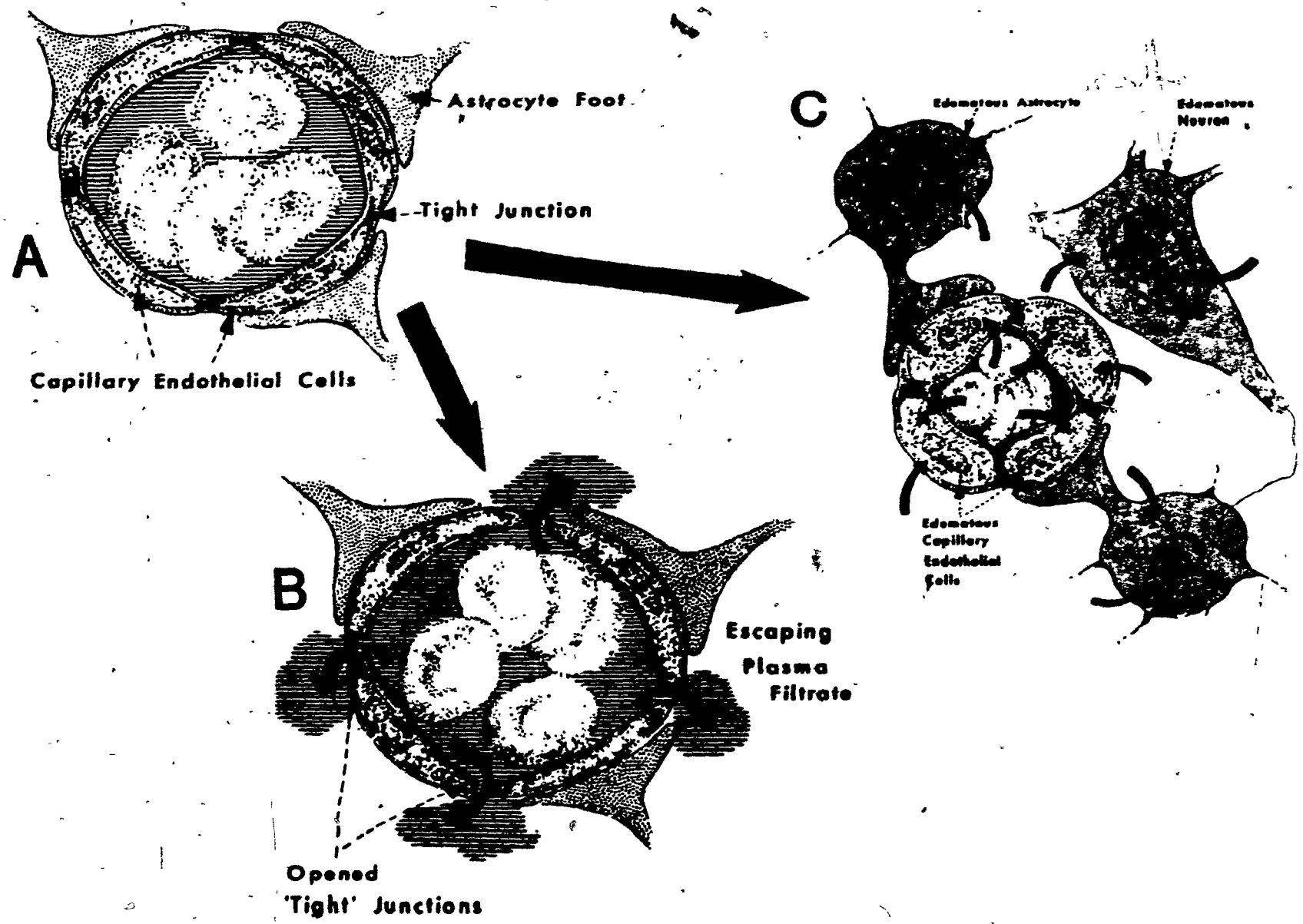


Figure 1.9: Schematic representation of the development of various types of brain edema. "A" depicts the normal situation. "B" depicts changes in permeability of the endothelial cells and tight junctions of the capillary wall in Vasogenic Brain Edema. "C" depicts swelling of the endothelial, glial, and neuronal cells during Cytotoxic Brain Edema (modified from Fishman, 1975).

TYPES OF BRAIN EDEMA



1969). Recently the development of brain edema has been implicated as a possible factor in the pathogenesis of hepatic coma (Crossley et al., 1983a; Goldstein, 1984). This, however, has yet to be proven.

The pathogenesis of brain edema during FHF is still obscure. Injury to the blood-brain barrier has been suggested to be a primary defect in the genesis of brain edema (Livingstone et al., 1977; Zaki et al., 1983). Using a partial hepatectomy model of FHF, Livingstone et al. (1977) and more recently Potvin et al. (1984) have demonstrated increases in the passive permeability of substances normally excluded from the brain by an intact blood-brain barrier. Another group, Zaki et al. (1984) has reported similar finds using the galactosamine-induced FHF rat model. Both of these groups conducted their experiments during the terminal stages of FHF (i.e. Grades III and IV hepatic coma) and have implicated the existence of vasogenic component for brain edema in this stage of liver failure. The existence of a vasogenic mechanism for brain edema formation is in contradiction to experimental and clinical observations where osmotherapy using mannitol have demonstrated beneficial effects in ameliorating cerebral edema in FHF (Zimmerli et al., 1981; Canalese et al., 1982).

Using a galactosamine-induced FHF model in rabbits, Horowitz et al. (1983) have demonstrated permeability changes in the blood-brain barrier even during very early stages (i.e. before the development of overt hepatic encephalopathy) of liver failure. It is possible that cytotoxic damage to the blood-brain barrier may be mediating these changes in barrier permeability. Recently Seda et al. (1984) have demonstrated that serum from FHF patients inhibited rat brain Na^+K^+-ATPase activity. Thus, toxins of liver failure could affect the blood-brain barrier and neuronal cell membranes, resulting in inhibition of Na^+K^+-ATPase and intracellular edema, or cytotoxic edema. This is as yet unconfirmed, as no direct evaluation of the integrity of the blood-brain barrier has been done during early stages of liver failure.

Thus, various groups have examined the existence of brain edema in FHF. These studies were performed during very early or late stages of liver failure and coma. Controlled studies evaluating the genesis and development of brain edema during progressive stages of FHF are lacking.

1.6. FULMINANT HEPATIC FAILURE

Fulminant hepatic failure has been a subject of several recent excellent reviews by renowned specialists in the field (Berk and Popper, 1978; Silk and Williams, 1979; Saunders et al., 1979; Jenkins and Williams, 1980; Knell, 1980; Jones and Schafer, 1982; Williams, 1983). The condition is defined as a clinical syndrome developing as a result of massive necrosis of liver cells in patients with no previous evidence of liver disease (Trey and Davidson, 1970). It is characterized by progressive and severe mental changes starting with confusion and often rapidly advancing to stupor, coma and death. A grading of the various stages of coma is shown in Table 1.3. The mortality in FHF is high and increases with age. FHF is also notable because it is associated with gross biochemical disturbances. These arise due to not only impairment of hepatic synthetic processes but also failure of the normal detoxification and excretory functions of the liver. Apart from their obvious involvement in the pathogenesis of hepatic coma, the biochemical malfunctions may form the basis of the various secondary complications (e.g. brain edema, bleeding, infection, etc.) that frequently occur in FHF (Ware et al., 1971; Murray-Lyon et al., 1975).

Lack of reliable epidemiologic data suggests that worldwide, the most frequent cause of FHF in the past may have been yellow fever, and that today one of the most frequent causes may be hepatitis B (Jones and Schafer, 1982). At King's College, Liver Failure Unit, the most frequent cause of FHF has been reported to be paracetamol overdose, taken with suicidal intent (Williams, 1983). Other causes of FHF have been associated with acute fatty liver of pregnancy, and other drug related liver injuries.

1.6.1 Animal Models of Fulminant Hepatic Failure

The syndrome of fulminant hepatic failure (FHF) is one of the most challenging in clinical medicine. FHF with Grade IV hepatic coma is associated with mortality rates of about 80 to 90 percent (Trey and Davidson, 1970; Rueff and Benhamon, 1973; Berk and Popper, 1978). Excluding standard intensive care, there are no general agreements in forms of active therapy (Starzl et al., 1983; Chang, 1984). Thus, in order to gain a better understanding of this condition, as well as evaluate appropriate treatment regimens, a need for a suitable animal model of FHF is of utmost importance.

1.6.2 Requirements of an Animal Model of Fulminant Hepatic Failure

In order to obtain a suitable animal model of fulminant hepatic failure, it is important to define the requirements of such a model. Five basic requirements have been described in the literature (Terblanche et al., 1975).

1) Similarity to human condition: A selective hepatic lesion (injury) should be produced which gives rise to death from liver failure, after a suitable time interval which is sufficiently long to allow experimental investigations to be carried out. The mortality rate should be comparable to that in human fulminant hepatic failure.

2) Reversibility: The hepatic failure produced should be potentially reversible to enable the animal to respond and recover with suitable treatment.

3) Reproducibility: The biochemical, neurological, and pathological (i.e. secondary complications, mortality, etc.), changes associated with FHF, should be consistent among animal groups.

4) Large animal model: The animal should be large enough to allow for repeated sampling (of blood, etc.) and enable the evaluation of experimental procedures to be applied directly to man.

5) Safety to personnel: Any drugs, techniques or micro-organisms used should endanger minimal risk to laboratory personnel.

1.6.3 Available Animal Models

Basically, there are two groups of available animal models that have been extensively used in the study of experimental fulminant hepatic failure. These are:

1) Surgically-induced model of FHF.

2) Drug-induced models of FHF.

A third group consisting of virus-induced models, e.g. murine hepatitis, has not been extensively studied. The contribution of viremia and possible neurotropic properties of the agent remain to be evaluated.

1.6.3.1 Surgically-Induced Models of Fulminant Hepatic Failure

Among the surgically-induced models of FHF basically three types exist: 1) anhepatic model (total hepatectomy), 2) partial hepatectomy model, and 3) devascularization model.

1) Anhepatic models: First described by Mann and Magath (1922), this model has been extensively studied by various groups (Terblanche et al., 1975; Livingstone et al., 1977;

Potvin et al., 1984); however, because a total hepatectomy is performed the model does not meet the requirement of reversibility. Furthermore, the absence of damaged liver cells make this model fundamentally different from human fulminant hepatic failure.

2)Partial (two-thirds) Hepatectomy: This model has also been extensively studied in various laboratories (Bollman and van Hook, 1968; Starzl et al., 1980; Ryan et al., 1982) and has been reported as a suitable model of FHF. However, the absence of damaged liver cells (i.e. necrotic liver tissue) within the body makes this model significantly different from most forms of human FHF. Nevertheless, its reproducibility and reversibility make it a suitable model for studying FHF.

3) Devascularization Model: Complete hepatic devascularization (i.e. complete deprivation of the blood supply to the liver) also produces a non reversible model, like the anhepatic models, and the graded hepatic ischaemia lacks reproducibility (Rappaport et al., 1953). This makes it an unsuitable model of FHF. Recently, with improved surgical techniques, temporary or partial devascularization has been successfully produced in the pig and other

laboratory animals (Denis et al., 1983) and has been demonstrated to be a well reproducible and satisfactory animal model of FHF.

1.6.3.2 Drug-Induced Models of Fulminant Hepatic Failure

Among the drug-induced animal models of FHF the 1) carbon tetrachloride model, and 2) the Galactosamine model have been extensively studied. Another model, the Dimethylnitrosamine model, has also been selectively studied.

1) Carbon tetrachloride model: The carbon tetrachloride model for producing FHF was first developed by Trey et al. (1969) in the monkey. It has now been successfully reproduced in several species including the rat and pig (van Leenhoff et al., 1974). Carbon tetrachloride produces liver damage in two stages in which hepatocyte necrosis is preceeded by a marked lobular degeneration. Although this model has been shown to be reversible, reproducible, and presenting features similar to human FHF, the popularity of the model is controversial. Carbon tetrachloride is highly volatile, making it very hazardous to laboratory personnel. Furthermore, the fact that carbon tetrachloride is known to also injure the lungs (by causing pulmonary edema, and

respiratory failure), and kidneys makes it unsuitable for use as an ideal model for FHF (Saunders, 1979; Chirito et al., 1978).

2) Galactosamine model: The galactosamine-induced FHF model is currently considered the one which most closely resembles human fulminant hepatic failure in practically all major aspects (Saunders, 1979). This model has been well characterized and has been studied in a variety of animals at various laboratories worldwide (Decker and Keppler, 1972; Pickering et al., 1975; Blitzer et al., 1978; Chirito et al., 1978, 1979; Niu et al., 1983; Zaki et al., 1984). It has been used extensively at the Artificial Cells and Organs Research Centre, McGill University, as a FHF model for the studies of artificial liver support systems (Chang, 1972, 1975, 1981, 1984; Chang and Lister, 1980, 1981; Chang and Migchelsen, 1973; Chang et al., 1978; Chirito et al., 1977, 1978, 1979; Dixit and Chang, 1981, 1982, 1985; Mohsini et al., 1980; Shi and Chang, 1982, 1984a,b; Shu and Chang, 1981, 1983; Tabata and Chang, 1980). The hepato-specificity of galactosamine was confirmed recently by Horowitz et al. (1983) at the N.I.H., who conclusively demonstrated that intravenously administered radiolabelled galactosamine was totally (100 percent) taken up in the liver within two hours of

administration. Virtually none of the hepatotoxin was localized in the brain, kidney, or in other organs tested.

In rats, galactosamine induces maximum liver injury 48 hrs. after its administration (Decker and Keppler, 1972; Chirito et al., 1979). During this time animals develop a characteristic hepatic encephalopathy as follows: Grade I coma -- lethargic movements and behaviour, Grade II coma -- confusion, stupor, but awake; Grade III coma -- sleeping most of the time but arousable, occasional convulsions; Grade IV coma -- unarousable and unresponsive to painful stimuli (Chang et al., 1978). Death follows rapidly after this stage, often preceeded by severe convulsions and behaviour resembling decerebrate rigidity. Changes in the biochemistry, histology, and hematology have been described and closely resemble human FHF (Blitzer et al., 1978; Chirito et al., 1978, 1979).

The biochemical basis of galactosamine hepatotoxicity lies in its metabolism by the hepatocytes. The primary lesion results from the rapid depletion of hepatic uridine nucleotides (UMP, UDP, and UTP) due to the formation of uridine derivatives of galactosamine. This leads to a secondary lesion which results from defects in macromolecular glycoprotein synthesis, giving rise to progressive organelle injury (mainly in the cell plasma

membrane) and subsequent cellular necrosis (Decker and Keppler, 1972; El-Mofty et al., 1975).

An alternate, concurrent, extrahepatic pathway for galactosamine-induced liver necrosis has also been described (Liehr et al., 1978). According to this pathway galactosamine is considered to activate a series of extra-hepatic events resulting in histaminemia, endotoxemia, and complement activation. Synergistically, these mechanisms lead to inflamation and liver cell necrosis. A summary outline of the possible events leading to galactosamine-induced hepatocyte necrosis is given in Figure 1.10.

The galactosamine-induced fulminant hepatic failure model meets all the requirements for an appropriate animal model as outlined by Terblanche et al., (1975) (cf. section I.6.2 above). It substantially represents human FHF in its clinical and biochemical manifestations, it is reproducible and potentially reversible, and not hazardous to laboratory personnel.

3) Dimethylnitrosamine model: The description of this model for the study of liver failure was first reported by Barnes and Magee in 1954. Since then dimethylnitrosamine has been shown to be a selective hepatotoxin which produces acute centrilobular necrosis of the liver in a variety of animals including mice, rats, guinea pigs, rabbits and dogs

GALACTOSAMINE INDUCED FULMINANT HEPATIC FAILURE

CLOSELY RESEMBLES HUMAN FHF
PRIMARY LESION IS CONNECTED WITH METABOLISM OF GAL N
MEMBRANE INJURY SECONDARY TO ALTERATION OF
GAL N INDUCED BIOCHEMICAL DEFECT IN GALACTOSE PATHWAY

PRIMARY LESION: GAL N ⟶ GAL N 1 PHOSPHATE ⟶ FORMATION OF UDP DERIVATIVES OF GAL N , DEPLETION OF HEPATIC UTP ,UDP-GLUCOSE , UDP-GALACTOSE

SECONDARY LESION: DEPRESSION OF URACIL NUCLEOTIDE-DEPENDENT BIOSYNTHESIS OF MACROMOLECULES (NUCLEIC ACID, GLYCOPROTEINS, GLYCOLIPIDS IN MEMBRANES, GLYCOGEN) ⟶ ORGANELLE INJURY IN VIABLE CELLS ⟶ NECROSIS OF THE LIVER

ALTERNATE (EXTRA HEPATOCELLULAR) PATHWAY:

GAL N ⟶ DEGRANULATION OF MAST CELLS ⟶

HISTAMINEMIA ⟶ EDEMA OF COLON WALL ⟶

ENDOTOXIN ABSORPTION ⟶ ENDOTOXEMIA ⟶

COMPLEMENT ACTIVATION ⟶ INFLAMMATION ⟶

LIVER CELL NECROSIS

Figure 1.10: A summary outline of the possible events involved in galactosamine-induced hepatocyte necrosis Decker and Keppler, 1972; Liehr et al., 1978).

(Madden et al., 1970; Levy, 1976, 1977). When administered intravenously, in a single bolus, dimethylnitrosamine produced uniformly lethal fulminant hepatic failure in dogs, with survival time of approximately 24 hours (Kuster and Woods, 1972). However, when orally administered, dimethylnitrosamine produces histological, biochemical, and physiological disturbances resembling those commonly observed in patients with advanced cirrhosis (Levy, 1976, 1977; Madden et al., 1970; Mortiz et al., 1973). Thus, more recently, this model has extensively been evaluated as a model for hepatic cirrhosis and ascites (Levy, 1976, 1977; Levy and Allotey, 1978). The suitability of using the dimethylnitrosamine model for studying fulminant hepatic failure requires more animal experiments, characterization, and critical evaluation.

1.6.4 Animal Model in the Present Study

The galactosamine-induced FHF model in the male Wistar rat was used in the present study for the reasons stated above. Furthermore, this model is well characterized and has been extensively studied as part of the ongoing investigations at the Artificial Cell and Organs Research Centre of McGill University (Chang, 1972, 1975, 1981, 1984; Chang and Lister, 1980, 1981; Chang and Migchelsen, 1973;

Chang et al., 1978; Chirito et al., 1977, 1978, 1979; Dixit and Chang, 1981, 1982, 1985; Mohsini et al., 1980; Shi and Chang, 1982, 1984a,b; Shu and Chang, 1981, 1983; Tabata and Chang, 1980).

1:7 AIMS AND OBJECTIVES

The aims and objectives of the present study are as follows:.

1) To obtain further basic information regarding physical, biochemical, and histological changes following galactosamine-induced hepatocyte injury in Wistar rats during various grades of hepatic coma.
2) To investigate the effects of Prostaglandin E_2 on hepatocytes following the development of galactosamine induced hepatocyte injury.
3) Fundamental studies on the evolution of brain edema in galactosamine-induced FHF are lacking. The present study was intended to obtain basic information regarding the changes in the blood-brain barrier and subsequent evolution and development of brain edema in rats following galactosamine-induced liver injury.
4) To investigate the effects of PGE_2 administration on the blood-brain barrier and the development (i.e. the progression) of brain edema in rats following galactosamine-induced hepatic injury.

CHAPTER II

MATERIALS AND METHODS

CHAPTER II
MATERIAL AND METHODS

2.1 ANIMAL MODEL

A galactosamine-induced fulminant hepatic failure (GalN-FHF) rat model was used in the following study. This model is well characterized (Decker and Keppler, 1972; Liehr et al., 1978; Saunders, 1979) and has been extensively studied at various centres world-wide (Blitzer et al., 1978; Chirito et al., 1979; Niu et al., 1983; Zaki et al., 1984). Galactosamine (GalN) is a highly specific hepatotoxin which has been shown to cause a selective hepatitis-like liver injury in various animal models (Blitzer et al., 1978; Chirito et al., 1979). Recent studies involving intravenous administration of radiolabelled (^{14}C) galactosamine have now conclusively demonstrated the hepatospecificity of the drug. Virtually 100% of the injected GalN is removed from the circulation and is sequestered in the liver within 2 hours of infusion. At this time virtually none of the radiolabelled galactosamine was detected in other organs such as the kidney or the brain (Horowitz et al., 1983). Thus galactosamine induces a consistently reproducible model for hepatocyte injury and is generally regarded as one that closely resembles human drug-induced FHF and viral hepatitis with necrosis (Saunders, 1979).

For a given strain of animal, GalN shows a consistently reproducible dose-effect relationship with regard to hepatocyte injury. In addition, the dosage of GalN required to produce the same degree of hepatocyte injury is inversely proportional to the age (weight) of the animal (Decker and Keppler, 1972).

2.1.1. Preparation of the Galactosamine-Induced Fulminant Hepatic Failure Rat Model for the Present Studies

In all of the experiments (unless otherwise stated) 63-77 day old (9-11 weeks) male Wistar rats (Canadian Breeding Farm and Laboratories Ltd., Montreal) weighing approximately 270-300g (279.4g $\pm$ 3.21g, mean $\pm$ SD) were used. Because of the age-dose relationship of GalN (Decker and Popper, 1972), it was necessary to strictly adhere to age/weight range in order to ensure a consistently reproducible liver injury. Female rats were excluded from these studies because GalN produces a diminished hepatotoxic effect during the estrus cycle (Pickering et al., 1975).

The dosage of GalN used in the following experiments was 850 mg GalN/kg body weight. This dosage was optimal in producing a mortality rate of 70% in the experimental animals described above.

All animals were housed at the animal room facility of the Artificial Cells and Organs Research Centre, McGill University. Standard size rat cages were used; one rat per cage. All animals were allowed a standard Purina Rat Chow (Ralston Purina Co., U. S. A.) diet with water ad libitum. Liver injury was induced by a single intraperitoneal injection of GalN at the above mentioned dosage. Following liver injury by GalN, the water was replaced with 0.55M (10g%) Dextrose solution which was available to the rats ad libitum. The ambient temperature was maintained at all times at 25°C.

2.1.2 Preparation of Galactosamine

Just prior to injection, D(+) galactosamine hydrochloride (GalN) (Sigma Chemical Co., St. Louis, Mo., U.S.A.) was dissolved in 300mM sodium chloride (isotonic saline), the pH was adjusted to 7.4 with 2.5N sodium hydroxide and then saline was added to obtain a final concentration of 400mM GalN, (85 mg GalN/ml saline).

2.1.3 Dose-Response of Galactosamine

In order to assess varying degrees of GalN-induced hepatocyte injury and necrosis, a dose response study of GalN was carried out. Male Wistar rats weighing 270-300g

(i.e. 63-77 days old) were used in this study. GalN doses ranging from 650-1250 mg/kg body weight were injected intraperitoneally into the rats in order to test the varying extent of hepatocyte injury.

Hepatocyte injury was measured as a function of mortality (or its reciprocal, the survival rate). That is to say the greater the liver injury the greater the mortality. A mortality rate of 70% produced a potentially reversible model for liver injury while allowing the animal to be large enough to facilitate repeated blood sampling (Chirito et al., 1977, 1978; Chang et al., 1978). On the basis of this dose response study, a dosage of 850 mg GalN/kg body weight was adopted for subsequent experiments.

2.1.4 Age-Dose Relationship of Galactosamine

The age of the animal and the dose of GalN used are critical factors in GalN-induced hepatocyte injury. Thus, in order to minimize the amount of GalN used while still maintaining an optimal size of the rats (for sampling, etc.), it was important to establish a proper age-dose response for the model used in these experiments.

As part of the present study, the age-dose relationship of GalN was assessed to further characterize

the present GalN-FHF rat model. This relationship was evaluated in two different ways as follows.

2.1.4.1 Degree of Hepatocyte Injury Constant

In this set of experiments, the age-dose relationship was assessed while keeping the mortality rate constant. In these experiments the dose of galactosamine was varied while maintaining a mortality rate of 70% in different age groups of rats.

2.1.4.2 Dose of Galactosamine Constant

In these experiments, the dosage of GalN was kept constant at 850 mg/kg body weight. At this fixed dosage, the degree of GalN-induced liver injury was assessed in different age groups of rats. N.B. -- In this and subsequent discussion hepatic injury has been considered a function of mortality (due to liver injury). Also since age and body weight are so intimately related, I have, for ease of discussion, used the rat's body weight when referring to the various age groups of rats. Table 2.1 gives age-weight conversions for the Wister rats used in these studies.

Table 2.1

Growth Tables*

Species : Wistar Rats
Sex : Male
Supplier: Canadian Breeding Farm and Laboratories, Montreal

Age											
	Days	21	28	35	42	49	56	63	70	77	84
	Weeks	3	4	5	6	7	8	9	10	11	12
Body weight (g)		35	65	100	140	185	225	260	285	300	325

* Adopted from Canadian Breeding Farm and Laboratories Ltd., Montreal, Quebec, Canada, Customer Service Catalogue.

2.1.5 Galactosamine-Induced Hepatic Injury and Accompanying Neurological Changes

Galactosamine induced liver injury (FHF) has been well documented in various animal models (Decker and Keppler, 1972; Pickering et al., 1975; Blitzer et al., 1978; Chirito et al., 1979). In the rat model maximum liver injury, as assessed by biochemical and histological evidence, occurred 48 hours after GalN injection (Decker and Keppler, 1972; Chirito et al., 1979). At these times the major secondary complications of FHF (such as neurological impairment (coma) and bleeding complications) were also evident. The development of hepatic coma in the GalN-FHF model has already been discussed in the previous chapter (Table 1.3). Briefly, the animals develop a characteristic hepatic coma as follows:

Grade I Coma:	Lethargy	24 hours after GalN injection
Grade II Coma:	Confusion and stupor, but awake	48 hours after GalN injection
Grade III Coma:	Sleeping most of the time, but arousable. Occasional convulsions.	
Grade IV Coma:	Unarousable and unresponsive to pain. Occasional convulsions.	

The first clearly distinct symptoms of neurological deterioration are apparent approximately 48 hours after GalN injection when the animals are in Grade II coma. At this stage maximum liver injury, due to GalN, has also taken place (Chirito et al., 1979). Grade II hepatic coma is rapidly followed by Grade III and Grade IV hepatic coma as the rat's level of consciousness (arousal) deteriorates precipitously. Death finally occurs within 4-6 hours after the onset of Grade II hepatic coma.

In the present study this deterioration of consciousness, as a result of GalN-FHF, has been quantitatively assessed as a function of time after GalN injection.

2.1.6 Assessment of Galactosamine-Induced Liver Injury

As previously mentioned, neurological impairment (i.e. the development of hepatic coma) is the hallmark of FHF due to acute liver injury (Rueff and Benhamou, 1973; Knell, 1980). An intimate inter-relationship also exists between the severity of GalN induced liver injury (GalN-FHF) and the deterioration of consciousness/arousal (i.e. the development of hepatic coma) in this animal model (Chang et al., 1978; Ede et al., 1982; Crossley et al., 1983; Schafer et al., 1984). The severity of GalN induced liver injury, as judged by histological, biochemical, and EEG parameters,

has been shown to correlate well with the development of hepatic coma in this animal model (Chirito et al., 1978; Pappas et al., 1984; Seda et al., 1984). Thus the grade of coma can be regarded as a convenient non-invasive technique to estimate the severity of GalN induced liver injury.

In this thesis, the severity of liver injury was estimated from the animal's grade of coma. Grade I hepatic coma is very difficult to accurately determine in the GalN-FHF rat model. It represents a stage 24 hours after GalN liver injury. At this time maximum liver injury has not yet taken place and it is possible for the animal to spontaneously recover. Galactosamine inflicts maximum injury 48 hours after injection (Decker and Keppler, 1972; Chirito et al., 1978). At this time the majority of the animals are in Grade II coma (Chang et al., 1978). Thus Grade II coma represents a stage of maximum liver injury in this animal model. Grades III and IV hepatic coma represent the terminal stages of this animal model. During these terminal stages, maximum liver injury results in various secondary complications due to a severely diminished functioning liver biomass. Death usually ensues Grade IV hepatic coma and is often preceeded by severe convulsions and loss of response to any stimuli.

In this project the GalN-FHF rat model has been further characterized by obtaining additional basic information regarding the physiological, biochemical, and histological changes which occur during various stages of GalN induced liver injury. Brain edema, a major secondary complication in FHF, was also studied. Samples from the blood, liver, and brain were obtained at various coma stages for this purpose. The detailed methodology for the various techniques and procedures that were employed have been described in relevant sections of this chapter.

2.2 PROSTAGLANDIN STUDIES

The hepatoprotective effects of PGE_2 against various hepatotoxins including GalN has been well documented (Stachura et al., 1980; Robert, 1981; Ruwart et al., 1981a,b; Ruwart et al., 1982; Miyazaki et al., 1983). However, it is as yet still unclear whether PGE_2 has any hepatoprotective or other beneficial effects after the development of GalN induced hepatic injury and ensuing coma. The prostaglandin (PG) studies in this thesis involved the investigation of the effects of PGE_2 on GalN-FHF rats after maximum GalN induced hepatocyte injury had already occurred. Because of the intimate liver-brain interrelationship following massive hepatocyte necrosis

(FHF), the following experiments were designed to study the effects of PGE_2 on both these organs.

2.2.1 Preparation of Prostaglandin E_2

Prostaglandin E_2 (PGE_2) (Sigma Chemical Co., St. Louis, Missouri, U.S.A.) was dissolved in 300mM sodium chloride to obtain a final concentration of 700mM PGE_2 (250 μg PGE_2/ml saline). A fresh solution of PGE_2 was prepared just prior to use. In the present experiments PGE_2 was injected intraperitoneally into Grade II coma GalN-FHF rats at a dosage of 100 μg PGE_2/100g body weight of rat.

2.2.2 Dose-Response of Prostaglandin E_2

The optimal dosage of PGE_2 was determined by observing its dose-response effect on GalN-FHF rats in Grade II hepatic coma. Any significant effects of PGE_2 on the GalN-FHF rats would be reflected as statistically significant changes in the survival time or survival rate of the FHF rats.

In these experiments, survival time has been defined as the duration of time (in hours) the rat is alive following GalN injection. The Survival Rate (%) is defined as the percentage of animals that survive following GalN induced

hepatocyte necrosis. The overall survival rate for a given group of animals was determined as follows:

$$\% \text{ Survival Rate (for a given group)} = \frac{\text{Number of animals that recover following GalN-FHF*}}{\text{Total number of GalN-FHF animals}} \times 100$$

*animals alive and free from coma at 96 hours after injection of GalN.

The survival rate, for a given age/weight range, is a function of the degree of hepatocyte injury (i.e. dosage of GalN). In the present experiment, the dosage of GalN was carefully adjusted to obtain a survival rate of approximately 30% (Mortality rate of 70%).

Controlled experiments were carried out using GalN-FHF rats in Grade II hepatic coma. For each experiment, the rats were randomly divided into treated and control groups containing 8-12 rats each. The former received intraperitoneal injections of PGE_2 while the latter received an equivalent volume of saline. Results were tabulated from a mean of 3-6 experiments for each of the following doses of PGE_2.

The following doses of PGE_2 were tested: 1) 1 μg PGE_2/100g body wt.; 2) 10 μg PGE_2/100g body wt.; 3) 50 μg PGE_2/100g body wt.; 4) 100 μg PGE_2/100g body wt.; 5) 200 μg PGE_2/100g body wt.

The PGE_2 was freshly prepared in physiological saline (300mM NaCl; pH 7.4) just prior to use. The PGE_2 or saline was administered intraperitoneally.

2.2.2.1 Effects of Prostaglandin E_2 During Terminal Stages of Galactosamine-Induced Fulminant Hepatic Failure

The effects of PGE_2 on the survival time and survival rate were observed in GalN-FHF animals during late stages of liver injury. In the present study, Grades III and IV hepatic coma are considered to be late or terminal stages of GalN-FHF. Prostaglandin E_2 (100 μg PGE_2/100 g body weight or equivalent volume of saline) was intraperitoneally injected into GalN-FHF rats in Grades III and IV hepatic coma. Survival time and survival rate statistics were recorded as described above.

2.2.3 Experimental Protocol

The following experimental protocol was designed to study the effects of PGE_2 on the liver and brain of GalN-FHF rats in Grade II hepatic coma. Grade I coma rats were excluded from the study because it represents a very early stage of GalN induced liver injury. The effects of PGE_2 in early stages of hepatic injury have also recently been demonstrated (Stachura et al., 1980; Ruwart et al., 1981; Miyazaki et al., 1983). Grades III and IV coma FHF

rats were similarly excluded because they represented a terminal stage of FHF.

FHF was induced in male Wistar rats as described earlier. To ensure batch to batch uniformity, animals in Grade II coma were identically matched and paired. One rat from each pair was then randomly selected to receive either PGE_2 or an equivalent volume of saline as control. All animals were sacrificed by decapitation (or by glutaraldehyde fixation in electron microscopy studies) at either 4 or 8 hrs. after PGE_2 or saline injection. Blood biochemistry, and brain and liver histology were also carried out at these times in both the PGE_2 and saline injected FHF animals. At these specific times, changes in brain water content and brain tissue swelling were also determined in the experimental animals by standard procedures and have been described in the relevant section of this thesis. Since the development of brain edema is regarded as a hallmark feature of FHF from a variety of etiologies, the integrity of the blood-brain barrier was also determined in the PGE_2 and saline injected animals. The various blood and tissue (liver and brain) samples were grouped as follows: Group A -- Normal control (no GalN); Group B -- Grade II coma GalN-FHF rat (just prior to PGE_2 or saline injection, i.e. 0 hours); Group C -- GalN-FHF rat

- 4 hrs. after saline injection; Group D -- GalN-FHF rat - 4 hrs. after PGE_2 injection; Group E -- GalN-FHF rat - 8 hrs. after PGE_2 injection. No samples were obtained for the saline injected rats at 8 hrs. because there was no survival of these rats at this time.

As an adjunct to the PGE_2 studies, similar control experiments were conducted using GalN-FHF rats with increasing severity of hepatic injury (i.e. Grades I-IV hepatic coma). As was for the PGE_2 studies, similar measurements were made to evaluate the nature of GalN-FHF and its ensuing secondary complications.

2.3 BLOOD BIOCHEMISTRY AND LIVER ENZYME ANALYSIS

2.3.1 Serum Biochemistry Analysis

A standard, routine, serum biochemistry analysis was performed using a Sequential Multiple Autoanalyzer with Computer (SMAC) (SMAC, serial #367, Technion Instrument Corp., Tarrytown, New York, U.S.A.) at the Division of Clinical Biochemistry, Royal Victoria Hospital, Montreal, Canada. At specific times (i.e. in various grades of hepatic coma during GalN-FHF, or at "0," "4," and "8" hrs. after PGE_2/saline injection -- described above, cf. Section 2.2, blood samples were obtained from sodium pentobarbitol

anesthetized animals, by heart puncture. The blood was allowed to clot in glass tubes. Following retraction of the clot, the tubes were centrifuged (at 12,000g for 5 minutes) to obtain the serum. Five hundred microlitre aliquots of serum sample were transferred to 1.0ml polypropylene Eppendorf tubes, wrapped in aluminum foil, and immediately taken to the Royal Victoria Hospital for analysis by the SMAC.

3.2.2 Liver Enzyme Analysis

Serum samples were obtained as described above. Serum samples were immediately refrigerated and analyzed, within 3-4 hrs. of sampling, for the following liver enzymes: 1) Aspartate aminotransferase (ASAT/GOT, EC 2.6.1.1); 2) Alanine aminotransferase (ALAT/GPT, EC. 2.6.1.2); 3) Alkaline phosphatase (Alk. P., EC 3.1.3.1); and 4) Lactic dehydrogenase (LDH, EC 1.1.1.27). Alk. P and LDH were measured by SMAC (described above). ASAT and ALAT were measured using commercially available kits (Boehringer Mannheim GmbH Diagnostica, Mannheim, West Germany).

2.3.2.1 Assay System For ASAT and ALAT

The assay system for both ASAT and ALAT involves a colorimetric determination (by spectrophotometer) of the

decrease in level of NADH, in the assay medium, at 340 nm (Bergmeyer et al., 1978). The assay reaction is as follows:

(1) for ASAT:

$$\alpha\text{-oxoglutarate} + \text{L-aspartate} \underset{}{\overset{\text{ASAT (GOT)}}{\rightleftharpoons}} \text{L-glutamate} + \text{oxaloacetate}$$

$$\text{oxaloacetate} + \text{NADH} + \text{H}^+ \overset{\text{MDH}}{\rightleftharpoons} \text{L-Malate} + \text{NAD}^+$$

MDH: Malate dehydrogenase

(2) for ALAT:

$$\alpha\text{-oxoglutarate} + \text{L-alanine} \overset{\text{ALAT (GPT)}}{\rightleftharpoons} \text{L-glutamate} + \text{pyruvate}$$

$$\text{pyruvate} + \text{NADH} + \text{H}^+ \overset{\text{LDH}}{\rightleftharpoons} \text{L-Lactate} + \text{NAD}^+$$

LDH: Lactate dehydrogenase

The rate of consumption of NADH, as absorbance change per minute, was measured using a Beckman DB-GT Grating Spectrophotometer and Beckman 10" Chart Recorder (Beckman Instruments Inc., Fullerton, Ca., U.S.A.).

2.4 LIVER AND BRAIN HISTOLOGY

Light and electron microscopy (LM & EM) studies were carried out to study the histological changes occurring during progressive stages of GalN-FHF (i.e. Grades 0-IV hepatic coma). Detailed tissue histology (LM/EM) of the

liver and brain was also carried out on the animals used in the PGE_2 studies. At various stages of GalN-FHF (i.e. Grades 0-IV hepatic coma) and at specific times during the PGE_2 studies (i.e. 0, 4, and 8 hrs. after PGE_2/saline injection), the animals were fixed for histology as described below. Unless otherwise indicated, all tissues (liver or brain) were handled and fixed in a similar fashion. All the animals used in this study were fixed for histology while they were still alive.

2.4.1 Light Microscopy

Light microscopy studies were carried out using standard laboratory histology techniques (Emmel and Cowdry, 1964). Briefly, the samples (liver/brain) were collected from the animals immediately following decapitation by a guillotine (Harvard Apparatus Ltd., Kent, England). Samples of liver tissue (approximately 5mm x 5mm x 2mm in size) were dissected, rinsed in isotonic saline, and fixed in Carnoy's Fluid (Willey, 1971) for 2-3 hrs. The brain tissue was excised by cracking open the cranium with a pair of miniature bone cutters. After a brief rinse in isotonic saline (to remove any blood and debris) the whole brain was fixed by immersion in Carnoy's Fluid for 2-3 hours.

Following fixation, the tissue (liver and brain) was processed through a series of dehydration, clearing, and paraffin impregnation steps using the Fisher Tissuemation tissue processor (Fisher Scientific Co., Montreal, Canada). This is an automated tissue processor requiring the following steps:

1)	70% ethanol	- 1.5 hrs.
2)	80% ethanol	- 1.5 hrs.
3)	90% ethanol	- 1.5 hrs.
4)	100% ethanol-I	- 1.5 hrs.
5)	100% ethanol-II	- 1.5 hrs.
6)	100% ethanol-III	- 1.5 hrs.
7)	100% ethanol and 100% xylene (1:1 ratio)	- 1.5 hrs.
8)	100% xylene-I	- 1.5 hrs.
9)	100% xylene-II	- 1.5 hrs.
10)	100% xylene-III	- 1.5 hrs.
11)	Molten paraffin wax I	- 2 hrs.
12)	Molten paraffin wax II	2 hrs.

After step 12 (above) the tissue was removed from the machine and embedded in fresh wax using a Thermoline PD-11625E (Sybron Corporation, Dubuque, Iowa, U.S.A.) paraffin dispenser.

The tissue blocks were fine trimmed and cut into 5 micron thin sections using an American Optical model 820 microtome (American Optical Company, Buffalo, N.Y., U.S.A.).

The slides were stained using Delafield's Hematoxylin and Eosin Y staining techniques (Willey, 1971).

2.4.2 Electron Microscopy

Ultrastructural observations of the liver and different regions of the brain were assessed in normal and comatose GalN-FHF rats in progressive stages of liver necrosis. Similar observations were also carried out in the PGE_2 studies at the specific times (i.e. 0, 4, 8 hrs. after PGE_2/saline) described above.

Using standard vascular perfusion techniques (Hayat, 1970), animals were fixed using 3.5% glutaraldehyde in 0.1M sodium cacodylate buffer, pH 7.4. Each perfusion, via heart puncture, was carried out for at least 20-25 min. using a Gilson Minipuls 2, peristaltic pump (Gilson Medical Electronics, Villiers le Bel, France). The flow rate for the fixation was 1ml/min. The liver was perfused via a separate cannula inserted into the portal vein.

To ensure complete tissue fixation, samples from the liver, cerebral cortex, cerebral white matter, cerebellum and brain stem were re-immersed in fresh ice-cold

glutaraldehyde for a further 2 hrs. This was followed by a 2 hrs. post fixation of the tissue in ice-cold 1% osmium tetroxide in 0.1M sodium cecodylate buffer, pH 7.4. Osmium tetroxide is a strong lipid fixative and is rapidly degraded by room light. The substance is highly toxic and volatile. Extreme precautions were taken to ensure the fixation was carried out in the dark and under a well ventilated fume hood.

Following fixation, the tissue samples were dehydrated in progressively increasing ethanol concentrations as was done during the light microscopy sample preparation. After dehydration the tissue samples were finally embedded in low viscosity Spurr plastic resin (J. B. EM Services, Montreal, Canada) and cured at 70°C for a minimum of 14 hrs.

Ultrathin "silver" sections (0.07 m) were obtained using a LKB ultramicrotome and diamond knife. Tissue sections were finally post stained with 4% uranyl acetate and Reynolds' lead citrate and examined under a Phillips 410 electron microscope.

