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ADAPTIVE GROWTH IN THE LIVER

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ABSTRACT

The control of DNA synthesis in epithelial cells undergoing hyperplasia is not understood and although concurrent cellular hypertrophy sometimes occurs it appears that these two processes are independent. Recently the administration of chemicals which stimulate hepatic growth in hypophysectomised rats has further underlined their independence. It was suggested that DNA synthesis was inhibited by the presence of a "relatively" increased amount of DNA in the atrophied liver of the hypophysectomised rat. The effect of these agents to induce DNA synthesis was restored after partial hepatectomy. The level of hepatic nuclear DNA appeared to regulate this response.

The challenging of the hypothesis formed the basis of this project. The capacity of phenobarbitone to stimulate DNA synthesisin hypophysectomised rats was examined where the total hepatic DNA was not reduced.

Hypophysectomised male Wistar rats were divided into three major groups. One group received phenobarbitone treatment alone and in the other two groups partial hepatectomy or portal vein ligation was performed prior to phenobarbitone treatment and their effects on the liver compared to controls.

In hypophysectomised animals phenobarbitone stimulated cell hypertrophy but not significantly cell replication. However following partial hepatectomy a situation where both cytoplasm and nuclear DNA were reduced phenobarbitone administration induced cell multiplication and liver growth involved both cell hypertrophy and hyperplasia. Similarly following portal vein ligation in which cell atrophy occurred

without alteration in hepatic DNA content both cell replication and hypertrophy were induced in the unligated lobes.

This study demonstrated that DNA synthesis could be stimulated by chemicals in the presence of "relative" DNA excess. Because of the results with procedures which reduced cytoplasm only, the possibility is canvassed that factors concerned with chemical stimulation of DNA synthesis in the rat may be associated with or processed by cytoplasmic or plasmalemmal components of the hepatocyte.

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LIST OF ABBREVIATIONS USED IN TEXT

AFP	Alpha-fetoproteins
A.U.	Arbitrary units of integrated nuclear density
cAMP	Cyclic AMP
CPA	Cyproterone acetate
DNA	Deoxyribonucleic acid
EGF	Epidermal growth factor
HCH	Alpha-hexachlorocyclohexane
Individual liver lobes	L.L. Left lateral lobe M.L. Median lobe R.P. Right posterior lobe C.L. Caudate lobe
Intact	Sham hypophysectomised animals
3-MC	3-methylcholanthrene
ODC	Ornithine decarboxylase
PB	Sodium phenobarbitone (Johnson & Johnson)
PCN	Pregnenolone-16-carbonitrile
SER	Smooth endoplasmic reticulum
TAGH	Isotonic solution of triiodothyronine (T_3), amino acids, glucagon and heparin
[^{3}H]TdR	Tritiated thymidine
VLDL	Very low density protein

CHAPTER 1

ADAPTIVE GROWTH IN THE LIVER

1.1 General review

The mechanisms underlying the regulation of DNA synthesis in the liver are not identified and may be different for varieties of growth processes. Different circumstances attend these varieties and it may be necessary to define them separately.

In post-natal life it is likely that developmental or somatic liver growth, a process accompanied by growth in the whole organism, is regulated differently from adaptive growth which results from alterations induced by experimental manipulation, by some disease processes or by administration of therapeutic drugs or other xenobiotics leading to an increase mainly in the organ itself. The induction of adaptive growth in the liver may follow loss of functioning liver tissue for example by surgical manipulation such as partial hepatectomy (Harkness,1957; Weinbren,1959; Bucher, 1963; Grisham, 1973; Weinbren, 1979), chemical damage induced by hepatotoxins such as chloroform or carbon tetrachloride (Feuer et al., 1965; Golberg, 1966; Platt and Cockrill, 1969a; Grisham, 1973) and disease including viral hepatitis (Peters, 1975; Ishak, 1976). Adaptive growth without destruction of hepatocytes may be induced by chemicals such as polycyclic hydrocarbons, phenobarbitone (PB) or alpha-hexachlorocyclohexane (HCH) and often involves induction of enzymes (Conney and Burns, 1972; Koransky et al., 1966; Conney, 1967; Schlicht et al., 1968; Platt and Cockrill, 1969; Schulte-Hermann, 1974; Schulte-Hermann et al., 1974; Argyris, 1974).

The increase in mass which occurs after damage to or loss of a part of the liver, is associated with increases in DNA content and in numbers of liver cells and is referred to as compensatory hyperplasia whilst an increase in liver mass where tissue is neither removed nor damaged is referred to here as adaptive growth. In experimentally induced adaptive growth following administration of many drugs, insecticides and food additives, both hyperplasia (involving DNA synthesis) and cell hypertrophy (involving protein synthesis) can usually be detected (Weinbren, 1975; Schulte-Hermann, 1974). While some authors conclude they are part of the same process (Baserga, 1976; Schneyer et al., 1967; Hamilton, 1968; Johnson, 1969), others consider them to be independent (Malamud, 1972; Weinbren et al., 1972; Weinbren and Washington, 1976; Schulte-Hermann et al., 1977).

Several studies demonstrate that xenobiotics produce liver enlargement by stimulating:

1) Cell enlargement involving increases in main cell constituent proteins, RNA, lipid, glycogen and water and increases in microsomal enzymes and endoplasmic reticulum (ER) (Koransky et al., 1966; Staubli et al., 1969; Glaumann, 1970; Glazer and Sartorelli, 1972; Schulte-Hermann, 1974). The cell enlargement is largely due to increase in proteins, proliferation of ER and enzymes with a morphological decrease in nucleus/cytoplasm ratio (Schlicht et al., 1968; Kunz et al., 1966; 1966a; Staubli et al., 1969). Hypertrophy is considered to be cell enlargement without the increase in DNA content produced by an increase in ploidy or numbers of nuclei (Schulte-Hermann, 1974; 1979).

2) Cell multiplication shown by increase in number of cells involved in DNA synthesis and mitosis. The total DNA content of the organ is increased (Bucher, 1963).

3) Increase in nuclear ploidy (Bucher, 1963; Schulte-Hermann, 1974; 1979). This is produced by treatment with, for example, HCH and cyproterone acetate (CPA) and is accompanied by a decrease in binuclear cells. This shift in binuclearity probably results from replication of binuclear cells which after cytokinesis produce 2 mononuclear cells of increased ploidy (Schulte-Hermann, 1979). Hyperplasia is regarded as an increase in genetic material whether derived from an increase in ploidy or from cell division (Schulte-Hermann, 1974; 1979).

Other general characteristics of liver enlargement are:

a) The effects of xenobiotics are self-limiting. Active liver growth and cell multiplication occur in the early phase of treatment and the rate of cell proliferation returns towards normal even if the treatment is continued (Schulte-Hermann et al., 1968; Schlicht et al., 1968; Schulte-Hermann, 1979). For example, following treatment with HCH the DNA content of the liver increases only in the initial stage of exposure and thereafter it remains constant (Schulte-Hermann, 1974; 1979).

b) The amount of hyperplasia or hypertrophy contributing to the adaptive cell growth depends on the compound administered, the dose of the drug administered and the age of the animal. For example, HCH, CPA and low doses of PB induce liver growth in young rats predominantly by hyperplasia, the result of cell multiplication.

In contrast liver enlargement induced by higher doses of PB in young rats and by PB, HCH or CPA in adult rats is the result of a combination of hypertrophy and hyperplasia, where hyperplasia is represented by an increase in polyploid cells (Schulte-Hermann et al., 1968; Schulte-Hermann, 1974; 1979; Argyris, 1974; Augenlicht and Argyris, 1975).

c) Following the cessation of drug administration, liver enlargement is readily reversible except for the raised DNA content and/or increased nuclear ploidy which persists for several weeks (Schulte-Hermann et al., 1971; Schulte-Hermann, 1979; Schulte-Hermann and Parzefall, 1981).