2.5 BRAIN EDEMA MEASUREMENTS

Brain edema is best defined as an increase in brain volume (i.e. brain swelling) owing to an increase in its water content (Fishman, 1975; Rapoport, 1976). Since

cerebral edema is now acknowledged as a major manifestation and primary cause of death in patients with FHF (Ware et al., 1971; Ede et al., 1982), experiments were designed to determine the nature and extent of brain edema, and subsequent brain swelling, in the GalN-FHF rat model. The progression of brain edema was also studied in the animals involved in the PGE_2 studies to determine its effects (if any) on the development of brain edema in Grade II hepatic coma FHF animals.

2.5.1 Brain Water Content Measurements

Various methods of measuring brain edema have been described in the literature (Go, 1980). Of these, the most direct and simple method is by physically measuring the water content of the brain (Stewart-Wallace, 1939). Thus, the brain water content was quantitatively determined to assess the extent of brain edema in comatose FHF rats as well as those rats used in the PGE_2 studies. GalN-FHF rats in progressive grades of hepatic coma (Grades 0-IV hepatic coma) or at specific times during the PGE_2 studies (0, 4, and 8 hrs. after PGE_2/saline injection) were sacrificed by decapitation. The complete brain from each rat was carefully excised from the cranium and its "wet" weight determined using a Mettler H20T analytical balance (Fisher

Scientific Co., Montreal, Canada). Brain samples were then placed in a ventilated oven (Blue M Electric Co., U.S.A.) at 65° for at least 2 weeks to ensure complete dehydration to constant weight. Its "dry" weight thus determined, was used to calculate the brain water content (g H_2O/g dry wt.) as follows:

$$\text{Brain Water Content} = \frac{\text{Wet weight} - \text{dry weight}}{\text{Wet weight}}$$

2.5.2 Brain Swelling Analysis

As already stated in the preceding section, brain swelling is the result of increased brain volume owing to an increase in its water content. The phenomenon was first quantified in classical experiments by Elliot and Jasper in 1949 while working at the Montreal Neurological Institute. The percent swelling of the tissue caused by brain edema is calculated from changes in brain water content as follows:

$$\% \text{ Swelling} = \frac{100(E-C)}{100-E}$$

where E = % brain water content in experimental animal

C = % brain water content in normal animal

From the above equation (formula modified from Elliot and Jasper, 1949) it is apparent that small increases in brain water content represent much larger volume increases

of the brain. Thus, seemingly minor increases in brain water content can manifest as substantial brain swelling. Significant brain swelling can result in increased intracranial pressure, brain compression, altered cerebral blood flow, deranged neurological function, and coma. All of these are common symptoms of FHF patients of various etiologies (Ede et al., 1982; Berndt, 1982).

2.6 TEST FOR THE STRUCTURAL INTEGRITY OF THE BLOOD-BRAIN BARRIER

The blood-brain barrier is a regulatory interface which allows the entry of only specific substances into the brain (Rapoport, 1976). An intact blood-brain barrier normally prevents dyes such as trypan blue or Evans blue from entry into the brain to stain it. Any staining of the brain would reflect an increased permeability or physical disruption of the blood-brain barrier. With this in mind, the integrity of the blood-brain barrier was tested, at various stage of GalN-FHF, to determine the nature of the evolution of brain edema in the present animal model. The integrity of the blood-brain barrier was also tested in the animals involved in the PGE_2 studies to determine whether PGE_2 affected the integrity of this blood-brain interface following the development of GalN-FHF and coma.

Animals in progressive stages of GalN-FHF (i.e. Grades 0-IV hepatic coma) as well as those rats in the PGE_2 studies were tested for the integrity of the blood-brain barrier at specific times (i.e. 0, 4, 8 hrs. after PGE_2/saline injection), using trypan blue dye infusion techniques. In these animals, a 2% solution of trypan blue was administered intravenously via the right common carotid artery in a single bolus given at a dosage of 0.35 ml/100g body weight. The animals were anesthetized with sodium pentabarbitol prior to trypan blue infusion. Thirty minutes after the infusion of trypan blue the animals were sacrificed by guillotine and the brain was examined for any staining by the trypan blue dye.

2.7 STATISTICS

All results (where applicable) unless otherwise indicated, are expressed as mean $\pm$ standard error. Statistical significance was analyzed by the Student 't' test, using a Texas Instruments TI-55 programmable calculator.

CHAPTER III

RESULTS

CHAPTER III
RESULTS

The results presented in this chapter are basically divided into two groups: 1) characterization of the galactosamine-induced fulminant hepatic failure (GalN-FHF) rat model (Section 3.1), and 2) the studies of the effects of PGE_2 on this FHF rat model (Sections 3.2-3.3).

Section 3.1 (and its sub-sections) deals with the results from basic studies which investigate the physiological, biochemical, and histological changes occuring during progressive stages (i.e. Grades 0-IV hepatic coma) of GalN-induced hepatocyte injury. Since brain edema is often a major secondary complication of fulminant hepatic failure (FHF), a detailed study investigating the nature and evolution of this phenomenon is also presented.

The second set of results, Sections 3.2-3.3 (and their respective sub-sections) deal with the PGE_2 studies. In this section, similar biochemical and histological data (as for the previous control study) are presented to evaluate the effects of PGE_2 on Grade II coma FHF rats. The effects of PGE_2 on the development and progression of brain edema in Grade II coma FHF rats is also presented.

3.1 BASIC STUDY ON THE GALACTOSAMINE-INDUCED FULMINANT HEPATIC FAILURE RAT MODEL

In this study physiological, biochemical, and histological observations were carried out to characterize and further elucidate the nature of GalN-FHF and its major secondary complication, brain edema. A unique aspect of this study was that samples were obtained from the FHF animals during progressive stages (i.e. Grades 0-IV hepatic coma) of GalN-induced hepatic injury, to study the hepatocellular changes occurring during progressive grades of hepatic coma.

3.1.1 Dose Response of Galactosamine

The dose response curve of GalN-FHF is presented in Figure 3.1. It was observed that for a given age (weight) range, GalN produced a consistently reproducible hepatocyte injury in a positive dose-effect relationship. In the present group of animals (270-300g; 63-77 day old male Wistar rats) it was seen that 850mg GalN/kg body weight consistently produced a mortality rate of 70%. Since this was the desired mortality rate, the dosage of 850mg GalN/kg body weight was adopted for all subsequent experiments in this study.

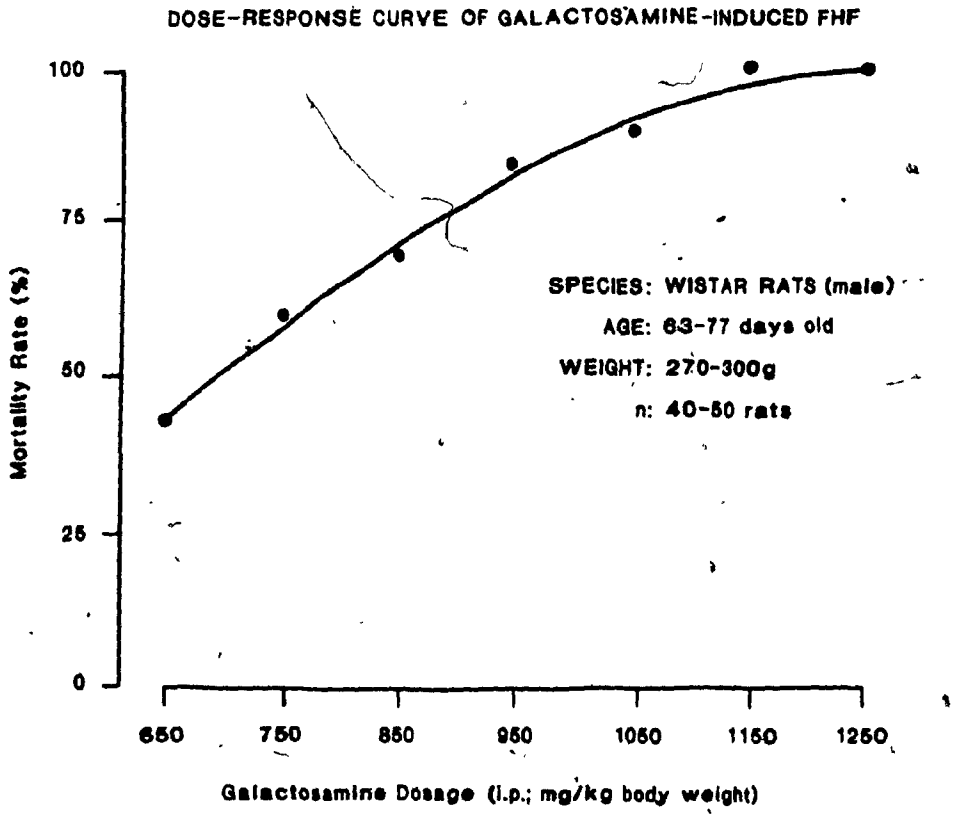


Figure 3.1: Dose-response curve of galactosamine. A positive dose-effect relationship was observed with increasing dosage of galactosamine. In the present experiment (unless otherwise indicated), a dose of 850 mg GalN/kg body weight, administered intraperitoneally, achieved a consistently reproducible mortality rate of 70%. Each point on the curve represents data from 40-50 rats.

3.1.2 Age-Dose Relationship of Galactosamine

To further characterize the GalN-FHF rat model, the age-dose relationship of GalN was evaluated in two different ways; first, by maintaining the degree of hepatocyte injury (i.e. mortality rate) constant (Figure 3.2) and second, by keeping the GalN dosage constant (Figure 3.3).

3.1.2.1 Degree of Hepatocyte Injury Constant

Figure 3.2 illustrates the relationship between the GalN dosage and the age (weight) of the animal when the mortality rate was maintained at 70%. In such conditions, it was observed that the amount of GalN required to produce a given degree of hepatic injury was inversely proportional to the age of the animal. Thus, it was seen that the younger the animal the greater the amount of GalN was required to produce the same degree of liver injury (Figure 3.2).

Since GalN is a very expensive drug, it was necessary to optimize the dose requirement while still maintaining an optimal animal size for sampling, etc. For this reason, a GalN dosage of 850 mg/kg body weight and average body weight of 275 g was adopted for all experiments in this study.

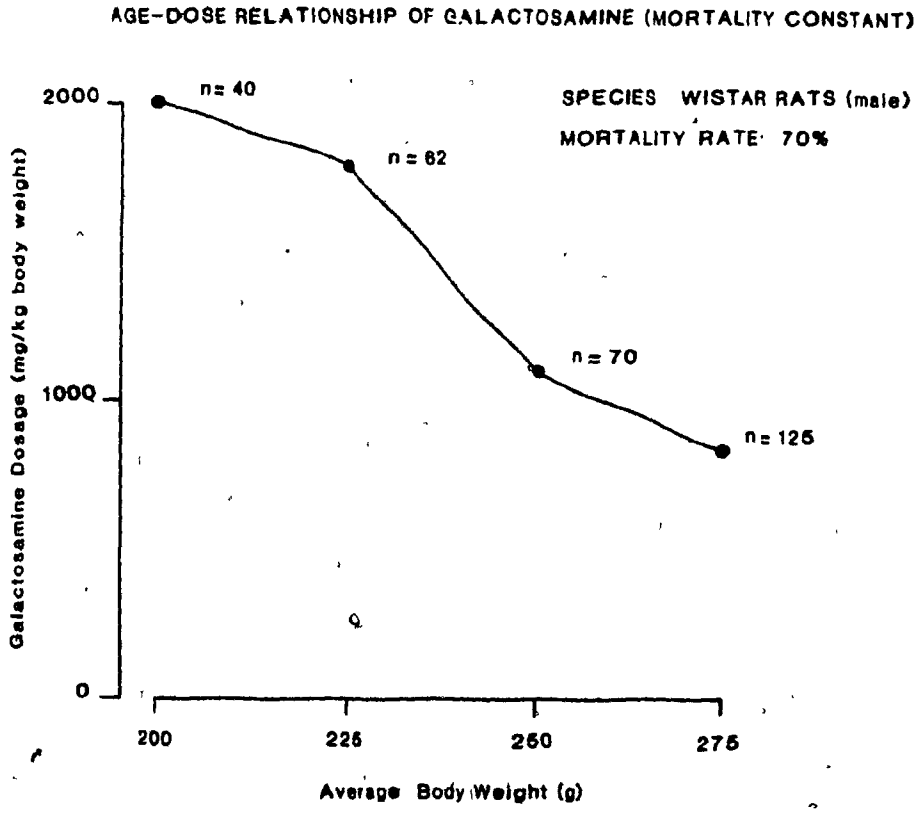


Figure 3.2: Age-dose relationship of galactosamine (mortality constant). Various doses of galactosamine were administered intraperitoneally to rats of different age (weight) groups, while maintaining the mortality rate constant at 70%. In such conditions, it was observed that the degree of galactosamine-induced hepatic injury was inversely proportional to the age of the animal. Thus, it was seen that the younger the animal, the greater the amount of galactosamine was required to produce the same degree of liver injury, and vice versa.

3.1.2.2 Dose of Galactosamine Constant

Figure 3.3 illustrates the relationship between the mortality rate and the age (weight) of the rat when the GalN dose was fixed at 850 mg/kg body weight. Under these conditions, it was observed that GalN-induced hepatotoxicity was directly proportional to the age of the animals (Figure 3.3). Thus, for a given dosage of galactosamine, the older or younger rats produced very severe, or mild degrees of hepatic injuries respectively.

For this reason it was critical to strictly adhere to the 270-300g body weight range for the experimental animals.

3.1.3 Neurological Changes Following Galactosamine Injection

Neurological changes (i.e. coma status) were assessed as a function of time after GalN injection. Figure 3.4 graphically illustrates the deterioration in consciousness following GalN injection. It was seen that during the first 24 hours after GalN injection, the neurological changes are mild (Grade I coma) and transition to deeper coma is slow. More profound neurological changes in the form of overt encephalopathy (Grade II coma) begin to take place approximately 48 hours after GalN injection. At this time the animals were in varying grades of coma (Grades

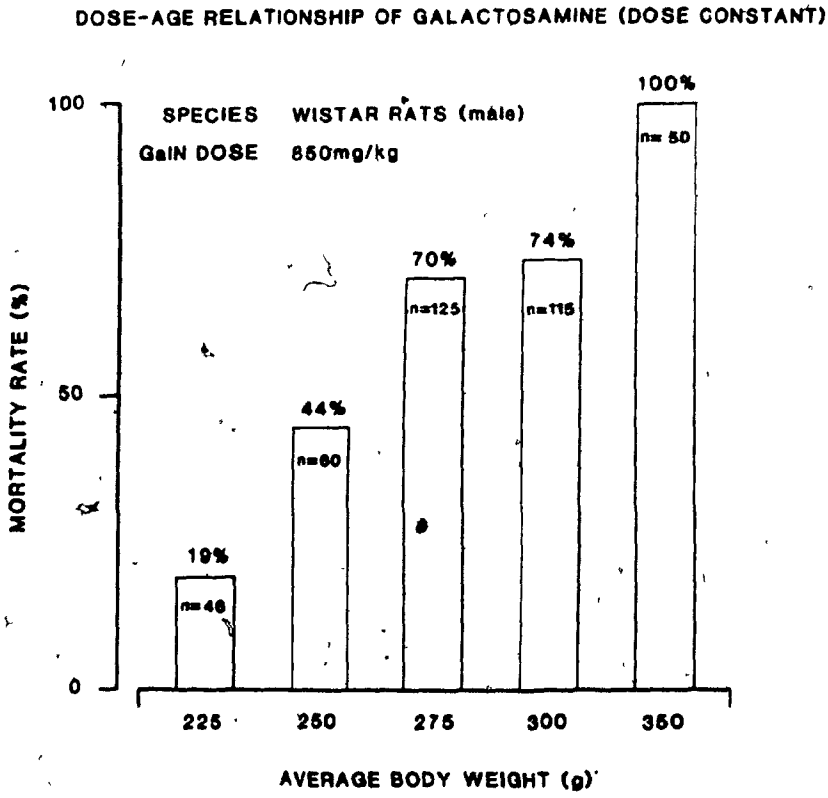


Figure 3.3: Age-dose relationship of galactosamine (GalN dose constant). A fixed galactosamine dosage (850 mg/kg body weight) was administered intraperitoneally to rats of different age (weight) groups to see its effect on the mortality rate (i.e. degree of hepatic injury). Under such conditions, it was observed that GalN-induced hepatotoxicity was directly proportional to the age of the animal. Thus, for the given dose of galactosamine (850 mg GalN/kg body weight), the older or younger rats produced very severe or mild degrees of hepatic injuries respectively.

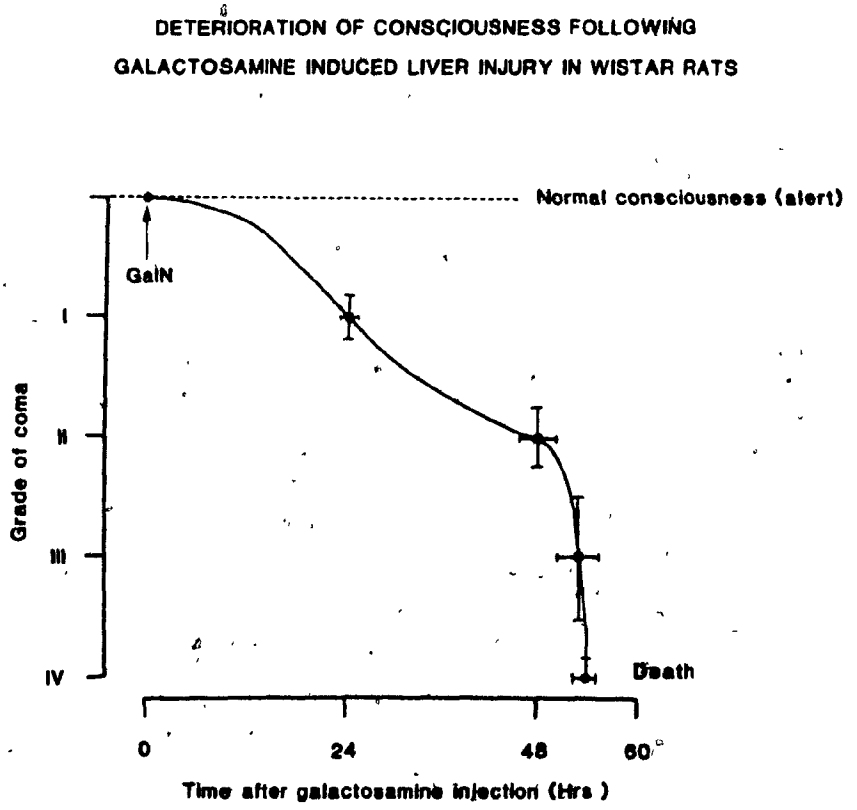


Figure 3.4: A graphic illustration of deterioration of consciousness, following galactosamine injection (arrow). During the first 24 hours after GalN injection, the neurological changes are mild (Grade I coma), and transition to deeper coma is slow. Overt encephalopathy (Grade II coma) became evident approximately 48 hours after GalN injection. At this time the animals were in varying grades of hepatic coma (i.e. Grades II-IV coma). Also noteworthy is that the transition from Grade II coma to deeper grades of hepatic coma (i.e, Grades III and IV coma) becomes highly precipitous and rapid at this time. Animals succumbed from GalN-FHF approximately 54-56 hours after GalN injection.

II-IV hepatic coma). Also at this time, the transition from Grade II coma to deeper grades of coma (i.e. Grades III and IV hepatic coma) becomes highly precipitous and rapid. Animals succumbed from GalN-FHF approximately 54-56 hours after GalN injection.

Thus, 48 hours after GalN injection represented a critical period during GalN-FHF as the animals presented varying severity of coma (and liver injury) at this time. Therefore, in order to standardize the selection of experimental animals, the animals were selected on the basis of their coma grade. This way any discrepancies arising due to varying degrees of GalN-induced hepatoctye injury were avoided.

3.1.4 Body Dehydration During Galactosamine-Induced Fulminant Hepatic Failure

Excluding the alteration of consciousness and the development of hepatic coma following GalN-FHF, the most striking observation in this animal model was a marked decrease in food and water intake following liver injury. This occurred as early as 24 hours after the injection of GalN (and before the development of overt encephalopathy, i.e. Grade I coma). This was manifested by marked dehydration and was measured as the decrease in body weight. In this

experiment, body dehydration has been expressed as the percent decrease in body weight (Table 3.1) and percent increase in hematocrit (Table 3.2). Figure 3.5 is a composite graph illustrating the respective changes of percent body weight and hematocrit of the animal following GalN-induced hepatocyte injury and development of coma. As early as Grade I coma (24 hours after GalN injection), significant dehydration was evident in the FHF rats. Significant decreases in body weight (6.28%; $p < 0.001$) were coupled by similar significant increases in hematocrit at this time. Marked dehydration was distinctly visible when the animals were in Grade II coma (48 hours after GalN injection). By this time the rat's body weight had dropped by 14.21% and its hematocrit had increased by more than 15% (of normal) to 62.62%. Although the extent of dehydration seemed to persist after Grade II coma and through to death (in Grade IV coma), there were no further significant increases in dehydration during Grades III and IV hepatic coma.

3.1.5. Blood Biochemistry During Progressive Stages of Galactosamine-Induced Fulminant Hepatic Failure

The blood biochemistry analysis was performed at the Division of Biochemistry of the Royal Victoria Hospital, Montreal, Quebec, Canada, using a Sequential Multiple

Table 3.1

Body Dehydration During Galactosamine-Induced Fulminant Hepatic Failure

Grade of Coma	Time After Galactosamine Injection (hrs)	Percent Decrease in Body Weight (mean ± SD)	n
0	0	0	70
I	24	6.28 ± 2.03*	63
II	48	14.21 ± 1.07**	43
III	48	14.92 ± 1.44$^{\Delta}$	32
IV	48	16.66 ± 1.52$^{\Delta}$	31

Statistical significance

* $p<0.001$
** $p<0.02$
} with respect to proceeding coma grade

Δ $p<0.001$ with respect to normal (Grade 0 coma) values

Table 3.2

Hematocrit Values During Galactosamine-Induced Fulminant Hepatic Failure

Grade of Coma	Time After Galactosamine Injection (hrs)	Hematocrit mean ± SD	n
0	0	46.31 ± 0.60	16
I	24	55.80 ± 1.37*	15
II	48	62.62 ± 1.99**	16
III	48	62.86 ± 1.35$^{\Delta}$	15
IV	48	64.26 ± 1.70$^{\Delta}$	15

Statistical significance

* $p<0.001$
** $p<0.02$
} with respect to proceeding coma grade

Δ $p<0.001$ with respect to normal (Grade 0 coma) values

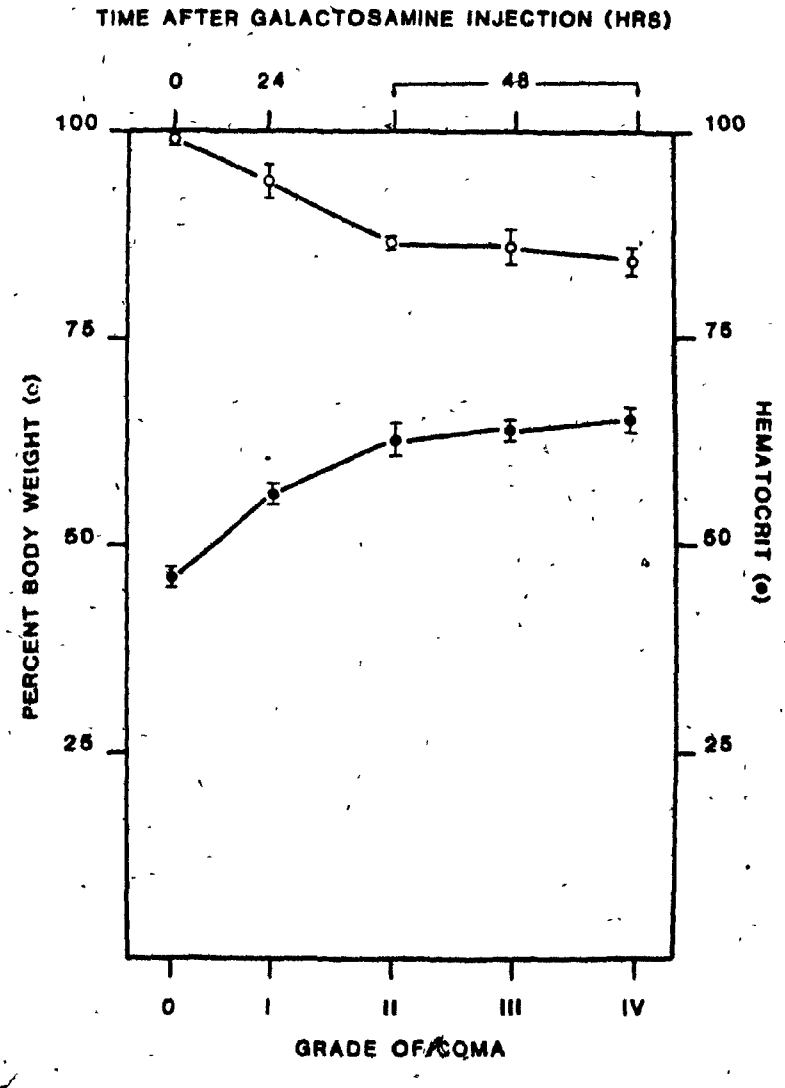


Figure 3.5: Body dehydration during galactosamine-induced fulminant hepatic failure. A composite graph illustrating the marked dehydration that occurs following GalN-FHF. Here dehydration has been measured as a function of change in body weight and hematocrit. Grade 0 coma represents the normal value before the injection of galactosamine.

Autoanalyzer with Computer (SMAC-II). The serum samples were obtained before GalN injection (i.e. Grade 0 coma) and during progressive stages of GalN-FHF (i.e. Grades I-IV coma) for biochemistry and electrolyte analysis by the SMAC.

3.1.5.1. Clinical Biochemistry Analysis

Rat sera from normal (i.e. before GalN injection) and GalN-FHF rats in progressive grades of hepatic coma were analyzed by SMAC for the following biochemical parameters: 1) Glucose (mg/dl); 2) Blood Urea Nitrogen (BUN) (mg/dl); 3) Creatinine (mg/dl); 4) Uric Acid (mg/dl); 5) Total Proteins (g/dl); 6) Albumin (g/dl); 7) Total Bilirubin (mg/dl); 8) Cholesterol (mg/dl). The clinical serum biochemical analysis of these animals is presented in Figure 3.6 and Table 3.3. A severe alteration of the rat's clinical biochemistry was seen following GalN induced hepatocyte injury and the onset of coma. Depressed levels of total proteins and albumin coupled with a precipitous fall in glucose and uric acid levels were seen with progressive GalN induced hepatocyte damage and coma. Similarly there were significant elevations in BUN, creatinine, total bilirubin, and cholesterol levels with progressive hepatocyte injury and coma (Figure 3.6 and

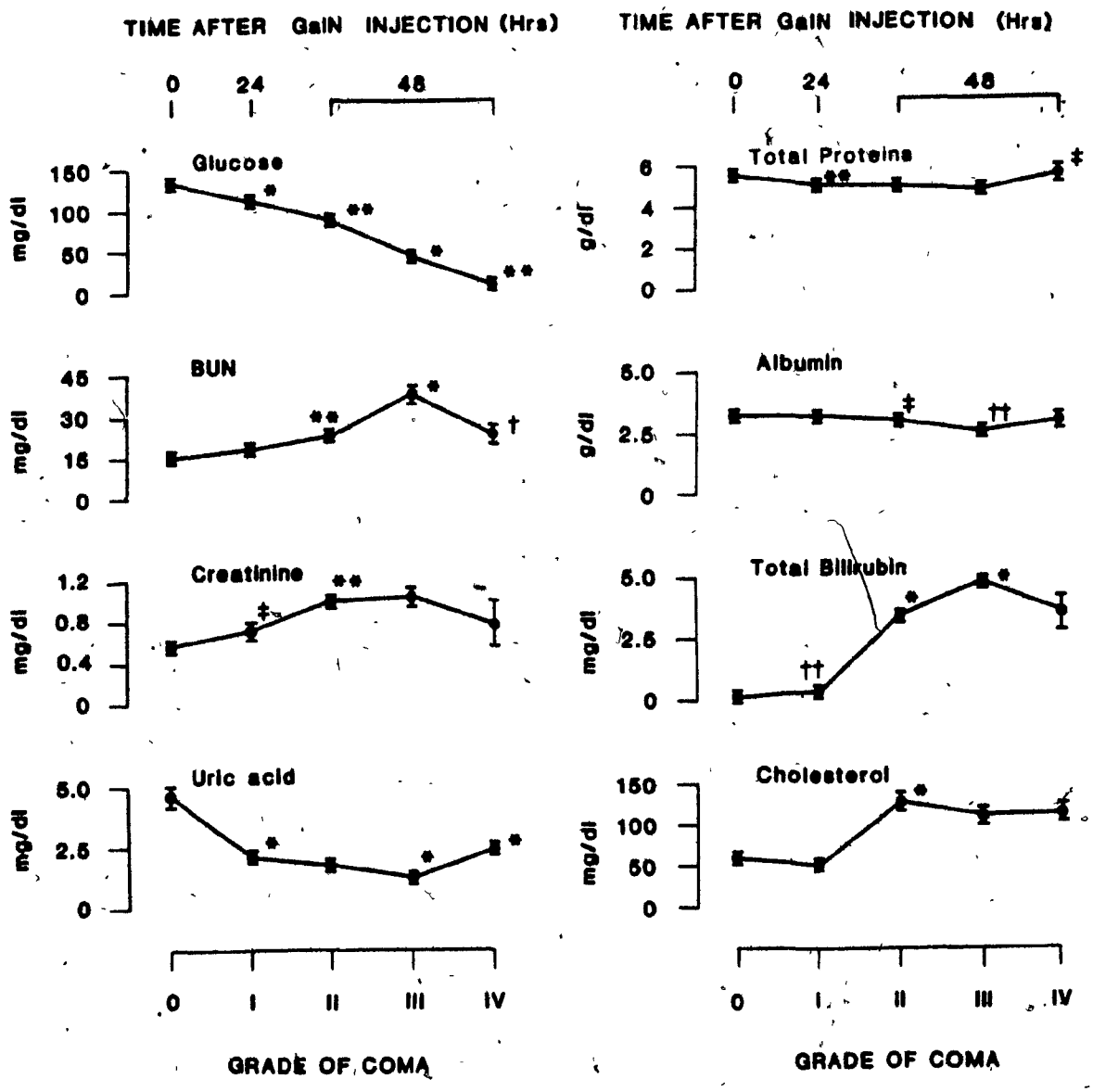


Figure 3.6: Serum biochemistry analysis during progressive stages of galactosamine-induced hepatocyte injury. Serum samples were obtained before galactosamine injection (Grade 0 coma) and during progressive stages of hepatic coma (i.e. Grades I-IV coma). Samples were analyzed by the SMAC II autoanalyzer. Statistical significance was determined using the Student 't' test.

Table 3.3). Specifically these fluctuations in clinical biochemistry were recorded as follows:

1) Glucose: A precipitous fall in glucose levels from 137.4 ± 1.75 mg/dl in normal animals (before GalN injection) to 15.0 ± 2.0 mg/dl in FHF rats (Grade IV hepatic coma) was measured during the course of GalN-FHF in this rat model. A significant ($p < 0.001$) decrease in glucose levels was measured as early as 24 hours after GalN induced liver damage, when the animals were in Grade I coma. Subsequent significant ($p < 0.01$ to $p < 0.001$) decreases in glucose levels were also measured during each progressive stage of GalN-FHF to death in Grade IV hepatic coma (Figure 3.6 and Table 3.3).

2) BUN: Galactosamine-induced hepatocyte injury resulted in a steady increase in BUN levels, by nearly 2.5 fold, which peaked at 37.0 ± 2.6 mg/dl during Grade III coma. Increased BUN levels (23.3 ± 1.47 mg/dl) first became statistically significant ($p<0.005$) during Grade II coma, 48 hours after GalN injection. The level of BUN finally fell to 24.0 ± 4.7 mg/dl just prior to death in Grade IV coma (Figure 3.6 and Table 3.3).

3) Creatinine: A steady rise in serum creatinine levels was observed following GalN-induced liver injury. Significant ($p<0.05$) elevations in serum creatinine levels

Table 3.3
Clinical Biochemistry Analysis During Galactosamine-Induced Hepatocyte Injury

Time After Galactosamine (hrs.)	0	24	48	48	48
Grade of Coma	0	I	II	III	IV
n	13	9	7	14	6
Glucose (mg/dl)	137.40 ± 1.75	115.00 ± 2.3*	97.00 ± 4.9**	44.50 ± 8.2*	15.00 ± 2.0**
BUN (mg/dl)	15.00 ± 0.48	17.00 ± 1.25	23.30 ± 1.47**	37.00 ± 2.6*	24.00 ± 4.7'
Creatinine (mg/dl)	0.58 ± 0.03	0.75 ± 0.07'	1.03 ± 0.04**	1.04 ± 0.09	0.77 ± 0.18
Uric Acid (mg/dl)	4.71 ± 0.37	2.17 ± 0.16*	1.90 ± 0.09	1.26 ± 0.05*	2.43 ± 0.17*
Total Proteins (g/dl)	5.61 ± 0.08	5.07 ± 0.15**	4.96 ± 0.10	4.75 ± 0.19	5.57 ± 0.30◇
Albumin (g/dl)	3.27 ± 0.05	3.28 ± 0.06	3.07 ± 0.07''	2.70 ± 0.11''	3.07 ± 0.24
Total Bilirubin (mg/dl)	0.10 ± 0.00	0.43 ± 0.11''	3.38 ± 0.10*	4.80 ± 0.30*	4.37 ± 0.63
Cholesterol (mg/dl)	58.50 ± 2.10	53.70 ± 4.3	129.00 ± 6.5*	118.30 ± 3.25	115.00 ± 2.03

Statistical significance with respect to preceeding coma grade
* $p<0.001$ ' $p<0.025$ ◇ $p<0.05$
** $p<0.005$ '' $p<0.01$

were first evident when the rats were in Grade I coma, 24 hours after GalN injection. At this time, a mild rise in creatinine levels to 0.75 ± 0.07 mg/dl, from 0.58 ± 0.03 mg/dl (normal value before GalN injection) was observed. The rise in creatinine levels finally peaked during Grades II and III coma at 1.03 ± 0.04 mg/dl. During the terminal stage of GalN-FHF, in Grade IV coma, creatinine levels fell to 0.77 ± 0.18 mg/dl. Owing to large standard error, this decrease in creatinine levels was not statistically significant when compared to either the peak value (1.03 ± 0.04 mg/dl during Grades II and III coma) or to the pre GalN levels (Figure 3.6 and Table 3.3).

4) Uric Acid: A significant ($p<0.001$) fall in uric acid levels was recorded 24 hours after GalN injection, when the animals were in Grade I coma. Uric acid levels continued to fall (during Grade II coma) and finally bottomed out at 1.26 ± 0.05 mg/dl in Grade III coma ($p<0.001$). An approximately two fold increase in uric acid levels ($p < 0.001$) was measured just prior to death in Grade IV coma (Figure 3.6 and Table 3.3).

5) Total Proteins: A significant decrease in total protein concentration from 5.61 ± 0.08 g/dl to 5.07 ± 0.15 g/dl was observed as early as Grade I coma, 24 hours after GalN-induced hepatocyte injury. Subsequently, the protein

levels fell only slightly and remained depressed throughout Grades II and III hepatic coma. Just prior to death in Grade IV coma, however, a significant ($p<0.05$) increase in the level of total protein to near normal (pre GalN) values was observed (Figure 3.6 and Table 3.3).

6) Albumin: As was observed for the total protein concentration, albumin concentrations were similarly depressed following GalN-FHF and coma. However, unlike total protein concentrations, the decrease in the albumin levels first became statistically significant ($p < 0.05$) during Grade II coma. At this time serum albumin levels fell from 3.27 ± 0.05 g/dl (pre GalN values) to 3.07 ± 0.07 g/dl. The serum albumin levels continued to fall ($p < 0.01$) to as low as 2.70 ± 0.11 g/dl during Grade III coma. Although a mild rise in albumin concentrations was observed just prior to death in Grade IV coma, the increase was not statistically significant (Figure 3.6 and Table 3.3).

7) Total Bilirubin: Highly significant ($p < 0.001$) increases in total bilirubin levels were recorded following GalN induced hepatocyte injury and development of hepatic coma. Increased total bilirubin levels first became evident during Grade I coma, 24 hours after GalN injection. Total bilirubin levels increased to more than 300% over normal control (no GalN) values (0.1 ± 0.00 g/dl) to $3.38 \pm$

0.10 mg/dl in Grade II coma. Total bilirubin levels finally plateaued during Grade III coma with concentrations almost as high as 50 times that of normal control values. Total bilirubin levels decreased only slightly during Grade IV coma but remained elevated until death (Figure 3.6 and Table 3.3).

8) Cholesterol: More than two fold increases in serum cholesterol levels were measured following GalN-induced hepatocyte injury. During early stages of GalN-induced liver injury (i.e. Grade I coma) no significant changes in cholesterol levels were observed. With the progression of hepatocyte injury and the development of overt hepatic coma, however, serum cholesterol levels significantly ($p < 0.001$) increased by more than two fold to 129 ± 6.5 mg/dl in Grade II coma. Cholesterol levels remained elevated throughout subsequent stages of hepatocyte necrosis to death in Grade IV coma (Figure 3.6 and Table 3.3).

3.1.5.2 Serum Electrolyte Analysis

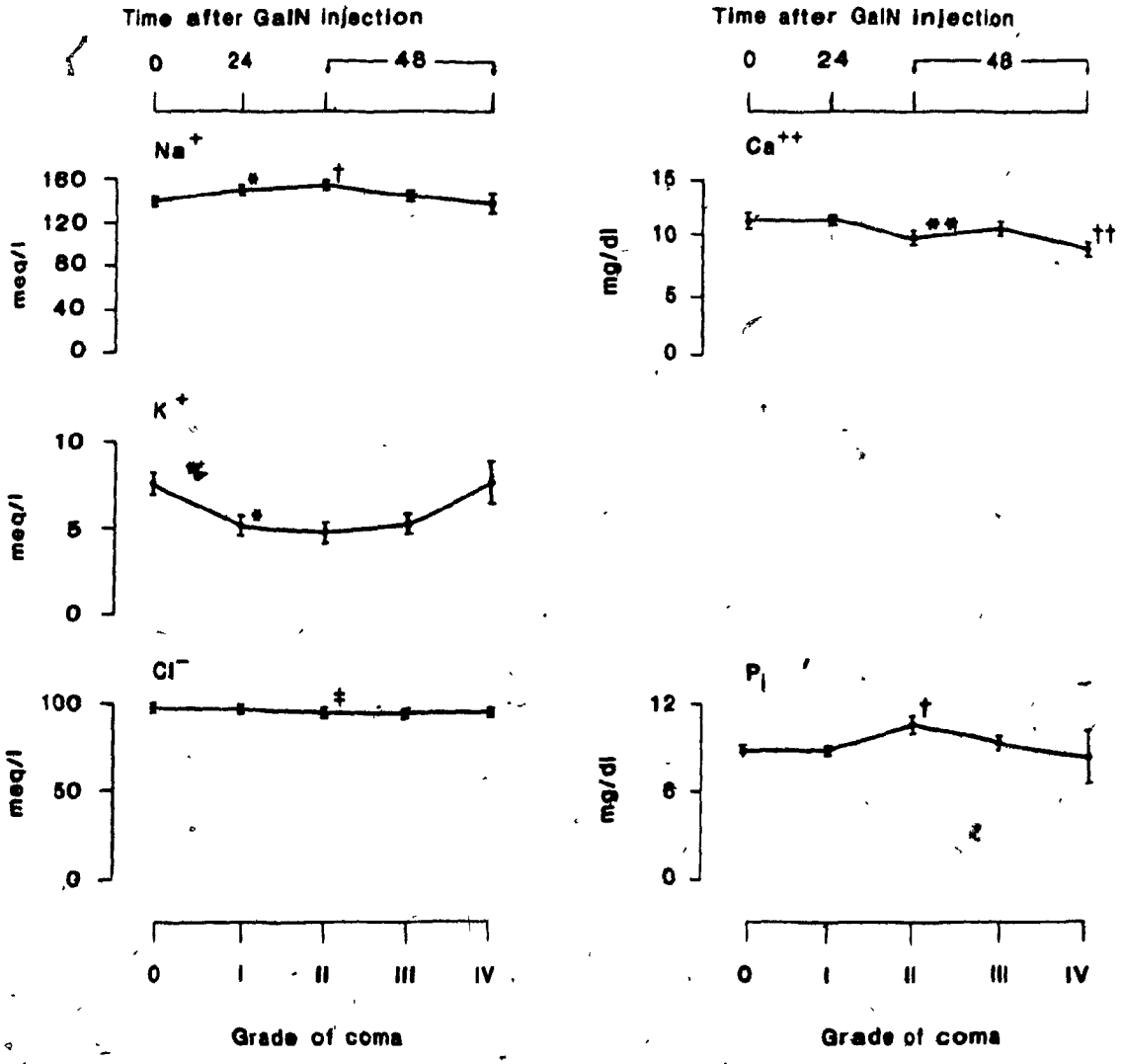
Serum electrolyte analysis was performed using the SMAC II autoanalyzer. Serum samples from normal control rats (i.e. No GalN, Grade 0 coma) and GalN-FHF rats in progressive stages of hepatic coma (i.e. Grades I-IV coma) were analyzed for the following serum electrolytes:

1) Sodium (Na^+) (meq/l); 2) Potassium (K^+) (meq/l); 3) Chloride (Cl^-) (meq/l); 4) Calcium (Ca^{++}) (mg/dl); 5) Inorganic Phosphorous (P_i) (mg/dl). Results of the serum electrolyte# analysis are presented in Figure 3.7 and Table 3.4.

Galactosamine-induced hepatocyte injury resulted in the alteration in the levels of all the electrolytes analysed. Significant ($p<0.001$) changes in Na^+ and K^+ levels were evident during the first 24 hours after GalN injections, when the rats where in Grade I coma. Levels of Cl^-, Ca^{++}, and P_i were also altered, however, these changes were statistically significant only during Grade II coma, 48 hours after GalN injection. Specific electrolyte alterations are described below;

1) Sodium: Significant ($p < 0.001$) increase in Na^+ levels from 141.4 $\pm$ 0.49 meq/l (before GalN injection, Grade 0 coma) to 147.8 $\pm$ 0.49 meq/l was detected before the onset of overt encephalopathy when the rats were in Grade I coma. With the progression of the coma to Grade II hepatic coma, a further significant ($p < 0.01$) increase in Na^+ levels was observed. In late stages, Na^+ levels appeared to decrease and approached normal control values (pre GalN levels). However, because of the large standard deviation, these latter changes were not statistically significant (Figure 3.7 and Table 3.4).

SERUM ELECTROLYTE ANALYSIS DURING PROGRESSIVE STAGES OF GALACTOSAMINE-INDUCED HEPATOCYTE INJURY



Statistical significance with preceeding coma grade

* P < 0.001 † P < 0.01 ‡ P < 0.05

** P < 0.005 †† P < 0.02

Figure 3.7: Serum electrolyte analysis during progressive stages of galactosamine-induced hepatocyte injury. Serum samples were obtained before galactosamine injection (Grade 0 coma) and during progressive stages of hepatic coma (i.e. Grades I-IV coma). Samples were analyzed by the SMAC II autoanalyzer. Statistical significance was determined by the Student 't' test.

2) Potassium: Like sodium, potassium levels were also altered during the first 24 hours after GalN injection (i.e. Grade I coma). At this time, K^+ concentrations decreased by nearly 25% of normal control values (7.4 ± 0.27 meq/l) to 5.21 ± 0.30 meq/l. During Grades II and III hepatic coma, potassium levels decreased slightly and remained depressed throughout these stages. Just prior to death, during Grade IV coma, K^+ levels were seen to approach normal control values (i.e. pre GalN levels). However, because of the large fluctuations in K^+ measurements, this restoration of K^+ to normal levels was not statistically significant (Figure 3.7 and Table 3.4).

3) Chloride: Chloride levels remained more or less stable throughout the course of GalN-FHF. Only a slight ($p < 0.05$) decrease in chloride concentration was seen during Grade II coma when chloride levels briefly fell to 95.7 ± 0.99 meq/l from normal control values of 97.8 ± 0.37 meq/l (Figure 3.7 and Table 3.4).

4) Calcium: Calcium levels remained unaffected during the first 24 hours after GalN-induced hepatic injury (Grade I coma). A significant ($p < 0.005$) decrease in Ca^{++} was first observed during Grade II coma when Ca^{++} levels fell to 9.88 ± 0.40 mg/dl from normal control values of 11,5 ± 0.22 mg/dl. No significant changes in Ca^{++} were seen during

Table 3.4

Serum Electrolyte Analysis During Galactosamine-Induced Hepatocyte Injury

Time After Galactosamine (hrs.)	0	24	48	48	48
Grade of Coma	0	I	II	III	IV
n	13	9	7	14	6
Sodium (meq/l)	141.40 ± 0.49	147.80 ± 0.49*	150.80 ± 0.87''	145.70 ± 2.6	139.30 ± 3.38
Potassium (meq/l)	7.40 ± 0.27	5.21 ± 0.30*	4.78 ± 0.38	5.30 ± 0.30	7.33 ± 1.17'
Chloride (meq/l)	97.80 ± 0.37	98.30 ± 0.37	95.70 ± 0.99◇	95.30 ± 1.30	96.00 ± 1.00
Calcium (mg/dl)	11.50 ± 0.22	11.37 ± 0.18	9.88 ± 0.40**	10.59 ± 0.46	9.13 ± 0.32'
Inorganic Phosphorous (g/dl)	9.90 ± 0.37	9.81 ± 0.08	11.84 ± 0.62''	10.45 ± 0.40	9.17 ± 1.83

Statistical significance with respect to preceeding coma grade

* $p<0.001$ '' $p<0.01$ ◇ $p<0.05$
** $p<0.005$ ' $p<0.02$

Grade III coma. Just prior to death in Grade IV coma, a further significant ($p < 0.02$) decrease in Ca^{++} levels (to 9.13 $\pm$ 0.32 mg/dl) was observed (Figure 3.7 and Table 3.4).

5) Inorganic Phosphorous (P_i): Levels of inorganic phosphorous were unaffected during the first 24 hours after GalN-induced hepatic injury. A significant ($p < 0.01$) increase in P_i levels was observed during Grade II coma when P_i levels peaked at 11.84 $\pm$ 0.62 mg/dl. A subsequent decline in P_i levels towards normal values (i.e. 9.9 $\pm$ 0.37 mg/dl) was seen during Grades III and IV coma (Figure 3.7 and Table 3.4).

3.1.6 Liver Enzyme Analysis During Progressive Stages of Galactosamine-Induced Fulminant Hepatic Failure

Serum samples from normal (Grade 0 coma) and GalN-FHF rats in progressive grades of liver injury (i.e. Grades I-IV coma) were analyzed for the following liver enzymes: 1) Aspartate Aminotransferase (ASAT); 2) Alanine Aminotransferase (ALAT); 3) Alkaline Phosphatase (Alk.P); and 4) Lactic Dehydrogenase (LDH). Liver enzyme determination from these animals is presented in Figure 3.8 and Table 3.5.

Without exceptions, GalN-induced hepatocyte injury resulted in significantly ($p<0.001$) elevated serum enzyme levels. A 2.5 to 120 fold increase (for alkaline

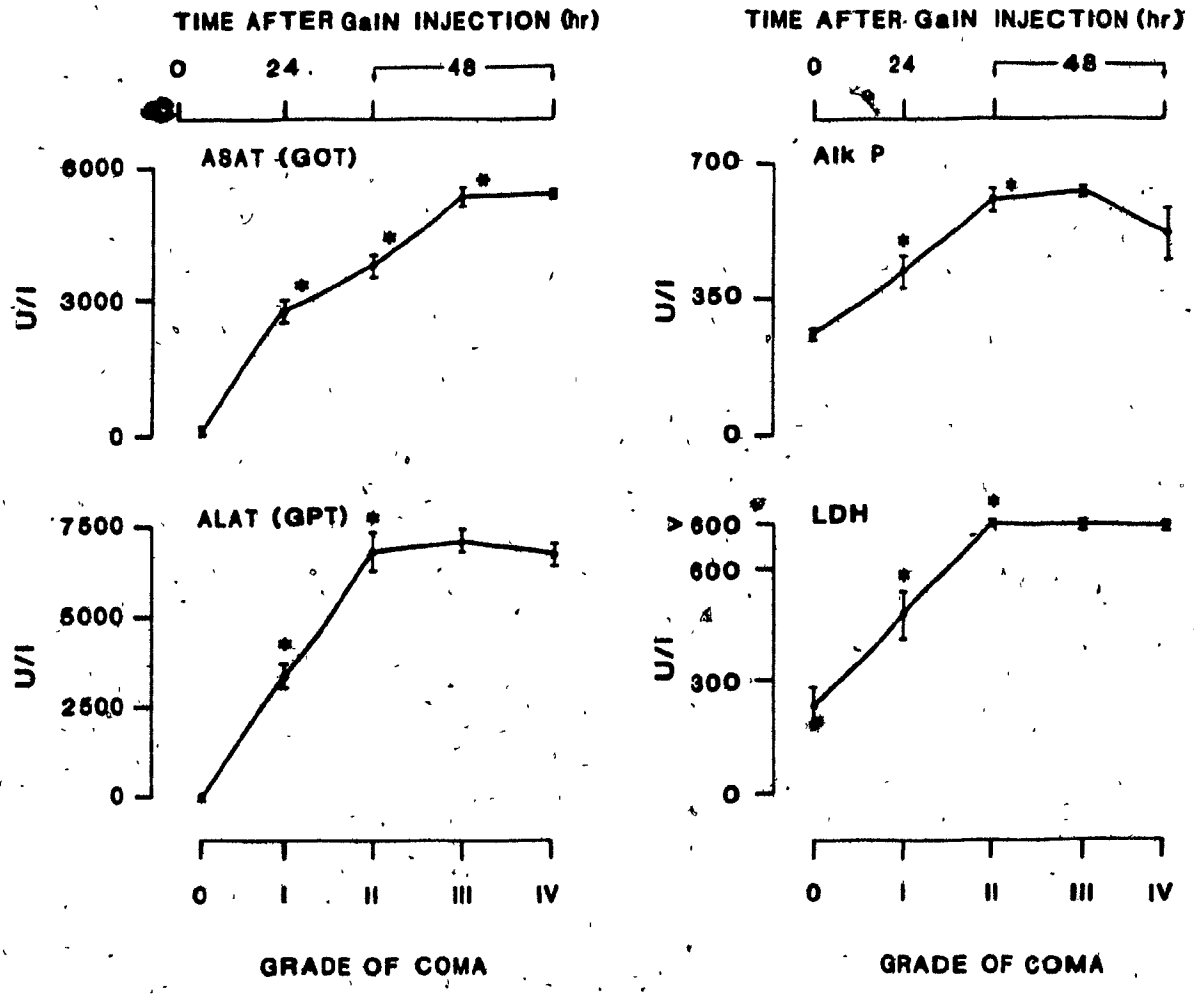
phosphatase and alanine aminotransferase respectively) in enzyme activities was seen following GalN-induced hepatic injury and coma. Increased enzyme levels correlated well with the extent of hepatic injury and grade of coma. Activities of all enzymes, with the exception of ASAT, peaked during Grade II coma, 48 hours after GalN injection. This peak also corresponded to the maximum hepatic damage taking place at this time. ASAT activity peaked during Grade III coma. All enzymes maintained their elevated levels until death in Grade IV coma.

Specific changes in serum enzyme levels, presented in Figure 3.8 and Table 3.5, are described below:

1) Aspartate Aminotransferase: Progressive increases in serum ASAT activity were observed with progressive stages of GalN-induced hepatocyte injury and subsequent development of coma. ASAT levels finally peaked during Grade III coma (5391.1 $\pm$ 138.2 U/l) when a greater than 67 fold increase in activity, over control (Grade 0 coma) levels (i.e. 80.1 $\pm$ 3.5 U/l) was observed. ASAT remained elevated till death in Grade IV coma (Figure 3.8 and Table 3.5).

2) Alanine Aminotransferase: Significant ($p < 0.001$) increases in ALAT levels were seen with progressive GalN-induced hepatic injury and the development of coma.

LIVER ENZYME ANALYSIS DURING PROGRESSIVE STAGES OF GALACTOSAMINE-INDUCED HEPATOCYTE INJURY



Statistical significance with respect to preceeding coma grade

* P< 0.001

Figure 3.8: Liver enzyme analysis during progressive stages of galactosamine-induced hepatocyte injury. Serum samples were obtained before galactosamine injection (Grade 0 coma) and during progressive stages of hepatic coma (i.e. Grades I-IV coma). Statistical significance was determined by the Student 't' test.

ALAT levels peaked during Grade II coma when a greater than 120 fold increase in activity (over control levels of 57.3 ± 4.1 U/l) was observed. ALAT remained elevated at this level (7012.0 ± 232.5 U/l) during Grade III and Grade IV coma (Figure 3.8 and Table 3.5).

3) Alkaline Phosphatase: Significant ($p < 0.001$) increases in serum Alk. P levels were observed with progressive GalN-induced hepatocyte injury and the development of hepatic coma. Alk. P levels peaked during Grade II coma when levels increased by nearly 2.5 fold to 606.3 ± 34.4 U/l. No further significant changes in Alk. P levels were seen during Grades III and IV coma (Figure 3.8 and Table 3.5).

4) Lactic Dehydrogenase: LDH levels were also significantly ($p < 0.001$) elevated with progressive GalN-induced hepatocyte injury and development of coma. LDH, like ALAT and Alk. P, also peaked during Grade II coma, when maximum GalN-induced hepatocyte injury had occurred. LDH remained at this elevated level throughout Grades III and IV coma until death (Figure 3.8 and Table 3.5).

Table 3.5

Liver Enzyme Analysis During Galactosamine-Induced Hepatocyte Injury

Time After Galactosamine (hrs.)	0	24	48	48	48
Grade of Coma	0	I	II	III	IV
n	13	9	7	14	6
ASAT (GOT) (U/l)	80.1 ± 3.5	2867.6 ± 137.1*	3756.8 ± 197.2*	5391.1 ± 138.2*	5384.3 ± 150
ALAT (GPT) (U/l)	57.3 ± 4.1	3490.7 ± 257.7*	6899.7 ± 415.8*	7012.0 ± 232.5	6678.6 ± 239
Alk. P (U/l)	269.3 ± 15.1	422.6 ± 40.6*	606.3 ± 34.4*	617.0 ± 27.6	517.7 ± 61.3
LDH (U/l)	233.8 ± 49.3	495.3 ± 49.7*	>600 (4800*)	>600 (4700)	>600

Statistical significance with respect to preceeding coma grade

* $p<0.001$

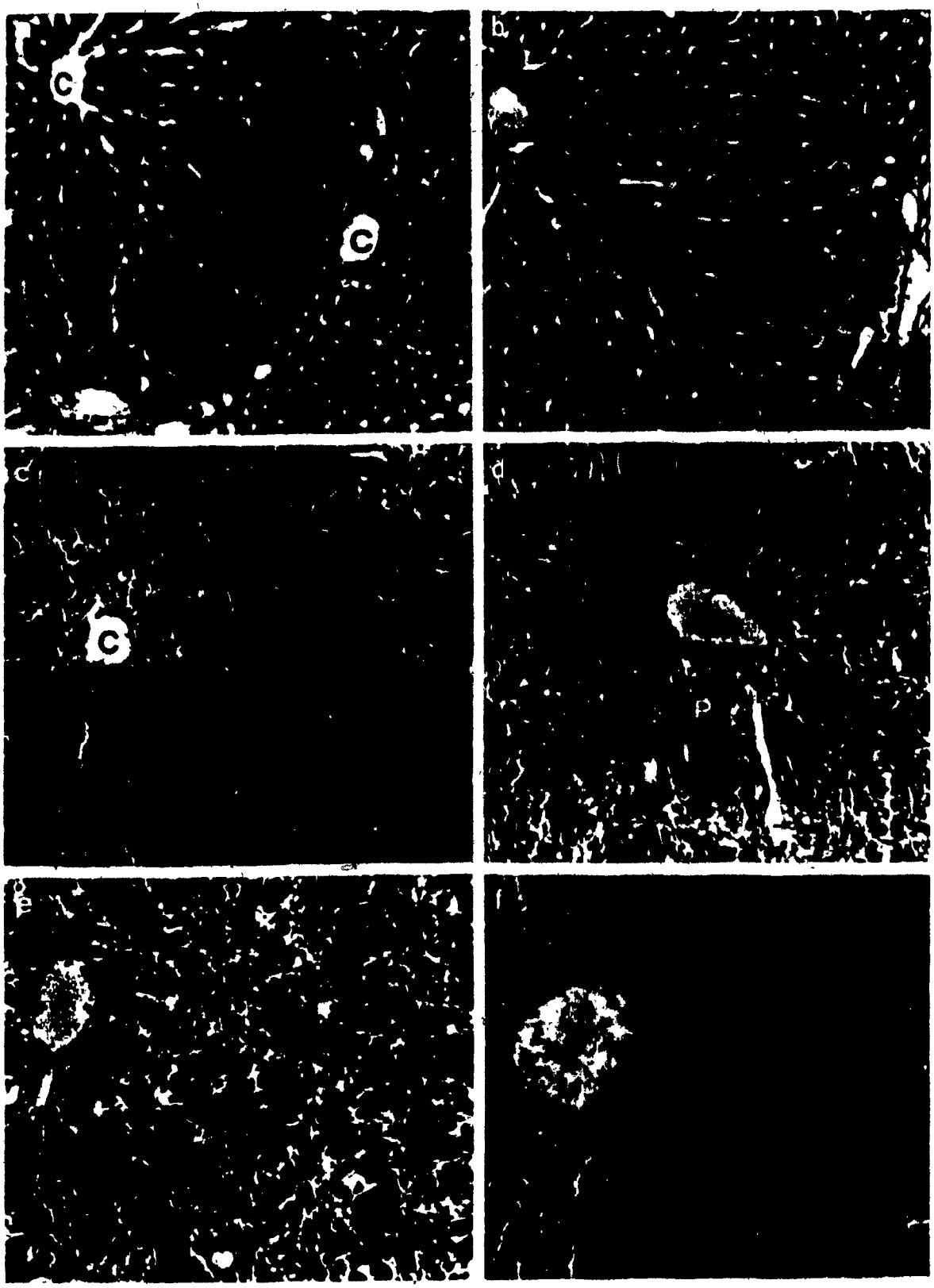
3.1.7 Liver Histology Following Galactosamine-Induced Hepatocyte Injury

Light and electron microscopy of the liver tissue were performed to study the cellular events occurring during GalN-induced hepatocyte injury. Samples of liver tissue were obtained from normal (No GalN, Grade 0 coma) and GalN-FHF animals during specific stages of liver injury (i.e. Grades I-IV coma). This has already been described in detail in the previous chapter. The histology results from these studies are presented below.

3.1.7.1 Light Microscopy

Light microscopy of the liver tissue was performed on 31 normal and GalN-FHF rats in progressive grades of hepatic coma. Each group of animals (i.e. Grades 0-IV coma) consisted of a minimum of 6 animals. Representative results of liver histology are presented in Figure 3.9. Figure 3.9a shows a liver section from a normal rat, before GalN insult (Grade 0 coma). The normal cord-like arrangement of the hepatocytes (H) within the hepatic lobule is distinctly visible. The central vein (C) and the portal area (P) are also free from any inflamatory infiltrations. During Grade I coma, 24 hours after GalN insult, no significant changes in the hepatic lobule were

Figure 3.9: Representative light microscopy observations of the liver before and after galactosamine injection. a: Normal (Grade 0 coma) liver lobule. The normal cord-like arrangement of the hepatocytes (4) within the hepatic lobule is distinctly visible. The central vein (c) and portal areas (P) are also free from any inflamatory infiltrations. b: Hepatic lobule during Grade I coma, 24 hours after GalN injection. c & d: Hepatic lobule during Grade II coma, 48 hours after GalN injection. An almost complete dissociation of the normal cord-like arrangement of the hepatocytes (H) is visible. Extensive areas of hepatocellular necrosis (H), karyolysis, and portal inflamation (P) is also evident at this time. e & f: Hepatic lobule during Grades III and IV hepatic coma respectively. Extensive hepatocellular necrosis (H), karyolysis, and inflamatory infiltrations are evident. Tissues were fixed in Carnoy's Fluid and stained, using Delafield's hematoxylin and eosin Y staining techniques. Magnification 360x.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evident (Figure 3.9b). Liver injury was maximal 48 hours after GalN injection when the animals were in Grade II coma. Figure 3.9c shows the hepatic lobule during Grade II coma. At this time, there was an almost complete dissociation of the normal cord-like arrangement of the hepatocytes (H) (Figure 3.9c). Extensive areas of hepatocellular necrosis, karyolysis, and portal inflamation were also evident at this time (Figure 3.9d). No further significant changes in hepatic histology were seen in the liver samples obtained during Grades III and IV coma (Figure 3.9e,f). Using a double-blind analysis technique, it was impossible to categorically distinguish between the hepatic histology of rats in Grades II, III, and IV hepatic coma.

3.1.7.2 Electron Microscopy

The ultrastructural events which occur following GalN-induced liver injury were studied in 25 normal (i.e. Grade 0 coma) and GalN-FHF rats in progressive stages of liver injury (i.e. Grades I-IV hepatic coma). Each group (i.e. Grades 0-IV hepatic coma) consisted of at least 5 animals. Representative electron micrographs of the liver sections from these groups of rats are presented in Figure 3.10.

Figure 3.10a depicts a normal hepatocyte before GalN injury (i.e. Grade 0 coma). A solitary nucleus (N) with numerous surrounding mitochondria (M) and endoplasmic reticulum (ER) are visible throughout the cytoplasm. The interdigitating cristae within the mitochondria is also visible at this time. Figure 3.10b depicts a hepatocyte from a FHF rat 24 hours after GalN-induced liver injury (i.e. Grade I coma). At this time, the onset of a close association between the nucleus and ER was clearly evident. The ER, once dispersed throughout the cytoplasm, now appeared to closely align itself with the nuclear membrane (Figure 3.10b). Although no significant mitochondrial aberrations were evident at this time, the interdigitation of mitochondrial cristae appeared less prominent.

With progression of hepatic injury and the development of overt encephalopathy during Grade II coma (48 hours after GalN injection), the ER was seen becoming closely associated with the nuclear membrane such that it was completely wrapped around the nucleus to envelop it (Figure 3.10c). At places, the "nucleus-ER complex" was seen to completely detach itself from the cytoplasm (Figure 3.10d). Other ultrastructural aberrations seen during Grade II coma were the abnormal elongation of the mitochondria. Three to five fold elongation of the mitochondria

Figure 3.10: Representative electron micrographs of the liver tissue from normal and GalN-FHF rats in progressive grades of hepatic coma. a: Normal (Grade 0 coma) hepatocyte before galactosamine injection. A solitary nucleus (N) with numerous surrounding mitochondria (M) and endoplasmic reticulum (ER) are visible throughout the cytoplasm. The interdigitating cristae within the mitochondria are also visible at this time. b: A hepatocyte from a GalN-FHF rat during Grade I coma. The ER, once dispersed throughout the cytoplasm, now appears to align itself with the nucleus. c & d: A hepatocyte from a GalN-FHF rat in Grade II coma. The ER becomes closely associated with the nuclear membrane such that it is completely wrapped around the nucleus to envelop it. Abnormal elongation of the mitochondria and loss of cristae are also evident at this time. e: A hepatocyte from a GalN-FHF rat in Grade III coma. Complete detachment of the nucleus from the cytoplasm and the onset of karyolysis is evident. f: A hepatocyte from a GalN-FHF rat in Grade IV coma. Complete cellular necrosis resulting from karyolysis can be seen. All samples were fixed using glutaraldehyde- and osmium tetroxide, and were post-stained with uranyl acetate and lead citrate. Bar represents 1μ.

were observed as they became stretched to assume a "dumb-bell" configuration. By this time all the mitochondria had migrated towards the nucleus, many sandwiched between the ER and the nucleus (Figure 3.10d). In the distorted mitochondria a disintegration of the cristae was clearly evident. The interdigitation of the mitochondrial cristae was also totally absent (Figure 3.10d).

During Grade III coma, the detachment of the "nucleus-ER complex" was more prominent and eventually became completely free from the remainder of the cytoplasm (Figure 3.10c). Mitochondrial aberrations during Grade III coma were similar to those observed during Grade II coma. During Grade IV coma, the complete destruction of the nucleus or karyolysis was observed (Figure 3.10f). The karyolysis resulted in the creation of a large lacunae in the place previously occupied by the nucleus and necrosis soon ensued.

3.1.8 Brain Histology Following Galactosamine-Induced Hepatocyte Injury

Brain edema has been previously reported to be the major secondary complication of fulminant hepatic failure (Ware et al., 1971). In the present study brain edema has been elaborately studied in the GalN-FHF rat model.

Extensive histological studies of the brain tissue were carried out by both light and electron microscopy. The brains from normal (i.e. Grade 0 coma) and GalN-FHF rats in progressive stages of liver injury (i.e. Grades I-IV coma) were prepared for tissue histology as previously described. Representative results from these studies are presented below.

3.1.8.1 Light Microscopy

Light microscopy of the brain tissue was performed on a total of 31 normal (Grade 0 coma) and GalN-FHF animals in progressive stages of liver injury (Grades I-IV coma). Each group of animals (Grades 0-IV coma) consisted of a minimum of 6 rats. Representative light microscopy results are presented in Figures 3.11-3.13.

The dense or compact tissue structure of the normal brain parenchyma (i.e. in Grade 0 coma rats) is shown in Figure 3.11a and 3.12a,b. Evidence of brain edema was confirmed by the presence of large ovoid spaces in the brain parenchyma (Geschickter and Cannon, 1963; Triep, 1978). No distinct evidence of brain edema was visible in Grade I coma rats and their brain (cerebral and cerebellar) histology was indistinguishable from control animals receiving no GalN (i.e. Grade 0 coma) (Figures 3.11a,b and

3.12a-d). In contrast, extensive evidence of brain edema was found in all the GalN-FHF animals displaying signs of overt encephalopathy (i.e. Grades II-IV coma).

In these animals the presence of extensive brain edema (E) was visible in the form of large ovoid spaces which were seen scattered throughout the cerebral and cerebellar parenchyma, creating swelling and distortion of the brain tissue (Figures 3.11c-f, 3.12e,f, and 3.13a-d). Although a progressive increase in the presence of brain edema (as estimated by the number and size of the edematous spaces (E) was seen with increasing severity of coma (Figures 3.11-3.13), it was not possible to adequately elucidate the mechanism(s) involved in the development of brain edema by light microscopy alone. To further study this, electron microscopic investigations were carried out to observe the ultrastructural events involved in the development of brain edema during liver failure.

3.1.8.2 Electron Microscopy

The ultrastructural changes occurring during the development of brain edema were examined by electron microscopy studies performed on 25 normal (i.e. Grade 0 coma) and GalN-FHF rats in progressive stages of liver injury (i.e. Grades I-IV). Each group of animals (i.e.

Figure 3.11: Representative light microscopy observations of the cerebral tissue from normal and GalN-FHF rats. a: Cerebral parenchyma of a normal (Grade 0 coma) rat before galactosamine injection. A dense and compact tissue structure of the cerebral parenchyma can be seen. b: Cerebral tissue from a GalN-FHF rat in Grade I coma. Evidence of very mild brain edema was visible at this time. c: Cerebral tissue from a GalN-FHF rat in Grade II coma. Extensive evidence of cerebral edema (E) was visible at this time. d: Cerebral tissue from a GalN-FHF rat in Grade III coma. Extensive widespread cerebral edema (E) can be seen throughout the cerebral parenchyma. e & f: Cerebral parenchyma from a GalN-FHF rat in Grade IV coma. Extensive brain edema, in the form of large ovoid spaces (E), was seen scattered throughout the cerebral parenchyma, creating swelling and distortion of the brain tissue. Tissues were fixed in Carnoy's Fluid and stained using Delafield's hematoxylin and eosin Y staining techniques. Magnification 360x.

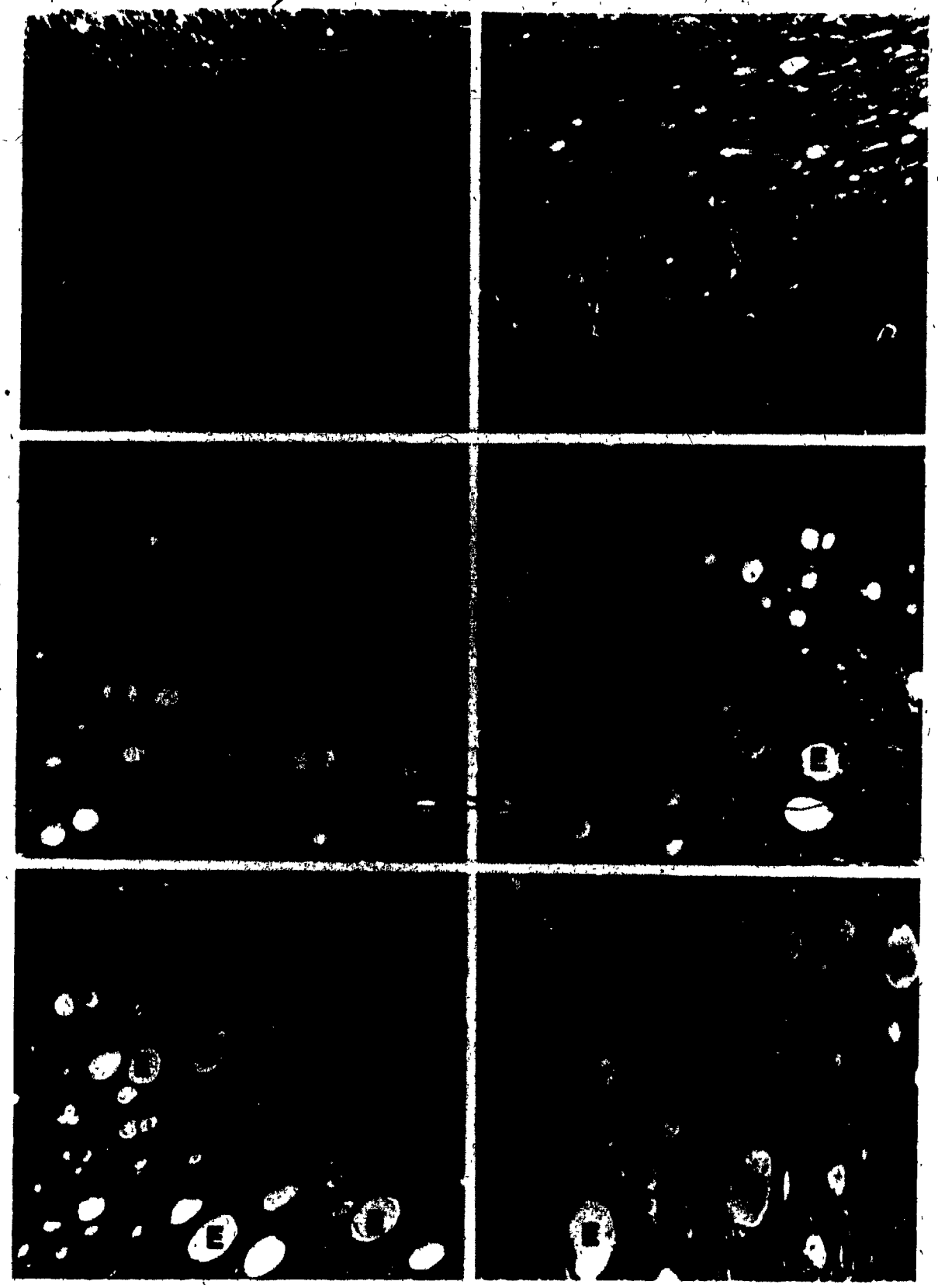


Figure 3.12: Representative light microscopy observations of the cerebellar tissue from normal and GalN-FHF rats. a & b: Cerebellar parenchyma of a normal (Grade 0 coma) rat before galactosamine injection. A dense and compact tissue structure of the cerebellar parenchyma can be seen. c & d: Cerebellar tissue from a GalN-FHF rat in Grade I coma. Evidence of only very mild brain edema was visible. e & f: Cerebellar tissue from a GalN-FHF rat in Grade II coma. Extensive cerebellar edema (E) was visible at this time. Evidence of tissue compression and distortion is also visible. Tissues were fixed in Carnoy's Fluid and stained using Delafield's hematoxylin and eosin Y staining techniques. Magnification 360x.

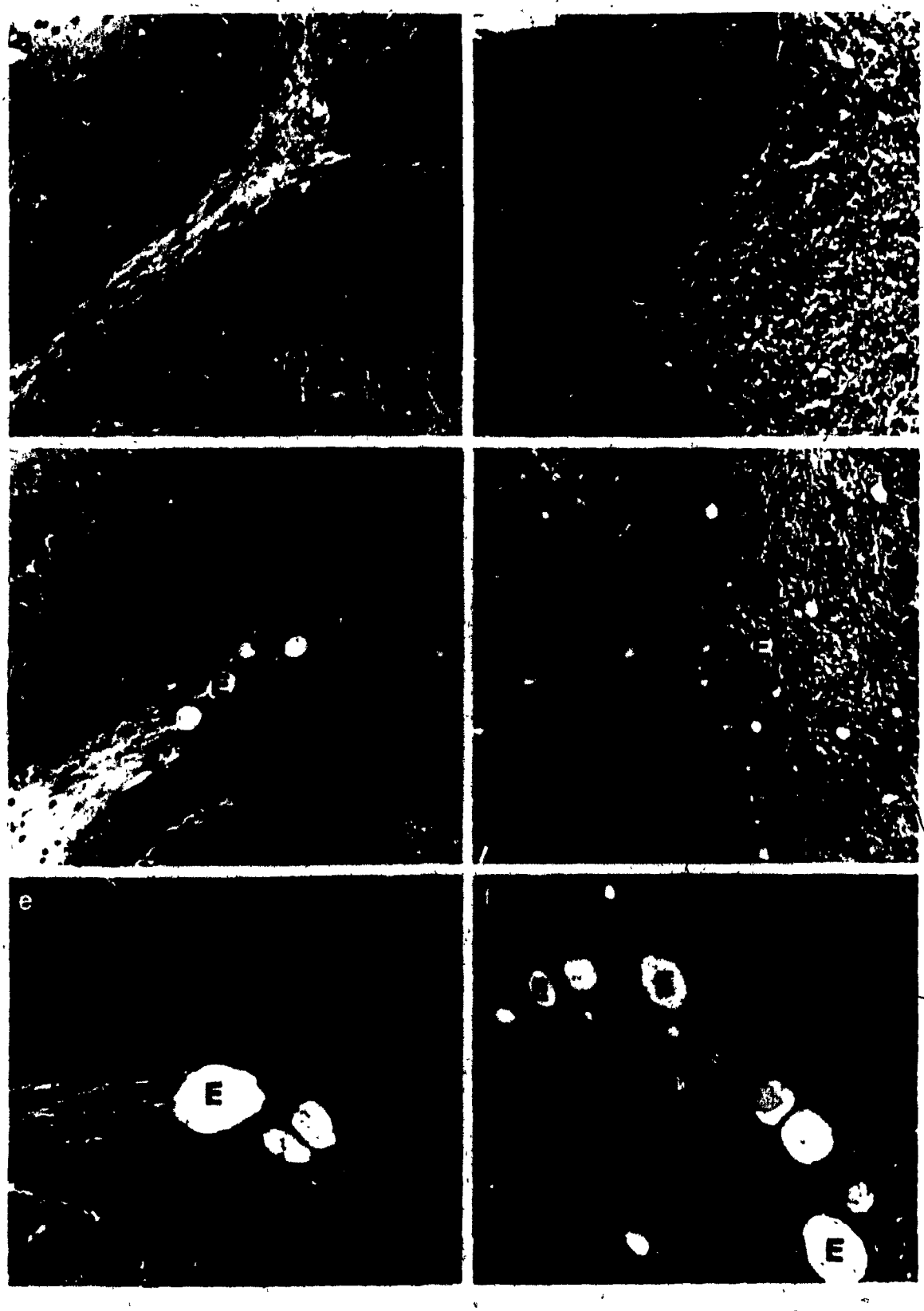
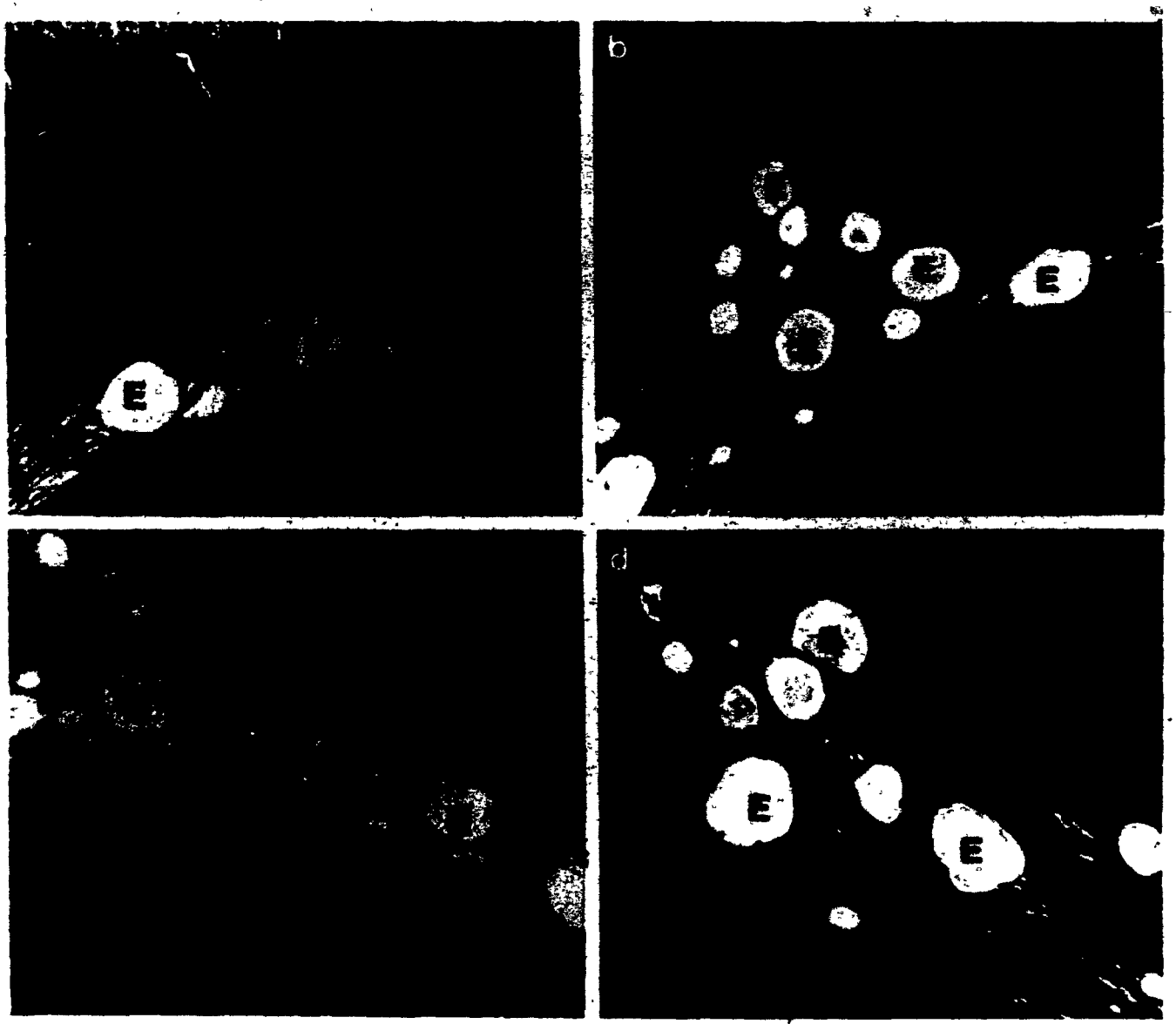

e
E
E

Figure 3.13: Representative light microscopy observations of the cerebellar tissue from GalN-FHF rats. a & b: Cerebellar tissue from a GalN-FHF rat in Grade III coma. Marked tissue edema (E) is visible throughout the cerebellar parenchyma. c & d: Cerebellar tissue from a GalN-FHF rat in Grade IV coma. Extensive tissue edema (E) can be seen throughout the cerebellar parenchyma. Tissue distortion and compression is also distinctly visible. Tissues were fixed in Carnoy's Fluid and stained using Delafield's hematoxylin and eosin Y staining techniques. Magnification 360x.


b
E
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E

Figure 3.14: Capillary from the cerebral cortex of a normal rat, before galactosamine injection and no liver injury or coma (Grade 0 coma). A compact cerebral parenchyma with no visible evidence of any abnormal astroglial (AG) swelling is evident. L: capillary lumen. Sample was fixed using glutaraldehyde and osmium tetroxide, and was post-stained with uranyl acetate and lead citrate. Bar represents 1μ.

Grades 0-IV coma) consisted of 5 animals. Representative results from these groups are presented in Figure 3.14 and Figure 3.15. Figure 3.14 depicts a cerebral capillary of a normal rat with no liver injury or coma (Grade 0 coma). A compact cerebral parenchyma with no evidence of any abnormal astroglial (AG) swelling is visible. Figure 3.15a shows a typical cerebral capillary of rats in Grade I coma, 24 hours after GalN-induced liver injury. At this time, despite slight astroglial swelling, no major changes were evident in comparison with normal rats.