It has been suggested that the increase in cell size induced by phenobarbitone (PB) is due to an increase in smooth endoplasmic reticulum (SER) (Barka and Popper, 1967; Staubli et al., 1969; Schulte-Hermann et al., 1972; Schulte-Hermann, 1974; Boger et al., 1978; Hardwick, 1983) and that protein synthesis is controlled by microsomal enzymes found within the SER (Kato et al., 1966; Argyris, 1974). Many drugs are classified according to which enzymes they induce. Several drugs including PB, can be separated into five different classes as they induce five different subsets of enzymes collectively known as cytochrome P450, each class of inducer modulating gene expression through different molecular mechanisms (Conney, 1967; Argyris and Magnus, 1968; Argyris, 1969; Hodgson and Dauterman, 1980; Goldstein, 1984; Lechner, 1987).

The increase in microsomal enzymes due to enzyme synthesis along with some enzyme stabilization of the smooth endoplasmic reticulum

(Kuriyama et al., 1969; Matsumura and Omura, 1973; Schulte-Hermann, 1974) is paralleled with an increased liver weight, but on cessation of the drug both return to normal. This appears to be an adaptive response by the liver to an increased work load (Gilbert and Goldberg, 1967; Platt and Cockerill, 1969; 1969a) which led Argyris (1974) to propose that this increase was coupled with the triggering of liver growth.

However cell enlargement is independent of these activities as hypertrophy induced by 3-methylcholanthrene (3-MC) is not accompanied by, or is only associated with an insignificant increase in the smooth endoplasmic reticulum and no measurable change in microsomal protein per gram of liver (Conney and Gilman, 1963; Fouts and Rogers, 1965; Glaumann, 1970). In addition high doses of HCH suppress microsomal enzymes but continue to induce liver growth (Schulte-Hermann et al., 1974) and diethylaminoethylphenyldiallyl-acetate (CFT-1201), an inhibitor of cytochrome P450, has no effect on DNA synthesis and mitotic activity in the remaining lobes of partially hepatectomised rats (Schulte-Hermann et al., 1972). These and more recent studies, identifying specific factors which lead to protein synthesis entirely independent of drug metabolising enzymes or endoplasmic reticulum, (Schulte-Hermann et al., 1972; Koransky et al., 1966; Schlicht et al., 1968; Ragnotti and Aletti, 1974; 1978; Schulte-Hermann et al., 1982; Hardwick, 1983; Osorio-Almeida et al., 1986; Lechner et al., 1987) suggest that while liver enlargement is the result of an increase in total protein accompanied by an increase in total RNA, DNA and nuclear count (Conney, 1967; Argyris, 1969; 1974) the mechanisms responsible for cell enlargement are independent of those resulting in cell multiplication.

Following administration of a xenobiotic the proportion by which hyperplasia and/or hypertrophy contributes to the adaptive response depends on the type and dose of xeniobiotic and the age of the animal. For example, while liver growth induced by repeated injections of 50 mg/kg body weight of PB in immature rats is largely due to an increase in cell number, at higher doses (100 mg/kg body weight) cell enlargement is also a significant contribution. In adult rats however, liver weight is increased to a lesser degree at this higher dose and is a combination of cell enlargement and increase in DNA due to an increase in polyploidy (Paulini et al., 1970; Staubli et al., 1969; Schulte-Hermann et al., 1968; Argyris, 1974; Argyris and Magnus, 1968; Augenlicht and Argyris, 1975; Koransky et al., 1966; Schlicht et al., 1968). Furthermore 20 mg/kg body weight of 3-MC in the immature male rat will stimulate liver growth by cell hypertrophy alone (Augenlicht and Argyris, 1975) whereas treatment with CPA or pregnenolone-16 -carbonitrile (PCN) to young female rats will induce liver enlargement, a result of DNA synthesis and cell multiplication (Schulte-Hermann et al., 1980).

In adult rats while cell multiplication is less pronounced after administration of drugs such as HCH (Schulte-Hermann et al., 1968; Argyris, 1974), on cessation of the drug hepatocyte enlargement regresses (Schulte-Hermann et al., 1968; Schlicht et al., 1968; Argyris, 1969; 1971) while the hyperplastic component within the enlarged liver remains (Schulte-Hermann et al., 1971). These workers also showed that inhibitors of DNA synthesis such as CFT 1201, SKF 525A and actinomycin D, acted early in the cell cycle before the onset of DNA synthesis and when given in conjunction with HCH, cellular enlargement

or increased microsomal activity were unaffected but if given after this early sensitive period did not prevent DNA synthesis and cell proliferation. These and other factors, including the influence of food on DNA synthesis rather than cellular enlargement following HCH treatment, suggest that the mechanisms responsible for cell proliferation are independent of those responsible for cell enlargement (Pardee, 1974; Schulte-Hermann et al., 1976a; Schulte-Hermann, 1977; Schulte-Hermann and Dorffler, 1979; Kallenbach et al., 1983).

However while enzyme synthesis, cell enlargement and proliferation in the liver can be induced by drugs independently of each other (Golberg, 1966; Koransky et al., 1969; Schulte-Hermann, 1977; 1979; Schulte-Hermann et al., 1980), the mechanism by which these xenobiotics induce liver growth is still unknown. It is also unknown whether the primary target for these xenobiotics is intra- or extrahepatic (Schulte-Hermann, 1974). The hypophysis appeared a candidate since hypophysectomy leads to atrophy of the liver (Weinbren, 1959; Szabo et al., 1973), pituitary hormones induce liver growth in hypophysectomised or intact rats (Di Stefano et al., 1955; Echave Llanos et al., 1971; Schulte-Hermann, 1974) and following hypophysectomy there is a reduction in cytoplasmic volume and RNA content in the rat liver, while the DNA content remains unchanged, (Di Stefano et al., 1955; Schulte-Hermann et al., 1977) thus leading to an increase in hepatic DNA concentration and a decrease in the RNA:DNA ratio (Schulte-Hermann et al., 1977).

Following partial hepatectomy liver weight is restored to control values in intact rats but only restored to 75%-85% of pre-operation levels following hypophysectomy (Franseen et al., 1938; Doljanski and Novogrotzky, 1959; Schulte-Hermann et al., 1977). This is a similar

effect to starvation in intact rats and is the result of anorexia as hypophyseal hormones control food intake and body growth. Their absence causes cessation of body growth and diminished food intake which alters the functional load imposed on the liver (Franseen et al., 1938; Schulte-Hermann et al., 1977). DNA synthesis is not prevented in the absence of the hypophysis following partial hepatectomy (Doljanski and Novogrotzky, 1959; Schulte-Hermann et al., 1977) but stimulation of liver growth by HCH or PB in hypophysectomised rats results in an increase in liver mass by cell hypertrophy alone as an increase in DNA failed to occur, as shown using ^{3}H-thymidine uptake into hepatic DNA as an index of DNA synthesis (Schulte-Hermann et al., 1977). Hypophysectomy alone results in a decrease in liver size and RNA content while DNA content remains unchanged. This altered DNA:RNA ratio they proposed led to a "relative DNA surplus", which on elimination by partial hepatectomy restored the ability by these xenobiotics to induce DNA synthesis (Schulte-Hermann et al., 1977). Such responses led these authors to suggest that stimulation of DNA synthesis by these drugs is controlled by an autoregulatory feedback system which monitors an excess of DNA and suppresses cell replication if DNA content exceeds the normal level. They explained that this was why cell enlargement alone occurs in hypophysectomised animals (Schulte-Hermann et al., 1977; Schulte-Hermann and Schmitz, 1980).

These studies clearly demonstrated that stimulation of cell enlargement and cell multiplication can be separated (Schulte-Hermann et al., 1977; Schulte-Hermann and Schmitz, 1980) and support the concept that these anabolic processes involve two separate biochemical compartments in the cell.

The postulate that DNA 'surplus' in hypophysectomised rats prevents stimulation of DNA by chemicals implies that the molecule whose presence or absence constitutes the signal for DNA synthesis resides in nuclear DNA or some closely associated entity. The challenging of this hypothesis forms the basis of this project.