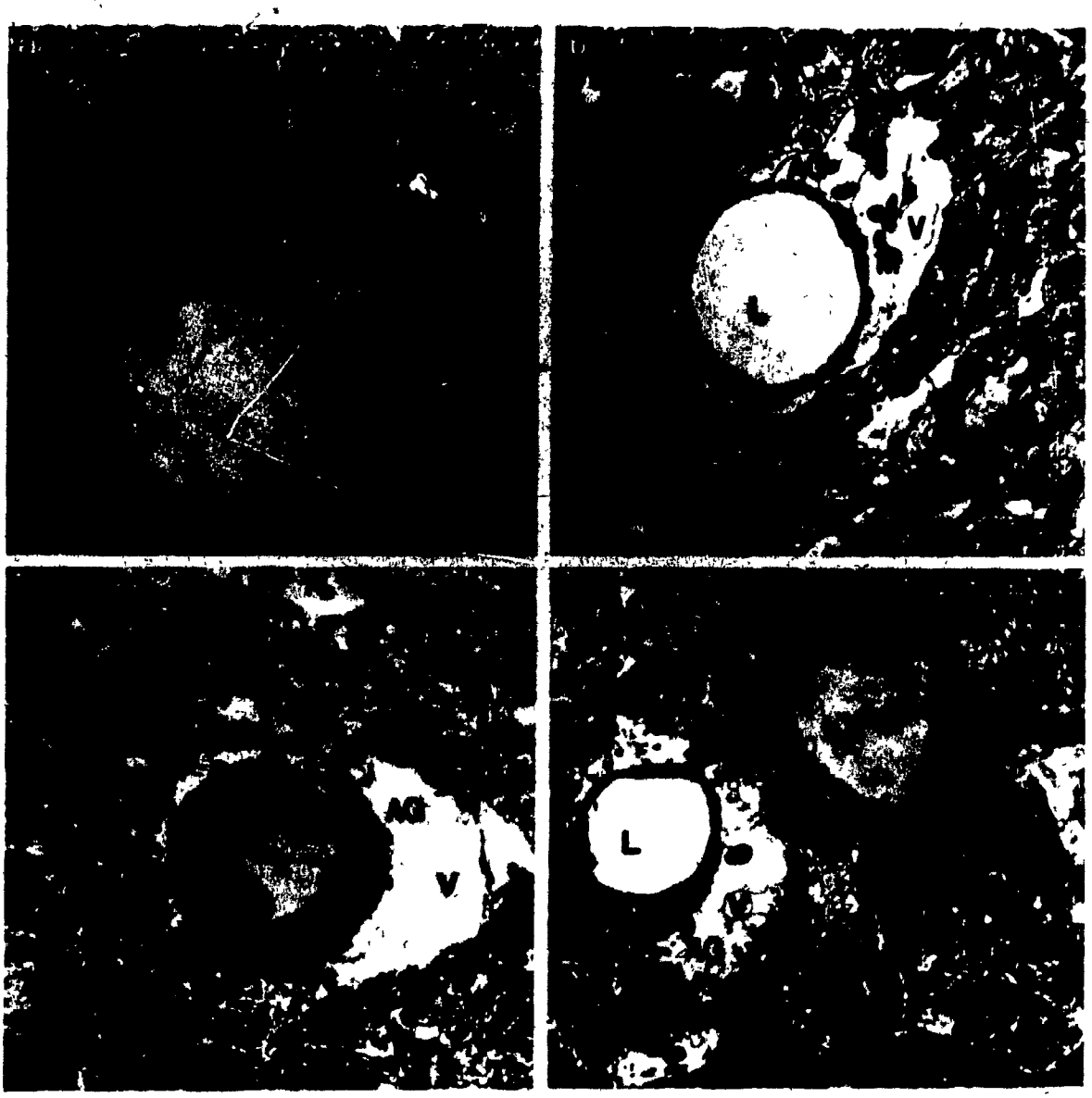
Galactosamine results in maximum liver damage 48 hours after its administration. At this time, neurological abnormalities such as coma are also distinctly visible. Figure 3.15b shows a typical cerebral capillary of a rat in Grade II hepatic coma. Extensive swelling of the astroglial (AG) cytoplasm can be seen in the pericapillary astrocyte. Abnormal presence of cytoplasmic vacuoles (V) and swollen mitochondria were seen throughout the brain tissue, especially in the grey matter in the region of the brain stem and candate nucleus. Figure 3.15c depicts a cerebral capillary from a rat in Grade III hepatic coma. Extensive astroglial swelling and tissue necrosis can be seen in the pericapillary astrocyte (AG). Evidence of large cytoplasmic vacuoles as well as tissue distortion was

also prominent at this stage (i.e. Grade III coma). Figure 3.15d depicts a cerebral capillary of GalN-FHF rats in Grade IV coma. As was the case during Grade III coma, animals in Grade IV coma also showed evidence of extensive astroglial swelling with tissue necrosis (Figure 3.15d, see arrow). Mitochondrial swelling, abnormal presence of vacuoles (V), and other cytoplasmic aberrations were evident throughout the cerebral parenchyma. Evidence of tissue compression and distortion due to brain edema and swelling was similar in animals manifesting Grade III and Grade IV hepatic coma.

3.1.9 Evaluation of the Evolution of Brain Edema During Galactosamine-Induced Fulminant Hepatic Failure

In the present section, a quantitative evaluation of the evolution of brain edema following GalN-induced liver injury is presented. Since brain edema is best defined as an increase in brain volume (swelling) owing to an increase in its water content (Fishman, 1975; Rapoport, 1976; Go, 1980), this was determined. The development of brain edema has been studied in terms of both brain water content and percent brain swelling as follows:

Figure 3.15: Representative electron micrographs of the cerebral cortex of GalN-FHF rats in progressive grades of hepatic coma. a: A typical cerebral capillary of a GalN-FHF rat in Grade I coma. At this time, despite slight astroglial (AG) swelling, no major changes were evident in comparison with normal rats. b: A typical cerebral capillary of a GalN-FHF rat in Grade II hepatic coma. Extensive swelling of the astroglial (AG) cytoplasm can be seen in the pericapillary astrocyte. Abnormal vacuoles (V) and swollen mitochondria are also evident. c: A typical cerebral capillary of a GalN-FHF rat in Grade III hepatic coma. Extensive astroglial swelling and tissue necrosis can be seen in the pericapillary astrocyte (AG). Evidence of large cytoplasmic vacuoles (V), as well as tissue distortion, was also prominent at this stage. d: A typical cerebral capillary of a GalN-FHF rat in Grade IV hepatic coma. Evidence of extensive astroglial (AG) swelling, tissue necrosis (see arrow), mitochondrial swelling, abnormal presence of vacuoles, and other cytoplasmic aberrations were seen throughout the cerebral parenchyma at this time. L-capillary lumen. Samples were fixed using glutaraldehyde and osmium tetroxide, and were post-stained with uranyl acetate and lead citrate. Bar represents 1μ.


V
AG
V
L

3.1.9.1 Brain Water Content Measurements

Brain water content (g H_2O/g dry weight of brain) measurements were determined in normal (Grade 0 coma) and GalN-FHF rats in progressive stages (Grades I-IV coma) of liver injury. Results from a total of 53 animals are presented in Figure 3.16 and Table 3.6. A steady increase in brain water content was observed as the animals progressed through increasing severity of coma (i.e. Grades 0-IV coma). Increase in brain water content first became statistically significant ($p<0.02$) with the development of overt encephalopathy during Grade II coma. At this time brain water content increased from 3.474 $\pm$ 0.12 g H_2O/g dry weight (normal levels) to 3.531 $\pm$ 0.018 g H_2O/g dry weight (Grade II coma). With increasing severity of coma further increases in brain water content were observed. Just prior to death in Grade IV coma, brain water content had increased by over 5.41% of control values to 3.663 $\pm$ 0.027 g H_2O/g dry weight (Figure 3.16 and Table 3.6).

3.1.9.2 Estimation of Brain Swelling During Brain Edema

Increases in brain water content (i.e. brain edema) during progressive stages of liver injury resulted in substantive swelling of the brain tissue. It was observed that relatively small increments in water content

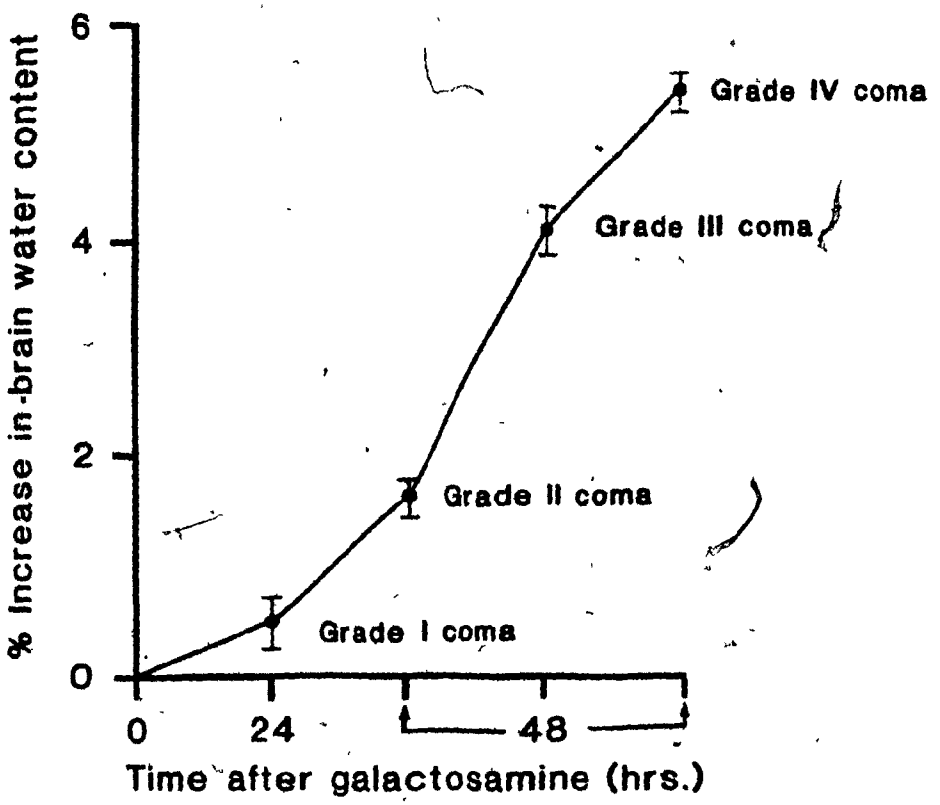


Figure 3.16: The percent increase in brain water content vs the time (in hours) after galactosamine injection. The graph also depicts the progressive increases in brain edema, as measured by increase in brain water content, with increasing severity of hepatic coma.

Table 3.6

Measurements of Brain Edema in Normal and Galactosamine-Induced FHF Rats

Group No.	Grade of Coma	Number of Animals	Brain Water Content g H_2O/g dry wt. of brain (mean ± SE)	% Brain Water Content (mean ± SE)	% Swelling of Brain (mean ± SE)
1	0 (Normal)	11	3.474 ± 0.012	77.649 ± 0.061	0.000
2	I	16	3.492 ± 0.009	77.736 ± 0.041	0.393 ± 0.196*
3	II	11	3.531 ± 0.018**	77.835 ± 0.022	1.277 ± 0.410***
4	III	6	3.618 ± 0.039◇	78.500 ± 0.162	3.677 ± 0.783△
5	IV	9	3.662 ± 0.027○	78.544 ± 0.125	4.343 ± 0.595

Determination of brain edema (in terms of brain water content and brain swelling) in various stages of hepatic coma (i.e. Grade I through Grade IV coma). Normal animals, receiving no galactosamine injection, are designated as Grade 0 coma. A progressive increase in brain water content was observed which became significant in Grade II coma, 48 hours after galactosamine injection. Significant brain swelling was evident as early as Grade I coma, 24 hours after galactosamine injection.

Statistical significance by Student 't' test:

* $p<0.05$
** $p<0.02$
*** $p<0.005$
} with respect to normal (Grade 0 coma) rats

◇ $p<0.05$
△ $p<0.02$
○ $p<0.001$
} with respect to Grade II coma rats

represented much larger volume increases of the brain (i.e. brain swelling) (Table 3.6 and Figure 3.17). For instance, an increase of the brain's percent water content from a normal value of 77.649% to 78.544% in Grade IV coma resulted in swelling of the brain by 4.34% (Figure 3.16 and Table 3.6). Thus, significant ($p < 0.05$) swelling of the brain was evident as early as Grade I coma, 24 hours after GalN liver injury. With the further progression of liver injury and the development of coma, substantive swelling of the brain was observed with subsequent grade of coma. Although increase in brain swelling was observed with severity of coma, no statistically significant difference in brain swelling was observed between Grade III and Grade IV hepatic coma.

3.1.10 Test for the Structural Integrity of the Blood-Brain Barrier During the Development of Brain Edema in Galactoamine-Induced Fulminant Hepatic Failure Rats

Brain histology and brain water measurements conclusively proved the presence and development of brain edema during GalN-induced liver injury. However, these studies could not provide the basic information regarding the nature of the evolution of brain edema during GalN-induced acute liver failure. To determine this the physical integrity of the blood-brain barrier was tested

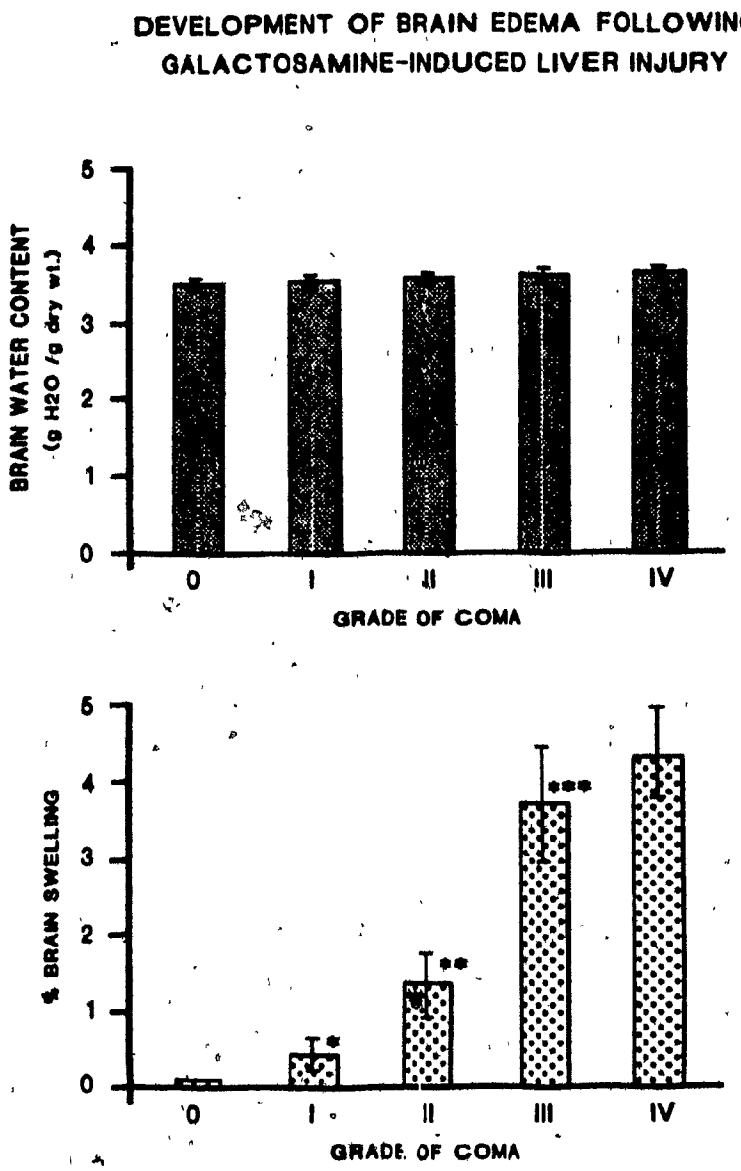


Figure 3.17: The development of brain edema following galactosamine-induced liver injury. Top panel: Changes in brain water content with the development of hepatic coma. Bottom panel: The percent swelling of the brain (due to brain edema) with the progressive development of hepatic coma. From this figure it is clear how small increases in brain water content represent much larger increases in brain swelling. Statistical significance (by Student 't' test) with preceeding grade of hepatic coma:

* $p < 0.05$
** $p < 0.005$
*** $p < 0.02$

during progressive stages of GalN-induced liver injury. Trypan blue dye infusion studies were carried out on 25 normal and comatose GalN-FHF rats (5 in each group -- Grades 0-IV hepatic coma). Representative results are presented in Figure 3.18. An intact blood-brain barrier normally prevents entry of the dye to stain the brain. Any staining of the brain would reflect an increase in permeability, or the total breakdown of the blood-brain barrier.

As was expected, normal animals (i.e. no liver damage, Grade 0 coma) showed no significant coloration of the brain tissue following intravenous trypan blue infusion (Figure 3.18). With progression of liver injury but before the development of overt encephalopathy (i.e. Grade I coma) only a slight bluish coloration of the cerebral cortex was observed. During Grade II coma, when liver injury was also maximal (i.e. 48 hours after GalN injection), both the cerebrum and cerebellum were only slightly tainted blue. However, between the two regions of the brain, the cerebellum showed deeper staining (Figure 3.18). With the progression to deeper grades of coma (i.e. Grades III and IV hepatic coma), a distinct deep blue coloration of the total brain was seen. Upon sectioning the stained brain at this time, the distinct deep blue staining by trypan blue was visible in the cerebrum, cerebellum, and regions of the brain stem.

Figure 3.18: Representative results showing the integrity of the blood-brain barrier during progressive stages of GalN-FHF. A 2% solution trypan blue dye was infused into the carotid artery of normal (Grade 0 coma) and GalN-FHF rats in progressive grades of hepatic coma. An intact blood-brain barrier normally prevents entry of the dye to stain the brain. Any staining would reflect an increase in permeability or the total breakdown of the blood-brain barrier. Animals receiving no galactosamine injection (Grade 0 coma) showed no staining of the brain with trypan blue. GalN-FHF animals in Grades I and II hepatic coma showed only slight coloration of the brain tissue. With progression to deeper grades of coma (Grades II and IV hepatic coma) a distinct deep blue coloration of the brain was seen. Upon sectioning the stained brain at this time, the distinct deep blue staining of the brain was visible in the cerebrum, cerebellum, and regions of the brain stem.

0 I II III IV

GRADE OF COMA

3.2 PROSTAGLANDIN E_2 STUDIES ON GALACTOSAMINE-INDUCED HEPATIC INJURY

The effects of PGE_2 on GalN-FHF rats in Grade II coma were studied as described in Chapter II of this thesis. Only rats in Grade II coma were used in this study as PGE_2 had no significant effects on the survival time of rats in later grades of coma (figure 3.22). Grade II coma also represents the stage of maximum liver injury by GalN. PGE_2 has been reported to significantly increase the survival time of GalN-FHF rats in Grade II coma (Dixit and Chang, 1982). It is as yet still unclear what role PGE_2 plays in prolonging the survival time in these rats. In the present study, the effects of PGE_2 on the serum biochemistry and liver histology has been studied following GalN-induced hepatocyte injury and the development of Grade II coma. Since the evolution brain edema is a major feature during GalN liver injury, the effects of PGE_2 on its development was also investigated. Qualitative (brain histology) as well as quantitative (brain water content and brain swelling) studies were performed and are presented in subsequent sections of this chapter.

3.2.1 Dose-Response of Prostaglandin E_2

The dose-response curve of PGE_2 is shown in Figure 3.19. Of the range of PGE_2 doses tested, it was seen that

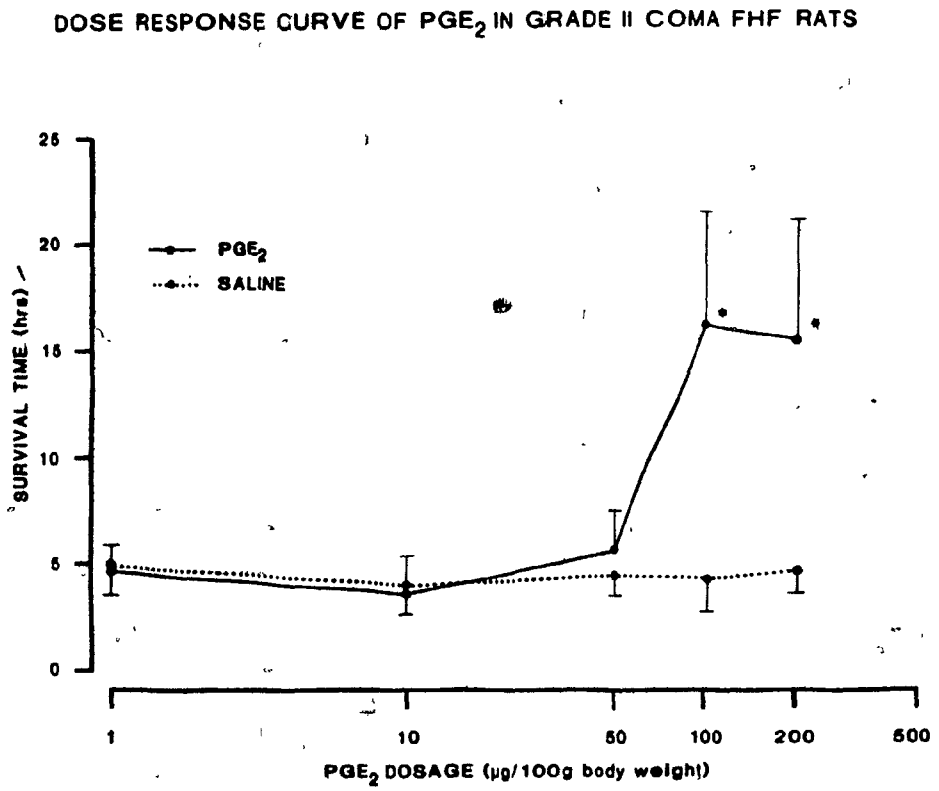


Figure 3.19: Dose-response curve of PGE_2 in Grade II coma GalN-FHF rats. Various doses of PGE_2 were intraperitoneally injected into GalN-FHF rats in Grade II hepatic coma. Any significant effects of PGE_2 on these rats reflected as significant changes in the survival time of the FHF rats. From these studies, it was found that a PGE_2 of dose 100 µg PGE_2/100g body weight was optimal in its effect.

the PGE_2 dose of 100 μg PGE_2/100g body weight was optimal in its effects. Table 3.7 and Figure 3.20 show the effects of various doses of PGE_2 on the survival time of Grade II coma FHF rats. A PGE_2 dose of 50 μg/100g body weight, or lower, had no significant effects on the survival time of the rats tested. PGE_2 dosage of 100 μg and 200 μg/100g body weight had similar beneficial effects of increased survival time in the GalN-FHF animals tested. No significant beneficial effects of PGE_2 were observed with respect to survival rate of the GalN-FHF animals, for the range of PGE_2 doses tested (Figure 3.21 and Table 3.8). On the basis of these observations, the optimal dose of 100 μg PGE_2/100g body weight was selected for all subsequent experiments involving PGE_2.

3.2.2 The Effects of PGE_2 During Terminal Stages of Galactosamine-Induced Fulminant Hepatic Failure

Unlike the response in Grade II hepatic coma GalN-FHF rats, it was observed that PGE_2 had no beneficial effects in Grades III and IV hepatic coma. There were no significant effects in either the survival time or survival rate in these latter rats (Figure 3.22 and Tables 3.9, 3.10). It was concluded that Grade III and IV hepatic coma represented terminal stages of GalN-FHF and were therefore excluded from the present study. Unless otherwise indicated,

Table 3.7

Dose-Response of PGE_2 in Grade II Coma Galactosamine-Induced Fulminant Hepatic Failure Rats: Survival Time Studies

PGE_2 Dosage (g/100g b. wt.)	n*	Survival Time (Hrs ± SE) PGE_2	saline	Statistical Significance (Student t-test)**	
1	30	4.6 ± 1.2	4.8 ± 1.0	$p<0.90$	N.S.
10	36	3.5 ± 1.0	4.0 ± 1.5	$p<0.80$	N.S.
50	44	5.5 ± 2.0	4.5 ± 1.1	$p<0.50$	N.S.
100	66	16.2 ± 5.2	4.3 ± 1.6	$p<0.05$	S
200	48	15.6 ± 5.4	4.6 ± 1.1	$p<0.05$	S

* Total number of rats (from 3-6 experiments) for each of the PGE_2 treated and saline control groups.

** With respect to corresponding "matched" saline controls

S = significant.
N.S. = not significant

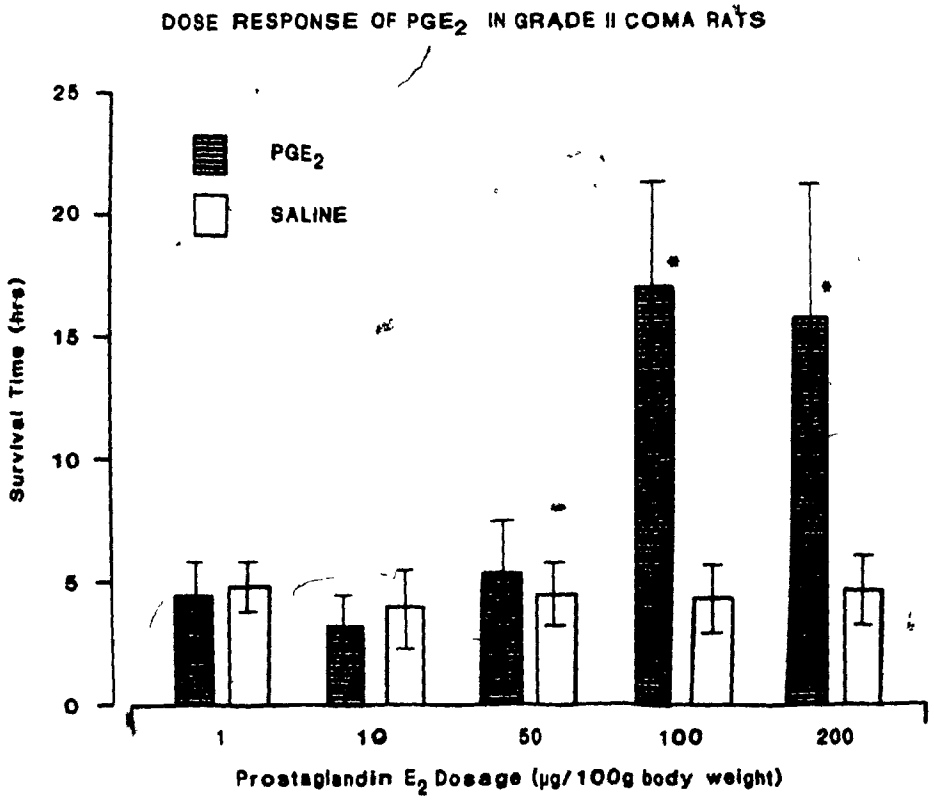


Figure 3.20: The effect of PGE_2 on the survival time of GalN-FHF rats in Grade II hepatic coma. PGE_2 doses ranging from 1-200 μg PGE_2/100g body weight were injected intraperitoneally into GalN-FHF rats in Grade II coma. Any significiant effects of PGE_2 were reflected as significant improvements in the survival time of these rats. In these studies PGE_2 dose of 100 μg PGE_2/100g body weight was optimal in its effects.

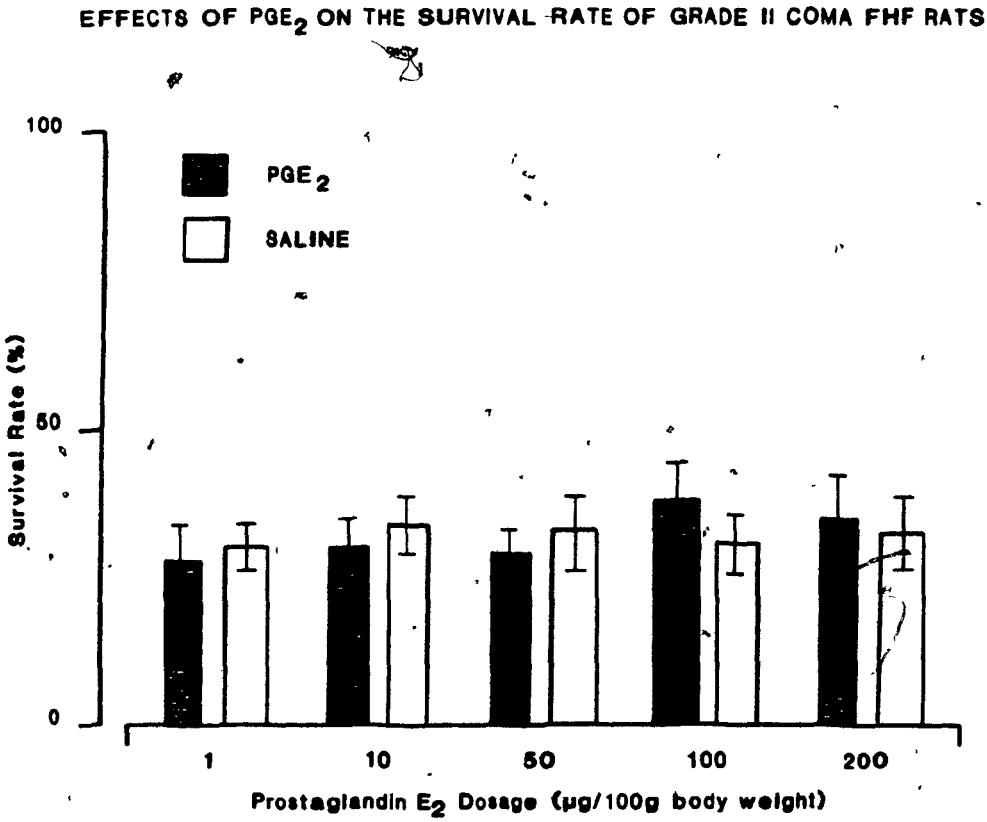


Figure 3.21: The effects of PGE_2 on the survival rate of GalN-FHF rats in Grade II hepatic coma. Various doses of PGE_2 ranging from 1-200 μg PGE_2/100g body weight were injected intraperitoneally into GalN-FHF rats in Grade II coma. Any significant effect of PGE_2 were reflected as significant improvements in the survival rates of these rats. In the present studies PGE_2 did not significantly improve the survival rates of GalN-FHF rats in Grade II coma.

Table 3.8

Dose-Response of PGE_2 in Grade II Coma Galactosamine-Induced Fulminant Hepatic Failure Rats: Survival Rate Studies

PGE_2 Dosage (g/100g b. wt.)	No. of Experiments*	Survival Rate** (%) PGE_2	saline	Statistical Significance (Student t-test)***
1	3	28.3 ± 5.1	30.8 ± 3.3	$p<0.80$ N.S.
10	3	30.4 ± 5.0	33.0 ± 5.0	$p<0.70$ N.S.
50	4	29.2 ± 4.2	32.4 ± 5.3	$p<0.65$ N.S.
100	6	38.3 ± 5.2	31.3 ± 4.9	$p<0.30$ N.S.
200	4	35.6 ± 6.0	33.3 ± 4.8	$p<0.80$ N.S.

* Each experiment involved 8-12 rats for each of the PGE_2 treated and saline control groups.
** Calculated from a mean of 3-6 experiments
*** With respect to corresponding "matched" saline controls

S = significant
N.S. = not significant

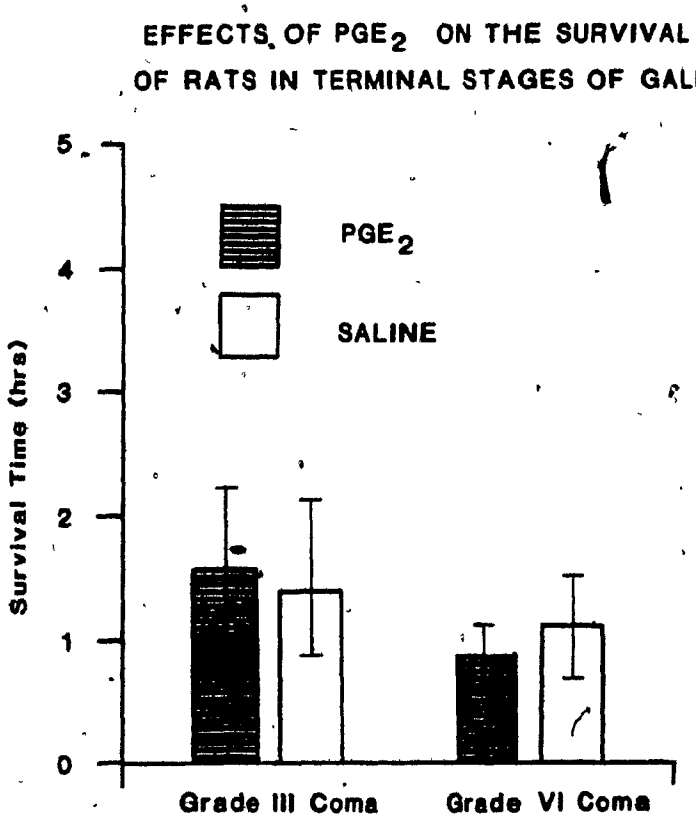


Figure 3.22: The effects of PGE_2 on the survival time of rats in terminal stage of GalN-FHF. Terminal stages of GalN-FHF have been described as the stages when the rats were in Grades III and IV hepatic coma. PGE_2 was injected intraperitoneally at a dose of 100 μg PGE_2/100g body weight. No significant improvements in the survival time was observed following the injection of PGE_2 in these rats.

Table 3.9

The Effects of PGE_2 on the Survival Time of Galactosamine-Induced Fulminant Hepatic Failure Rats in Grades III and IV Hepatic Coma

Grade of Coma	PGE_2 Dosage (g/100g b. wt.)	n*	Survival Time (Hrs) PGE_2	Survival Time (Hrs) saline	Statistical Significance (Student t-test)**	
III	100	30	1.6 ± 0.6	1.4 ± 0.5	$p<0.80$	N.S.
IV	100	32	3.5 ± 1.0	4.0 ± 1.5	$p<0.80$	N.S.

* Total number of rats (from 3-6 experiments) for each of the PGE_2 treated and saline control groups.

** With respect to corresponding "matched" saline controls

S = significant
N.S. = not significant

Table 3.10

The Effects of PGE_2 on the Survival Rate of Galactosamine-Induced Fulminant Hepatic Failure Rats in Grades III and IV Hepatic Coma

Grade of Coma	PGE_2 Dosage (g/100g b. wt.)	No. of Experiments	Survival Rate** (%) PGE_2	Survival Rate** (%) saline	Statistical Significance 't' test***
III	100	4	0	0	N.S.
IV	100	4	0	0	N.S.

* Each experiment involved 8-12 rats for each of the PGE_2 treated and saline control groups

** Calculated from a mean of 4 experiments

*** With respect to corresponding "matched" saline controls

S = significant
N.S. = not significant

the present study investigated the effects of PGE_2 (dosage 100 μg PGE_2/100g body weight) on GalN-FHF rats in Grade II hepatic coma.

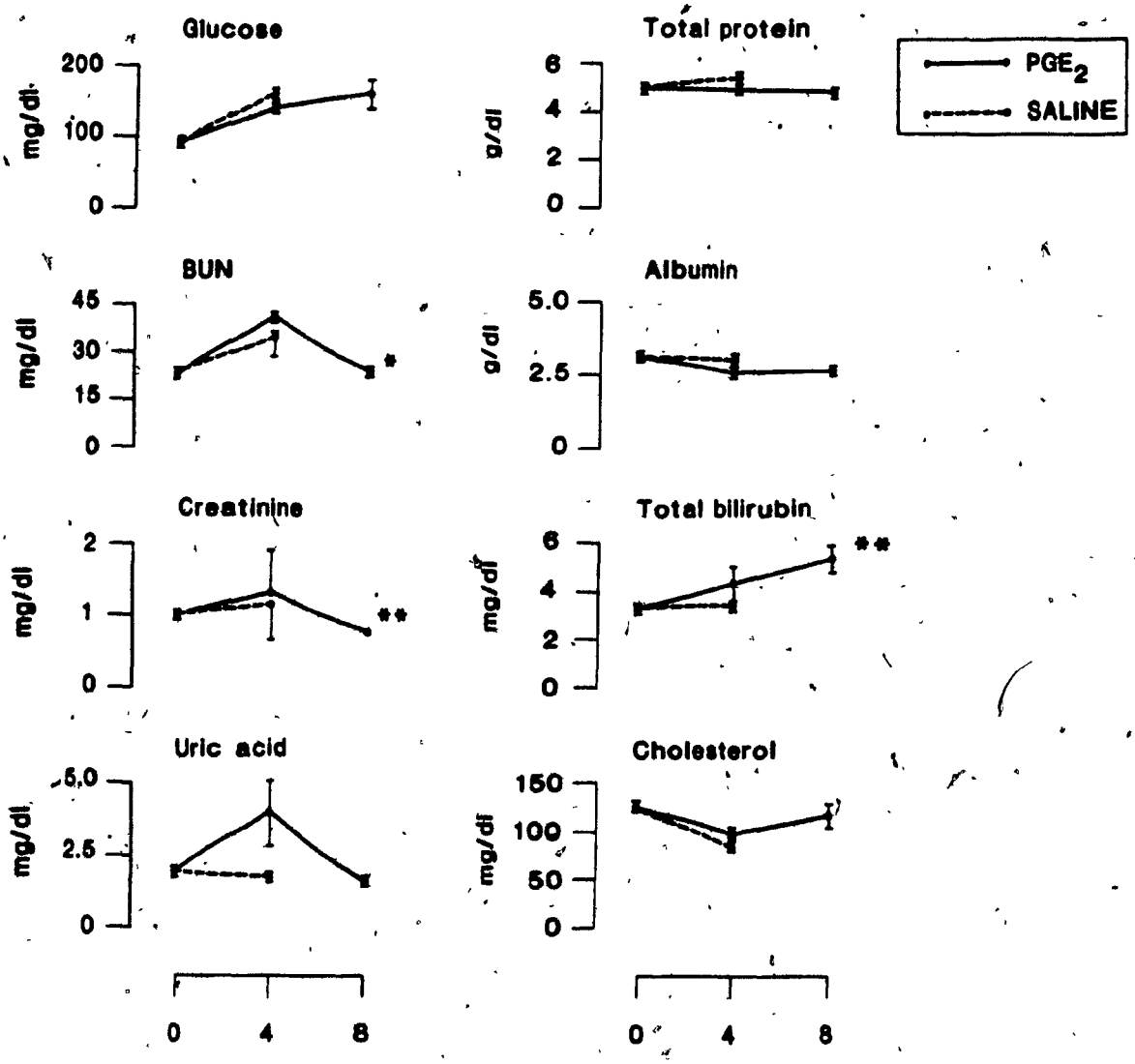
3.2.3 Blood Biochemistry Analysis in Prostaglandin E_2 Injected Galactosamine-Induced Fulminant Hepatic Failure Rats

The effect of PGE_2 was studied on GalN-FHF rats in Grade II hepatic coma. Following the development of GalN-FHF, only those animals which had developed symptoms of Grade II hepatic coma were selected for the PGE_2 studies. This time was designated as "0 hour." Subsequently, PGE_2 or physiological saline (as control) was injected intraperitoneally into the Grade II coma rats. At specific times, i.e. 4 or 8 hours after PGE_2 (or saline) injection, blood and tissue samples were obtained for serum biochemistry and liver histology. Since the mean survival time in the saline injected control animals was only 4.3 $\pm$ 1.6 hrs. (Table 3.7 and Figure 3.20) no samples could be obtained for the animals in this group after 4 hours. The samples obtained were designated as follows: 1) 0 hr. (initial sample, before PGE_2/saline injection); 2) 4 hrs. after saline injection; 3) 4 hrs. after PGE_2 injection; and 4) 8 hrs. after PGE_2 injection. Serum samples were analyzed

for clinical biochemistry and electrolytes by the SMAC-II autoanalyzer as follows:

3.2.3.1 Clinical Biochemistry Analysis in PGE_2 Studies

SMAC results of the clinical serum biochemistry analysis of the PGE_2/saline injected rats are presented in Figure 3.23 and Table 3.11. No statistically significant biochemical differences were observed in all parameters between the PGE_2 and saline groups at 4 hours after PGE_2/saline injection. The increase in glucose levels is due to the sub-cutaneous injection of 50% dextrose given to all animals in both groups to prevent the hypoglycemia observed after GalN-induced hepatocyte injury. Eight hours after PGE_2 injection, the elevated serum BUN levels were reduced significantly ($p < 0.001$) in comparison to levels observed at 4 hours in the same group. When compared to 0 hr. (pre-PGE_2/ saline injection levels), the elevated serum creatinine levels in PGE_2 injected animals were also significantly ($p < 0.001$) reduced (Figure 3.23 and Table 3.11). Total bilirubin levels were significantly ($p < 0.001$) increased in the PGE_2 animals 8 hours after PGE_2 injection. A detailed analysis of the clinical biochemistry of PGE_2/saline injected animals is as follows:



* P< 0.001 Compared to 4hrs PGE_2

** P< 0.001 Compared to 0 hrs (i.e. before PGE_2 /saline injection)

Figure 3.23: Serum biochemistry analysis, as performed by the SMAC II autoanalyzer, during the PGE_2 studies. PGE_2 was intraperitoneally injected into GalN-THF animals in Grade II hepatic coma. Serum samples were obtained for analysis before PGE_2 injection (i.e. 0 hr.), and at specific times (i.e. 4 and 8 hrs.) after PGE_2 (or saline) injection. Statistical significance was determined by the Student 't' test.

Table 3.11

Serum Biochemistry Analysis: PGE_2 Studies

Time of Sampling (hrs.)	0 hrs (Grade II Coma)'	4 hrs after PGE_2 injection	4 hrs after saline injection	8 hrs after PGE_2 injection
n	13	9	7	14
Glucose (mg/dl)	97.00 ± 4.9	146.70 ± 5.8	153.10 ± 6.1	160.90 ± 23.7
BUN (mg/dl)	23.30 ± 1.47	42.20 ± 3.48	36.67 ± 6.9	24.80 ± 1.3*
Creatinine (mg/dl)	1.03 ± 0.04	1.30 ± 0.06	1.20 ± 0.5	0.78 ± 0.04**
Uric Acid (mg/dl)	1.91 ± 0.09	3.92 ± 1.2	1.70 ± 0.2	1.60 ± 0.12
Total Proteins (g/dl)	4.96 ± 0.10	4.94 ± 0.13	5.13 ± 0.17	4.74 ± 0.15
Albumin (g/dl)	3.07 ± 0.07	2.76 ± 0.07	2.97 ± 0.14	2.78 ± 0.08
Total Bilirubin (mg/dl)	3.38 ± 0.10	4.42 ± 0.59	3.50 ± 0.26	5.39 ± 0.5**
Cholesterol (mg/dl)	129.00 ± 6.5	98.00 ± 4.5	89.70 ± 8.2	120.60 ± 12.6

' 48 hrs after Galactosamine injection
* $p<0.001$ compared to 4 hrs PGE_2
** $p<0.001$ compared to 0 hrs (i.e. before PGE_2/saline injection)

1) Glucose: PGE_2 had no effect on preventing the fall, or causing the elevation of serum glucose levels in Grade II coma FHF rats. Since severe hypoglycemia is a major symptom of GalN induced hepatocyte injury, all animals were given 2 ml of 50% dextrose by sub-cutaneous injection. This was sufficient to prevent the hypoglycémia and restore serum glucose to pre-GalN levels. The increase in serum glucose from 97.0 $\pm$ 4.9 mg/dl to 146.7 $\pm$ 5.8 mg/dl in PGE_2 animals at 4 hours PGE_2 was due to the sub-cutaneous dextrose injection. Control FHF animals receiving saline had similar increases in glucose levels following subcutaneous injections of 50% dextrose (Figure 3.23 and Table 3.11).

2) Blood Urea Nitrogen: A nearly 1.5 to 2.0 fold increase in BUN levels was seen 4 hours after saline and PGE_2 injection respectively. This elevation of serum BUN corresponded to the similar BUN increase seen when the rats progressed from Grade II to Grade III coma (Figure 3.6 and Table 3.3). A significant ($p < 0.001$) fall in BUN levels was observed 8 hours after PGE_2 injection. At this time BUN levels were similar to those seen during Grade II coma, just prior to PGE_2 injection (Figure 3.23 and Table 3.11). Incidentally, this was also the level observed during Grade IV coma, just prior to death (Figure 3.6 and Table 3.3).

3) Creatinine: No significant increases in serum creatinine levels were observed in either the PGE_2 or saline injected animals at 4 hours (Figure 3.23 and Table 3.11). However, at 8 hours after PGE_2 injection a significant ($p<0.001$) decrease in creatinine was observed and was similar to the creatinine level seen during Grade IV coma, just prior to death (Figure 3.6 and Table 3.3).

4) Uric Acid: A greater than 2 fold increase in uric acid levels was observed in the PGE_2 injected rats after 4 hours. Control rats injected with only saline showed no such elevation in uric acid levels at this time. At 8 hours after PGE_2 injection, uric acid levels declined to those seen in Grade II coma, prior to PGE_2/saline injection (Figure 3.23 and Table 3.11).

5) Total Proteins: No significant differences in total protein concentrations were observed after PGE_2/saline injection (Figure 3.23 and Table 3.11).

6) Albumin: No significant alterations in albumin concentrations were observed following PGE_2/saline injection (Figure 3.23 and Table 3.11).

7) Total Bilirubin: A steady increase in total bilirubin levels was seen in the PGE_2 injected rats. The increase became statistically significant 8 hours after PGE_2 injection. At this time a nearly 1.6 fold increase in

total bilirubin was observed over those measured during Grade II coma (i.e. at 0 hr.). Control animals receiving only saline showed no such increases (Figure 3.23 and Table 3.11).

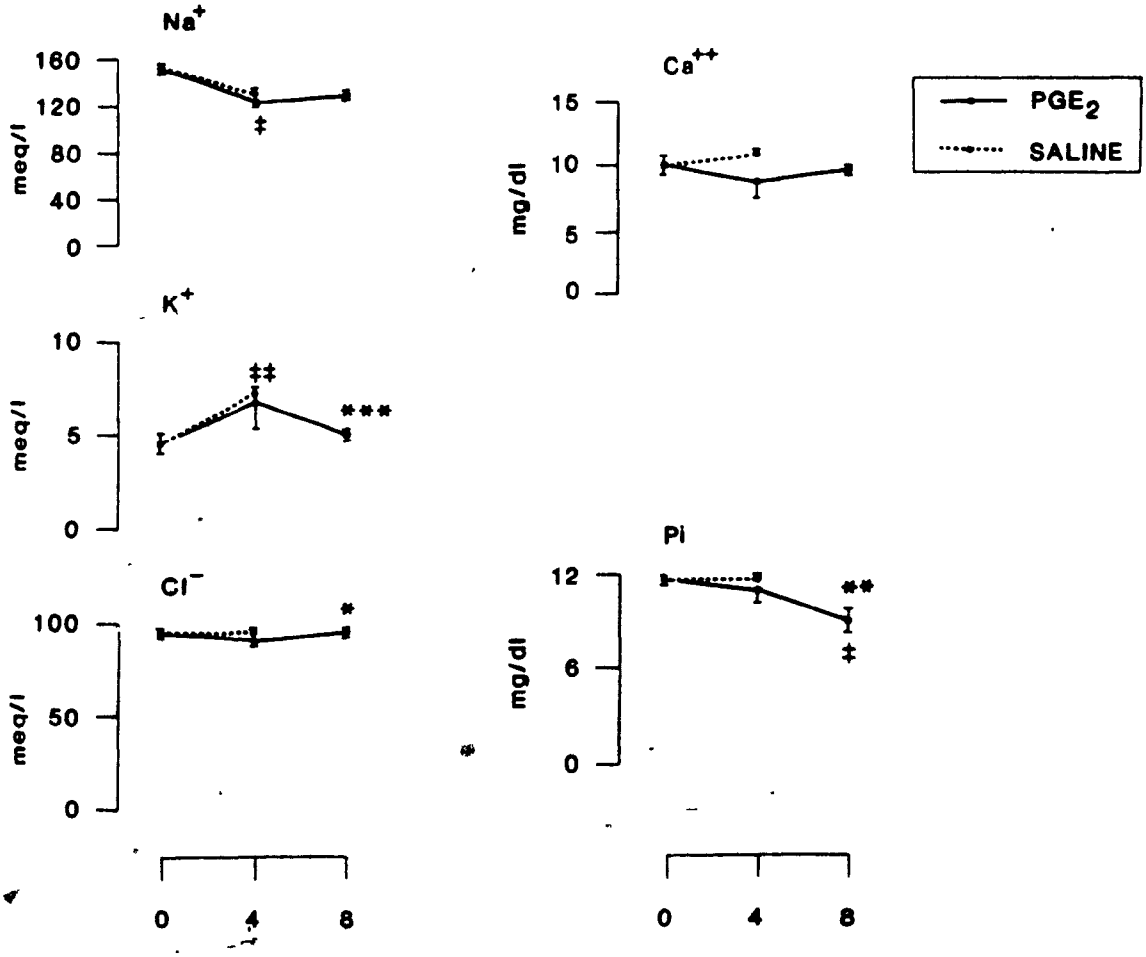
8) Cholesterol: No significant differences in serum cholesterol levels were observed between the PGE_2 and saline injected groups. Despite a slight fall (at 4 hrs. PGE_2/saline injection), cholesterol levels remained elevated at 8 hours after PGE_2 injection (Figure 3.23 and Table 3.11).

3.2.3.2 Serum Electrolyte Analysis in PGE_2 Studies

Serum electrolyte measurements obtained during the PGE_2 studies are presented in Figure 3.24 and Table 3.12. Alterations in all ions, with the exception of Calcium, were seen during the course of this study. Specific electrolyte changes are described as follows:

1) Sodium: A significant ($p<0.01$) decrease in sodium was seen as early as 4 hrs. after PGE_2 injection. No further change in sodium was observed at 8 hours PGE_2 (Figure 3.24 and Table 3.12). In the control rats receiving only saline, sodium levels followed the normal patterns observed during the transition from Grade II to Grade III coma (Figure 3.7 and Table 3.4).

PGE_2 STUDIES - SERUM ELECTROLYTE ANALYSIS



* P < 0.01 compared to 4hrs PGE_2

** P < 0.05 compared to 4hrs PGE_2

*** P < 0.02 compared to 4hrs saline

‡ P < 0.005 compared to 0 hrs (i.e. before PGE_2 /saline injection)

‡‡ P < 0.01 compared to 0 hrs (i.e. before PGE_2 /saline injection)

Figure 3.24: Serum electrolyte analysis, as performed by the SMAC II autoanalyzer, during the PGE_2 studies. PGE_2 was intraperitoneally injected into GalN-FHF animals in Grade II hepatic coma. Serum samples were obtained for analysis before PGE_2 injection (i.e. 0 hr.), and at specific times (i.e. 4 and 8 hrs.) after PGE_2 (or saline) injection. Statistical significance was determined by the Student 't' test.

Table 3.12

Serum Electrolyte Analysis: PGE_2 Studies

Time of Sampling (hrs)	0 hrs (Grade II Coma)'	4 hrs after PGE_2 injection	4 hrs after saline injection	8 hrs after PGE_2 injection
n	13	9	7	14
Sodium (meq/l)	150.80 ± 0.87	142.40 ± 1.9	145.00 ± 4.70	144.60 ± 1.6
Potassium (meq/l)	4.78 ± 0.38	6.75 ± 1.1	7.00 ± 0.67◇	4.95 ± 0.36***
Chloride (meq/l)	95.73 ± 0.99	92.60 ± 0.87	95.00 ± 2.60	96.00 ± 0.66*
Calcium (mg/dl)	9.88 ± 0.40	8.82 ± 1.7	10.60 ± 0.3	9.46 ± 0.47
Inorganic Phosphorous (g/dl)	11.84 ± 0.62	11.22 ± 0.8	11.73 ± 0.73	9.10 ± 0.49**,△

' 48 hrs after Galactosamine injection

* $p<0.001$ compared to 4 hrs PGE_2
** $p<0.001$ compared to 0 hrs (i.e. before PGE_2/saline injection)
*** $p<0.02$ compared to 4 hrs saline
△ $p<0.005$ compared to 0 hrs (i.e. before PGE_2/saline injection)
◇ $p<0.01$ compared to 0 hrs (i.e. before PGE_2/saline injection)

2) Potassium: Although no significant difference in potassium levels was observed between the PGE_2 and saline groups at 4 hours after PGE_2/saline injection, serum potassium was significantly ($p < 0.01$) elevated in the control group (receiving only saline). Eight hours after PGE_2 injection, the potassium level was significantly ($p < 0.02$) lower when compared to its levels in the control (saline) group at 4 hours after saline injection (Figure 3.24 and Table 3.12).

3) Chloride: No significant change in chloride levels was seen in the PGE_2 or saline control groups at 4 hours after PGE_2/saline injection. At 8 hours after PGE_2 injection a slight rise in chloride levels was observed. The fluctuations in chloride levels remained within the normal physiological range (Figure 3.24 and Table 3.12).

4) Calcium: Despite minor fluctuations, no significant change in calcium levels was seen during the course of this study (Figure 3.24 and Table 3.12).

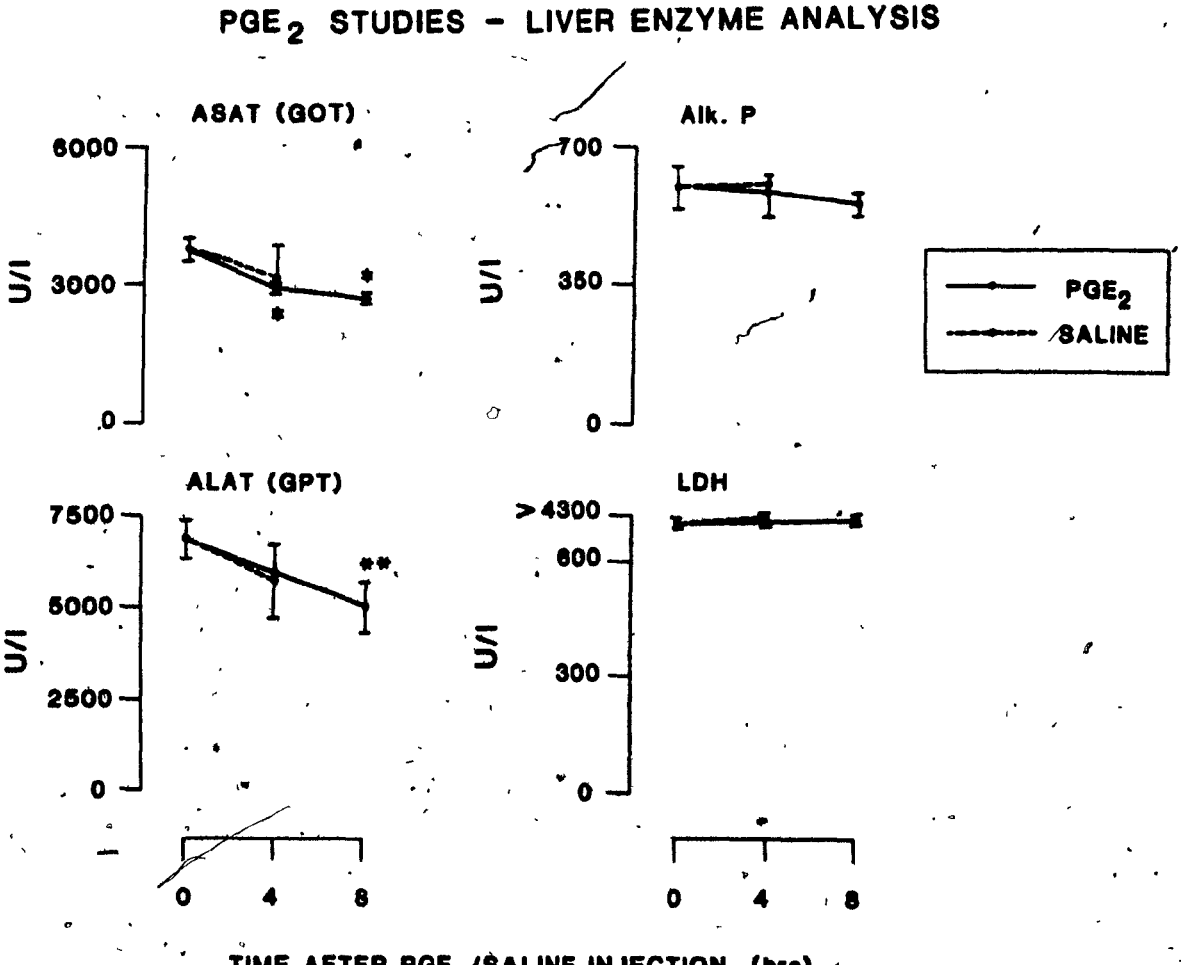
5) Inorganic Phosphorous: A steady decline in inorganic phosphorous was seen during the course of this study. The decrease in phosphorous became statistically significant ($p < 0.005$) 8 hours after PGE_2 injection. Control animals receiving only saline showed no significant variations in phosphorous levels (Figure 3.24 and Table 3.12).

3.2.4 Liver Enzyme Measurements During PGE_2 Studies

The measurements of liver enzymes obtained during the PGE_2 studies are presented in Figure 3.25 and Table 3.13. A significant decrease in enzyme levels was seen only in the PGE_2 injected group. In particular, serum ASAT and ALAT showed significant decrease during the course of this study. No significant changes were observed in AP and LDH in this study. Detailed assessments of enzyme changes are presented below.

1) **Aspartate Aminotransferase:** A significant ($p < 0.01$) decrease (17%) in ASAT levels was observed in the PGE_2 injected animals as early as 4 hours after PGE_2 injection. ASAT levels remained depressed at this level even at 8 hours after PGE_2 injection. There were no statistically significant variations in ASAT levels in the control rats receiving only saline (Figure 3.25 and Table 3.13).

2) **Alanine Aminotransferase:** As was for ASAT (above), ALAT levels also steadily fell after PGE_2 injection. The decrease in ALAT became statistically significant ($p < 0.05$) 8 hours after PGE_2 injection. In control rats receiving only saline, ALAT levels remained statistically unchanged when compared to levels seen during "0" hour (Figure 3.25 and Table 3.13).



* $P < 0.01$ compared to 0 hrs (i.e. before PGE_2 /saline injection.)

** $P < 0.05$ compared to 0 hrs (i.e. before PGE_2 /saline injection)

Figure 3.25: Liver enzyme analysis during the PGE_2 studies. PGE_2 was intraperitoneally injected into GalN-FHF animals in Grade II hepatic coma. Serum samples were obtained for analysis before PGE_2 injection (i.e. 0 hr.), and at specific times (i.e. 4 and 8 hrs.) after PGE_2 (or saline) injection. Statistical significance was determined by the Student 't' test.

Table 3.13

Liver Enzyme Analysis: PGE_2 Studies

Time of Sampling (hrs)	0 hrs (Grade II Coma)'	4 hrs after PGE_2 injection	4 hrs after saline injection	8 hrs after PGE_2 injection
n	13	9	7	14
ASAT (GOT) (U/l)	3756.8 ± 197.7	3101.1 ± 124*	3252.0 ± 534.7	2983.3 ± 184*
ALAT (GPT) (U/l)	6899.7 ± 415.8	5970.9 ± 636.7	5949.2 ± 977.2	5057.3 ± 721.6**
AP (U/l)	606.3 ± 34.4	598.6 ± 66.9	603.3 ± 17.2	579.4 ± 31.9
LDH (U/l)	4800	4300	4700	4700

' 48 hrs after Galactosamine injection

* $p<0.01$ compared to 0 hrs (i.e. before PGE_2/saline injection)
** $p<0.05$ compared to 0 hrs (i.e. before PGE_2/saline injection)

3) Alkaline Phosphatase: No significant changes in serum Alk. P were observed in either the PGE_2 or saline (control) groups in this study (Figure 3.25 and Table 3.13).

4) Lactic Dehydrogenase: Similar to Alk. P, LDH levels remained unchanged in both the PGE_2 and saline injected (control) groups throughout the course of this study (Figure 3.25 and Table 3.13).

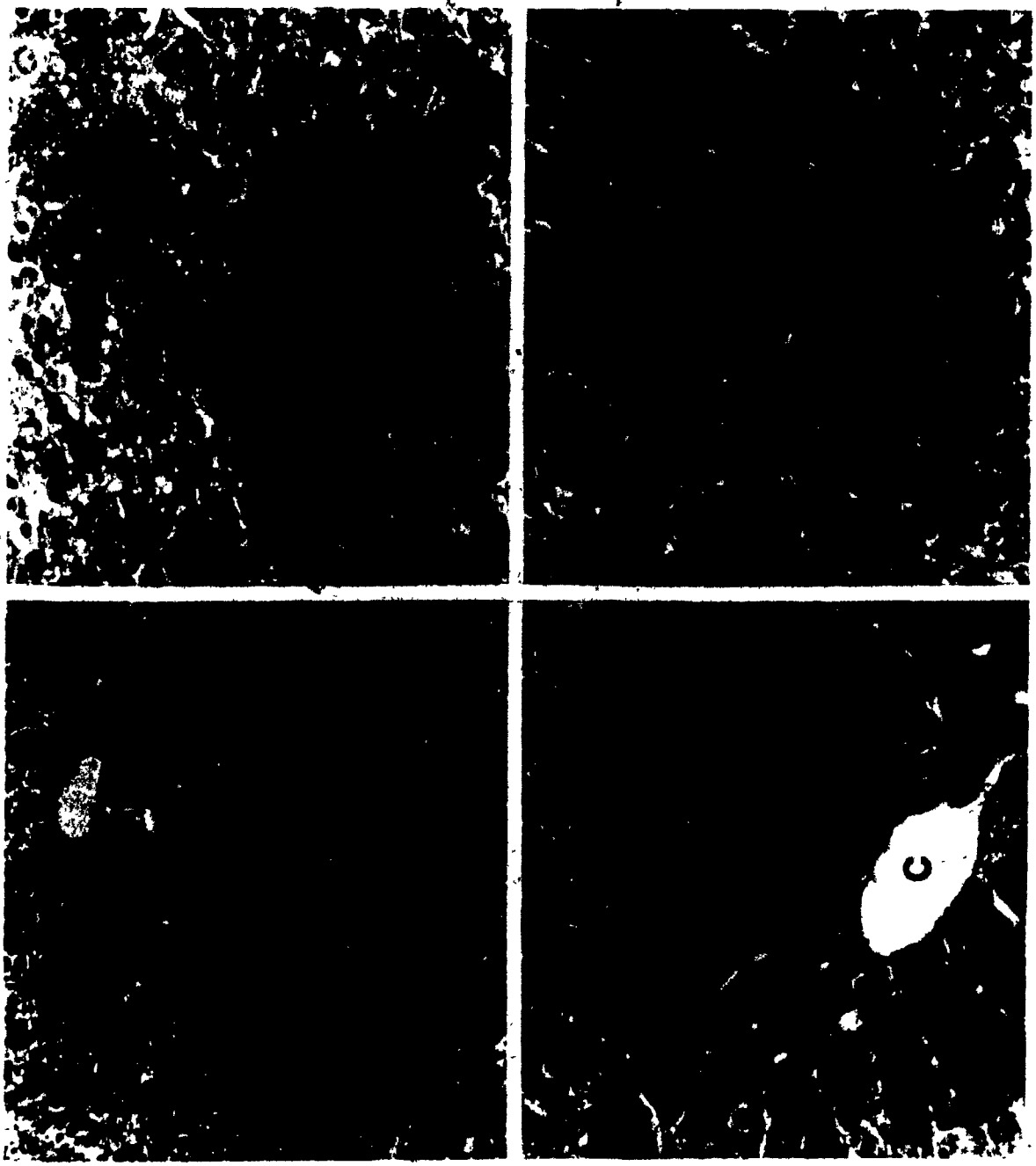
3.2.5 Histological Studies of the Liver During PGE_2 Studies

Light and electron microscopy of liver tissue were performed at specific times (i.e. 0, 4, and 8 hours after PGE_2/saline injection) during the PGE_2 studies. Representative results from more than 50 rats fixed for light and electron microscopy are presented below.

3.2.5.1 Light Microscopy of the Liver During PGE_2 Studies

The following are representative observations from approximately 30 rats which were fixed for light microscopy. Light microscope histology results are presented in Figure 3.26. Figure 3.26a represents a hepatic lobule during Grade II coma (48 hours after GalN induced hepatocyte injury), just prior to PGE_2/saline injection. The mottled appearance of hepatocytes coupled with the total disarray of the normal cord-like arrangement

Figure 3.26: Representative histological observations of the light microscopy performed on the liver of rats in the PGE_2 studies. PGE_2 was intraperitoneally injected into GalN-FHF animals in Grade II hepatic coma. Liver tissue samples were obtained for histology before PGE_2 (or saline) injection (i.e. 0 hr.) and at specific times (i.e. 4 and 8 hrs.) after PGE_2 (or saline) injection. a: A hepatic lobule from a rat in Grade II coma just prior to PGE_2 injection (0 hr.). Mottled appearance of the hepatocytes coupled with the total disarray of the normal cord-like arrangement of the hepatocytes is evident. b: Section of a hepatic lobule 4 hours after saline injection (control group). Massive hepatic necrosis with evidence of karyolysis is present. Differential staining distinguishes viable from necrotic hepatocytes. c: Section of hepatic lobule 4 hours after PGE_2 injection. Presence of intact viable hepatocytes are more prominent with respect to corresponding rats injected with only saline. d: Section of hepatic lobule 8 hours after PGE_2 injection. Necrotic hepatocytes undergoing karyolysis and inflamatory infiltrations between adjacent hepatocytes can be seen throughout the hepatic lobule. All tissues were fixed in Carnoy's Fluid and stained using Delafield's hematoxylin and eosin Y staining techniques. Magnification a: 360x; b-d: 400x.

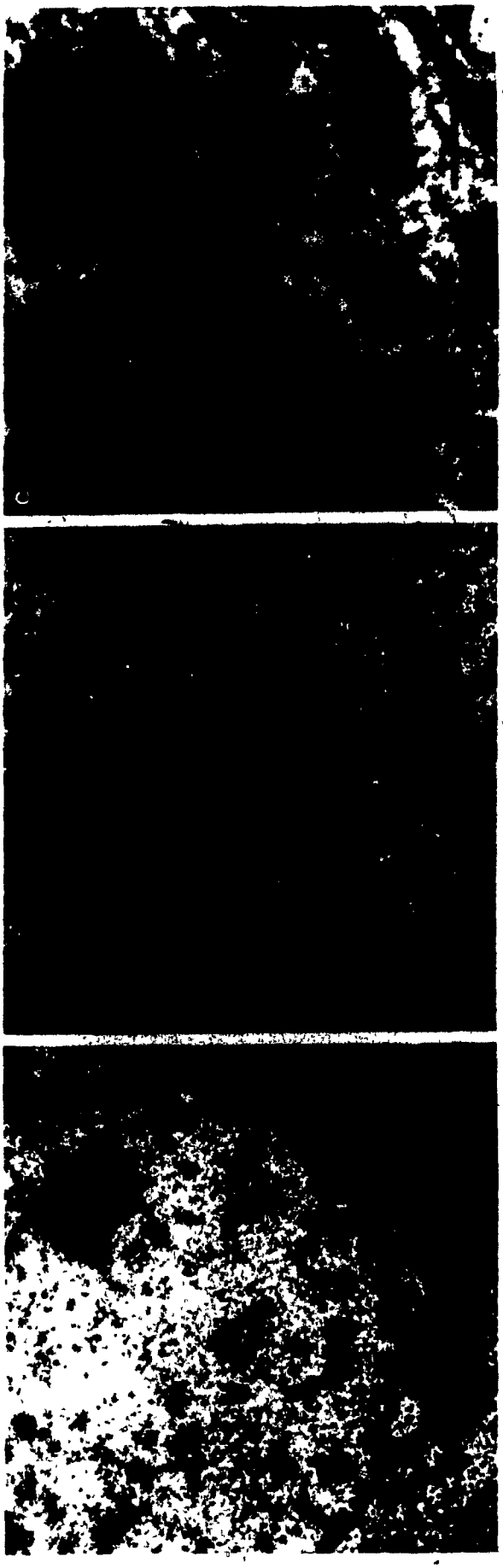


of hepatocytes is evident. At this time maximum liver damage had also occurred. Figure 3.26b shows a section of a hepatic lobule 4 hours after saline injection (control group). Massive hepatic necrosis with evidence of karyolysis is clear. The differential staining distinguished viable from necrotic hepatocytes. The hepatic lobule, 4 hours after PGE_2 injection is shown in Figure 3.26c. The presence of intact viable hepatocytes is more prominent with respect to corresponding rats injected with only saline. Although some necrotic hepatocytes are visible, the majority of the hepatocytes still appear viable. Figure 3.26d shows a section of a hepatic lobule 8 hours after PGE_2 injection. Total disarray of the cord-like arrangement of the hepatocytes is distinctly evident. Necrotic hepatocytes undergoing karyolysis and inflammatory infiltrations between adjacent hepatocytes can be seen throughout the hepatic lobule. The mottled appearance due to differential staining separate viable from necrotic hepatocytes. The hepatic lobule shown in Figure 3.26d shows marked similarity to the hepatic lobule 4 hours after saline injection (Figure 3.26b).

3.2.5.2 Ultrastructural Observations of the Liver During PGE_2 Studies

Liver ultrastructure was studied at specific times (i.e. 0, 4, and 8 hours after PGE_2/saline injection) during the PGE_2 studies. Electron microscopy of the hepatocytes was performed on at least 20 rats involved in these studies. Representative ultrastructural observations of the hepatocytes are presented in Figure 3.27 and 3.28. Figure 3.27a depicts a normal hepatocyte before GalN-induced hepatocyte injury. The location of the endoplasmic reticulum (ER) and the mitochondria (M) can be seen distinct and separate from each other and the nucleus (N) (Figure 3.27a). In this figure, the oval-shaped mitochondria with interdigitating cristae are also clearly visible. Figure 3.27b shows a typical hepatocyte during Grade II coma (48 hours after GalN-induced liver injury), just prior to PGE_2/saline injection, i.e. at 0 hour. The characteristic migration of the ER and mitochondria to align itself with the nuclear membrane is evident at this time (Figure 3.27b). Severe mitochondrial aberrations (e.g. elongated and dumbbell-shaped appearance) with loss of cristae are also evident at this time (Figure 3.27c). Figure 3.28a depicts a hepatocyte 4 hours after control saline injection. The onset of hepatocyte karyolysis is apparent. At this time, severely distorted mitochondria

Figure 3.27: Some representative ultrastructural observations from normal, and GalN-FHF rats in Grade II hepatic coma just prior to PGE_2 injection. a: A typical normal hepatocyte before GalN-induced hepatocyte injury. The location of the endoplasmic reticulum (ER) and the mitochondria (M) can be seen distinct and separate from each other and the nucleus (N). b: A typical hepatocyte during Grade II coma, just prior to PGE_2 injection. The characteristic migration of the ER and mitochondria (M) to align themselves with the nuclear membrane is evident at this time. c: Severe mitochondrial aberrations (elongation and dumbbell-shaped appearance) with loss of cristae are also evident at this time. Samples were fixed using glutaraldehyde and osmium tetroxide, and were post-stained with uranyl acetate and lead citrate. Bar represents 1μ.



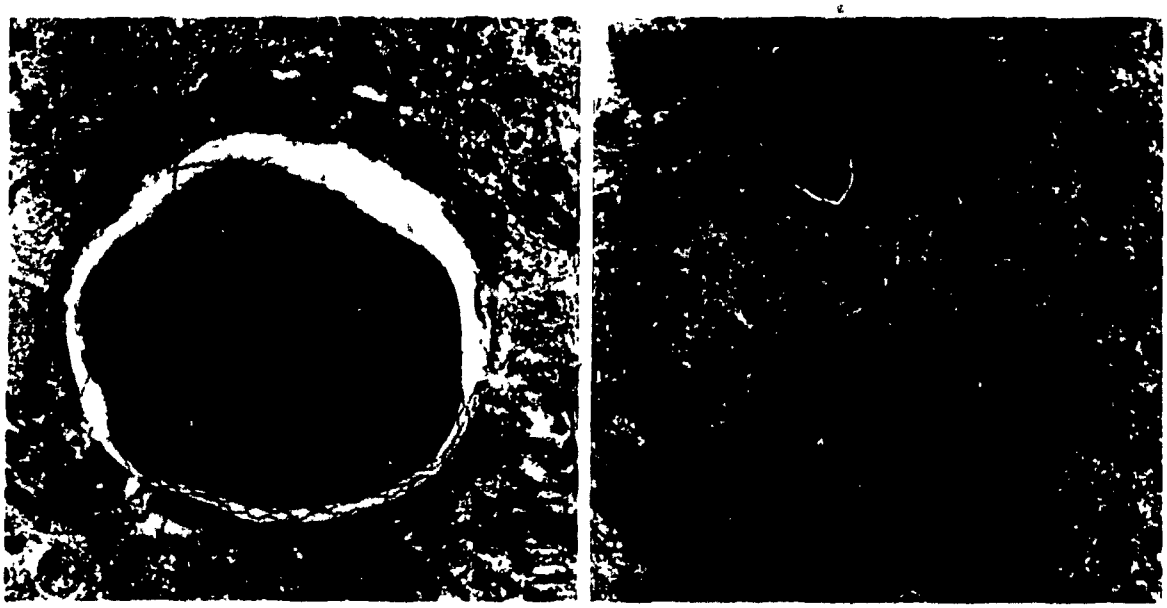


Figure 3.28: Some representative ultrastructural observations of hepatocytes during PGE_2 studies. PGE_2 was injected intraperitoneally into GalN-FHF rats in Grade II hepatic coma. a: A typical hepatocyte 4 hours after saline injection (control). the onset of hepatocyte karyolysis is apparent. At this time the endoplasmic reticulum (ER) can be seen surrounding the nucleus in a ring-like formation. Severely distorted mitochondria (M) are also visible in the hepatocyte cytoplasm. b: A typical hepatocyte from the group of rats receiving PGE_2, 4 hours after its injection. The ER can be seen circling the nucleus but no evidence of hepatocyte karyolysis was visible in the PGE_2 treated group. Eight hours after PGE_2 injection, the majority of the hepatocytes appeared to undergo karyolysis and appeared similar to that shown in Figure 3.28a. Tissue samples were fixed in glutaraldehyde and osmium tetroxide, and were post-stained with uranyl acetate and lead citrate. Bar represents 1μ.

were visible in the hepatocyte cytoplasm. The ER can also be seen surrounding the nucleus in a ring like formation (Figure 3.28a). Figure 3.28b shows a typical hepatocyte from the group of rats receiving PGE_2, 4 hours after its injection. The ER can be seen circling the nucleus but no evidence hepatic karyolysis was seen in the PGE_2 treated group at this time. Mitochondrial distortions were still evident and a greater number of them were seen in the close proximity of the ER and nucleus (Figure 3.28b). Eight hours after PGE_2 injection, the majority of the hepatocytes appeared to undergo karyolysis and appeared similar to that shown in Figure 3.28a.

3.3 THE EFFECTS OF PGE_2 ON THE DEVELOPMENT OF BRAIN EDEMA

During the course of this study, it was evident that brain edema was a critical and dominant secondary complication during GalN-FHF. It was also seen that PGE_2 significantly prolonged the survival time of rats in Grade II hepatic coma. The following study was executed to determine whether PGE_2 had any effects on the development of brain edema in these rats. Brain edema was assessed in terms of brain water content, brain swelling, as well as by tissue histology. Additional studies involving intravenous trypan blue dye infusions were also carried out to determine

the structural integrity of the blood-brain barrier during the PGE_2 studies.

3.3.1. Brain Water Content Measurements During PGE_2 Studies

Brain water content measurements were obtained at specific times (i.e. 0, 4, and 8 hours after PGE_2 or saline injection) during the PGE_2 studies. Table 3.14 contains the brain water content measurements (gH_2O/g dry wt. of brain tissue) of the experimental animals in these PGE_2 studies. Prostaglandin E_2 or physiological saline as control was injected intraperitoneally during Grade II coma, i.e. 0 hour. At 4 hours (after saline injection) a significant ($p<0.01$) increase in the water content of the brain was observed in the control animals receiving saline. In contrast, a similar matched group of rats receiving PGE_2, instead of saline, showed no such increases in brain water content at this time (Table 3.14). Eight hours after PGE_2 injection, the brain water content had decreased below initial levels seen prior to PGE_2 injection (i.e. at 0 hour). This decrease, however, was not statistically significant when compared to brain water content values obtained 4 hours after PGE_2 injection. It was, however, a significant ($p<0.02$) decrease with respect to the control group receiving saline only.

Table 3.14

Effects of PGE_2 on Brain Edema in Grade II Coma Galactosamine-Induced Fulminant Hepatic Failure Rats: Brain Water Content Measurements

Group No.	Experimental Condition	Number of Animals	Brain Water Content g H_2O/g dry wt. of brain (mean ± SE)	Statistical Significance*
A	Normal-No GalN-FHF	11	3.474 ± 0.012	A vs B ($p<0.2$)
B	0 hrs (Grade II coma)	11	3.531 ± 0.018	B vs C ($p<0.01$)
C	4 hrs after saline	5	3.660 ± 0.037	C vs D ($p<0.02$)
D	4 hrs after PGE_2	5	3.535 ± 0.024	B vs D (N.S.)
E	8 hrs after PGE_2	5	3.480 ± 0.042	C vs E ($p<0.02$) B vs E (N.S.) D vs E (N.S.)

Effects of PGE_2 on the evolution of brain edema in Grade II coma FHF rats. PGE_2 prevented the progression of brain edema in rats, 4 hours after its administration. A significant increase in brain water content was observed in the control rats in the same time. Brain water content was reduced to near normal levels 8 hours after PGE_2 injection. There was no survival in the control (i.e. saline injection) groups at this time.

* Statistical significance was determined using Student 't' test.
NS = not significant.

3.8.2 Estimations of Brain Swelling During the PGE_2 Studies

Since small increments in brain water content measurements may not relate the actual magnitude of brain edema and subsequent brain swelling, the latter was estimated using the formula described in Chapter II of this thesis. Brain swelling measurements are presented in Figure 3.29 and Table 3.15.

What may appear minor increases in percent brain water content (Table 3.15) are reflected as major increases in percent brain swelling. Four hours after PGE_2/saline injection, a more than 3 fold increase in brain swelling was seen in the saline injected control rats. No such increases in percent brain swelling were seen in the PGE_2 injected group at this time (Figure 3.29 and Table 3.15). Furthermore, 8 hours after PGE_2 injection the brain swelling had subsided to near normal values (Table 3.15).

A composite graph relating percent swelling of the brain to percent increase in brain water content is shown in Figure 3.30. From this graph, it can be clearly seen how small increments in brain water content can result in substantive swelling of the brain. This is especially true after Grade II coma. For example, when percent brain water content increased from 77.835 $\pm$ 0.022% in Grade II coma to 78.544 $\pm$ 0.125% in Grade IV coma, brain swelling increased

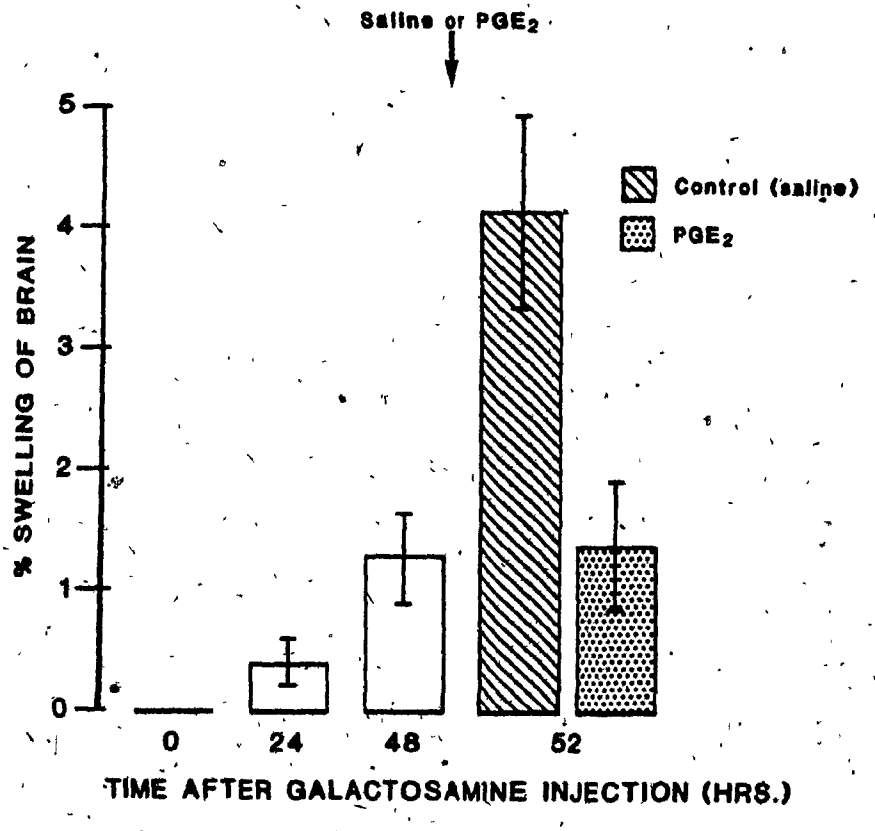


Figure 3.29: The effects of PGE_2 on brain edema. PGE_2 was injected intraperitoneally into GalN-FHF rats in Grade II hepatic coma. A steady and massive increase in brain swelling was seen in the control animals receiving only saline. PGE_2 injected animals did not show such increases in brain swelling.

Table 3.15

Effects of PGE_2 Brain Edema in Grade II Coma Galactasamine-Induced Fulminant Hepatic Failure Rats: Brain Swelling Measurements

Group No.	Experimental Condition	Number of Animals	% Brain Water Content (mean ± SE)	% Swelling of the Brain (mean ± SE)
A	Normal-No GalN-FHF	11	77.649 ± 0.061	0.000
B	0 hrs (Grade II coma)	11	77.835 ± 0.122	1.277 ± 0.41
C	4 hrs after saline	5	78.531 ± 0.171	4.134 ± 0.829*
D	4 hrs after PGE_2	5	77.946 ± 0.118	1.356 ± 0.543**
E	8 hrs after PGE_2	5	77.845 ± 0.244	0.1274 ± 0.9263***

animals were given PGE_2/saline during Grade II coma, 48 hours after GalN-induced liver injury

Statistical significance was determined using the Student 't' test.

* $p<0.01$ with respect to "0 hrs"
** $p<0.02$ with respect to "4 hrs saline" group
*** $p<0.001$ with respect to "4 hrs saline" group

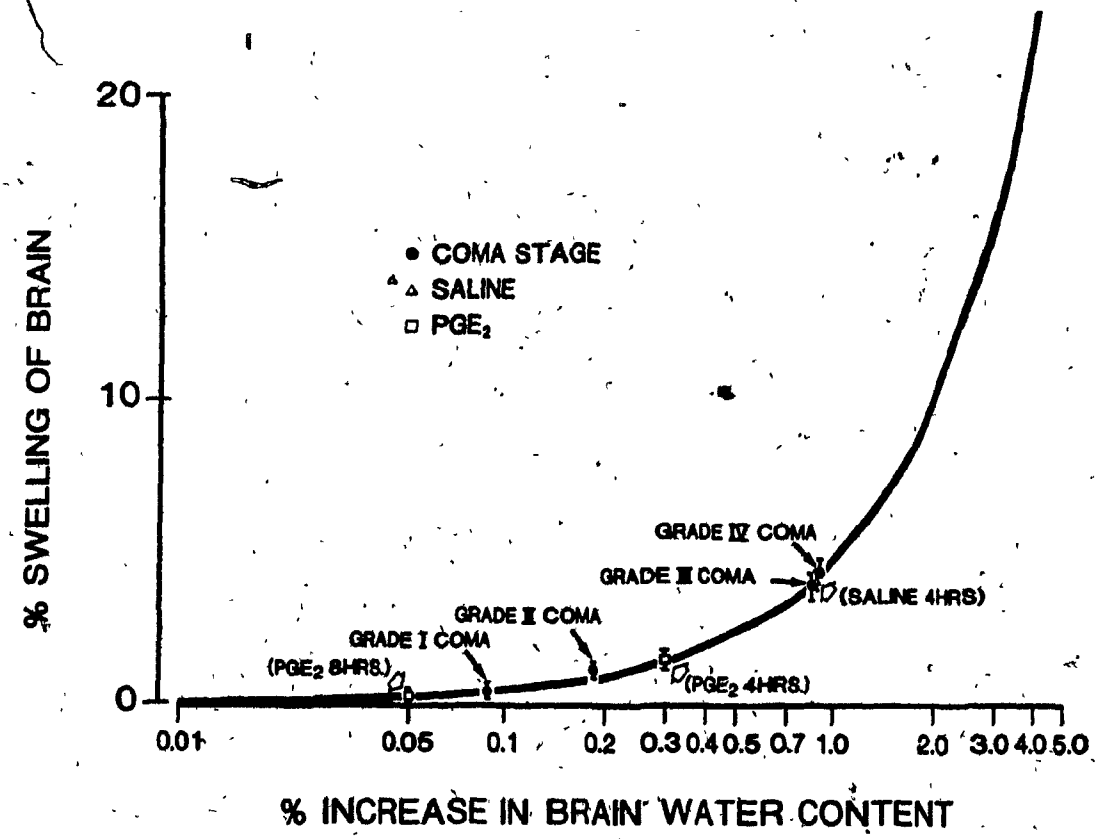


Figure 3.30: A graph relating percent swelling of the brain to the percent increase in brain water content. From this graph it is clear that small increases in brain water content can result in substansive swelling of the brain. It can be seen here that PGE_2 given during Grade II coma prevented the progression of brain edema and brain swelling from reaching the steep portion of the curve. Furthermore, 8 hours after PGE_2 injection brain swelling had subsided to near normal values.

by nearly 3.5 fold. It was seen that PGE_2 injection prevented the progression of brain edema (brain swelling) from reaching this critical stage.

3.3.3 Brain Histology During PGE_2 Studies

In addition to the quantitative measurements (described above), brain edema was also examined qualitatively by histology. At specific times (i.e. 0, 4, and 8 hours post PGE_2/saline injection) during the PGE_2 studies, tissue samples were obtained for both light and electron microscopy. Representative observations from approximately 50 rats fixed for light and electron microscopy are presented below.