However, in order to understand more of the possible mechanisms involved in the response by the liver to xenobiotics, which result in DNA synthesis, it is necessary to consider briefly other experimental situations in which a similar response is induced. These include studies into the mechanisms which regulate the phenomena involved in compensatory hyperplasia and have involved the identification of both endogenous and extrahepatic factors (such as hormones and growth factors), the possibility of endogenous liver growth inhibitors, i.e. chalones, consideration of various biochemical changes which accompany cell proliferation following partial hepatectomy and evaluation of modifying factors which may influence the proliferative response.

These investigations have in general involved

a) observations made on in vivo changes in animals undergoing compensatory hyperplasia,
b) observations on attempts to modify the response,
c) identification of possible growth factors by in vitro methods, and
d) attempts to correlate both in vitro and in vivo studies (Alison, 1986; Weinbren and Hadjis, 1988).

After partial hepatectomy, the parenchymal cells in the rat liver remnant undergo metabolic changes which culminate in the initiation of DNA synthesis and mitosis approximately 16-18 and 24 hours later,

respectively. This is followed by an increase in mean nuclear ploidy with a concomitant fall in the proportion of binucleated cells (Bucher, 1963; Grisham, 1962; Fabrikant, 1967; 1968; Nadal and Zajdela, 1966; Gerhard, 1975). Hepatocyte cell size also changes by 12 hours following partial hepatectomy, thus leading to increased size of the lobules and architecturally a crowded appearance, but returns to normal after a few days (Harkness, 1957). Partial hepatectomy in effect triggers hepatocytes, which are in the temporary resting phase G_0 of the cell cycle, back into the proliferative cycle which results in the ultimate restoration of the liver mass by 10-15 days post-hepatectomy (Harkness, 1957; Bucher, 1963).

Following partial hepatectomy, arterial levels of hormones such as insulin and thyroxine fall and glucagon levels rise, the changes correlating with the amount of liver removed (Leffert et al., 1975; Bucher et al., 1978; 1978a). Serum levels of adrenalin increase following partial hepatectomy (Marotta et al., 1978) and hepatic regeneration is accelerated by stress (Sakamoto et al., 1979).

Intrahepatic hepatotrophic substances have been identified in rats following partial hepatectomy in cross-circulation studies and extracts from weanling rats act specifically on DNA synthesis during rapid or regenerative growth both in vitro and in vivo (Cristenson and Jacobsen, 1949; Sigel et al., 1963; Moolten and Bucher, 1967; Fisher et al., 1971; Short et al., 1972; La Breque and Pesch, 1975; La Breque, 1979). It has been found that similar extracts isolated from regenerating dog and rat liver and at lower concentrations from non-regenerating rat liver, augment rather than initiate liver regeneration in collaboration with extrahepatic factors (Starzl et al., 1980; Terblanche et al., 1980;

Michalopoulos et al., 1982; 1984; Schwarz et al., 1985; Diaz-Gil et al., 1986; 1986a). This suggested that multiple factors may control liver regeneration.

Growth inhibitors known as "chalones" (Saetren, 1956; Bullough, 1962; Glinos, 1967; Verly et al., 1971; Verly, 1973; Sekas and Cook, 1976) including VLDL (very low density lipoprotein) (Leffert and Weinstein, 1976) have been identified in adult rat serum and liver supernatants. They have been shown to reduce DNA synthesis in young proliferating or regenerating rat liver and to inhibit the initiation of DNA synthesis in fetal rat hepatocyte cultures. These inhibitors are absent in baby rats, reduced in proliferating adult hepatocytes and they are reduced proportionally to the amount of tissue removed following partial hepatectomy (Maugh, 1972; Nadal, 1973; 1975; Pietu et al., 1978; Leffert et al., 1978). DNA synthesis is also reduced following partial hepatectomy in hyperlipoproteinemic rats compared with normal rats (Leffert and Weinstein, 1976). An inhibitory activity was considered to be removed from rat serum following two-third partial hepatectomy thus allowing unidentified hepatotrophic substances to act on primary hepatocyte cultures (Michalopoulos et al., 1982). Alteration of VLDL levels by an isotonic saline solution of triiodothyronine (T_3), amino acids, glucagon and heparin (TAGH) would, however, suggest that VLDL modifies rather than totally inhibits DNA synthesis (Leffert, 1974a; b) and more recently the concept of "chalones" being involved in the control of DNA synthesis has been questioned (Alison, 1986).

Investigations into the kinetics of experimentally induced proliferation have identified biochemical changes which are associated with DNA synthesis and cell proliferation. Evidence has accumulated

supporting a role of non-histone chromosomal proteins in the control of cell proliferation in mammalian cells (Baserga, 1974). For example their synthesis is increased in quiescent cells following stimuli to proliferate, such as following partial hepatectomy (Stein and Baserga, 1970; Smith et al., 1970; Kostraba and Wang, 1973). In addition non-histone proteins migrate to the nucleus (Baserga, 1976) alter chromatin activity (Marks and Rifkind, 1972; Baserga, 1974), control transcription during the cell cycle (Stein and Baserga, 1970; Stein et al., 1974; 1974a; c; Stein, 1975) and initiate gene activation that triggers DNA synthesis (Baserga, 1974). Levels of these proteins can be altered by starvation, thyroid hormones (De Groot et al., 1977; Wimpfheimer et al., 1979; Barsano et al., 1980) and EGF (Kaneko, 1983). However, as yet the exact role of non-histone nuclear proteins in the control of DNA synthesis and cell proliferation remains unclear.

An increase in ornithine decarboxylase (ODC), the rate-limiting enzyme in the polyamine pathway, occurs during rapid growth and cell proliferation in mammalian systems and following partial hepatectomy (Janne and Raina, 1968; Inoue et al., 1974; Takigawa et al., 1977; Russell and Durie, 1978; Russell and Snyder, 1968; Janne et al., 1977; Williams-Ashman and Canellakis, 1979; Nagarajan and Gospalakrishna, 1982). Although this association is not understood, elevated ODC levels are thought to be related to subsequent DNA synthesis and cell proliferation in various tissues in vivo (Kato et al., 1978; Poso and Janne, 1976; Takigawa et al., 1977; Kato et al., 1978; Danzin et al., 1979; Mann and Wright, 1984; Kato et al., 1978; Williams-Ashman and Canellakis, 1979), especially as such increases may be blocked by inhibitors of ODC such as diaminopropane, DL-hydrazino-aminovaleric acid

(DL-AVA) and α-difluoromethylornithine (DFMO), thereby preventing DNA synthesis (Poso and Janne, 1976; Kato et al., 1978; Poso and Pegg, 1982). However whilst the onset of DNA synthesis and ODC activity appear independent (McGowan and Fausto, 1978), studies suggest that polyamines may be essential for the maintenance of cell proliferation induced by other factors (Holtta et al., 1979; Goyns, 1982).

Alpha-fetoproteins (AFP) are found in the serum of growing neonatal and pregnant rats and following partial hepatectomy in rats and dogs (Gitlin and Boesman, 1967; Perova et al., 1971; Leffert and Sell, 1974; Madsen, 1980) with increases paralleling DNA synthesis (Madsen, 1980). However fewer hepatocytes are engaged in AFP synthesis compared with DNA synthesis following partial hepatectomy, indicating only an association between the two (Leffert et al., 1978; Tuczek et al., 1981).

Recently studies have demonstrated a sequential and transient expression of certain proto-oncogenes including c-myc and c-fos after partial hepatectomy. Stimulation of c-fos mRNA levels in quiescent adult rat hepatocytes by EGF, followed by c-ras mRNA between 6 and 24 hours later (paralleling the delayed hepatic response that occurs _in vivo_ following partial hepatectomy) suggests that this sequential proto-oncogene expression during liver regeneration is caused by hepatocellular interactions with specific mitogens (Fausto and Shank, 1983; Huber et al., 1986; Fausto, 1986; Kruijer et al., 1986). These authors suggest, in the light of the relationship which exists between some proto-oncogenes and growth factors, that once hepatocytes become competent to progress through the cell cycle, further progression may occur by the products of the myc and fos genes inducing synthesis of hepatocyte growth factors. These would then control the continued

progression through the cell cycle. Alternatively these products may stimulate receptors in the liver which permit the cell to respond to factors which circulate in the blood (Fausto, 1986).