3.3.3.1 Light Microscopy of the Brain During PGE_2 Studies

Representative observations from approximately 30 rats fixed for light microscopy are presented in Figures 3.31 and 3.32. The former depicts sections from the cerebrum and the latter from the cerebellum. The basic observations were strikingly similar in both regions of the brain. Presence of brain edema was evident before the onset of the PGE_2 studies when the rats were in Grade II hepatic coma (Figure 3.31a and Figure 3.32a). It was observed that brain edema developed similarly in both groups of rats

Figure 3.31: Representative histological observations of the light microscopy performed on the cerebrum of rats in the PGE_2 studies. PGE_2 was injected intraperitoneally into GalN-FHF rats in Grade II hepatic coma. Samples of the cerebrum were obtained for histology before PGE_2 (or saline) injection (i.e. 0 hr.) and at specific times (i.e 4 and 8 hrs.) after PGE_2 (or saline) injection. a: Cerebrum of a rat in Grade II coma just prior to PGE_2 injection (0 hr.). Extensive brain edema (E) is evident at this time. b: Cerebrum of a rat 4 hours after saline injection. Massive brain edema (E) with compression and distortion of adjacent brain tissue is evident at this time. c: Cerebrum of a rat 4 hours after PGE_2 injection. The presence of brain edema (E) is still evident, however, much less than in the corresponding control group of rats which received only saline. d: Cerebellum of a rat 8 hours after PGE_2 injection. A total remission of brain edema was observed at this time. All tissues were fixed in Carnoy's Fluid, and stained using Delafield's Hematoxylin and eosin Y staining techniques. Magnification a: 360x, b-d: 400x.

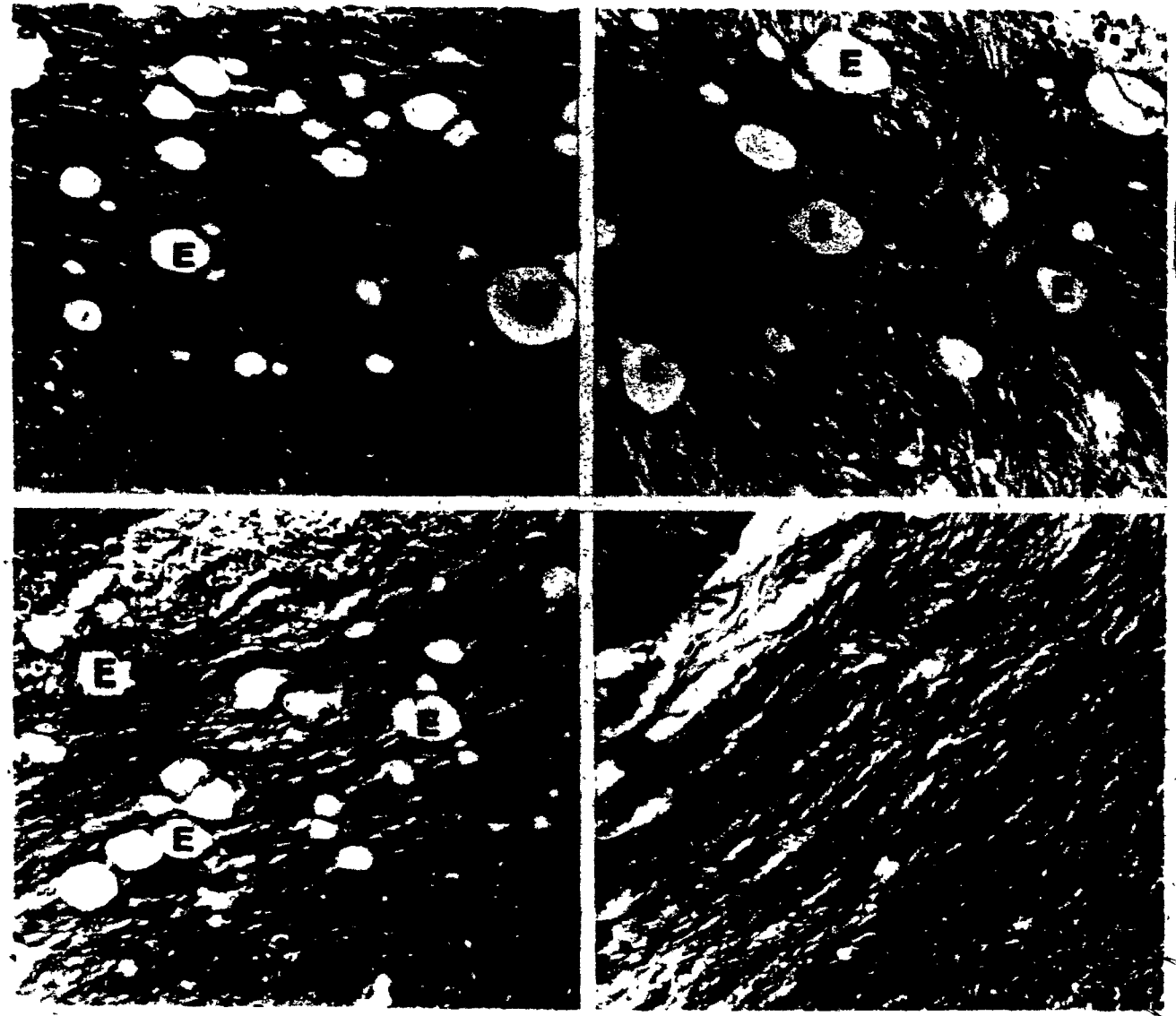

E
E
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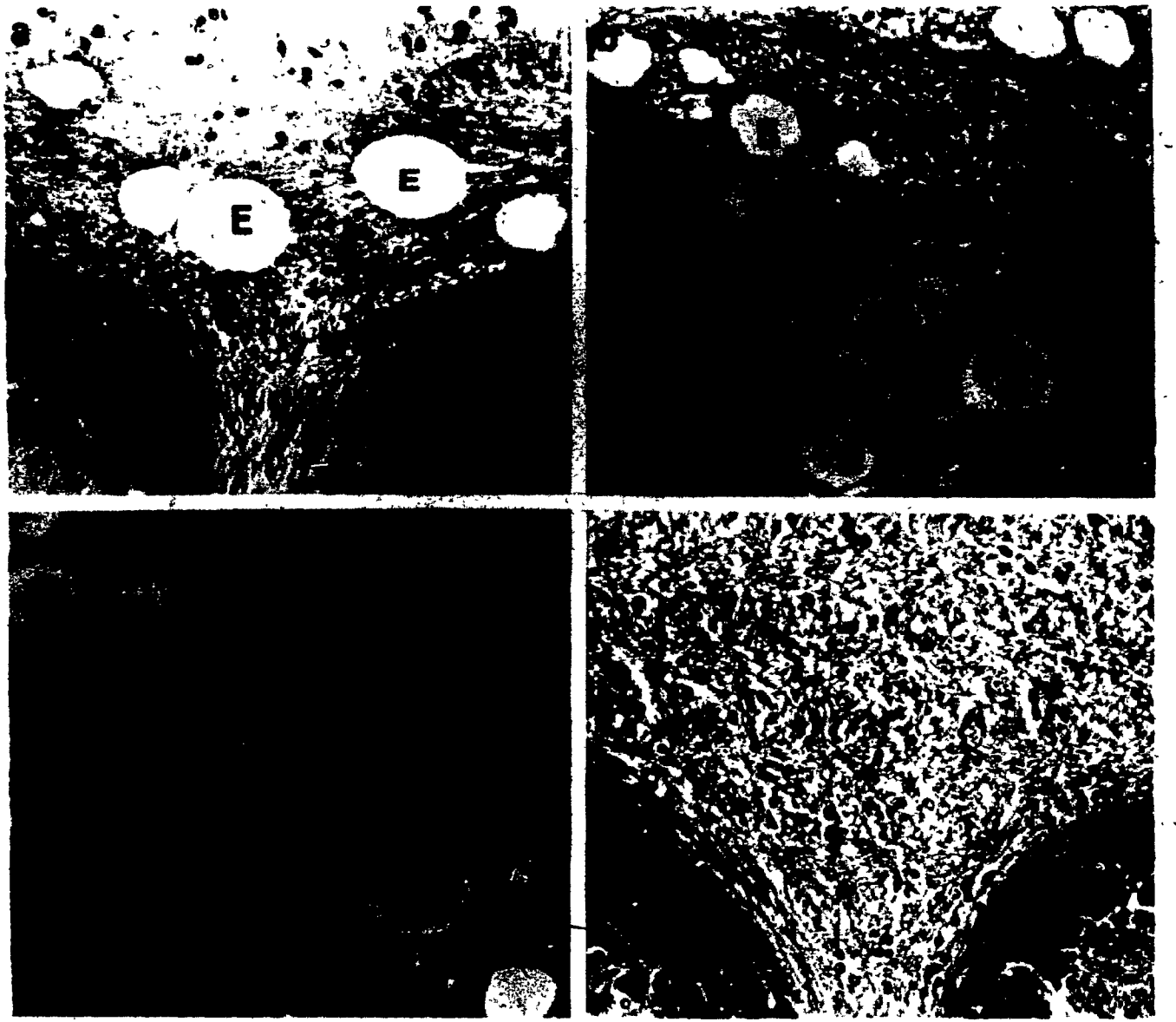
Figure 3.32: Representative histological observations of the light microscopy performed on the cerebellum of rats in the PGE_2 studies. PGE_2 was injected intraperitoneally into GalN-FHF rats in Grade II hepatic coma. Samples of the cerebellum were obtained for histology before PGE_2 (or saline) injection (i.e. 0 hr.) and at specific times (i.e 4 and 8 hrs.) after PGE_2 (or saline) injection. a: Cerebellum of a rat in Grade II coma, just prior to PGE_2 injection (0 hr.). Extensive brain edema (E) is evident at this time. b: Cerebellum of a rat 4 hours after saline injection. Massive brain edema (E) with compression and distortion of adjacent brain tissue is evident at this time. c: Cerebellum of a rat 4 hours after PGE_2 injection. The presence of brain edema (E) is still evident; however, much less than in the corresponding control group of rats which received only saline. d: Cerebellum of a rat 8 hours after PGE_2 injection. A total remission of brain edema was observed at this time. All tissues were fixed in Carnoy's Fluid, and stained using Delafield's Hematoxylin and eosin Y staining techniques. bv = blood vessel. Magnification a: 360x, b-d: 400x.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during the 4 hours period following the injection of either saline or PGE_2 (Figure 3.31b,c and Figure 3.32b,c). It was surprising, however, to note that in animals receiving PGE_2 a total remission of brain edema was observed 8 hours after PGE_2 injection (Figure 3.31d and Figure 3.32d). Numerous observations in other similar rats have confirmed the validity of these findings. Since it was difficult to derive further information regarding the development of brain edema and its subsequent regression 8 hours after PGE_2 injection by light microscopy, ultrastructural studies involving electron microscopy were carried out.

3.3.3.2 Electron Microscopy of the Brain During PGE_2 Studies

Representative observation of the cerebral cortex of approximately 20 rats is presented in Figures 3.33 to 3.35. Figure 3.33a depicts a normal cerebral capillary before GalN-induced hepatic injury. A cerebral capillary within a compact cerebral parenchyma is visible. A perivascular astroglial cell (AG) is also visible. Figure 3.33b shows a typical cerebral capillary of a GalN-FHF rat in Grade II hepatic coma, just prior to PGE_2/saline injection. A swollen (edematous) perivascular astroglial cell (AG) containing abnormal vacuoles (V) can be seen surrounding the cerebral capillary.

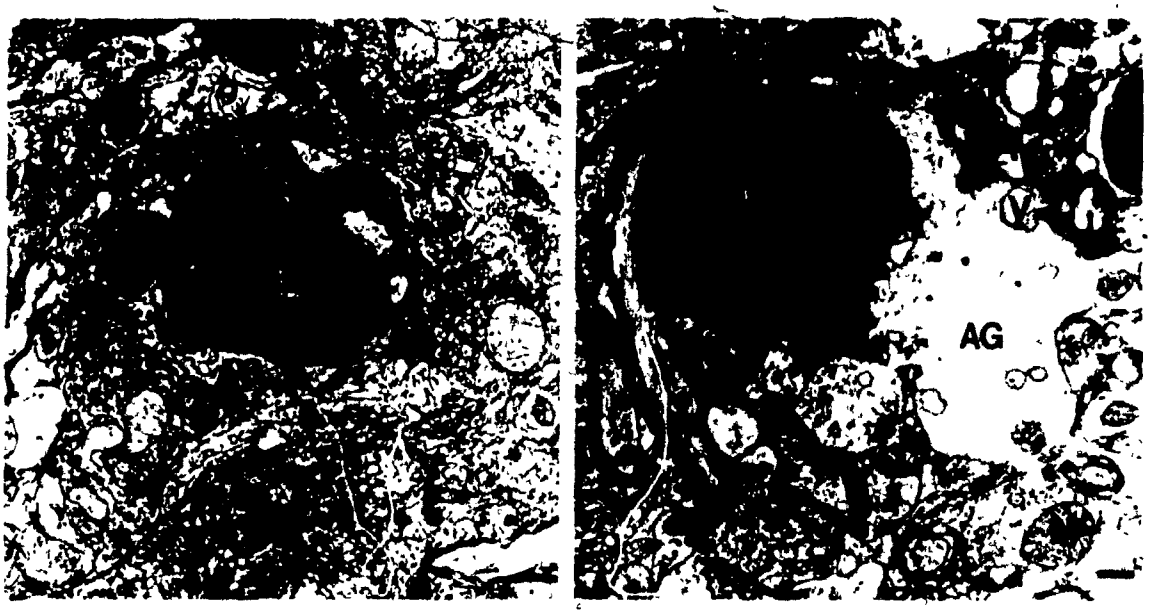

Figure 3.33: A typical cerebral capillary from a normal(a) and GalN-FHF rat in Grade II hepatic coma (b). The dense and compact cerebral parenchyma and perivascular astroglial cell (AG) is visible in the section from a normal rat (a). Extensive astroglial (AG) swelling containing abnormal vacuoles and swollen mitochondria (M) is clearly visible in (b). E = erythrocyte. Tissues were fixed in glutaraldehyde and osmium tetroxide, and post-stained with uranyl acetate and lead citrate. Bar represents 1μ.

Figure 3.34a depicts a cerebral capillary from a rat 4 hours after control saline injection. Extensive perivascular astroglial (AG) swelling with compression and distortion of adjacent cerebral parenchyma is markedly evident. In contrast Figure 3.34b depicts a cerebral capillary from a PGE_2 injected animal after 4 hours. A small amount of astroglial (AG) swelling can be seen in the perivascular region of the blood vessel. Unlike its matched control (which received only saline), the PGE_2 injected animal showed a marked control of brain swelling at this time (i.e. 4 hours post PGE_2 injection).

Figure 3.35 is an electron micrograph of a rat cerebral capillary 8 hours after PGE_2 injection. Only a slight astroglial (AG) swelling is evident at this time. There are no abnormal vacuoles (within the astroglial cell) or the evidence of any compression of adjacent cerebral parenchyma at this time (Figure 3.35).

3.3.4 Test for Blood-Brain Barrier Integrity During PGE_2 Studies

Because of the apparent control of brain edema and its resultant brain swelling during the PGE_2 studies (above), the structural integrity of the blood-brain barrier was tested to determine whether PGE_2 had any effects on the physical integrity of this regulatory blood-brain interface.

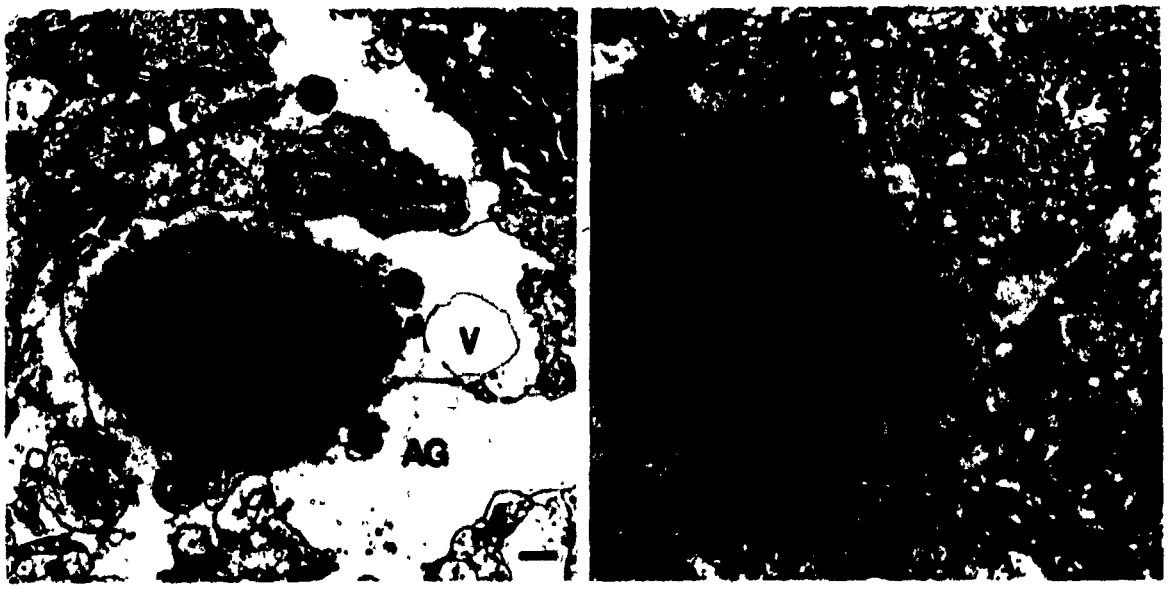

Figure 3.34: Cerebral capillary from rats used in the PGE_2 studies. a: A cerebral capillary from a rat 4 hours after saline injection (control). Extensive perivascular astroglial (AG) swelling with compression and distortion of adjacent cerebral parenchyma is markedly evident. b: A cerebral capillary from a PGE_2 injected animal after 4 hours. A small amount of astroglial (AG) swelling can be seen in the perivascular region of the blood vessel. Unlike its matched control (which received only saline), the PGE_2 injected animal showed marked control of astroglial swelling. Tissues were fixed in glutaraldehyde and osmium tetroxide, and post-stained with uranyl acetate and lead citrate. Bar represents 1μ.

Figure 3.35: A cerebral capillary from a PGE_2 injected rat, 8 hours after PGE_2 injection. Only slight astroglial (AG) swelling is evident at this time. There are no abnormal vacuoles within the astroglial cell or any evidence of compression of adjacent cerebral tissue. L: lumen. Tissues were fixed in glutaraldehyde and osmium tetroxide, and post-stained with uranyl acetate and lead citrate. Bar represents 1μ.

Trypan blue dye infusion studies were carried out at specific times (i.e. 0, 4, and 8 hours post PGE_2/saline infusion) as described previously. Representative results from 25 rats (5 rats per group shown) are presented in Figure 3.36. The brain from a normal rat (with no liver damage) is not stained by trypan blue, indicating an intact blood-brain barrier. During Grade II coma (48 hrs. after GalN-induced hepatic injury) and just prior to PGE_2/saline injection (i.e. 0 hour in PGE_2 studies), the brain appeared partially stained, indicating partial barrier breakdown at this stage. In the group of rats receiving only saline (control) a total and complete blood-barrier breakdown is evident after 4 hours. The deep blue staining indicates the ability of the dye, normally prevented from crossing the intact blood-brain barrier, to traverse it to stain the cerebral and cerebellar tissue. In the PGE_2 injected rats no such extensive barrier breakdown is observed after 4 hours. The rat brains resemble those of Grade II coma rats and reveal only slight cerebral and cerebellar staining. PGE_2 rats at 8 hours showed evidence of blood-brain barrier breakdown; however, it was not as extensive as that seen in the saline injected control group at 4 hours post saline injection (Figure 3.36).

Figure 3.36: Test for the structural integrity of the blood-brain barrier. Representative results from normal and Grade II coma GalN-FHF rats which were injected with PGE_2. Trypan blue dye was injected via intracarotid infusion to determine BBB integrity. Normally an intact BBB prevents entry of the dye to stain the brain. Any staining of the brain would indicate an increase in permeability, or total breakdown of the BBB.

PGE_2 STUDIES

NORMAL

GRADE II COMA

0 HRS

4 HRS SALINE

4 HRS PGE_2

8 HRS PGE_2

CHAPTER IV

DISCUSSION

CHAPTER IV
DISCUSSION

4.1 ON THE BASIC PREAMBLE

Investigations concerning the cytoprotective property of prostaglandins have stimulated extensive research since its first observations in the canine gastric mucosa nearly a decade ago (Robert, 1976). Cytoprotection by prostaglandins is now recognized as separate from, and unrelated to, its inhibitory effects on gastric secretion (Robert, 1979). In recent years the property of cytoprotection by prostaglandins has been extended to include a variety of other gastrointestinal organs including the liver (Stachura et al., 1980; Reber et al., 1980; Ruwart et al., 1981a). The concept of hepato-cytoprotection (Miyazaki, 1983) now includes not only hepatocytes, but other liver cells including those of the biliary epithelium as well (Ruwart et al., 1984).

It has been extensively reported that prostaglandin E_2 (PGE_2) is successful in preventing hepatocyte necrosis against a variety of noxious stimuli ranging from thermal injury to specific hepatotoxins such as carbon tetra-chloride and galactosamine (Stachura et al., 1980, 1981; Ruwart et al., 1981b, 1982a,b, 1984; Miyazaki, 1983). In these studies PGE_2 was administered either just before or immediately after the exposure of the liver to the

necrotizing agent. It is not known whether PGE_2 is capable of providing any cytoprotection after the development of hepatocyte injury. With this as an initial curiosity, the present project was embarked upon. The galactosamine-induced fulminant hepatic failure (GalN-FHF) rat model was selected to provide a suitable model for hepatocyte injury and necrosis. This model has been extensively used at the Artificial Cells and Organs Research Centre, McGill University, as well as other similar major centres for studies concerning liver failure and artificial liver support (Chang et al., 1978; Chirito et al., 1978, 1979; Blitzer et al., 1980; Tabata and Chang, 1980; Dixit and Chang, 1981, 1982, 1985; Shu and Chang, 1983; Shi and Chang, 1982, 1983, 1984a,b; Horowitz et al., 1983; Niu et al., 1983; Zaki et al., 1984; Shaffer et al., 1984). This model is also considered as the one which most closely resembles human FHF in its biochemical, neurological, and pathological features (Saunders, 1979).

Given the highly complex physiology of the liver and its even more complex pathophysiology during liver failure, it was not expected that a single chemical compound, in this instance PGE_2, would be capable of protecting the liver against severe necrosis during fulminant hepatitis. Various doses of PGE_2 were tested on FHF rats, in

progressive stages of liver failure, after the development of maximal liver injury and coma. As was expected, there was no significant increase in the overall survival rate of these animals. However, a surprising observation was made that PGE_2 injected animals had significantly longer survival times when compared to saline injected control animals (Dixit and Chang, 1982). Furthermore, upon routine autopsy and histological examination of these animals, it was noticed that the PGE_2 injected rats also had less or even no brain edema (Dixit and Chang, 1985). Corresponding control animals receiving only saline exhibited marked evidence of extensive brain edema. With these preliminary findings, further detailed investigations were carried out here to study the effects of PGE_2 on both the liver and brain of rats with liver failure. The results of these studies have been described in earlier sections of this thesis.

Concurrent to the PGE_2 studies, the basic GalN-FHF rat model has been re-evaluated in terms of its neurological status (i.e. grade of hepatic coma). In earlier studies, the characterization of this model was based upon the time elapsed (in hours) following galactosamine injury (injection). This time scale was used to assess the extent of hepatocyte injury (Decker and Keppler, 1972; Pickering

et al., 1975; Chirito et al., 1979). However, keeping in mind the distinct and progressive deteriorations in consciousness, which resulted from progressive liver failure, it was considered more appropriate to evaluate GalN-FHF in terms of grades of coma. Thus, when using the earlier "time scale" classification, all animals 48 hours after GalN injection were considered as having the same degree of hepatocyte injury and necrosis. However, in practice, it was seen that at this time (i.e. 48 hours after GalN injection) the animals were in varying stages (grades) of hepatic coma (Figure 3.4). Thus, by using a "time scale" evaluation system, it was difficult and even misleading in the accurate assessment of hepatic injury (Chang et al., 1978). A grading system based on the assessment of neurological status may better reflect the extent of hepatic injury.

4.2 THE BASIC GALACTOSAMINE-INDUCED FULMINANT HEPATIC FAILURE RAT MODEL

The justification for re-evaluating the basic GalN-FHF rat model has been considered in the preamble of this chapter. However, it may be noteworthy to once again emphasize that hepatocellular necrosis results in profound neurological alterations. Since the severity of coma

reflects a serious impairment in normal hepatic physiology, it seems more appropriate to evaluate acute hepatocyte necrosis or FHF in terms of grades of coma. In order to facilitate comparison of the two grading systems, the results of the present study are presented in terms of both the time elapsed after GalN injection, as well as the grade of hepatic coma.

Galactosamine was shown to produce acute hepatocellular injury in a consistently reproducible dose-effect relationship (Figure 3.1). Furthermore, GalN produced hepatocyte injury which showed an inverse relationship with the age of the animal (Figure 3.2). When the dosage was kept constant, the GalN-induced hepatic injury was directly proportional to the age of the animal (Figure 3.3). Thus, it was necessary to strictly adhere to a fixed animal age group in order to maintain a consistently reproducible model for GalN-induced liver injury.

Following GalN injection and the onset of hepatocyte injury, one of the first observable changes was marked pilo-erection and jaundice (during Grade I and II coma). Significant ($p < 0.001$) decline in body weight ($6.28 \pm 2.03\%$) was evident before the development of overt encephalopathy (i.e. Grade I coma), i.e. 24 hours after galactosamine. The body weight of the animals fell

rapidly, by nearly 15 percent, till the development of Grade II coma 48 hours after GalN injection. No further significant decreases in body weight were seen with subsequent grades of coma (Table 3.1 and Figure 3.5). Coinciding with the decreases in body weight were marked increases in hematocrit values (Tables 3.2 and Figure 3.5). As in the case of body weight, hematocrit values rose significantly till Grade II coma. In subsequent coma grades, only very minor increases in hematocrit values were observed. Thus, liver failure resulted in marked decreases in body weight as well as extensive dehydration. These observations were probably the result of diminished food and water intake by the liver failure rats.

4.2.1 Serum Biochemistry Analysis

Serum biochemical analysis, as performed by the SMAC analysis and by commercially available kits, is presented in Figures 3.6 - 3.8 and Tables 3.3 - 3.5. A dramatic derangement of all the biochemical parameters was seen as a result of GalN-induced hepatocellular injury. Of major significance was a steady and significant decline in serum glucose levels following hepatocyte injury. Serum glucose levels fell from 137.4 $\pm$ 1.73 mg/dl to 15.0 $\pm$ 2.0 mg/dl during the course of liver failure following GalN injection.

Severe hypoglycemia can cause seizures and depressed neurological states often associated with hepatic coma (Duffy and Plum, 1976; Bradbury, 1984; Pardridge, 1984). However, although the extent of hypoglycemia seemed to correlate with the severity of coma, it has not been implicated as a major factor contributing to coma or death in this model (Blitzer et al., 1978). Furthermore, glucose infusions during deep or severe grades of coma do not seem to ameliorate hepatic encephalopathy or survival (Blitzer et al., 1978). Nevertheless, because of the severe glycogen depletion seen following hepatocellular injury (Decker and Keppler, 1972; Chirito et al., 1979), it was considered important to maintain normoglycemia. In all liver failure studies being carried out at this centre, a 20 to 50 percent solution of glucose was routinely administered to all GalN-FHF rats via subcutaneous injections (Chirito et al., 1977; Chang et al., 1978).

The inability of the hepatocytes to conjugate bile salts is a major feature of the failing liver (Rueff and Benhamou, 1973; Saunders et al., 1979). In accordance, the total serum bilirubin levels increased steadily (by approximately 50 fold) with progressive severity of coma (Figure 3.6 and Table 3.3). Despite this seemingly direct correlation between the levels of total bilirubin and the

grade of coma, it is generally recognized that serum bilirubin levels bear no relation to coma (Knell, 1980; Jones and Schafer, 1982); the deepest jaundice is often seen during recovery (Knell, 1980). However, continuing coma and deep jaundice usually imply a fatal outcome (Saunders et al., 1979).

Elevation of serum cholesterol levels first became apparent at Grade II coma and remained elevated through progressive grades of coma until death (Figure 3.6 and Table 3.3). The greater than two fold elevation in serum cholesterol levels observed during overt encephalopathy is most likely the reflection of massive hepatocellular membrane injury and necrosis taking place at this time. It has been previously reported that, in the GalN-FHF rat model, hepatic injury was maximal at 48 hours after GalN injection (Decker and Keppler, 1972; Chirito et al., 1979). In the present study, this was also the time when encephalopathy (Grade II coma) first became apparent.

Impaired hepatocellular protein synthesis was evident ($p<0.005$) as early as Grade I coma, 24 hours after GalN injection. The level remained depressed until Grade IV coma, at which time a significant ($p<0.05$) increase in the level of total proteins was seen (Figure 3.6 and Table 3.3). Serum albumin levels behaved in a similar fashion (Figure

3.6 and Table 3.3). Depressed fibrinogen levels have been reported in the GalN-FHF rabbit model; however, it was not clear whether the animal was comatose or not at this stage (Blitzer et al., 1978). Depression of specific coagulation proteins, as well as albumin during liver failure, can seriously impair normal hemostasis and affect serum osmolarity.

Uric acid is formed in the liver as the final degradation product of purines (Lehninger, 1982). Thus, as a result of GalN-induced hepatocyte injury, there was a progressive decrease in serum uric acid levels with increasing severity of hepatic coma (Figure 3.6 and Table 3.3). A nearly 50 to 75 percent decrease in uric acid levels was observed during the progression from Grade I to Grade III coma.

Elevated serum creatinine and blood urea nitrogen (BUN) are characteristic features of impaired renal function (Guyton, 1981). In the present study, mild but significant ($p<0.05$) increases of both serum creatinine and BUN were observed (Figure 3.6 and Table 3.3). However, in the present study, it was unlikely that any major impairment of renal function was the cause of the observed increases in serum creatinine and BUN levels. Earlier studies at this centre and elsewhere have conclusively

shown that galactosamine itself was not nephrotoxic (Blitzer et al., 1978; Chirito et al., 1979; Horowitz et al., 1983). Furthermore, these studies also documented that it was unlikely that GalN-FHF produced any impairment of renal function based on histological observations. The mild rise in serum creatinine and BUN levels may be attributed to the extensive dehydration taking place during progressive stages of liver failure.

Serum electrolyte analyses of the GalN-FHF rats were carried out during progressive grade of hepatic coma and are presented in Figure 3.7 and Table 3.4. Mild fluctations in the serum levels of all the electrolytes were seen as a result of acute hepatocyte injury. Williams' group at King's College, Cambridge, has reported incidences of hypokalemia during early stages of liver failure (Williams, 1975). The present studies on the GalN-FHF rat model confirm such findings.

El-Mofty et al. (1975) have reported that GalN produces characteristic alterations in the hepatocyte plasma membrane, such that intracellular accumulation of calcium was seen as a result of membrane injury (by GalN) to the hepatocytes. In the present study, calcium levels were significantly ($p < 0.005$) depressed during Grade II coma (Figure 3.7 and Table 3.4). Thus, it is quite possible

that the reduced calcium levels (as observed in the present study) may be a consequence of similar membrane injury described by El-Mofty et al. (1975). If this were the case, then Grade II hepatic coma may, in fact, represent one of the first stages of significant membrane injury to the hepatocytes by galactosamine.

Liver enzyme analysis was also performed during progressive stages of hepatic coma following GalN-FHF. Results are tabulated and presented in Figure 3.8 and Table 3.5. An enormous increase in the levels of all enzymes was seen as a result of GalN-induced hepatocyte injury. For all the enzymes, with the exception of aspartate amino-transferase (ASAT), the levels peaked during Grade II coma and remained elevated throughout the successive coma grades till death. ASAT levels peaked during Grade III coma. The enormous magnitude of the elevation in serum enzyme activity reflects the extensive hepatocellular injury taking place following GalN injection. In all cases (except for ASAT), it appears that hepatocyte injury was maximal during Grade II coma. Earlier studies using this rat model have also reported similar findings showing that maximum hepatocyte injury occurred 48 hours after GalN injection (Decker and Keppler, 1972; Chirito et al., 1979). This is also the approximate time when the first symptoms

of overt hepatic coma (i.e. Grade II hepatic coma) became apparent. Thus, from the forgoing discussion, it can be reasonably concluded that GalN-induced hepatocellular injury and necrosis was maximal during Grade II hepatic coma.

4.2.2 Histological Analysis of the Hepatocytes Following Galactosamine-Induced Liver Injury

Galactosamine-induced hepatocyte injury was assessed during progressive stages of liver injury and hepatic coma by both light and electron microscopy. Some representative histological observations are presented in Figures 3.9 and 3.10 respectively. Light microscopy histology results showed progressive hepatocellular injury with increasing severity of hepatic coma. There were, however, no major distinguishable histological abberations during very early stages of GalN-induced hepatocyte injury, i.e. during Grade I coma (Figure 3.9b). With the development of Grade II coma there was an almost complete dissociation of the normal cord-like arrangement of the hepatocytes (Figure 3.9a,c). Evidence of the onset of mottled hepatocellular necrosis, as well as the presence of extensive periportal inflammatory infiltrations, were also evident at this time (i.e. Grade II coma) (Figure 3.9c,d). During the terminal stages of GalN-FHF, i.e. Grades III and IV hepatic coma,

there was increased evidence of hepatocyte karyolysis and cellular necrosis (Figure 3.9e,f). However, due to the inherently poor resolution of a light microscope it was not possible to clearly distinguish between the various cellular events that may have been taking place during the progressive stages of GalN-induced hepatocellular injury. Thus, in order to observe the ultrastructural events involved during GalN-induced hepatocyte injury and necrosis, electron microscopy studies were carried out. Some representative ultrastructural observations are presented in Figure 3.10.

Electron microscopy of the liver revealed that GalN-induced hepatocyte injury resulted due to a characteristic pattern of hepatocellular events which lead to eventual hepatocellular necrosis. Furthermore, it was observed that the sequence of these hepatocellular events correlated with the progressive development of hepatic coma. Thus, the normal arrangement of the hepatocyte's sub-cellular organelles was dramatically altered, as early as Grade I coma, such that there was a generalized migration of the mitochondria and endoplasmic reticulum (ER) towards the nucleus (Figure 3.10a,b). This was followed by a stage during Grade II coma, where there was a close association between the ER and the nucleus, such that

the ER was almost completely wrapped around the nucleus (Figure 3.10c). At this time the mitochondria also appeared abnormally elongated and sandwiched between layers of ER (Figure 3.10d). The final stages of GalN-induced hepatocyte necrosis occurred between Grades III and IV hepatic coma (Figure 3.10e,f). During these stages the ER-enveloped nucleus appeared to dissociate from the cytoplasm to result in karyolysis and cellular necrosis.

A sequential account of the cellular events involved in GalN-induced hepatocyte necrosis is lacking in the literature. Earlier biochemical studies have suggested that GalN-induced liver cell death results from plasma membrane alterations as well as changes in the calcium content of the injured cells (Decker and Keppler, 1972; El-Mofty et al., 1975; Liehr et al, 1970). According to these biochemical studies the hepatotoxicity of GalN lies in its metabolism by the hepatocytes. A primary lesion results from a very rapid depletion of hepatic uridine nucleotides due to the formation of uridine derivatives of GalN. A secondary lesion subsequently results from defects in macromolecular glycoprotein synthesis, giving rise to progressive organelle injury, membrane injury, and cellular necrosis. It is, however, still unclear as to whether plasma membrane injury is a cause or an effect of cell death.

The present study suggests that hepatocyte karyolysis may be a major factor in GalN-induced hepatocellular necrosis. Thus, this observation would implicate hepatocyte plasma membrane injury (and cell necrosis) by GalN to be secondary to cellular karyolysis. Furthermore, the present as well as other studies have also confirmed that structural alterations, in the form of distorted mitochondria and hypertrophied smooth ER, are taking place as early as 24 - 25 hours after GalN-induced hepatocyte injury (Medline et al., 1971; Scharnbeck et al., 1972). These observations do not support the assumption of the involvement of early membrane injury in GalN-induced hepatocyte necrosis.

The present study has demonstrated a unique and characteristic pattern of cellular events taking place during GalN-induced hepatocyte injury and necrosis. Furthermore, the severity of hepatocyte injury also appeared to correlate with the severity of hepatic coma. Previous biochemical and histological studies using this animal model have demonstrated maximum hepatic injury taking place approximately 48 hours after GalN injection (Decker and Keppler, 1972; Pickering et al., 1975; Chirito et al., 1979). The present study, in principle, confirms these earlier observations. However, the departure in the

present study points out that at 48 hours after GalN injection the rats are in different grades of hepatic coma, depending on the degree of hepatocyte injury and necrosis (Figure 3.4). That is to say that the grade of coma reflects the degree of hepatocyte injury. Thus, it was seen both biochemically and histologically that GalN injection resulted in hepatocyte injury before Grade I coma. Maximum liver injury seemed to coincide with the first manifestation of Grade II coma. During Grades III and IV hepatic coma the hepatocellular injury appeared to reach an irreversible stage. At these grades of coma, fulminant hepatocellular injury and necrosis resulted in dramatic alterations in serum biochemistry (Figures 3.6 - 3.8) as well as a rapid deterioration in coma (Figure 3.4). Massive hepatocellular necrosis during these stages has already been demonstrated to result in the gross accumulation of various toxic metabolites and middle molecules (Zieve, 1979; Chang and Lister, 1980; Popper, 1981; Fischer, 1982). In addition, toxins generated from necrotic hepatocytes may also build up to complicate coma and other secondary complications (e.g. brain edema, bleeding, etc.) of FHF. By using a grading system (for hepatocellular necrosis) based on the grade of hepatic coma, it may be possible to more accurately assess

GalN-induced hepatocyte injury and necrosis and thus may serve to better comprehend the pathophysiology of FHF and its secondary complications.

4.2.3 The Evolution and Development of Brain Edema Following Acute Hepatocyte Necrosis

The genesis of cerebral edema is perhaps the major life threatening secondary complication of fulminant hepatic failure. Clinically, several authors have found high incidences of cerebral edema during autopsy of patients who succumbed to acute liver failure and coma (Pirola et al., 1969; Ware et al., 1971; Tholen, 1972; Silk et al., 1977; Hoyumpa, 1979; Ede et al., 1982). In patients with severe cerebral edema, death may be due to brain swelling and subsequent uncal or cerebellar herniation. From various recent studies (Groflin and Tholen, 1978; Hanid et al., 1979; Zimmerli et al., 1981; Potvin et al., 1984), it can also be concluded that, in cases of experimental (surgical and drug-induced) hepatic failure, cerebral edema is one of the major causes of death.

Although the presence of cerebral edema in FHF has been well documented, the possibility of its involvement as a major factor in the pathophysiology of acute liver failure and hepatic coma has only recently been seriously considered (Ede et al., 1982; Crossley et al., 1983b;

Goldstein, 1984; Dixit and Chang, 1985). The pathogenesis of brain edema during conditions of acute hepatocellular necrosis is as yet unclear. Earlier studies evaluating the mechanism of brain edema in FHF have resulted in conflicting results. Where some have implicated a vasogenic mechanism (Livingstone et al., 1977) others have proposed a cytotoxic mechanism for the genesis of brain edema during FHF (Hanid et al., 1979; Zimmerli et al., 1981). Various explanations for these conflicting observations are possible. The fact that different animal models for FHF were employed, or that brain edema was studied during either very late or early stages of FHF is often pointed out (Crossley et al., 1983a). In the opinion of many authors (Chang et al., 1978; Chirito et al., 1977, 1979; Saunders, 1979; Horowitz et al., 1983; Niu et al., 1983; Shaffer et al., 1984; Zaki et al., 1984) the galactosamine-induced FHF model is considered as the one which most closely resembles human FHF. With this in mind, controlled studies were performed using the GalN-FHF rat model to try and elucidate the mechanism involved in the genesis of brain edema during progressive stages of FHF.

In the present study, the evolution and development of brain edema has been assessed both histologically and by physical measurements of brain water content during

progressive grades of hepatic coma in the GalN-FHF rat model. Initial qualitative evidence of brain edema during FHF was distinctly evident from the gross physical examination of the brain at autopsy. At this time the brain appeared swollen and edematous. The edema did not appear localized to any one particular region of the brain but was widespread throughout the brain tissue.

In order to quantify the evolution and development of the brain edema of FHF, the increase in water content of the brain was physically measured by using an analytical balance. Furthermore, brain edema has been determined as an increase in brain volume (i.e. brain swelling), owing to an increase in its water content by standard procedures (Fishman, 1975; Rapoport, 1976). The brain water contents of animals in progressive grades of hepatic coma have been presented in Table 3.6. At first glance, the absolute changes in brain water content (or the percent water content of the brain) between successive grades of coma may appear minor (Figure 3.17). However, if one were to calculate the percent increase in the brain water content (Figure 3.16), it was seen that the water content of the brain had increased by more than 5 percent during the course of GalN-FHF. Similar increases in brain water content have been repeated during fatal brain edema of

various etiologies (Johansson, 1976; Hossmann, 1976; Groflin and Tholen, 1978).

As mentioned earlier, the definition of brain edema implies an increase in brain volume (i.e. brain swelling). In the early literature, brain edema and brain swelling were differentiated. These terms are now considered synonymous. Thus, brain edema during GalN-FHF was also quantified in terms of brain swelling (Table 3.6 and Figure 3.17). Increases in the brain water content and brain swelling became statistically significant during Grade II coma when the first distinct symptoms of neurological impairment (i.e. coma) were also visibly evident. As the animals progressed into deeper grades of coma, further significant increases in brain swelling were observed (Table 3.6 and Figure 3.17). Thus, the formation of brain edema closely paralleled the deterioration in the level of arousal in these animals. A theoretical curve relating percent brain swelling to percent increase in brain water content (i.e. brain edema) is shown in Figure 3.30. The experimental results, when fitted on the theoretical curve, show the following. During the early stages of hepatic coma (i.e. Grades I and II hepatic coma) the amount of brain swelling was relatively minor. The time required to reach Grade II coma after GalN-induced hepatic injury is approximately 48 hours.