Many experiments have been carried out which have attempted to modify the proliferative response in order to identify possible initiating factor(s) of compensatory hyperplasia. These include studies involving hormones. Following perfusion experiments in the dog in which the right and left liver received portal blood draining from various splanchnic organs (Starzl et al., 1973; 1975) or porta-caval shunts (Starzl et al., 1976), with graded non-hepatic splanchnic or total splanchnic evisceration (Starzl et al., 1978), insulin was found largely to protect against atrophy and have a modifying effect on DNA synthesis, although glucagon had a less consistent effect (Starzl et al., 1973; 1975; 1978; 1978a). Some studies in the rat have demonstrated that glucagon and insulin act in a synergistic manner during hepatic regeneration (Bucher and Swaffield, 1973; Bucher et al., 1978a; Takatsuki et al., 1981). However, these hormones fail to excite DNA synthesis in non-hepatectomised eviscerated or normal livers following portacaval shunt or enhance regeneration following partial hepatectomy in rats (Bucher and Swaffield, 1973; Bucher et al., 1978; 1978a; Junge and Creutzfeldt, 1978; Freise et al., 1982). These and cross-circulation studies in which DNA synthesis was stimulated in rats in cross-circulation with partially hepatectomised (Fisher et al., 1971; Short et al., 1972; Moolten and Bucher, 1967) or total portally-eviscerated rats (Greisler et al., 1979), indicated that DNA synthesis was modified rather than initiated by these factors (Starzl et al., 1978; 1978a; Bucher et al., 1978; 1978a; Malamud and Perrin, 1974).

Similarly, whilst having distinct biological activity and being capable of modulating hepatic proliferation, neither epidermal growth factor (EGF) (Bucher et al., 1978; 1978a), thyroxine (Moolten and Bucher, 1967; Short et al., 1972); Leffert and Alexander, 1976; Leffert et al., 1979), heparin (Short et al., 1972) nor adrenalin (Bullough and Lawrence, 1964; Marotta et al., 1978; Sakamoto et al., 1979; Kato and Shimazu, 1983) appear to be initiators of compensatory hyperplasia. The difference in the capacity of these growth stimulators, e.g. EGF and thyroxine to act, appears to depend on which other stimulators are present (Short et al., 1972; Bucher et al., 1978; 1978a) and also suggests that these hormones function through different mechanisms.

Other studies which have modified the environment of the hepatocyte during liver regeneration indicate that the response may be altered by environmental circumstances. These have included interference with nervous activity (Kato and Shimazu, 1983; Sobczak and Duguet, 1986), the administration of stimulatory substances as already discussed (Terblanche et al., 1980; Michalopoulos et al., 1982; 1984) and alteration of blood volume (Weinbren, 1955; Blumgart, 1978; Rozga et al., 1985). This is emphasised by observations that while acetylcholine is not necessary but modifies DNA synthesis, noradrenalin inhibits DNA synthesis <u>in vivo</u> but stimulates it <u>in vitro</u> (Cruise et al., 1985; Sobczak and Duguet, 1986). Following haemodynamic experiments neither an intact portal blood supply nor hepatotrophic factors were shown to be required to induce DNA synthesis in the regenerating liver (Weinbren, 1955; Weinbren et al., 1972; Blumgart, 1978; Short et al., 1980). Similarly they are not required during transhepatic perfusion or hepatic arterial recirculation following portal vein ligation (Rozga et al.,

1985). This last study emphasised however, that while compensatory hyperplasia was independent of the quality of the blood supply, the quantity of blood may modify the degree of compensatory hyperplasia in the unligated lobes (Castaing et al., 1983; Takeshige et al., 1982; Rozga et al., 1985).

Several studies demonstrate that DNA synthesis and cell multiplication can be induced and modulated in the intact and regenerating rat liver by altering the concentration of dietary proteins or more specifically essential amino acid levels (Leduc, 1949; Short et al., 1973; 1974; Gershbein, 1980; Kallenbach, 1983; McGowan and Fausto, 1978). Some of these are essential, combined with the presence of T_3 for DNA synthesis to occur in protein-deprived animals (Short et al., 1973; 1974). In addition it appears that there are two populations of hepatocytes: one which proceeds to DNA synthesis without food intake and a second requiring dietary protein in the early pre-replicative (G_0) phase and late pre-replicative phase 3-8 hours before DNA synthesis, following partial hepatectomy (Kallenbach et al., 1983).

It is recognised that cells in culture provide an experimental system for studying the mechanism by which factors may control DNA synthesis and cell proliferation without many of the complexities and other factors of whole animal experimentation (Rozengurt and Collins, 1983). Many postulates have been derived from these in vitro studies, some of which suggest that liver regeneration in adult rats is hormonally controlled (Cohen, 1965; Glinos, 1967; Frank et al., 1975; Leffert, 1974a; b; Koch and Leffert, 1974; 1976; 1979; Bucher and Swaffield, 1973; Bucher et al., 1969; 1978; Leffert et al., 1975; 1976; 1979; Leffert and Weinstein, 1976; Gospodarowicz, 1983). Hormones

including growth hormone, thyroid hormone, glucagon and insulin are all known to stimulate adult or neonatal rat hepatocyte DNA synthesis or CH_3-[^{3}H] thymidine ([^{3}H]dT) incorporation into DNA (Leffert, 1974; Leffert et al., 1979; Junge and Creutzfeldt, 1978). Thyroid and growth hormones enhance the effects of insulin on DNA synthesis while glucagon inhibits the insulin-stimulated response and one hormone may obviate the necessity for another (Leffert, 1974b; Leffert et al., 1982). However these multiple factors mediate rather than initiate DNA synthesis and led Leffert to propose that these hormones may act via the cell membrane (Leffert, 1974).

Several in vitro studies have demonstrated that hormones, nutrients and serum proteins act on the cell membrane, alter permeability, Ca^{++} concentration and reduce intracellular cyclic AMP (cAMP) levels or may modulate other factors such as highly phosphorylated nucleotide regulatory compounds ("HPNRC") which act at the cell membrane and lead to DNA synthesis (Hechter, 1957; Holley and Kienan, 1974; Herschko et al., 1971; Paul and Walter, 1975; Frank et al., 1975; Koch et al., 1976; Koch and Leffert, 1976; 1979).

EGF is a strong mitogen in culture (Richman et al., 1976; Leffert and Koch, 1977; 1978; Koch and Leffert, 1979) and binds in rat liver to specific EGF receptors (O'Keefe et al., 1974; Jacobs et al., 1980; Johnson et al., 1981) to form complexes (Fabrikant et al., 1977; Haigler et al., 1979; Gospodarowicz, 1983) which are endocytosed (Haigler et al., 1979; Gordon et al., 1978; Carpenter and Cohen, 1979). Associated intracellular changes include rapid activation of a cyclic-AMP-independent phosphorylating system (Carpenter et al., 1979; Buhrow et al., 1982; Yarden et al., 1982) thought to reside in the receptor itself

(Buhrow et al., 1982) stimulation of Na^+, K^+, ATPase and cAMP levels, activation of ODC (Schreiber et al., 1981; Yarden et al., 1982) and morphological changes in the cell related to re-organisation of the actin microfilament cytoskeleton (Schlessinger and Geiger, 1981; Yarden et al., 1982). However the exact relationship between these membrane-associated changes and mitogenic activity by EGF remains unclear. Various ligands are known to stimulate this activity but if specific receptor sites are blocked by related molecules occupying the receptor sites, this "down-regulation" will affect cellular activity (Earp and O'Keefe, 1981).

Other factors which stimulate hepatocyte DNA synthesis in culture and share a number of common properties with epidermal growth factor (EGF) include insulin-like growth factors IGF I and IGF II and somatomedin C (Cohen, 1959; 1962; Rinderknecht and Humbel, 1978; 1978a; Zapf et al., 1978; Svoboda et al., 1980; Gospodarowicz, 1983). They are similar in structure to insulin and combined, obviate the requirement for other factors (Leffert and Koch, 1980; Leffert et al., 1982). Alone however, they are weak mitogens. As with the previously described hormones, these are supporters of cell proliferation rather than initiators, because without them cells may not progress through their cell cycle (Stiles et al., 1979). Another group of proliferogenic factors includes rat platelet-derived growth factor (PDGF) which increases hepatocyte DNA synthesis in vitro (Strain et al., 1982), enhances the action of somatomedin C (Stiles et al., 1979) and is thought to render cells competent to the action of later proliferogenic factors (Kaplan et al., 1979). All these studies confirm previous in vivo observations that the degree to which individual factors are

hepatotrophic depends on their concentration and combination with others (Leffert et al., 1982).

Several _in vitro_ studies show "paradoxes" regarding the control of hepatic proliferation observed _in vivo_. For example, insulin promotes DNA synthesis in rat hepatocytes _in vitro_ within chemically defined medium (Leffert, 1974; Leffert and Koch, 1977), while after partial hepatectomy rat hepatic insulin levels fall (Leffert et al., 1975). Glucagon antagonises _in vitro_ DNA synthesis promoted by insulin under chemically defined medium (Leffert, 1974), whereas _in vivo_ arterial glucagon levels rise in partially hepatectomised rats.

However, alterations in liver tissue undergoing compensatory hyperplasia are similar in several respects to those noted in culture systems. These include increases in the ODC putrescine system (Russell and Snyder, 1968), non-histone nuclear proteins (Kostraba and Wang, 1973; Stein, 1975; Kaneko, 1983) and AFP levels (Leffert and Sell, 1974; Madsen, 1980), proliferogenic activity in recipients of liver extracts whether _in vitro_ or _in vivo_ (La Breque and Pesch, 1975; La Breque, 1979) and decreases in VLDL (Leffert and Weinstein, 1976; Leffert et al., 1978). Studies which demonstrated that hepatic plasma membranes become partially resistant to the binding of glucagon during regeneration, but insulin binding remains altered could account for the altered insulin, thyroxine and glucagon levels following partial hepatectomy (Leffert et al., 1975).

Other _in vitro_ studies can explain certain changes associated with _in vivo_ cell proliferation. For example, the prereplicative interval both _in vitro_ and following partial hepatectomy, characterised by

preferential response to EGF or similar peptides, coincides with an increased Na^+ influx across the cell membrane required for amino acid transport (Koch and Leffert, 1979; Leffert et al., 1982; Le Cam et al., 1979; Fehlman et al., 1981; 1981a; Dolais-Kitabgi, 1981). This leads to RNA and protein synthesis which is potentiated in late G_I phase by CA^{++} dependent cyclic AMP (Ca/cAMP) and together with Ca/cAMP dependent DNA substrates, culminates in DNA synthesis. This latter phase is dependent on insulin and glucagon, explaining the delayed prereplicative requirement for these peptides both in vitro and following partial hepatectomy (Bucher and Swaffield, 1975; Leffert et al., 1982). However all these changes appear to reflect the proliferating state of the cells involved either in vivo or in a monolayer system and it is not likely that these changes represent the factor(s) which initiate(s) DNA synthesis and cell multiplication. This is emphasised by, for example, DNA synthesis not being prevented following partial hepatectomy in rats with congenitally high levels of VLDL (Leffert and Weinstein, 1976) and that raised levels of AFP both in vivo and in vitro are recognised as an early post-mitotic marker (Sell et al., 1974; Madsen et al., 1980).

Studies do however show that when comparing changes which occur during compensatory hyperplasia (seen following partial hepatectomy) or during adaptive liver growth (induced by, for example, HCH or PB), various responses are similar and culminate in liver enlargement either in the remaining remnant or throughout the intact liver. These include the timing and magnitude of DNA synthesis (Bucher, 1963; Weinbren and Woodward, 1964; Schulte-Hermann, 1974) the spatial distribution of the hepatocytes exhibiting mitoses (Harkness, 1957; Schlicht et al., 1967; Schulte-Hermann, 1974), water and lipid content (Simek et al., 1968;

Schlicht et al., 1968; Schulte-Hermann, 1974), increase in cell size (Bucher, 1963; Schulte-Hermann, 1974) and many biochemical changes (Schulte-Hermann, 1974; Francavilla et al., 1978).

The frequent occurrence of cell hypertrophy and hyperplasia (Weinbren, 1975) observed in experimentally induced liver enlargement has also been demonstrated in other organs including rat salivary glands and in the human heart (Sandritter and Scomazzoni, 1964; Barka, 1965; 1965a; Schneyer et al., 1967). Following studies where DNA synthesis was blocked, subsequent to an injection of isoproterenol, by inhibiting protein synthesis with puromycin (Baserga and Sasaki, 1969), not only did this further emphasise the intimate association between DNA and protein synthesis but it led Johnson to suggest that in the regenerating liver, cell mass and the control of DNA synthesis were related and that hypertrophy appeared to be a prerequisite of cell division (Johnson, 1969).

However, separation of DNA and protein synthesis has clearly been achieved by several different techniques. For example, following partial hepatectomy DNA synthesis was blocked by azathioprine but regeneration occurred by hypertrophy as assessed by RNA and protein content and hepatocyte volume (Malamud et al., 1972). Following induction of hepatocyte atrophy by portal vein ligation or starvation, partial hepatectomy stimulated DNA synthesis at a time when cytoplasmic mass was less than in normal cells (Weinbren et al., 1972; Doljanski et al., 1966), discounting the cytoplasmic mass hypothesis (Johnson, 1969). The independence of these two processes was further suggested following portacaval anastomosis in the rat liver. This procedure induced liver atrophy and on reconstitution of the portal vein, the portal blood flow

induced hepatocyte hypertrophy with minimal DNA synthesis in comparison to animals subjected to partial hepatectomy following portal caval anastomosis (Weinbren et al., 1975). While this study did not entirely support the suggestion that cellular hypertrophy and hyperplasia are different processes, their independence was demonstrated in a later study, following long term portacaval anastomosis. Here hyperplastic nodules developed in the atrophied liver (Weinbren and Washington, 1976). However both these studies show that the liver is capable of synthesising DNA and undergoing hyperplasia in the total absence of portal blood flow, in a situation where parenchymal protein (but not DNA) is substantially reduced (Weinbren et al., 1975; Weinbren and Washington, 1976).

It appears therefore that portal blood flow, with its circulating hormones and nutrients, is related to hepatocyte size and factors involved in regulating DNA synthesis include loss of hepatocyte cytoplasm (Weinbren, 1955; Weinbren and Dowling, 1972; Weinbren et al., 1975; Weinbren, 1978; 1979; Castaing et al., 1983; Rozga et al., 1986). It is clear that reduction of parenchyma including DNA, (as in hepatectomy), represents a signal similar to that generated by reduction of parenchyma without loss of DNA, as occurs following deprivation of portal flow. This is also true in chronic circumstances where long term parenchymal atrophy regularly stimulates compensatory hyperplasia. The common factor to all of these signals which triggers DNA synthesis is the reduction of the amount of cytoplasm (Weinbren and Washington, 1976; Weinbren, 1979; Weinbren, 1982).