This is represented by the relatively flat portion of the curve. During the later or terminal stages of GalN-induced hepatocyte injury (i.e. Grades III and IV hepatic coma) the extent of brain swelling was both large and rapid. For example, when coma progressed from Grade II to Grade IV brain swelling increased by nearly 3.5 fold within 3 to 4 hours.

Such substantial swelling of the brain tissue within the rigid confinements of the cranium can lead to brain compression and cause symptoms such as generalized brain dysfunction, medullary failure, and coma (Fishman, 1975; Berndt, 1982). Extensive brain swelling can also cause various types of brain herniation (Fishman, 1975). Clinically, brain edema and subsequent brain herniation is presently considered one of the major complications of FHF (Ware et al., 1971; Murray-Lyon et al., 1975; Ede et al., 1982). Recently, it has also been observed that progressive increases in intracranial pressure (up to 4 fold) can occur in patients and experimental animals dying from liver failure (Hanid et al., 1979; Ede et al., 1982; Swedlow et al., 1984). The Monro-Kellie Doctrine, which professes that the blood, cerebrospinal fluid (CSF), and brain tissue (enclosed within the rigid confines of the cranium) are in a reciprocal quantitative relationship to each other, may

account for the various complications of brain edema (e.g. increased intracranial pressure (ICP), brain compression, coma, brain herniation, etc.) during FHF. In accordance to this principle, the volume of any one component (i.e. the blood, CSF, or brain tissue) can only be altered at the expense of the other compartments. Thus, in liver failure an increase in the mass of the brain (i.e. due to brain swelling [edema]) must lead an increase in ICP and subsequent decrease in the cerebral volume or altered blood flow (Fishman, 1975; Hanid et al., 1979; Ede et al., 1982). Slight fluctuation in ICP (up to 30 Torr) can be compensated by the auto regulation of the cerebral circulation (Rapoport, 1976; Ede et al., 1982; Berndt, 1982). However, major increases in ICP (as seen during FHF), in addition to cerebral compression, could lead to tissue ischemia, depressed cerebral energy levels, generalized brain dysfunction and coma (Berndt, 1982; Holmin et al., 1983; Brunner, 1984). Various models of experimental brain edema support these possibilities (Rapoport, 1976; Marshall et al., 1976; Goldstein, 1984).

Having established and confirmed the consistent presence of brain edema during GalN-FHF, further histological studies were carried out to try to elucidate the basic mechanism for evolution of brain edema in liver

failure. For this, both light and electron microscopy of the brain tissue were carried out during progressive grades of hepatic coma. Representative light microscopy results are presented in Figures 3.11 - 3.13. In these studies, the presence of extensive cerebral (Figure 3.11), as well as cerebellar edema (Figure 3.12 - 3.13) was evident, especially after the onset of Grade II coma and onwards. During Grade I, the presence of brain (cerebral as well as cerebellar) edema was only minor (Figure 3.11 - 3.13). These observations complemented the brain water content measurements (mentioned above) which showed similar progression of brain edema. Although these studies verified the presence of brain edema during GalN-FHF, the mechanism of its genesis was still not clear. In order to observe the ultrastructural changes occurring in the brain during progressive grades of hepatic coma, electron microscopy was carried out.

Electron micrographs of cerebral blood vessels of FHF rats revealed evidence of intracellular swelling of pericapillary astrocytes (Figure 3.14, 3.15) even during Grade I coma. In contrast, electron micrographs of the normal animal (i.e. without GalN-induced hepatic injury) showed a compact cerebral parenchyma and no astroglial swelling (Figure 3.14). Thus, with the development of

GalN-induced hepatic injury and coma, a distinct and progressive brain edema in the form of perivascular astroglial swelling was seen (Figure 3.15). During Grade I coma, the brain edema was relatively minor; however, with increasing severity of coma, the astroglial swelling became more and more distinct (Figure 3.15b-d). During terminal stages of GalN-induced liver cell necrosis (i.e. Grade III and IV coma), extensive swelling eventually resulted in astroglial necrosis and what appeared to be extensive vasogenic edema (Figure 3.15c,d; see arrow). Thus, from the electron microscopy observations, it appeared that Grade II coma represented the stage of maximal astroglial swelling, prior to its eventual necrosis in later grades of hepatic coma. Electron microscopic studies on experimental metabolic brain edema have demonstrated similar findings in astroglial cells (Lee and Bakay, 1967).

These foregoing histological results suggest the presence of a cytotoxic brain edema, at least during early stages of GalN-FHF. Excessive astroglial swelling (as seen during Grades III and IV hepatic coma) would eventually lead to disruption in the astroglial cell metabolism to cause cellular necrosis and eventual breakdown of the blood-brain barrier. In such circumstances, an emergence of a vasogenic component is highly likely. To verify this,

the integrity of the blood-brain barrier was assessed by trypan blue infusion studies during progressive grades of hepatic coma. Representative results from 25 animals are presented in Figure 3.18.

The blood-brain barrier (BBB), by sheer virtue of its definition, is a highly selective regulatory interface between the blood and the brain (Rapoport, 1976). Under normal circumstances, the intact BBB is impermeable to substances such as trypan blue. However, with increasing severity of liver injury and hepatocyte necrosis, a progressive staining of the brain tissue was observed. During the early stages of hepatic coma (i.e. Grades I and II coma), the injury to the BBB appeared minimal (although some staining of the brain, especially in the cerebellum, during Grade II coma, was seen) (Figure 3.18). In contrast, however, a total BBB breakdown was seen during the terminal stages of liver failure and coma (i.e. Grades III and IV coma) (Figure 3.18).

These and previous EM observations suggest that the evolution of brain edema during GalN-FHF may follow a biphasic pattern, such that a cytotoxic component preceeds a vasogenic edema formation. In recent studies, using the GalN-FHF model, the Liver Group at the National Institute of Health, USA, as well as the Liver Unit at King's College,

Cambridge, have shown evidence of endotoxin accumulation and cytotoxic injury to the BBB (by affecting its permeability) during early stages of FHF (Horowitz et al., 1983; Zaki et al., 1983). Such insults to the BBB have been shown to cause substantial intracellular astroglial swelling and subsequent metabolic and transport abnormalities across the BBB (Hanid et al., 1979; Zaki et al., 1984). Eventually, in terminal stages of liver failure and coma, total astroglial cellular necrosis would result in total BBB breakdown and the formation of vasogenic brain edema (Livingstone et al., 1977; Zaki et al., 1984; Potvin et al., 1984). The results of the present study in this thesis support this hypothesis. The pathogenesis of metabolic brain edema (which is caused by the accumulation of metabolic endotoxins) has been reported to evolve by a similar biphasic mechanism, i.e. transition from cytotoxic to vasogenic edema due to tissue (astroglial) necrosis (Hossmann, 1982). The biphasic development of brain edema has also been demonstrated in various other models of cytotoxic as well as ischemic brain edema (Spatz et al., 1976; Little, 1976; Brock, 1982; O'Brien, 1982).

The precise cause-effect relationship for the development of brain edema during hepatic coma is still speculative. However, one cannot overlook the fact that

during acute liver failure there is a dramatic alteration in serum biochemistry, as well as the accumulation of various metabolic toxins (and some as yet unknown middle molecules) which are normally detoxified by the functioning liver (Zieve, 1979; Chang and Lister, 1980, 1981). Furthermore, the substances released from the necrotic hepatocytes may themselves be the source of additional endotoxins.

Recent studies have implicated several potential toxic substances of liver failure as contributors in the development of brain edema because of their deleterious effects on the BBB's membrane bound enzyme systems (Foster et al., 1974; Bazan et al., 1980; Baethmann et al., 1980). In this regard the Na^+-K^+ ATPase system has been extensively studied (Mrsulja et al., 1980; Fishman and Chan, 1980; Zaki et al., 1983, 1984; Seda et al., 1984). In the studies carried out by Williams' group, at King's College Liver Unit, it was demonstrated that serum from FHF patients severely inhibited the Na^+-K^+ ATPase system as well as other nutrient transport systems of the BBB (Zaki et al., 1983, 1984; Seda et al., 1984). These changes severely compromised the integrity, as well as the permeability, of the BBB to substances normally excluded from the brain. Inhibition of the Na^+-K^+ ATPase pump by

toxins such as ouabain have been demonstrated to cause cytotoxin brain edema by poisoning the Na^+-K^+ ATPase pump (Cornog et al., 1967). A similar mechanism may also be involved in the formation of cytotoxic edema in the present studies. Thus, a progressive accumulation of "cerebrotoxins" during GalN-FHF (Chang and Lister, 1980) may cause a progressive inhibition of the Na^+-K^+ ATPase pump to result in the progressive cerebral edema observed in the present study.

Similar defects in membrane-bound Na^+-K^+ ATPase activity have also been observed in Reye's Syndrome. In this syndrome, the endogenous toxins have been shown to have their major effect at the membrane level, in association with membrane-bound Na^+-K^+ ATPase, to cause the uncoupling of oxidative phosphorylation from the respiratory chain (De Vivo, 1978). In other studies, Holmin et al. (1983) have demonstrated a decrease in cerebral energy levels during liver failure as a result of such changes. The abnormal swelling of the astroglial mitochondria with progressive severity of coma, as seen in the present study (Figure 3.11), may explain such observations.

During the final stages of hepatic encephalopathy, sufficient accumulation of endogenous toxins would result in

total BBB breakdown and a transition from cytotoxic to vasogenic brain edema would ensue (Spatz et al., 1976; Hartmann et al., 1982). In such a situation, large quantities of blood-borne metabolites would freely enter the brain to augment brain edema and cause immediate death by brain herniation (Fishman, 1975; Rapoport, 1976; Livingstone et al., 1977).

The fact that early treatment with charcoal hemoperfusion resulted in increased survival in both animals and patients (Chang, 1972; Chang et al., 1978; Tabata & Chang, 1982; Gimson et al., 1982) could be due to the removal of endogenous toxins by hemoperfusion, thereby preventing the progression of cerebral edema in these cases. Hemoperfusion in late stages of hepatic coma, although beneficial in the recovery of consciousness (Chang, 1972; Weston, 1982) did not reduce the formation of cerebral edema unless treatment was initiated earlier (Chang et al., 1978; Tabata and Chang, 1982; Gimson et al., 1982). Furthermore, if breakdown of the BBB had already occurred, death would result despite corticosteroid or osmotherapy to reduce brain edema (Hoyumpa et al., 1979; Livingstone et al., 1977).

Thus, from the foregoing discussion, it can be concluded that brain edema during acute liver failure is a

serious complication. The development of brain edema in GalN-FHF appears to follow a biphasic pattern in which an initial cytoxic brain edema develops into vasogenic brain edema. The transition from cytotoxic to vasogenic brain edema may most likely be due to excessive astroglial swelling (and its subsequent necrosis) to eventually result in BBB breakdown. Inhibition of brain Na^+K^+ ATPase activity by endogenous toxins of liver failure may most likely be the underlying reasons for the evolution of brain edema and depressed neurological state (i.e. coma). In GalN-FHF the transition from cytotoxic to vasogenic brain edema may be a critical turning point with respect to prognosis of survival in FHF. In the present studies Grade II coma appeared to be this critical turning point in the transition from cytotoxic to vasogenic brain edema.

4.3 PGE_2 STUDIES -- ITS EFFECTS ON GALN-INDUCED HEPATOCYTE INJURY

The numerous physiological properties of prostaglandins are as diverse as their ubiquitous occurrence in the body. Of these, the cytoprotective property of several prostaglandins is truly unique. The cytoprotective effects of prostaglandins against a variety of noxious agents were first observed in the gastrointestinal mucosa (Robert, 1978). However, in recent years cytoprotection (against a variety of necrotizing agents) by prostaglandins (especially PGE_2) has been extended to include a variety of organs including the liver (Robert, 1979; Reber et al., 1980; Stachura et al., 1981; Ruwart et al., 1982b). These studies demonstrated that several prostaglandins (and especially PGE_2) protected hepatocytes from a wide range of necrotizing agents, including GalN, when administered either a few minutes before or immediately after the necrotizing stimulus. It is not known whether prostaglandins E_2 is (hepato) cytoprotective after the onset of hepatocyte injury and necrosis. The present PGE_2 studies were carried out to determine whether PGE_2 was capable of imparting "hepato-cytoprotection" after the onset of GalN-induced acute hepatocyte injury. Galactosamine-induced FHF animals in Grade II coma were selected for this study because Grade II coma represents the stage of maximum

hepatocyte injury in this model. Grades III and IV coma represent the terminal stages of hepatocellular injury and necrosis (Figure 3.10).

Of the various dosages of PGE_2 that were tested, 100 μg PGE_2/100g body weight was optimal in its effect in significantly improving the survival time but not the survival rate of Grade II coma rats (Figures 3.19-3.21 and Table 3.7-3.8). In later grades of coma (i.e. Grades III and IV coma) this dosage (100 μg PGE_2/100g body weight) did not improve the survival time nor the survival rate (Figure 3.22 and Table 3.9, 3.10). It is likely that during Grades III and IV hepatic coma, irreversible injuries to hepatocyte and blood brain barrier may be taking place and improvement in survival time or rate could not be expected (Figure 3.10).

4.3.1 The Effects of Prostaglandin E_2 on Serum Biochemistry

Since acute, hepatocyte injury and necrosis severely affects serum biochemistry (Figures 3.6 - 3.8), the effects of PGE_2 on hepatocyte function were assessed by analyzing the serum biochemistry at specific times (i.e. 0, 4, 8 hours) after PGE_2 injection. Routine serum biochemical analysis (during PGE_2 studies), as performed by the SMAC

and commercially available kits, is presented in Figures 3.23 - 3.25 and Tables 3.11 - 3.13.

Generally speaking, the serum biochemical analysis did not reveal any major differences between the PGE_2 treated group and the control animals. The increase in serum glucose levels seen in Figure 3.23 was due to a subcutaneous glucose injection given to all experimental animals. This was done to prevent the extreme hypoglycemia which is experienced by all GalN-FHF animals (Figure 3.6).

In the case of liver enzyme analysis (Figure 3.25 and Table 3.13), a significant improvement in the level of both ASAT and ALAT was observed in the PGE_2 injected animal over saline injected control animal. No significant changes were observed with respect to alkaline phosphatase or LDH in either PGE_2 or saline injected animals (Figure 3.25). Significantly reduced enzyme levels (ASAT and ALAT) in the PGE_2 injected animals suggest an improvement in liver function in this group. During acute liver failure, endogenous prostaglandin (E and F) levels are severely depressed (Trewby et al., 1975; Loginov and Markova, 1979). In these patients infusion of prostaglandin precursors improved liver function (as assessed by ASAT and ALAT levels) and brought back to normal the levels of various endogenous prostaglandins, including PGE_2 (Loginov and

Markova, 1979). Thus, in the present study, improvement in liver function may be attributed to the supplementation of exogenous PGE_2.

4.3.2 The Effects of Prostaglandin E_2 on Hepatocytes: A Histological Study

The effects of PGE_2 on GalN-injured hepatocytes were examined histologically by both light and electron microscopy (Figures 3.26 - 3.28). Light microscopy of the liver tissue showed similar evidence of extensive mottled hepatocyte necrosis and karyolysis in both the PGE_2 and saline (control) injected animals (Figure 3.26b-d). These light-microscopic studies were not sufficient to show any clear distinction between the PGE_2 treated and the saline injected control animals.

Electron microscopic studies showed much better details. Galactosamine-induced acute hepatocyte injury has been shown to result in a characteristic pattern of hepatocyte necrosis which directly correlated with the severity of coma grade (Figure 3.10). Ultrastructural studies using electron microscopy revealed that, during Grade II coma, the endoplasmic reticulum (ER) became intimately associated with the nucleus (i.e. the ER was wrapped around the nucleus) to ultimately cause karyolysis and cellular necrosis (Figure 3.10, 3.27). At this time

(Grade II coma), the mitochondria were also severely distorted (Figure 3.27). PGE_2 treatment during Grade II coma appeared to prevent the progression of liver injury for up to 4 hours (Figure 3.28b). In contrast, the control animal which received only saline (instead of PGE_2) showed marked evidence of karyolysis (Figure 3.28a) at this time (i.e. 4 hours after saline injection). Eight hours after PGE_2 injection, the hepatocytes started to show evidence of karyolysis in a manner similar to that shown in Figure 3.28a. Although PGE_2 seemed to prevent or delay the progression of hepatocyte necrosis by preventing karyolysis, it did not appear to have any significant effect on the prevention of mitochondrial distortion. Thus, it appears that PGE_2 may somehow affect the hepatocyte's membrane system to prevent the progression of cellular damage. It has been suggested that PGE_2 may help to stabilize the hepatocyte plasma membrane and thereby improve its integrity to cellular necrosis in the event of toxic injury (Stachura et al., 1980; Miyazaki et al., 1983). Additionally, PGE_2 may also have some effects on the hepatocellular metabolism to prevent the progression of liver cell necrosis.

The precise physiological role of prostaglandins in liver physiology and more specifically in hepatic

regeneration and as hepatocytoprotective agents is still unclear (Lifschitz, 1983). It has been demonstrated that hepatocyte plasma membrane possesses PGE receptors and that the mechanism of PGE action is mediated by adenylate cyclase (Kuehl and Humes, 1972; Simigel and Fleischer, 1974; Johansson and Bergstrom, 1982; Hammarstrom, 1982). It has been proposed that prostaglandins may have a regulatory effect on liver metabolism and function, as normalization of liver function accompanied a rise in the concentration of endogenous prostaglandins (Loginov and Markova, 1979). An increase in PGE was also observed during liver regeneration following partial hepatectomy in normal rats, suggesting that PGE may be of key importance in DNA synthesis (Macmanus and Braceland, 1976). A recent report confirmed this by showing that prostaglandins stimulate hepatic DNA synthesis and may seem a likely trigger for liver regeneration (Miura and Fukui, 1979). More recently it was shown that intraperitoneally administered exogenous PGE_2 prevented the inhibition of hepatic DNA synthesis by ethanol and also prevented the progression of liver injury due to ethanol (Mokowka et al., 1982; McNeil and Leevy, 1983; McNeil et al., 1985).

Since a defect or injury to the plasma membrane appears to be the final common pathway in nearly all forms

of hepatocellular necrosis (Popper, 1981), prostaglandins could impart hepatocyte protection by causing the "stabilization" of the hepatocyte's plasma membrane and, in this way, enhance the integrity of cellular membranes (Robert, 1981). The mechanism for this is not known. Prostaglandin I_2 has been demonstrated to stabilize cat liver lysomal membranes (Araki and Lefer, 1980) and by this mechanism protect hypoxic liver damage in the isolated perfused cat liver model. Moreover, PGE_2 completely prevented severe lysosomal damage of hepatocytes from a variety of noxious agents, including GalN (Stachura et al., 1980, 1981; Ruwart et al., 1982a,b; Miyazaki et al., 1983).

Recently it was proposed that, since PGE_2 stimulates cyclic AMP formation, this may be the mediator for cyto-protection (Stachura et al., 1980; Miyazaki et al., 1983; Ruwart et al., 1984). Alternatively, prostaglandins (E_2 and F_2) may also have important regulatory influences on cellular metabolism and thereby stimulate (as yet unknown) mechanisms to strengthen the integrity of the hepato-cellular plasma membranes. At the present time, however, there is no documented evidence for the mechanism(s) involved in hepato-cytoprotection by prostaglandins.

4.4 PROSTAGLANDIN E_2 STUDIES: ITS EFFECTS ON BRAIN EDEMA

The cytoprotective effects of PGE_2 on various organs including the liver has been extensively studied since its initial discovery nearly a decade ago (Robert, 1976, 1981; Stachura et al., 1980). Recently PGE_2 has been demonstrated to significantly prolong the survival time of GalN-FHF rats in Grade II hepatic coma (Dixit and Chang, 1982). However, PGE_2 given in later grades of coma did not improve the survival time or survival rate (Figure 3.22 and Table 3.10).

The precise mechanism for this has yet to be elucidated. Some effects of PGE_2 on GalN-injured hepatocytes have been discussed in the preceeding section. In the present section, the effects of PGE_2 on the development of brain edema will be considered.

Brain edema is now recognized as one of the major complications of fulminant hepatic failure (Ware et al., 1971; Hoyumpa, 1979; Ede et al., 1982). Thus, in the present study, the effects of PGE_2 on the development of brain edema in GalN-FHF rats has been investigated.

4.4.1 The Effects of Prostaglandin E_2 on Brain Edema

Evidence of the extensive development of brain edema and subsequent brain swelling during progressive stages of

GalN-induced hepatic injury has been discussed in a preceeding section of this chapter (Figures 3.11 - 3.18 and Table 3.6). From these control studies it was observed that Grade II coma represented a critical and pivotal stage in the development of brain edema. During deeper grades of coma (i.e. Grades III and IV hepatic coma), extensive cytotoxic injury to the blood-brain barrier was thought to result in the development of vasogenic edema (Figure 3.18).

In the PGE_2 studies, the effect of PGE_2 on the development of brain edema has been studied during Grade II coma. A quantitative study involving the effects of PGE_2 on brain water content and brain swelling has been presented (Tables 3.14, 3.15, and Figure 3.29). In addition, a histological study using light and electron microscopy has also been presented (Figures 3.31 - 3.35). Finally, a study involving the effects of PGE_2 on the integrity of the blood-brain barrier is also presented (Figure 3.36). In these studies, it was seen that animals which received PGE_2 did not show any significant increase in either brain water content or brain swelling (Tables 3.14, 3.15, and Figure 3.29) 4 hours after PGE_2 injection. In contrast, the control animals which received only saline and no PGE_2 showed a 3.5 fold increase in brain water content during this same time period (Figure 3.29 and Table

3.15). Furthermore, in the PGE_2 injected animals, a reduction in brain edema was observed 8 hours after PGE_2 injection (Table 3.15). At this time, both the brain water content and brain swelling subsided to near normal values (Tables 3.14, 3.15). In the control group, which received only saline, no survival was obtained 8 hours after saline injection. It is likely that in the control (saline injected) group, extensive hepatocyte necrosis and the development of severe brain edema may have been the cause of death.

In the present PGE_2 studies, the development of brain edema was also examined histologically using both light and electron microscopy (Figures 3.31 - 3.35). Light microscopy results (Figures 3.31 and 3.32) complemented the quantitative measurements of brain swelling (Tables 3.14, 3.15, and Figure 3.29). Light microscopic studies in control animals (i.e. saline injected) showed progressive and widespread cerebral and cerebellar edema 4 hours after saline injection (Figure 3.31b and 3.32b). During the same time period, the PGE_2 injected animals displayed somewhat less brain edema (Figures 3.31c and 3.32c). A distinct absence of brain edema was evident 8 hours after PGE_2 injection (Figures 3.31d and 3.32d). Electron microscopy studies showed consistent presence of brain edema in all

Grade II coma GalN-FHF animals (Figure 3.33). It was observed that in control animals, which received only saline, a further progression in brain edema occurred 4 hours after. At this time extensive swelling of the perivascular astroglial cells, as well as the presence of abnormal vacuoles and necrotic structures, were clearly evident in these animals (Figure 3.34a). In marked contrast, the PGE_2 injected animals showed a dramatic control of the perivascular astroglial swelling during the same time period (i.e. 4 hours after PGE_2 injection) (Figure 3.34b). Although some vacuoles and swollen mitochondria were still visible, the overall astroglial swelling was significantly reduced when compared to its corresponding control group. Eight hours after PGE_2 injection, an almost complete remission of astroglial swelling was seen (Figure 3.35). Furthermore, at this time, there was no evidence of any severe tissue compression or necrosis. Thus, as witnessed by electron microscopy, PGE_2 was able to reduce and nearly abolish the presence of brain edema during GalN-FHF. However, in this regard, it cannot be overlooked that PGE_2 may have an effect on the hepatocyte itself, thereby inducing the synthesis of some factor(s) which in turn may act on the blood-brain barrier to ameliorate the brain edema of

GalN-FHF. Recently, it has been demonstrated that exogenous PGE_2 had a beneficial effect on the hepatocyte plasma membrane such that it improved ATP dependent membrane function by improving high energy phosphate levels and slowly improve ATP-dependent intracellular function (Ghuman et al., 1982).

Finally, in order to investigate whether PGE_2 had any effects on the structural integrity of the blood-brain barrier (BBB), trypan blue dye infusion studies were carried out. Representative observations from 25 animals are presented in Figure 3.36. Normally, an intact BBB prevents dyes of large molecular weight, such as trypan blue, from staining the brain. In the present experiment, when trypan blue was injected into Grade II coma animals just prior to PGE_2 or control saline injection, a slight injury to the BBB was seen. Four hours after this, when trypan blue was injected into control animals there was deep blue staining of the complete brain, showing a total breakdown of the BBB. On the other hand, PGE_2 injected animals at 4 hours and 8 hours after did not reveal severe blood brain barrier breakdown. Thus, from these studies it appears that PGE_2 prevented the breakdown of the BBB and the transition of cytotoxic brain edema to vasogenic brain edema, at least for up to 8 hours.

The physiological action of prostaglandins (E) on brain capillary endothelium is uncertain. Conflicting reports of it having vasconstrictor as well as vasodilatory effect have added further controversy to its effect (Steiner et al., 1972; Wolfe, 1975). The effect of PGE_2 on BBB structural integrity is as yet unknown. The effects of PGE_2 on the Na^+K^+-ATPase pump, tissue adenyl cyclase activity, and cerebral vasculature is well documented (Wolfe, 1975; Rapoport, 1976; Greenberg et al., 1982). Depressed brain Na^+K^+-ATPase activity has been reported following various types of brain edema and FHF (De Vico, 1978; Mrsylja et al., 1980; Ede et al., 1982; Seda et al., 1984). PGE_2 has been shown to stimulate Na^+K^+-ATPase activity in the brain (Wolfe, 1975; Gilboe et al., 1976; Greenberg et al., 1982). Thus, it may be possible that stimulation of Na^+K^+-ATPase activity by exogenous PGE_2 may help to maintain the physical as well as the electrochemical integrity of the blood-brain barrier against the circulating toxins of liver failure. Furthermore, in brain capillaries it has been demonstrated that regulation of permeability is mediated by cyclic AMP (Joo et al., 1975; Joo, 1977). The fact that PGE_2 stimulates adenyl cyclase activity suggests that PGE_2 may play a role in maintaining the permeability of the blood-brain barrier which is often compromised in acute liver failure (Zaki et al., 1983, 1984).

CHAPTER V

CLAIMS TO ORIGINAL RESEARCH

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CLAIMS TO ORIGINAL RESEARCH

5.1 Summary of Claims to Original Research

1) Basic physiological, biochemical, and histological (LM/EM) studies were carried out to characterize the galactosamine-induced fulminant hepatic failure (GalN-FHF) model with respect to the progressive development of hepatic coma.

2) It was found that galactosamine induced a characteristic pattern of intracellular events which led to eventual hepatocyte necrosis. These events correlated to the severity of hepatic coma.

3) It was observed that significant changes in the orientation of the hepatocyte's endoplasmic reticulum (ER) and mitochondria started to take place before the onset of overt encephalopathy, i.e. as early as Grade I hepatic coma. At this time the ER and mitochondria became closely associated with each other and started to align themselves along the nuclear membrane.

4) During Grade II hepatic coma, gross structural alterations in the mitochondria were visible throughout the hepatocyte. Also at this time the ER had completely encircled the nucleus to envelop it. Thus, it was found that Grade II hepatic coma

represented a critical stage during GalN-FHF when cellular sequences leading to hepatocyte karyolysis had begun.

5) It was found that the hepatocyte's ER is intimately associated with Gal-induced liver cell necrosis. Massive hepatocyte necrosis appeared to take place during the terminal stages of GalN-FHF, i.e. during Grades III and IV hepatic coma. At this time it appeared that the ER, which had wrapped itself around the nucleus, resulted in the destruction of the nucleus. Following karyolysis, cellular necrosis ensued.

6) Galactosamine-induced FHF resulted in the progressive development of brain edema (as evaluated by tissue histology (LM/EM), and brain water content and brain swelling measurements) which correlated with the increasing severity of hepatic coma.

7) Galactosamine-induced FHF resulted in profound dehydration of the FHF rats. In these rats, despite general body dehydration, the development of brain edema remained a consistent pathological feature.

8) Evidence of brain swelling due to brain edema was evident before the onset of overt hepatic coma, i.e. when the animal was in Grade I hepatic coma.

9) Electron microscopy and trypan blue dye infusion studies revealed that the evolution of brain edema followed a biphasic pattern in which cytotoxic brain edema preceeded a vasogenic component.

10) Grade II coma appeared to be the critical and pivotal turning point in the evolution of brain edema. During the terminal stages of GalN-FHF (i.e. Grades III and IV coma) complete breakdown of the blood-brain barrier resulted in the development of vasogenic brain edema.

11) The breakdown of the blood-brain barrier was first evident in the cerebellum and rapidly followed to the cerebrum.

12) PGE_2, when injected into GalN-FHF rats in Grade II hepatic coma, significantly increased the survival time but not the survival rate in these rats.

13) PGE_2 had no significant effects on either the survival time or survival rate of GalN-FHF rats in terminal stages (i.e. Grades III and IV coma) of FHF.

14) Prostaglandin E_2 prevented the development of GalN-induced hepatocyte necrosis when given to rats in Grade II hepatic coma. It had no significant effects in later grades of coma.

15) PGE_2 prevented the progression of brain edema and brain swelling when given to GalN-FHF rats in Grade II hepatic coma.

16) PGE_2 injection significantly prevented the development of astroglial swelling for up to 4 hours; 8 hours after PGE_2 injection, brain edema, as evaluated by tissue histology and measurement of brain water content and brain swelling, had subsided to near normal values.

BIBLIOGRAPHY

BIBLIOGRAPHY

Adams RD, Foley JM. "The neurological disorder associated with liver disease." Res Publ Ass Res Nerv Ment Dis 32:198-237, 1953.

Agishi T, Yamashita N, Ota K. "Clinical results of direct charcoal hemoperfusion for endogenous and exogenous intoxication including hepatic failure." In Hemoperfusion, Kidney and Liver Support and Detoxification, ed. S Sideman and TMS Chang, Hemisphere Publ Corp, Washington, DC, pp. 255-263, 1980.

Amano I, Kano H, Takahira H, Yamamoto Y, Itoh K, Iwatsuki S, Maeda K, Ohta K. "Hepatic assist system using bead type charcoal." In Artifical Kidney, Artificial Liver, and Artificial Cells, ed. TMS Chang. Plenum Press, New York, 1978, pp. 89-98.

Andreis PG, Whitfield JF, Armato U. "Stimulation of DNA synthesis and mitosis of hepatocytes in primary cultures of neonatal rat liver by arachidonic acid and prostaglandins." Exp Cell Res 134:474-478, 1981.

Araki H, Lefer AM. "Cytoprotective action of prostacyclin during hypoxia in the isolated perfused cat liver." Am J Physiol 238:h176-183, 1980.

Baethmann A, Oettinger W, Rothenfuber W, Kempski O, Unterberg A, Gieger R. "Brain edema factors: current state with particular reference to plasma constituents and glutamate." In Advances in Neurology, Vol. 28: Brain Edema. eds. J Cervos-Navarro and R Ferszt, Raven Press, New York, pp. 171-195, 1980.

Barnes JM, Magee PN. "Some toxic properties of dimethyl-nitrosamine." Br J Ind Med 11:167, 1954.

Bartels O. "Hemoperfusion through activated carbon adsorbents in liver failure and hepatic coma." Acta Hepato-Gastroenterol 25:324-329, 1978.

Bazan NG, Rodriguez de Tusco EB. "Membrane lipids in the pathogenesis of brain edema: phospholipids and arachidonic acid, the earliest membrane components changed at the onset of ischemia." In Advances in Neurology, Vol. 28: Brain Edema, ed. J Cervos-Navarro and R Ferszt, Raven Press, New York, pp. 197-205, 1980.

Bergmeyer HU, Scheibe P, August WW. "Optimization of methods for aspartate aminotransferase and alanine aminotransferase." Clin Chem 24:58-73, 1978.

Bergstrom S, Carlson LA, Weeks JR. "The prostaglandins: a family of biologically active lipids." Pharmcol Rev 20:1-48, 1968.

Berk PD, Popper H. "Fulminant hepatic failure." Am J Gastroenterology 69(3):349-400, 1978.

Berndt SF. "Symptoms of cerebral edema." In Treatment of Cerebral Edema, eds. A Hartmann and M Brock. Springer-Verlag, Berlin, pp. 36-42, 1982 .

Blitzer BL, Waggoner JG, Jones EA, Gralnick HR, Towne D, Butler J, Weise V, Kopin IJ, Walters I, Teychenne PF, Goodman DG, Berk PD. "A model of fulminant hepatic failure in the rabbit." Gastroenterology 74(4): 664-671, 1978.

Bloch P, Delorme ML, Rapin Jr, Ghanger A, Boschat M, Opolon P. "Reversible modifications of neurotransmitters of the brain in experimental acute hepatic coma." Surg Gynecol Obstet 146:551-558, 1978.

Blume U, Helmstaedt D, Sybrecht G, Baldamus C, Submann P, Heyer U, Schmidt E, Schmidt FW. "Hemoperfusion therapy for acute hepatic failure." Dtsch Med Wochenschr 101:559, 1976.

Bohle E, May B. "Metabolic effects of prostaglandin E_1 upon lipid and carbohydrate metabolism." In Prostaglandin Symposium of the Worchester Foundation, eds. PW Ramwell and JE Shaw, Interscience, New York, pp. 115-124, 1968.

Bollman JL, van Hook E. "A simplified two stage hepatectomy in the rat." J App Physiol 24:722-723, 1968.

Bradbury MWB. "The structure and function of the blood-brain barrier." Fed Proc 43:186-190, 1984.

Brock M. "Is brain edema amenable to treatment." In Treatment of Cerebral Edema," eds. A Hartmann and M Brock, Springer-Verlag, Berlin, pp. 171-174, 1982.

Brown H. "Hepatic Failure." Charles C Thomas Publisher, Springfield, Ill, USA, 1970.

Brown IA. "Liver-Brain Relationships." Charles C Thomas Publisher, Springfield, Ill, USA, 1957.

Brunner G. personal communications, 1984.

Butcher RW, Sutherland EW. "The effects of catacholamines, adrenergic blocking agents, prostaglandin E_1 and insulin on cyclic AMP levels in rat epididymal fat pads in vitro." Ann NY Acad Sci 139:849-855, 1967.

Butcher RW, Baird CE. "Effects of prostaglandins on adenosine 3', 5'-monophosphate levels in fat and other tissues." J Biol Chem 243:1713-1717, 1968.

Bygdeman M, Gillespie A. "Prostaglandins in human reproduction." In Prostaglandins, ed. JB Lee, Elsevier North Holland, New York, pp. 215-249, 1982.

Canalese J, Gimson AE, Davis C, Mellon PJ, Davis M, Williams R. "Controlled trial of dexamethasone and mannitol for the cerebral oedema of fulminant hepatic failure." Gut 23(7):625-9, 1982.

Cao CM, Cepero EM. "Identification of prostaglandin-like substances in plants." In Advances in Prostaglandin and Thromboxane Research, Vol. 2, eds. B Samuelsson and R Paoletti, Raven Press, New York, p. 877, 1976.

Carlson LA, Michelli H. "Effect of prostaglandin E_1 on fat mobilizing lipolysis in rat adipose tissue in relation to the nutritional condition." Acta Physiol Scand 80:289-294, 1970.

Chang JA, Lewis GP, Piper PJ. "Inhibition by glucocortcoids of prostaglandin release from adipose tissue in vitro." Br J Pharmacol 59:425-432, 1977.

Chang TMS. "Hemoperfusion over microencapsultated adsorbent in a patient with hepatic coma," Lancet 2:1371-1372, 1972.

Chang TMS, Migchelsen M. "Characterization of possible toxic metabolites in uremia and hepatic coma based on the clearance spectrum for larger molecules by the ACAC microcapsule artificial kidney." Trans Am Soc Artif Intern Organs 19:314-319, 1973.

Chang TMS. "Artificial kidney and artificial liver." Lancet iv:1451-1452, 1975.

Chang TMS. "Hemoperfusion alone and in series with ultrafiltration on dialysis for uremia, poisoning, and liver failure." Kidney International 10:S305-S311, 1976.

Chang TMS, Lister C, Chirito E, O'Keefe P, Resurreccion E. "Effects of hemoperfusion rate and time of initiation of ACAC charcoal hemoperfusion on the survival of fulminant hepatic failure rats." Trans Am Soc Artif Intern Organs 24:243-245, 1978.

Chang TMS, Lister C. "Analysis of possible toxins in hepatic coma including the removal of mercaptan by albumin-collodion charcoal." Int J Artif Organs 3:108-112, 1980.

Chang TMS, Lister C. "Middle molecules in hepatic coma and uremia." Artifical Organs 4 (suppl):169-172, 1981.

Chang TMS. "Hemoperfusion, exchange transfusion, cross circulation, liver perfusion, hormones and immobilized enzymes." In Artiticial Liver Support, eds. G Brunner and FW Schmidt, Springer-Verlag, Berlin, pp. 126-133, 1981.

Chang TMS. "Early hemoperfusion in fulminant hepatic failure." Lancet 2 (8306):1039, 1982.

Chang TMS. "Liver support systems." In "Life Support Systems in Intensive Care," eds. Bartlett, Whitehouse WM, and JG Turcotte, Year Book Publ, pp. 461-485, 1984.

Chase RA, Trewby PN, Davis M, Williams R. "Serum octopamine, coma, and charcoal hemoperfusion in fulminant hepatic failure." Eur J Clin Invest 7:351-354, 1977.

Chaudhury TK, Jacobson ED. "Prostaglandin cytoprotection of gastric mucosa." Gastroenterology 74:59-63, 1978.

Chirito E, Reiter B, Lister C, Chang TMS. "Artificial liver: The effect of ACAC microencapsulated charcoal hemoperfusion on fulminant hepatic failure." Artif Organs 1:76-83, 1977.

Chirito E, Reiter B, Lister C, Chang TMS. "Assessment of two rat models of acute hepatic failure for testing artificial liver devices." In "Artificial Kidney, Artificial Liver and Artificial Cells," ed. TMS Chang, Plenum Press, New York, pp. 239-243, 1978.

Chirito E, Lister C, Chang TMS. "Biochemical, hematological, and histological changes in a fulminant hepatic failure rat model for artificial liver assessment." Artif Organs 3(1):42-46, 1979.

Christ EJ, van Dorp DA. "Comparative aspects of prostaglandin biosynthesis in animal tissues. In Advances in the Biosciences, Vol 9, ed. S Bergstrom, Pergamon Press, Oxford, pp. 35-38, 1973.

Cole M, Rutherford RB, Smith FO. "Experimental ammonia encephalopathy in the primate." Arch Neurol 26:130-136, 1972.

Conn HO, Lieberthal MM. The Hepatic Coma Syndromes and Lactulose. Williams and Wilkins Co., Baltimore, 1979, USA.

Contreras P, Later R, Navarro J, Touraine JL, Freyria AM, Traeger J. "Molecules in the middle molecular weight range." Nephron 32:193-201, 1982.

Cornog JL, Gonatas NK, Feierman JR. "Effects of intracerebral injection of ouabain on the fine structure of rat cerebral cortex." Am J Pathol 51:573-590, 1967.

Crabbé P. "Introduction, origin and properties." In Prostaglandin Research, ed P Crabbé, Academic Press, pp. 1-16, 1977.

Crawford JD, Haessler HA. "Insulin-like activities of prostaglandin E_1." In Prostaglandin Symposium of the Worchester Foundation, ed PW Ramwell and JE Shaw, Interscience, New York, pp. 103-112, 1968.

Crossley JR, Wardle EN, Williams R. "Biochemical mechanisms of hepatic encephalopathy." Clin Sci 64(3):247-52, 1983a.

Crossley JR, Wardle EN, Williams R. "Cerebral edema in fulminant hepatic failure." Clin Sci 65(4):445-446, 1983b.

Curro FA, Greenberg S, Gardier RW. "Effects of Prostaglandins on central nervous system function." In Prostaglandins -- Organs and Tissue Specific Actions, ed S Greenberg, PJ Kadowitz, and TF Burks, Marcel Dekker Inc, New York, pp. 367-406, 1982.

Curtis-Prior PB. Prostaglandins. North-Holland Publishing Co, Amsterdam, New York, Oxford, 1976.

Dawson W, Jessup SJ, McDonald-Gibson W, Ramwell PW, Shaw JE. "Prostaglandin uptake and metabolism by the perfused rat liver." Br J Pharmacol 39:585-598, 1970.

Decker K, Keppler D. "Galactosamine induced liver injury." In Progress in Liver Disease, Vol 4, eds H Popper and F Schaffner, Grune and Stratton, New York, pp. 183-199, 1972.

Denis J, Opolon P, Nusinovici V, Granger A, Darnis F. "Treatment of encephalopathy during fulminant hepatic failure by hemodialysis with high permeability membrane." Gut 19:787-793, 1978.

Denis J, Delorme ML, Boschat M, Nordlinger B, Opolon P. "Respective roles of ammonia, amino acids, and medium sized molecules in the pathogenesis of acute hepatic encephalopathy." J Neurochem 40:10-19, 1983.

De Vivo DC. "Reye syndrome: a metabolic response to an acute mitochondrial insult?" Neurology (Minneap) 28:105-108, 1978.

Dixit V, Chang TMS. "Soluable liver extracts as a supplementation to detoxification by hemoperfusion." Int J Artif Organs 4:291-294, 1981.

Dixit V, Chang TMS. "Effects of prostaglandin E_2 on the survival time of fulminant hepatic failure rats." Int J Artif Organs 5:388, 1982.

Dixit V, Chang TMS. "Preliminary report on the effects of prostaglandin E_2 on brain edema in fulminant hepatic failure rats." Int J Artif Organs 8:55-56, 1985.