To return to the concept proposed by Schulte-Hermann et al. (1977) in which they interpreted the response by hypophysectomised rats to

phenobarbitone and HCH following partial hepatectomy, to be the result of removal of a previous relative DNA 'surplus' preventing DNA synthesis by the xenobiotics, the work reported here investigates whether the capacity of the chemical inducers to stimulate DNA synthesis can be restored in hypophyectomised rats without reducing the total amount of hepatic DNA. This latter situation occurs following portal vein ligation with hepatic lobar atrophy in the intact rat associated with hyperplasia of the surgically unmanipulated liver, a response which in timing and extent is entirely comparable with the response associated with partial resection. The experiments therefore test whether reduction of cytoplasmic protein while retaining a relative excess of hepatic DNA, can restore the initiation of DNA synthesis by chemical inducers in hypophysectomised rats, indicating that the origin of the proliferative stimulus might be sought in cytoplasmic or plasmalemmal components.

The questions to be answered in this study are therefore:

(1) Do the measurements used in this study reflect DNA synthesis? This must be established in order to be able to detect the following:

(2) Whether DNA synthesis is induced when PB is given to

  (i) intact rats

  (ii) hypophysectomised rats

  (iii) hypophysectomised rats following one-third or two-third partial hepatectomy

  (iv) hypophysectomised rats after one-third or two-third portal vein ligation.

It is well known from other studies that in rats with or without pituitaries, partial hepatectomy results in DNA synthesis accompanied by a shift in nuclear ploidy classes. There is a concomitant decrease in the number of binucleate cells as well as an increase in cell numbers (Bucher, 1963; Geschwind et al., 1958; Doljanski and Novogrotzky, 1959). These responses are accompanied by hypertrophy which can be detected by a transient increase in cell size, returning to normal size within a few days (Bucher, 1963). Following partial hepatectomy the increased number of mitoses fall away over the next few days but polyploidy persists indefinitely (Sulkin, 1943; Bucher, 1963). Liver regeneration in hypophysectomised animals occurs at a slower rate, compatible with the overall depressed metabolic state of the animal (Harkness, 1957; Weinbren, 1959; Doljanski and Novogrotzky, 1959).

Hypophysectomy alone results in reduction of body, adrenal and testicular weight and in the liver reduced RNA levels and liver atrophy. Overall DNA content of the organ remains unchanged, resulting in the RNA:DNA ratio being decreased by approximately 25% and an increase in hepatic DNA concentration (Di Stefano et al., 1955; Weinbren, 1959; Bucher, 1963; Doljanski and Novogrotzky, 1959; Christensson et al., 1975; Schulte-Hermann et al., 1977). Progression of polyploidy is arrested by hypophysectomy and the DNA content of the nuclei remain unaltered (Alfert and Geschwind, 1958; Di Stefano et al., 1955; Di Stefano and Diermeir, 1956).

Similarly it is known that drugs such as HCH or PB cause liver enlargement in rats with or without pituitaries (Schulte-Hermann, 1974; Schulte-Hermann et al., 1977), PB inducing both cell hypertrophy and hyperplasia in the intact rat but hypertrophy alone in hypophysectomised

animals. Hyperplasia induced in the intact rat is the result of both an increase in cell numbers and shift in ploidy (Schulte-Hermann et al., 1968). While DNA synthesis and mitotic activity remain elevated for only a few days even with prolonged administration of some drugs (Schlicht et al., 1968), mitotic activity remains elevated following 15 days PB treatment (Schulte-Hermann et al., 1968; Gunther et al., 1967; Koransky et al., 1966; 1969; Schulte-Hermann, 1974). In Schulte-Hermann's study both the increased incidence of mitoses and polyploidy persisted for at least seven weeks following cessation of drug treatment (Schulte-Hermann et al., 1971). Herdson et al. (1964) reported that after 21 days PB treatment, mitoses were observed on an average in 2-3 hepatocytes in each lobule, in contrast to one mitosis every 2-3 lobules in normal resting liver. Others have also reported that mitoses are extremely rare in adult rat liver. For example, Brues and Marble (1937) found only one mitosis per 10,000-20,000 liver cells. The induction of DNA synthesis by PB however in hypophysectomised rats is blocked (Schulte-Hermann et al., 1977).

As there is a consistent shift in ploidy and cell proliferation following procedures such as partial hepatectomy or the administration of certain drugs it seemed reasonable to adopt methods in this study which would detect these changes, thereby reflecting DNA synthesis. It is clear from these studies that by using the regimen described by Schulte-Hermann et al. (1977), in which hypophysectomy was performed on day 0, partial hepatectomy 6 days later and animals sacrificed after another 21 days, changes in ploidy will still be detected at this late time. However mitotic activity, known to increase within 24 hours after partial hepatectomy, will have declined and therefore not be detected

21 days post-hepatectomy (Schulte-Hermann et al., 1968; Gunther et al., 1968; Schulte-Hermann et al., 1977). In some animal groups in these studies described by Schulte-Hermann and his colleagues (1977) PB was given for 8 days prior to death at 28 days. From the knowledge of their previous studies (Schulte-Hermann et al., 1971) it is reasonable to suggest that ploidy changes and increased mitoses might be detected 24 hours after 8 days of PB treatment in intact animals, but not in hypophysectomised animals.

Thus in the present study responses to partial hepatectomy and PB treatment in intact rats were checked, in order to demonstrate DNA synthesis, by investigating changes in nuclear ploidy and mitotic index. These parameters were then employed to compare DNA synthesis in hypophysectomised animals following partial hepatectomy and PB treatment with similarly treated intact animals in order to confirm responses described by Schulte-Hermann et al. (1977). Then these responses were compared with those following portal vein ligation in intact and hypophysectomised rats.

Following procedures such as partial hepatectomy, the proliferative response on the whole appears to be more active in the parenchymal than non-parenchymal cells (Bucher, 1963). Thus when choosing methods to detect shifts in ploidy and increased mitoses, it appeared reasonable to adopt methods in this study which would allow investigation of the parenchymal cells.

1.2 Methods used to detect polyploidy and cell proliferation

Some methods commonly used to detect DNA synthesis in light of these preceding comments would have certain disadvantages if used in

this study. For example, when using homogenates of liver for biochemical analysis of DNA and protein synthesis it is not possible to distinguish the parenchymal cell population from the other cellular types. The shift in ploidy which occurs following procedures such as partial hepatectomy cannot specifically be identified using biochemical assays, although an overall increase in DNA synthesis will be identified. Biochemical analysis does tend to eliminate any subjective disadvantage which may occur with other methods.

Similarly unless specific elution methods are used to obtain pure fractions of hepatocytes (Wanson et al., 1980) the determination of nuclear volume, analysis of nuclear ploidy and determination of DNA content using methods such as electronic particle counters and pulse cytophotometry or DNA assays (Romagna and Zbinden, 1981; Schulte-Hermann et al., 1976) are again obtained from mixed populations of cells.

Investigation using autoradiography can however define the cell populations in which DNA synthesis occurs. Because of its specificity for DNA, the incorporation of tritiated thymidine ([^{3}H]TdR) into DNA is the most widely used precursor for measurements of DNA synthesis and cell proliferation. However certain pitfalls may exist using this precursor. For example, non-specific labelling may occur in the presence of impure [^{3}H] thymidine (Wand et al., 1967; Diab and Roth, 1970), single intraperitoneal injections (1 uCi/g mouse; 5 Ci/mmol) may induce mitotic delay in labelled cells and mitotic activity in unlabelled cells arising from synchronization (Olsson, 1976) leading to inaccurate results, and doses of 1-2 uCi/g may be cytotoxic to certain cells (Cheng and Leblond, 1974). [^{3}H] TdR added to intact animal cells may only be minimally incorporated into DNA due to several enzymes of

TdR metabolism which degrade [^{3}H] Tdr, thus depleting the quantity available for DNA labelling(Maurer, 1981). In addition uptake of [^{3}H] TdR must not necessarily be correlated with cell division as [^{3}H] Tdr can be incorporated into non-proliferating cells which are repairing DNA (unscheduled DNA synthesis) (Harbers, 1975; Maurer, 1981). It is therefore recommended for in vivo studies that the suitability of using thymidine incorporation should be checked against other methods of measuring changes in DNA in order to determine whether any of these artefacts are causing inaccurate results (Maurer, 1981).

While morphometric studies may have a disadvantage as there may be a subjective element involved in the investigations, random and blind examination of histological slides and repeated investigations can overcome inadvertent bias. The parenchymal cell population can be distinguished from other cell populations in the liver and this allows data to be generated which is specific to parenchymal cells. Mitotic index has been a widely used histological criterion of adaptive cell growth resulting in cell proliferation (Bucher, 1963). However the mitotic peak may be short-lived and may be missed, or may differ depending on which part of the hepatic lobule is examined (Bucher, 1963) but for reasons already discussed this is a reasonable method to use to measure cell proliferation following PB treatment in this study.

The use of nuclear area (or volume) as an indicator of DNA content in hepatocytes is based on various studies which have shown that nuclear enlargement is proportional to DNA content and may reflect previous DNA synthesis. The nuclear enlargement associated with an increase in DNA content occurs when cells fail to undergo mitosis and shift to a higher nuclear ploidy class. It has been suggested that this occurs when the

cell cycle is blocked in G_2 and then when stimulated to divide, mitosis is bypassed and the cell enters a new S phase (McLean, 1970; Hendy and Grasso, 1977). Nuclear enlargement due to an increase in ploidy has been reported in the skin (Ingram and Grasso, 1985; 1987), in cell culture (Sutou and Tokuyama, 1974) and in the liver (Jackson, 1974; Ingram, 1979).

In order to confirm that the increase in nuclear area observed in rat hepatocytes treated with xenobiotics is due to an increase in ploidy rather than changes such as nuclear swelling, several authors have combined the determination of nuclear size with measuring DNA content. This has been achieved by combining the use of an electronic particle counter for determination of cell size with biochemical assay, microdensitometry or pulse cytophotometry methods (Schulte-Hermann et al., 1968; 1971; Crissman and Tobey, 1974; Barlogie et al., 1976; Wanson et al., 1980; Romagna and Zbinden, 1981). These authors have found that the two measurements are highly correlated, with PB treatment causing a shift from diploid to tetraploid (4n) nuclei and a small but significant increase in octoploid nuclei (Romagna and Zbinden, 1981). In addition it has been demonstrated following carcinogen-treatment to rats, that enlarged nuclei contain increased DNA proportionate to their increased volume. The enlarged nuclei indicate relative stepwise increases in ploidy corresponding to 2n, 4n, 8n, 16n and 32n (Christie and Le Page, 1961) with a shift towards the higher classes being increased following long-term carcinogen treatment (Hendy and Grasso, 1977). Changes in nuclear area have also been used to indicate alteration in ploidy in epidermis (Ingram and Grasso, 1985; 1987). This method is therefore considered relevant to the present study.

As already indicated, several authors have clearly confirmed using other methods that an increase in nuclear size correlates with an increase in DNA content. It was therefore decided to include a small study in this project to confirm that changes in nuclear size detected by morphometric analysis using the Quantimet 720 image analysing computer correlated with an increase in DNA content as indicated by shifts in ploidy. Using sections taken from the same sample of liver for each animal investigated, paraffin sections were stained with Feulgen and the DNA content of the parenchymal cell population investigated by integrated microdensitometry, using the M85 scanning microdensitometer.

The Quantimet 720 was also used to count the number of nuclei per 10 fields from each section.

1.3 Methods which detect cell enlargement

Liver enlargement is frequently observed in laboratory animals exposed to xenobiotic compounds (Schulte-Hermann, 1974) and investigations of liver enlargement following certain chemicals undertaken over three decades ago demonstrated that liver enlargement involved striking cellular hypertrophy associated with an increase in mitotic activity of hepatocytes (Brazda and Coulson, 1948; Wilson and Leduc, 1950; Rachmilewitz et al., 1950). This was confirmed later by several workers (Kunz et al., 1966; Schlicht et al., 1967; 1968; Schulte-Hermann, 1968; Conney, 1957; Herdson et al., 1964; Staubli et al., 1969).

The onset of liver enlargement following treatment with compounds such as HCH or PB occurs approximately 24 hours after treatment (Kunz et

al., 1966; Schulte-Hermann, 1974). With PB, DNA synthesis commences after 48 hours (Schlicht et al., 1968).

On cessation of treatment the hypertrophic component of the enlarged liver is reversed following PB treatment by about 5 days (Platt and Cockerill, 1969) and by 14 days using HCH (Schulte-Hermann et al., 1971). Thus in this study following 8 days PB treatment cellular enlargement may still be detected 24 hours after the final dose. Mean cell volumes were therefore estimated, by counting the number of cells per 10 fields using the nucleus of the cell as a guide. Binucleate cells, where present were included, in the one cell count.

In this study an increase in cell volume per unit area indicates that cell enlargement is induced by PB treatment compared with control animals. Reduction in cell size compared with intact control animals in hypophysectomised animals would indicate cell atrophy.

The transient cellular enlargement known to occur following partial hepatectomy and portal vein ligation (Duchen, 1961; Bucher, 1963; Weinbren and Tarsh, 1964) should not be detected in this study 21 days post-hepatectomy.

1.4 Relative liver weight

Relative liver weight to body weight is a recognised method of identifying restoration of liver mass following surgical procedures such as partial hepatectomy (Bucher, 1963; Argyris, 1974). This was calculated in this study from the weight of the remaining liver per 100 g body weight following partial hepatectomy or from the unmanipulated lobes following portal vein ligation. This established the degree to which compensatory growth restored the liver mass to that

of control levels. Changes in relative liver weight were also used to detect the adaptive response by the liver to PB treatment. The effects of hypophysectomy can also be detected using this method.

The relative weight of the right posterior lobe was also calculated as this allows a more specific estimation of the adaptive growth response in a constant part of the liver, which is part of the remnant following partial hepatectomy and one of the unmanipulated lobes following portal vein ligation.

Using these methods alone as an index of the adaptive response to these procedures may not be entirely reliable as liver weight may change with deposits of lipids, glycogen, water or other materials not directly related to the proliferative response (Bucher, 1963) but they are useful indicators of the response in conjunction with other methods which reflect changes in DNA synthesis and cell proliferation.

CHAPTER 2

MATERIALS AND METHODS

2.1 Rats

Male white Wistar rats bred in Nottingham Laboratories Field Station (University of Nottingham) weighing between 150-200 grams were kept on a balanced diet of standard rat cubes (Labsure 41B), given water ad libitum and housed in groups of six rats per 'closed bottom' cage.

2.2 Surgical procedures

All operations were conducted under ether anaesthesia and included (a) hypophysectomy, (b) sham hypophysectomy, (c) one-third partial hepatectomy (33%), (d) two-third partial hepatectomy (67%), (e) one-third portal vein ligation, (f) two-third portal vein ligation, (g) control laparotomy. Animals continued to feed ad libitum up to the time of each operation. All animals were weighed prior to each operation.

2.2.1 Hypophysectomy

Hypophysectomy was performed using a modified method based on those of Smith (1930) and Weinbren and Fitschen (1959). A parapharyngeal approach was used and details of the procedure are given in Appendix I, subsection A.I.1.

2.2.2 Sham hypophysectomy

Identical procedures were carried out for sham hypophysectomy excluding the removal of the pituitary after the dura mater was pierced. Post-operative procedures were also identical to those described in Appendix I.

2.2.3 Partial hepatectomy

One-third (33%) and two-thirds (67%) partial hepatectomy were performed under ether anaesthesia using a technique based on the method of Higgins and Anderson (1931), details of which appear in Appendix I, subsection A.I.2.

2.2.4 Portal vein ligation

One-third and two-thirds portal vein ligation were performed under ether anaesthesia employing the method of Weinbren and Tarsh (1964), using when necessary the aid of a Zeiss dissecting microscope. Details of the operations appear in Appendix I, subsection A.I.3.