Dozois RR, Donsa TP. "Interactions of prostaglandin E_2 and histamine with adenyl cyclase systems from canine gastric mucosa." In First International Symposium on Histamine H_2-Receptor Antagonist. eds. CJ Wood and MA Simkins, Smith Kline & French, Welwyn Garden City, England, pp. 331-340, 1973.

Duffy TE, Plum F. "Seizures and comatose states." In Basic Neurolochemistry. eds. GJ Siegal, RW Albers, R Katzman and BW Agranoff. Little, Brown and Co, Boston, pp. 646-667, 1976.

Dukes PP. "Effects of prostaglandins on erythropoiesis." In Prostaglandins -- Organs and Tissue-Specific Action, ed S Greenberg, PJ Kadowitz and TF Burks, Marcel Dekker, New York, pp. 283-295, 1982.

Ede R, Gimson AE, Cannalese J, Williams R. "Cerebral oedema and monitoring of intracranial pressure in fulminant hepatic failure." Gastroenterol Jpn 17(2);163-76, 1982.

Ehrlich P. Das Sauerstoff-Bedurfnis des Organismus. Eine Farbenanalytische Studie. Berlin, 1885.

Eliasson R. "Studies on prostaglandin occurrence, formation and biological actions." Acta Physiol Scand 46 (Suppl) 158:1-73, 1960.

Elliot KAC, Jasper H. "Measurement of experimentally induced brain swelling and shrinkage." Am J Physiol, 157:122-129, 1949.

El-Mofty SK, Scrutton MC, Serroni A, Nicolini C, Farber JL. "Early, reversible plasma membrane injury in galactosamine induced liver cell death." Am J Pathol 79:579-595, 1975.

Emmel VM, Cowdry EV. Laboratory Techniques in Biology and Medicine, Williams and Wilkins Co., Baltimore, 1964.

Faguer P, Delorme ML, Denis J, Cueille G, Boschat M, Opolon P. "Demonstration of middle molecules in plasma and brain during experimental acute hepatic encephalopathy." J Am Soc Artif Intern Organs, 3:153-159, 1980.

Ferenci P, Covell D, Schafer DF, Waggoner JG, Shrager R, Jones EA. "Metabolism of the inhibitory neurotransmitter gamma-aminobutyric acid in a rabbit model of fulminant hepatic failure." Hepatology 3(4):507-512, 1983.

Finlayson M. "Observations in the pathogenesis of hepatocerebral degeneration in cirrhosis." Bol Estud Med Biol Mex 32:3-11, 1982.

Fischer JE, Baldessarini RJ. "False neurotransmitters and hepatic failure." Lancet ii:75-80, 1971.

Fischer JE. "On the occurrence of false neurochemical transmitters." In Artificial Liver Support, eds. R Williams and IM Murray-Lyon, Pitman Medical Publishing Co, Tunbridge Wells, UK, pp. 31-50, 1975.

Fischer JE. "Amino acids in hepatic coma." Dig Dis Sci 27(2):97-102, 1982.

Fishman RA. "Brain edema." N Engl J Med 293:706-711, 1975.

Fishman RA and Chan PH. "Metabolic basis of brain edema." In Advances in Neurology, Vol. 28: Brain Edema. eds. J Cervos-Navarro and R Ferszt. Raven Press, New York, pp. 207-215, 1980.

Flower RJ. "Prostaglandins and related compounds." Agents Actions Suppl 3:99-105, 1977.

Foegh ML, Ramwell PW. "Physiological implications of products in the arachidonic cascade." In Prostaglandins and Related Substances, eds. C Pace-Asciak and E Granstrom, Elsevier, New York, pp. xii-xix, 1983.

Foster D, Ahmed K, Zieve L. "Action of methanethiol on Na^+K^+-ATPase: implications for hepatic coma." Ann N Y Acad Sci 242:573-576, 1974.

Frerichs FT. "A Clinical Treatise on Diseases of the Liver." New Sydeham Society, London, 238-241, 1860.

Gazzard BG, Weston MY, Murray-Lyon IM, Flax H, Record CO, Portman B, Langley PG, Dunlop EH, Mellon PJ, Ward MD, Williams R. "Charcoal hemoperfusion in the treatment of fulminant hepatic failure." Lancet 1:1301-1307, 1974.

Garrison A, Fielding H. History of Medicine, 4th ed, Saunders, Philadelphia, 1961.

Geiger A, Yamasaki S. "Cytidine and uridine requirement of the brain." J Neurochem 1:93-100, 1956.

Gelfand MC, Winchester JF, Knepschield JH, Cohen SL, Schreiner GE. "Biochemical correlates of reversal of hepatic coma with coated charcoal hemoperfusion." Trans Am Soc Artif Intern Organs 24:239-242, 1978.

Ghuman SS, Rush BF, Machiedo GW, LoVerme PJ, Kraven T, Dikdan G. "Effect of prostaglandin on cell membrane permeability and hepatic high energy stores following hemorrhagic shock." J Surg Res 32(5):484-8, 1982.

Gilboe DD, Drewes LR, Kintner D. "Edema formation in isolated canine brain: anoxia vs ischemia." In Dynamics of Brain Edema, eds HM Pappius and W Feindel, Springer-Verlag, Berlin, pp. 228-235, 1976.

Gimson AE, Braude S, Mellon PJ, Canalese J, Williams R. "Earlier charcoal hemoperfusion in fulminant hepatic failure." Lancet 2(8300):681-683, 1982.

Go KG. "Physical methods for study of brain edema." In Advances in Neurology, Vol. 28, Brain Edema, eds. J Cervos-Navarro and R Ferszt, Raven Press, New York, pp. 1-8, 1980.

Goldberg ND, Haddox MK, Hartle DK, Hadden JW. "The biological role of cyclic 3', 5' guanosine monophosphate." In Pharmacology and the Future of Man, Vol. 5, ed RA Maxwell and GA Acheson, Krager, Basel, pp. 146-169, 1973.

Goldblatt MW. "A depressor substance in seminal fluid." J Soc Chem Ind (Lond) 52:1056, 1933.

Goldblatt MW. "Properties of human seminal plasma." J Physiol (Lond) 84:208-218, 1935.

Goldmann EE. "Die aussere und innere Sekretion des gesunden und kranken Organismus in Lichte der 'vitalen Farbung'." Beitr Z Klin Chur 64:192-265, 1909.

Goldstein GW, Diamond I. Transport properties of capillaries isolated from brain." In Dynamics of Brain Edema. eds. HM Pappius and W Feindel. Springer-Verlag, Berlin, pp. 7-10, 1976.

Goldstein GW. "The role of brain capillaries in the pathogenesis of hepatic encephalopathy." Hepatology 4:565-567, 1984.

Greenberg S, Kadowitz PJ, Burks TF. Prostaglandins: Organ and Tissue-Specific Action. Marcel Dekker Inc, New York, 1982.

Grinde B, Ichihara A. "Effects of prostaglandins and divalent cations on ^{c}AMP production in isolated rat hepatocytes," Exp Cell Res 148:163-172, 1983.

Groflin UB, Tholen H. "Cerebral edema in the rat with galactosamine induced severe hepatitis," Experientia 34(11):1501-1503, 1978.

Guyton AC. Textbook of Medical Physiology, 6th edition, WB Saunders Co., Philadelphia, 1981.

Hall WJ, O'Donoghue JP, O'Regan MG, Penny WJ. "Endogenous prostaglandins, adenosine 3', 5'-monophosphate and sodium transport across isolate frog skin." J Physiol 258:731-753, 1976.

Hamberg, M. "A note on nomenclature." In Advances in Biosciences, Vol. 9: International Conference on Prostaglandins, eds. S Bergstrom and S Bernhard, Pergamon Press, New York, p. 847, 1973.

Hammarstrom S. "Biosynthesis and biological actions of prostaglandins and thrombroxanes." Arch Biochem Biophys 214(2):431-95, 1982.

Hanid MA, Makenzie RL, Jenner RE, Chase RA, Mellon PJ, Trewby PN, Janota I, Davis M, Silk DBA, Williams R. "Intracranial pressure in pigs with surgically induced acute liver failure." Gastroenterology 76(1):123-131, 1979.

Hartmann A, Menzel J, Buttinger C. "Effects of dexamethasone on regional cerebral blood flow on ischemic cerebral infarction." In Treatment of Cerebral Edema, eds. A. Hartmann and M Brock, Springer-Verlag, Berlin, pp. 157-170, 1982.

Hawkins RA, Mans AM, Biebuyck JF. "Alterations in amino acid transport across blood-brain barrier in rats following portacaval shunting." In Amino Acids: Metabolism and Medical Applications. eds. GL Blackburn, JP Grant, and VR Young, John Wright, PGS Inc., Boston, USA, pp. 239-253, 1983.

Hayat MAR. Principles and Techniques of Electron Microscopy. Van Nostrand Reinhold Co, New York, 1970.

Higgins CB, Braunwald E. "The prostaglandins," Am J Med 53:92-112, 1972.

Hillier K. "Prostaglandins and thrombonanes: Pharmacologic and biosynthetic aspects." Semin Perinatol 2:197-205, 1978.

Hittleman KJ, Butcher RW. "Cyclic AMP and the mechanism of action of the prostaglandins." In The Prostaglandins -- Pharmacological and Theraputic Advances. ed. MF Cuthbert, William Heinemann Medical Books Ltd, London, pp. 151-165, 1973.

Holloway CJ, Brunner G, Schmidt E, Schmidt FW. "Thiols and hepatic coma." Artif Organs 3(1):15-9, 1979.

Holmin T, Agardh CD, Alinder G, Herlin P, Hultberg B. "Influence of total hepatectomy on cerebral energy state, ammonia related amino acids of the brain and plasma amino acids in the rat." Europ J Clin Invest 13:215-220, 1983.

Horrobin DF. Prostaglandins: Physiology, Pharmacology and Clinical Significance, Eden Press, Montreal, 1978.

Horton EW. "Hypothesis on the physiological role of prostaglandins," Physiol Rev 49:122-161, 1969.

Horton EW. Prostaglandins -- Monograph on Endocrinology, Vol. 7, Springer-Verlag, New York, 1972.

Hossmann KA. "Development and resolution of ischemic brain swelling." In Dynamics of Brain Edema, eds. HM Pappius and W Feindel, Springer-Verlag, Berlin, pp. 219-227, 1976.

Hossmann KA. "Pathophysiology of vasogenic and cytotoxic brain edema." In Treatment of Cerebral Edema, eds. A Hartmann and M Brock. Springer-Verlag, Berlin, pp. 1-10, 1982.

Horowitz ME, Schafer DF, Molnar P, Jones EA, Blasberg RG, Patlak CS, Waggoner J, Fenstermacher JD. "Increased blood-brain transfer in a rabbit model of acute liver failure." Gastroenterology 84(5):1003-1011, 1983.

Hoyumpa AM, Desmond PV, Avant GR, Roderick KR, Schenker S. "Hepatic Encephalopathy." Gasteroenterology 76:184-195, 1979.

Hoyumpa AM Jr, Schenker S. "Perspectives in hepatic encephalopathy." J Lab Clin Med Oct; 100(4):477-87, 1982.

Hurd ER, Johnston JM, Okita JR. "Prevention of glomerulonephritis and prolonged survival in New Zealand black/New Zealand white F_1 hybrid mice fed an essential fatty acid deficient diet." J Lab Clin Invest 67:476-485, 1981.

Jaffe BM. "Radioimmunoassay: What it tells about prostaglandins." In Persepctives on Prostaglandins," ed. JB Lee, Medcom Press, New York, pp. 20-19, 1973.

James JH, Ziparo V, Jepson B, Fischer JE. "Hyperammonia, plasma amino acid imbalance, and blood-brain amino acid transport: a unified theory of portal systemic encephalopathy." Lancet 2:772-775, 1979.

Jenkins PJ, Williams R. "Fulminant viral hepatitis." Clinics in Gastroenterol 9:171-189, 1980.

Johansson BB. "Water content of rat brain in acute arterial hypertension." In Dynamics of Brain Edema. eds. HM Pappius and W Feindel, Springer-Verlag, Berlin, pp. 28-31, 1976.

Johansson C, Bergstrom S. "Prostaglandins and protection of the gastrointestinal mucosa." Scand J Gastroenterology S77:21-46, 1982.

Johnson M. "The mature erythrocyte." In The Prostaglandins, Vol 2, ed. PW Ramwell, Plenum Press, New York and London, pp. 75-98, 1974.

Joo F, Toth I, Jansco G. "Brain adenylate cyclase: its common occurrence in the capillaries and astrocytes." Naturwissenschaften 8:397, 1975.

Joo F. "Significance of adenylate cyclase in the regulation of the permeability of brain capillaries," In Pathophysiology of Cerebral Energy Metabolism, eds. BB Mrsulja, LM Rakic, I Klatzo, and M Spatz, Plenum Press, New York, pp. 211-237, 1977.

Jones EA, Schafer DF. "Fulminant Hepatic Failure." In Hepatology -- A Textbook of Liver Disease. eds. D Zakim and TD Boyer, WB Saunders C., Philadelphia, pp. 415-445, 1982.

Jones EA, Schafer DF, Ferenci P, Pappas C. "The neurobiology of hepatic encephalopathy." Hepatology 4:1235-1242, 1984.

Jones RL. "Pharmacology of the prostaglandins." In Prostaglandin Research, ed. P Crabbé, Academic Press, New York, pp. 65-88, 1977.

Klatzo I. "Neuropathological aspects of brain edema." J Neuropathol Exp Neurol 26:1-14, 1967.

Knell AJ. "Acute liver failure." J Roy Col Phys Lond 14:117-123, 1980.

Krnjevic K, Phillis JW. "Iontophoretic studies of neurons in the mammalian cerebral cortex." J Physiol 165:274-304, 1963.

Krumlowski FA, del Greco F, Niederman M. "Prolonged hemoperfusion and hemodialysis management of hepatic failure and hepatorenal syndrome." Trans Am Soc Artif Intern Organs 24:235-238, 1978.

Kuehl FA, Humes JL. "Direct evident for a prostaglandin receptor and its application to prostaglandin measurements." Proc Natl Acad Sci USA 69:480-484, 1972.

Kuehl FA. "Prostaglandins, cyclic nucleotides and cell function." In Les Prostaglandines 1973, Seminaire INSERM, Paris, pp. 55-80, 1973.

Kurzrok R, Lieb CC. "Biochemical studies of human semen. II. The action of semen on the human uterus." Proc Soc Exp Biol Med 28:268-272, 1930.

Lands W, Lee R, Smith W. "Factors regulating the biosynthesis of various prostaglandins." Ann NY Acad Sci 180:107-122, 1971.

Lands W. "The biosynthesis and metabolism of prostaglandins." Annu Rev Physiol 41:633-652, 1979.

Laursen H. "Cerebral vessels and glial cells in liver disease." Acta Neurol Scandinav 65:381-412, 1982.

Leber HW, Klausmann J, Goubeaud G, Schutterle G. "Middle molecules in the serum of patients and rats with liver failure: influence of sorbent hemoperfusion." In Artificial Liver Support, eds. G Brunner and FW Schmidt, Springer-Verlag, Berlin, pp. 96-102, 1981.

Lee JC, Bakay L. "Electron microscopic studies on experimental brain edema." In Brain Edema, eds. I Klatzo and F Seitelberger. Springer-Verlag, Berlin, pp. 590-597, 1967.

Lee JB. "The antihypertensive function of the kidney." In Perspectives on the Prostaglandins, ed. JB Lee, Medcom Press, pp. 48-70, 1973.

Lehninger A. Principles of Biochemistry. Worth Publishers, Inc, New York, 1982.

Levy M. "Observations on renal function and ascites formation in dogs with experimental portal cirrhosis." In The Kidney in Liver Disease, ed. M. Epstein, Elsevier, New York, pp. 131-142, 1976.

Levy M. "Sodium retention and ascites formation in dogs with experimental portal cirrhosis," Am J Physiol 233:F572-585, 1977.

Levy M, Allotey JBK. "Temporal relationship between urinary salt retention and altered systemic hemodynamics in dogs with experimental cirrhosis," J Lab Clin Med 92:560-569, 1978.

Liehr H, Grun M, Seelig HP, Seelig R, Reutter W, Heine WD. "On the pathogenesis of galactosamine hepatitis." Virchows Arch B Cell Path 26:311-344, 1978.

Lifschitz MD. "Prostaglandins in liver disease." In The Kidney in Liver Disease. ed. M Epstein, Elsevier Science Publishing Co, Inc, 2nd Edition, pp. 453-468, 1983.

Little JR. "Microvascular alterations and edema in focal cerebral ischemia." In Dynamics of Brain Edema, eds. HM Pappius and W Feindel, Springer-Verlag, Berlin, pp. 230-243, 1976.

Livingstone AS, Potvin M, Goresky CA, Finlayson MH, Hinchey EJ. "Changes in the blood-brain barrier in hepatic coma after hepatectomy in the rat." Gastroenterology 73(4):697-704, 1977.

Loginov AS, Markova MN. "Prostaglandins in liver diseases." Materia Medica Polona, 2(9):91-99, 1979.

MacManus JP, Whitfield JF. "Cyclic AMP, prostaglandins and control of cell proliferation." Prostaglandins 6:475-487, 1976.

MacManus JP, Whitfield BM. "A connection between the production of prostaglandin during liver regeneration and the DNA synthetic response." Prostaglandins 11:609-620, 1976.

Madden JW, Gertmann PM, Peacock EE. "Dimethylnitrosamine-induced hepatic cirrhosis: a new canine model for an ancient human disease." Surgery 68:260-268, 1970.

Makowka L, Falk RE, Cohen MM, Falk JA, Langer B, Blendis LM, Phillips JM. "Protective effect of 16, 16-dimethyl prostaglandin E_2 on acute ethanol induced inhibition of hepatic regeneration." Surg Forum 33:183-186, 1982.

Manabe T, Steer ML. "Protective effects of PGE_2 on diet-induced pancreatis in mice." Gastroenterology 78:777-781, 1980.

Manghani KK, Lunzer MR, Billings BH, Sherlock S. "Urinary and serum octopamine in patients with portal systemic encephalopathy." Lancet 2:943-946, 1975.

Mann FC, Magath TB. "Studies on the physiology of the liver: II. The effect of removal of the liver on the blood sugar level." Arch Int Med 30:73-84, 1922.

Marshall LF, Bruce DA, Graham DI, Langfitt TW. "Triethyltin-induced cerebral edema: Implications for determination of cerebral blood flow in edamatous tissue." In Dynamics of Cerebral Edema, eds. HM Pappius and W Feindel, Springer-Verlag, Berlin, pp. 83-86, 1976.

McNeil GE, Leevy CM. "Inhibition of ethanol-induced suppression of liver regeneration by 16, 16 dimethylprostaglandin E_2." Gastroenterology 84(5):1384, 1983.

McNeil GE, Chen TS, Leevy CM. "Reversal of ethanol and indomethacin-induced suppression of hepatic DNA synthesis by 16, 16-dimethyl prostaglandin E_2." Hepatology 5(1):43-46, 1985.

Medline A, Schaffner F, Popper H. "Ultrastructural features in galactosamine-induced hepatitis." Exp Molec Path 12:201-211, 1970.

Miller JD, Ganguli S, Sperling MA. "Prostaglandin synthesis inhibitors impair hepatic glucose production in response to glucagon and epinephrine stimulation." Diabetes 32(5):439-444, 1983.

Miller TA, Jacobson ED. "Gastrointestinal cytoprotection by prostaglandins." Gut 20:75-87, 1979.

Mitra A. The Synthesis of Prostaglandins. Wiley, New York, USA, 1977.

Miura Y, Fukui N. "Prostaglandins as possible triggers for liver regeneration after partial hepatectomy: A review." Cell Mol Biol 25:179-184, 1979.

Miyamoto T, Ogino S, Yamamoto S, Nayaishi OJ. "Purification of endoperoxide synthetase in bovine vesicular gland microsomes." J Biol Chem 251:2629-2636, 1976.

Miyazaki M, Makowka L, Falk RE, Falk JA, McDonell M, Venturi D. "Protection of thermochemotherapeutic-induced lethal acute hepatic necrosis in the rat by 16, 16-domethyl prostaglandin E_2." J Surg Res 34(5):415-26, 1983.

Mohsini K, Lister C, Chang TMS. "The effects of homologous cross-circulation and in-situ liver perfusion on fulminant hepatic failure rats." Artif Organs 4:171-175, 1980.

Moritz EW, Kreuzer A, Schenck WG. "Studies in experimental canine cirrhosis: haemodynamic alterations with emphasis on degree of spontaneous porto-systemic shunting," Ann Surg 177:503-506, 1973.

Mrsulja BB, Djuricic BM, Cvejic V, Branka J, Mrsulja KA, Spatz M, Klatzo I. "Biochemistry of experimental ischemic brain edema." In Advances in Neurology, Vol. 28: Brain Edema. eds. J Cervos-Navarro and R Ferszt. Raven Press, New York, pp. 217-230, 1980.

Murota SI, Morita I. "Prostaglandin-synthesizing system in rat liver: Changes with age and various stimuli." Adv Prostaglandin Thromboxane Res 8:1495-1506, 1980.

Murray-Lyon IM, Portman B, Gazzard BG, Williams R. "Analysis of the causes of death in the treatment failures." In Artificial Liver Support, eds. R Williams and IM Murray-Lyon, Pitman Medical Publishing Co, Tunbridge Wells, UK, pp. 242-248, 1975.

Murray-Lyon IM, Orr AH, Gazzard B, Kohn J, Williams R. "Prognostic values of serum alpha-fetoprotein in fulminant hepatic failure including patients treated by charcoal hemoperfusion." Gut 17(8):576-580, 1976.

Needleman P, Raz A, Minkes MS, Ferrendelli JA, Sprecher H. "Triene prostaglandins, prostacycline and thromboxane biosynthesis and unique biological properties." Proc Nat Acad Sci USA 76:944-948, 1979.

Nelson NA. "Prostaglandin nomenclature." J Med Chem 17(9):911-918, 1974.

Nezamis JE, Lancaster C, Robert A, Ruwart MJ. "Indomethacin-induced gastric ulcers and intestinal lesions in hamsters." Gastroenterology 82(5):1138, 1982.

Nezamis JE, Robert A. "Sodium salicylate, at a cytoprotective dose, stimulates gastric mucus formation; aspirin does not." Gastrenterology 82(5):1138, 1982.

Nishiki M, Kawanishi H, Kanao M, Sugiyama M, Tsuchiya T, Ezaka H. "Influence of hemoperfusion using coated bead-type charcoal on middle molecules of hepatic failure dogs." Hiroshima J Med Sci 33:493-497, 1984.

Niu Z, Shu CD, Jia SR, Li SM. "Factors possibly influencing the effect of hemoperfusion in fulminant hepatic failure rats." In The Past, Present, and Future of Artificial Organs. eds. E Piskin and TMS Chang, Meteksan Publishing Co, Ankara, Turkey, pp. 140-151, 1983.

Nugteren DH, Beerthuis RK, van Dorp DA. "Biosynthesis of prostaglandins. Prostaglandins, Proceedings of the Second Nobel Symposium, Stockholm, Almqvist and Wiksell Publishers, Stockholm, pp. 45-50, 1967.

O'Brien MD. "Ischemic cerebral edema." In Treatment of Cerebral Edema. eds. A Hartmann and M Brock, Springer-Verlag, Berlin, pp. 19-23, 1982.

Odaka M, Tabata Y, Kobayashi H, Nomura Y, Soma H, Hirasawa H, Sato. "Clinical experience of bead-shaped activated charcoal hemoperfusion in chronic renal failure and fulminant hepatic failure." In Artificial Kidney, Artificial Liver, and Artificial Cells, ed. TMS Chang, Plenum Press, New York, pp. 79-88, 1980.

Oliw E, Granstrom E, Anggard E. "The prostaglandins and essential fatty acids." In Prostaglandins and Related Substances, eds. C Pace-Asaik and E Granstrom, Elsevier, New York, pp. 1-44, 1983.

Opolon P, Huguet CI, Nusinovici V, Lavallard MC, Crubille C, Gallot D, Bloch P, Granger A. "Effect of middle molecule removal on encephalopathy during acute liver failure," Eur Soc Artif Organs 2:228-235, 1975.

Osuafor TOK. "Cerebral oedema of fulminant hepatic failure." Clin Sci 65(4):445-6, 1983.

Otto AM, Nilsen-Hamilton M, Boss BD, Ulrich M, Jimeneze de Asua L. "Prostaglandin E_1 and E_2 interact with prostaglandin F_2 to regulate initiation of DNA replication and cell division in Swiss 3T3 cells." Proc Natl Acad Sci USA 79:4992-4996, 1982.

Pappas SC, Ferenci P, Schafer DF, Jones EA. "Visual evoked potentials in a rabbit model of hepatic encephalopathy: II. Comparison of hyperammonemic encephalopathy, postictal coma, and coma induced by synergistic neurotoxins." Gastroenterology 86(3):546-551, 1984.

Pardridge WM, Oldendorf WH. "Transport of metabolic substrates through the blood-brain barrier." J Neurochem 28:5-12, 1977.

Pardridge WM. "Transport of nutrients and hormones through the blood-brain barrier." Fed Proc 42:201-204, 1984.

Parkes JD, Sharpstone P, Williams R. "Levodopa in hepatic coma." Lancet 2:1341-1343, 1970.

Pickering RW, James GWL, Parker FL. "An investigation of some parameters that affect the galactosamine model of hepatitis in the rat," Arzneim-Forsch (Drug Res) 25(6):898-901, 1975.

Pike JE. "Prostaglandins." Sci Am 225(5):84-92, 1971.

Piper PJ, Vane JR. "The release of prostaglandins from lung and other tissues." Ann NY Acad Sci 180:363-385, 1971.

Pirola RC, Ham JM, Elmslie RG. "Management of hepatic coma complicating viral hepatitis." Gut 10:898-903, 1969.

Popper H. "Mechanism of liver cell destruction in fulminant hepatic failure." In Artifical Liver Support. eds. G Brunner and FW Schmidt, Springer-Verlag, Berlin, pp. 1-7, 1981.

Potvin M, Finlayson MH, Hinchey EJ, Lough JD, Goresky CA. "Cerebral abnormalities in hepatectomized rats with acute hepatic coma." Lab Invest 50:560-564, 1984.

Rall TW, Sutherland EW, Bethet J. "The relationship of epinephrine to glucagon to liver phosphorylase. IV. Effect of epinepherine and glucagon on the reactivation of phosphorylase in liver homogenates." J Biol Chem 244:463-475, 1957.

Rapoport SI. Blood-Brain Barrier in Physiology and Medicine, Raven Press, New York, 1976.

Rappaport AM, Macdonald MH, Borowy ZJ. "Hepatic coma following ischemia of the liver." Surg Gynec Obst 97:748-762, 1953.

Reber HA, Tweedie JH, Mosley JG. "The cytoprotective effect of 16, 16-dimethylprostaglandin E_2 on bile salt induced damage to pancreas." Gastroenterology 78:1241, 1980.

Robert A, Nezamis JE, Phillips JP. "Inhibition of gastric secretion by prostaglandins." Am J Dig Dis 12:1073-1076, 1967.

Robert A. "An intestinal disease produced experimentally by prostaglandin deficiency." Gastroenterology 69:1045-1047, 1975.

Robert A. "Antisecretory, antiulcer, cytoprotective and diarrheogenic properties of prostaglandins." In Advances in Prostaglandin and Thromboxane Research, Vol. 2, eds. B Samuelsson and R Paolett, Raven Press, New York, pp. 507-520, 1976.

Robert A, Nezamis JE, Lancaster C, Hanchar AJ. "Cytoprotection by prostaglandins in rats." Gastroenterology 77:433-443, 1979.

Robert A. "Cytoprotection by prostaglandins." Gastroenterology 77:761-767, 1979.

Robert A. "Current history of cytoprotection." Prostaglandins 21:89-96, 1981.

Roberts E, Case TN, Tower DB. GABA in Nervous System Function. Raven Press, New York, 1981.

Robertson RP, Westcott KR, Storm DR, Rice MG. "Down regulation in vivo of PGE receptors and adenylate cyclase stimulation." Am J Physiol 239:E75-E80, 1980.

Roche-Sicot J, Peignous M, Bourdian D, Degos F, Degos J-D, Prandi D, Rueff B, Benhamou J-P. "Acute hepatic encephalopathy in the rat: The effect of cross-circulation." Clin Sci Mol Med 47:609-615, 1974.

Ruddon RW, Johnson JM. "The effect of prostaglandins on protein and nucleic acid synthesis in a cell-free system." Life Sci 6:1245-1252, 1967.

Rueff B, Benhamou J-P. "Acute hepatic necrosis and fulminant hepatic failure." Gut 14:805-815, 1973.

Ruwart MJ, Rush BD, Friedle NM, Piper RC, Kolaja GJ. "Protective effects of 16, 16-dimethyl PGE_2 on the liver and kidney." Prostaglandins 21:97-102, 1981a.

Ruwart MJ, Kolaja GJ, Friedle NM, Rush BD, Tarnawski J, Stachura J, Mach T, Ivey KJ. "Protection against CCl_4- induced liver damage by 16, 16-dimethyl PGE_2." Gastroenterology 80:1266, 1981b.

Ruwart MJ, Friedle NM, Rush BD. "16, 16-dimethyl PGE_2 protects in-vitro hepatocytes from carbon tetrachloride induced damage." Gastroenterology 82(5):1166, 1982a.

Ruwart MJ, Rush BD, Friedle NM. "16, 16-dimethyl PGE_2 partially prevents necrosis due to aflatoxin in rats." Gastroenterology 82(5):1167, 1982b.

Ruwart MJ, Rush BD, Friedle NM, Stachura J, Tarnawski A. "16, 16-dimethyl PGE_2 protection against alpha-nepthylisothiocynate-induced experimental cholangitis in the rat." Hepatology 4(4):658-660, 1984.

Ryan CJ, Courtney JM, Wood CB, Blumgart LH. "Hemoperfusion and DNA synthesis in the rat liver. Part I: the effect of hemoperfusion on the rate of DNA synthesis." Artif Organs 6(2):175-81, 1982.

Samuelsson BB. "Biosynthesis and metabolism of prostaglandins." In Les Prostaglandines 1973. Seminaire INSERM, Paris, pp. 21-41, 1973.

Samuelsson BB, Granstrom E, Green K, Hamberg M, Hammarstrom S. "Prostaglandins." Annu Rev Biochem 44:669-695, 1977.

Saunders SJ. "Experimental models of hepatic failure." In Hepatology IX, Freiberg, Falk Foundation, pp. ix-xi, 1979.

Saunders SJ, Seggie J, Kirsch RE, Terblanche J. "Acute liver failure." In Liver and Biliary Disease, eds. K Wright KGMM Alberti, S Karran, GH Millward-Sadler, WB Saunders, Philadelphia, pp. 569-584, 1979.

Schafer DF, Jones EA. "Potential neural mechanisms in the pathogenesis of hepatic encephalopathy." Prog Liver Dis 7:615-627, 1982.

Schafer DF, Jones EA. "Hepatic enephalopathy and the gamma-amino-butyric-acid neurotransmitter system." Lancet 1(8262):18-20, 1982.

Schafer DF, Pappas SC, Brody LE, Jacobs R, Jones EA. "Visual evoked potentials in the rabbit model of hepatic encephalopathy. I. Sequential changes and comparisons with drug-induced comas." Gastroenterology 86:540-545, 1984.

Scharnbeck H, Schaffner F, Keppler D, Decker K. "Ultrastructural studies on the effect of choline orotate on galactosamine-induced hepatic injury in rats." Exp Molec Path 16:33-46, 1972.

Schneider WP, Hamilton RD, Rhuland LE. "Occurrence of esters of (15s) PGA_2 and PGE_2 in coral." J Am Chem Soc 94:2122-2133, 1973.

Schreiner GE. "Blood and cerebrospinal-fluid changes associated with reversal of coma by charcoal hemoperfusion." Kidney Int 12:482, 1977.

Seda HWM, Hughes RD, Grove CD, Williams R. "Removal of inhibitors of brain Na^+, K^+-ATPase by hemoperfusion in fulminant hepatic failure." Artificial Organs 8(2):174-178, 1984.

Shaw JE, Ramwell PW. "Release of prostaglandins from rat epididymal fat pads on nervous and hormonal stimulation." J Biol Chem 243:1498-1503, 1968.

Shaw JE, Gibson W, Jessup S, Ramwell PW. "The effect of PGE_1 on cyclic AMP and ion movements in turkey erythrocytes." Ann NY Acad Sci 180:241-260, 1971.

Shi ZQ, Chang TMS. "The effects of hemoperfusion using coated charcoal or tyrosinase artificial cells on middle molecules and tyrosine in brain and serum of hepatic coma rats." Trans Am Soc Artif Intern Organs 28:205-209, 1982.

Shi ZQ, Chang TMS. "Amino acid disturbances in experimental hepatic coma rats." Int J Artif Organs 7:197-202, 1984a.

Shi ZQ, Chang TMS. "Effect of charcoal hemoperfusion on amino acid disturbances in experimental hepatic coma." Int J Artif Organs 7:203-208, 1984b.

Shu CD, Chang TMS. "Tyrosinase immobilized within artificial cells for detoxification in liver failure: II. In vivo studies in fulminant hepatic failure rats." Int J Artif Organs 4:82-84, 1981.

Shu CD, Chang TMS. "Middle molecules in the serum, brain, liver and urine of galactosamine-induced fulminant hepatic failure rats." Int J Artif Organs 6:273-277, 1983.

Sih CJ, Takeguchi C, Foss PJ. "Mechanism of prostaglandin biosynthesis III. Catecholamine and serotonin as co-enzymes." J Am Chem Soc 92:6670-6678, 1970.

Silk DBA, Hanid MA, Trweby PN, Davis M, Chase RA, Langley PG, Mellon PJ, Wheeler PG, Williams R. "Treatment of fulminant hepatic failure by polyacrylonitrile membrane hemodialysis." Lancet ii:1-3, 1977.

Silk DBA, Williams R. "The liver -- acute liver failure." Br J Hosp Med 22:437-446, 1979.

Simigel M, Fleischer S. "Characterization and localization of prostaglandin E_1 receptor in rat liver plasma membranes." Biochem Biophys Acta 332:358-373, 1974.

Spatz M, Fujimoto T, Go GK. "Transport studies in ischemic cerebral edema." In Dynamics of Brain Edema, eds. HM Pappius and W Feindel, Springer-Verlag, Berlin, pp. 181-186, 1976.

Stachura J, Tarnawski A, Szezudrawa J, Bogdal J, Mach T, Klinczyk B, Kirchmayer S. "Cytoprotective effects of 16, 16-dimethyl prostaglandin E_2 and some drugs on acute galactosamine-induced liver damage in rats." Folia Histochem Cytochem 9:311-318, 1980.

Stachura J, Tarnawski A, Ivey KJ, Ruwart MJ, Rush BD, Friedle NM, Szezudrawa J, Mach T. "16, 16-dimethyl prostaglandin E_2 protection of rat liver against acute injury by galactosamine, acetomenophen, ethanol, and ANIT." Gastroenterology 80:1349, 1981.

Starzl TE, Porter KA, Hayashida N, Schechter P, Terblanche J. "Further studies on hepatic stimulatory substances after partial hepatectomy." J Surg Res 29:471-474, 1980.

Starzl TE, Chang TMS, Williams R, Nose Y. "Liver support/transplants and artificial organs" -- Panel Conference. Trans Am Soc Artif Intern Organs 24:795-799, 1983.

Steinberg D, Vaughan M, Nestel PJ, Strand D, Bergstrom S. "Effects of prostaglandins on hormone-induced mobilization of free fatty acids." J Clin Invest 43:1533-1540, 1964.

Steiner L, Forester DMC, Bergvall U, Carlsson LA. "Effects of PGE_1 on cererbal circulatory disturbances." Proc 5th Int Symp Part II, Eur Neurol 8:23-31, 1972.

Stewart-Wallace AM. "A biochemical study of cerebral tissue and of the changes in cerebral oedema." Brain 62:426-438, 1939.

Swedlow D, Frewen T, Watcha M, Bruce DA. "Cerebral blood flow, AJD02 and CRM02 in comatose children." In Recent Progress in the Study and Therapy of Brain Edema, eds. KG Go and A Baethmann, Plenum Press, New York, pp. 365-371, 1984.

Tabata Y, Chang TMS. "Comparison of six artificial liver support regimes in fulminant hepatic coma rats." Trans Am Soc Artif Intern Organs 26:394-399, 1980.

Terblanche J, Hickman R, Miller DJ, Saunders SJ. "Animal experience with support systems: are there appropriate models of fulminant hepatic necrosis? In Artificial Liver Support, eds. R Williams and IM Murray-Lyon, Pitman Medical Publishing Co, Tunbridge Wells, UK, pp. 163-172, 1975.

Tholen H. "Hirondem. Eine Todesursache bie endogenem Liber-coma." Klin Wochenschr 50:296-301, 1972.

Treip CS. Color Atlas of Neuropathology. Year Book Medical Publishers, Inc, Chicago, USA, 1978

Trewby P, Bennett A, Murray-Lyon IM, Williams R. "Plasma prostaglandins in fulminant hepatic failure." Br Med J 4:442, 1975.

Trey C, Garcia FG, King NW, Lowenstein LM, Davidson CS. "Massive liver necrosis in the monkey: the effects of exchange blood transfusions on fulminant hepatic failure." J Lab Clin Med 73:784-794, 1969.

Trey C, Davidson CS. "The management of fulminant hepatic liver failure." In Progess in Liver Disease, eds. H Popper and F Schaffner, Vol. 3, Grune and Stratton, New York, pp. 282-298, 1970.

Tygstrup N, Gaub J, Ranek L. "Determination of liver function and regeneration in fulminant hepatic failure." In Artificial Liver Support, eds. G Brunner and FW Schmidt, Springer-Verlag, Berlin, pp. 39-45, 1981.

van Leenhoff JW, Hickman R, Saunders SJ, Terblanche J. "Massive liver cell necrosis induced in the pig with carbon tetrachloride." S Afr Med J 48:1201-1204, 1974.

Vane JR. "Inhibition of prostaglandin synthesis as a mechanism of action of aspirin like drugs." Nature New Biol 231:232-234, 1971.

Vane JR. "Inhibitors of prostaglandin, prostacyclin, and thromboxane synthesis." In Advances in Prostaglandin and Thromboxane Research, Vol. IX, eds. F Coceani and PM Olley, Raven Press, New York, pp. 27-44, 1978.

Vane JR. Prostacyclin in health and disease. Lilly Lecture 1981, Royal College of Physicians Edinburgh, 1982.

von Euler US. "Uber die spezifische blutdruksenkende Substanz des menschlichen Prosta-und Semenblasensekretes." Klin Worchenschr 14:1182, 1935.

von Euler US. "A depressor substance in the vesicular gland," J Physiol (Lond) 84:21P, 1935.

Wahren J, Denis J, Desurmont O, Eirksson LS, Escoffier J-M, Gauthier AP, Hagenfeldt L, Michel H, Opolon P, Paris J-C, Veyrac M. "Is intravenous administration of branched chain amino acids effective in the treatment of hepatic encephalopathy? A multicentre study." Hepatology 3(4):475-480, 1983.

Ware AJ, D'Agostino AN, Combes B. "Cerebral Edema: a major complication of massive hepatic necrosis." Gastroenterology 61(6):877-884, 1971.

Weston MJ. "Charcoal hemoperfusion for liver failure." Lancet 3(8305):983, 1982.

Willey RL. Microtechniques: A Laboratory Guide. Macmillian Publishing Co, Inc, New York, 1971.

Williams R. "Need for artificial liver support." In Artificial Liver Support, eds. R Williams and IM Murray-Lyon, Pitman Medical, Tunbridge Wells, England, pp. 3-10, 1975.

Williams R. "Fulminant hepatic failure." Post Grad Med J 59:33-41, 1983.

Wolfe LS. "Possible roles of prostaglandins in the nervous system." Adv Neurochem 1:1-50, 1975.

Wolfe LS, Rostorowski K, Marion J. "Endogenous formation of the prostaglandin endoperoxide metabolite thromboxane B_2 by brain tissue." Biochem Biophys Res Commun 70:907-913, 1976.

Yamamoto YL, Feindel W, Wolfe LS, Katoh HK, Hodge CP. "Effects of PGs on cerebral blood flow." Proc 5th Int Symp Part I. Eur Neurol 6:144-152, 1972.

Yamamoto YL, Feindel W, Wolfe LS, Katoh HK, Hodge CP. "Experimental vascoconstriction of cerebral arteries by prostaglandins." J Neurosurg 37:385-397, 1973.

Zaki AEO, Wardle EN, Canalese J, Ede RJ, Williams R. "Potential toxins of acute liver failure and their effects on blood-brain barrier permeability." Experientia 39(9):988-91, 1983.

Zaki AEO, Ede RJ, Davis M, Williams R. "Experimental studies of blood-brain barrier permeability in acute hepatic failure." Hepatology 4(3):359-363, 1984.

Zieve L, Olsen RI. "Can hepatic coma be caused by a reduction of brain norepinephrine or dopamine." Gut 18:688-691, 1977.

Zieve L. "Hepatic encephalopathy: Summary of present knowledge with an elaboration on recent developments." In Progress in Liver Diseases, Vol. 6, eds. H Popper and F Schaffner, Gune and Stratton Inc, New York, pp. 327-341, 1979.

Zieve L. "The mechanism of hepatic coma." Hepatology 1(4):360-365, 1981.

Zimmerli W, Grubinger CH, Tholen H, Oberholzer M, Bianchi L. "Mannitol treatment of cerebral oedema in rats with galactosamine-induced severe hepatitis." Experientia 37:1323-1325, 1981.

Zulch KJ. "Neurolopathological aspects and histological criteria of brain edema and brain swelling." In Brain Edema, eds. I Klatzo and F Seitelberger, Springer-Verlag, New York, pp. 95-116, 1967.

Zusman RM, Axelrod L, Rolkoff-Ruben N. "The treatment of the hepatorenal syndrome with intrarenal administration of prostaglandin E_1." Prostaglandins 13:819-830, 1977.