2.2.5 Control laparotomy

This was undertaken under ether anaesthesia and the animal was firstly weighed and the abdominal wall shaved. A median line incision was made from the xiphoid process extending 4 cm caudally. The liver was exposed and manipulated and ligaments severed to mobilise the liver more freely and then placed back into the peritoneal cavity. The abdominal wall was sutured with cat-gut suture material and the skin closed with surgical clips. Post-operative care was as previously described for other surgical procedures in Appendix I.

2.2.6 Control animals

2.2.6.1 Normal controls

Each animal underwent a sham hypophysectomy and laparotomy. This was in order to eliminate any of the possible effects of other factors, such as multiple anaesthetics, may have on the response of the animal to procedures such as hypophysectomy and partial hepatectomy. These animals may be referred to in the text as "intact" animals.

2.2.6.2 Hypophysectomy controls

Each animal underwent hypophysectomy and laparotomy operations under ether anaesthesia.

2.3 Drugs

Sodium phenobarbitone (PB) (Johnson & Johnson) was dissolved in water and administered orally by gavage using a flexible polystyrene catheter into the stomach. Administration of sodium phenobarbitone is referred to in the text as 'PB treatment'. Control gavage was by administration of an equal volume of normal saline into the stomach and will be referred to in the text as "saline treatment".

2.4 Experimental regimen

The experimental regimen was carried out as described by Schulte-Hermann et al. (1977). Hypophysectomy or sham hypophysectomy was carried out on day zero. Hepatectomy, portal vein ligation or laparotomy were performed six days later and on day 20 the drug regimen was commenced. 40 mg/kg of phenobarbitone was administered to animals for 4 consecutive days and 50 mg/kg for a further 4 days. Animals in groups which did not receive PB were administered an equal volume of normal saline over the same time period. The animals were killed 24 hours later. The dose of phenobarbitone was half that used by Schulte-Hermann et al. (1977) as the animals did not survive on higher doses, details of which are given in Appendix II, subsection A.II.2.

2.5 Macroscopic examinations

The animals were weighed prior to killing with ether anaesthesia. The liver, testes and adrenals were removed. All organs were weighed and in addition the individual lobes of the liver were weighed and weights

recorded. Samples were taken from the right posterior lobe of the liver and fixed in phosphate buffered formal alcohol for histological examination. Completeness of hypophysectomy was checked by dissection of the sella turcica and by body, adrenal and testicular weights. Where any remnant of the pituitary was found on dissection of the sella turcica, the animal was excluded from the study.

2.5.1 Relative liver weight and relative weight of the right posterior lobe

From body, liver and individual liver lobe weights, percentage liver to body weight, percentage right posterior lobe to body weight and percentage lobe to liver weight were calculated. Details for each animal per group and the mean value for each group appear in Tables 3.1, 3.2, 6.1, 6.2, 3.3 and 6.3. For the mean percentage each liver lobe contributed to the liver as a whole following PB treatment, partial hepatectomy or portal vein ligation per group compared with controls see Figures 3.3 and 6.3.

2.6 Histology

The right posterior lobe from the liver of each rat was paraffin wax embedded using routine laboratory procedures (RPMS Histopathology Department). Sections from the resulting blocks were cut 5 u in thickness and stained routinely with haematoxylin and eosin (H&E). Further sections were cut, 5 u in thickness and were stained to demonstrate deoxyribonucleic acid (DNA) using a Feulgen staining technique based on the original (Feulgen and Rossenbeck, 1924).

2.7 Microscopic examinations and determinations

2.7.1 Measurements used to reflect DNA synthesis and cell proliferation

Sections of the right posterior lobe of the liver from all experimental groups stained with H&E, were examined for the determination of hepatocyte mean nuclear area, the number of hepatocyte nuclei per 10 fields and distribution of their ploidy classes using a Quantimet 720 image analyser (Cambridge Instruments Ltd., Royston, Herts.).

H&E sections were also used to estimate mitotic index of the hepatocytes.

The Feulgen stained slides were used to determine the relative amount of hepatocyte DNA using a Vickers M85 scanning integrated microdensitometer (Vickers Instruments, York).

2.7.1.1 Quantimet 720 image analyser investigations

A Quantimet 720 image-analysing computer (Cambridge Instruments Ltd., Royston, Herts.), linked to a Hewlett Packard computer HP9830 was used for nuclear size measurement.

Slides were taken at random and using a x40 objective and a red filter in the light path, (to exclude the image of eosin stained material) areas were selected by tracking across the histological section from left to right. Careful observation separated parenchymal cell population from other cell populations and the former only, were included in the measurements. Repeat analyses on sections again chosen at random were undertaken to reduce any subjective element.

The area of individual clearly defined hepatocyte nuclei was outlined using the light pen (covered) on the television monitor screen. The programme was set to stop once 100 nuclei had been measured. Nuclei with ill-defined margins were avoided as these are frequently those that are cut tangentially and not sectioned through the centre (Ingram and Grasso, 1985). The mean nuclear area was obtained for each rat and the group mean nuclear area determined. Statistical analysis was undertaken. The mean nuclear area for each animal per group was ranked and compared between groups using the Wilcoxon 2 sample test.

The Quantimet was also programmed to give the frequency of nuclei in a sequence of size ranges in 5 um^2 classes from 10-120. By summing the size distributions of the nuclei of animals within a group, a mean group size percentage distribution was obtained and comparisons between the different experimental groups examined (see Figs. 3.1 and 6.1). Using individual animal nuclear size distribution by inspection of the computer print-out, peaks for 2n and 4n nuclei were identified. Where one peak was not obvious its position was estimated by calculating the relative nuclear area. This estimation was effected by converting the area of the identifiable peak into its volume, doubling or halving the volume as required and converting back into the area,

e.g. Peak area → Volume → Area

where the area for 2n nuclei = $\left(\sqrt[3]{\frac{(\sqrt{\text{Area } 4n})^3}{2}}\right)^2$ and the area for 4n nuclei = $\left(\sqrt[3]{2(\sqrt{\text{Area } 2n})^3}\right)^2$.

Peaks for 2n were found consistently within each group at the corresponding nuclear size range of 25-30 um^2 or 30-35 um^2. The 4n

peaks corresponded respectively at either 40-45 um^2 and 45-50 um^2. These peaks varied between different animals within the same group. It was considered whether to include all nuclei in the nuclear size range immediately before the 4n peak, i.e. 35-40 um^2 or 40-45 um^2 in order to include nuclei in the 4n range which may have been excluded due to experimental error. However as the 35-40 um^2 range is known to be closer to the range of 2n ploidy class (Christie and LePage, 1961) it was decided that in order to analyse the nuclei for shifts in ploidy class uniformly throughout the whole experiment a cut off point should be used at the nuclear size range 40-45 um^2 and that any nuclei measured within the range or above were considered in the 4n ploidy class and above. Those nuclei below this point were considered to fall within the 2n ploidy classes. These 2n/4n or above divisions were expressed as a percentage for the animal group (Tables 3.5 and 6.5) but the actual total numbers of nuclei falling within these divisions from individual animals were used to statistically analyse differences between experimental groups using the Wilcoxon 2 sample test.

Additionally the number of hepatocyte nuclei per 10 fields were counted. All hepatocyte nuclei were counted including binucleates.

These investigations using the Quantimet 720 were carried out in the major part of the study (Sections III and IV) as a means of estimating changes in DNA synthesis, following preliminary investigations in the pilot studies (I and II) which served as indicators of an adaptive growth response to inducers of such a response which included PB and partial hepatectomy.

Sections from the majority of animals from each group in Sections III and IV were investigated and the sizes of the group remained large enough to analyse statistically. At all times, the slides were chosen at random.

2.7.1.2 Integrated microdensitometry

A small study was carried out on a limited number of animals in order to confirm that the data generated by the use of the Quantimet 720 reflected the difference in hepatocyte DNA content between experimental groups.

This was undertaken using a photometric technique employing a Vickers M85 scanning integrating microdensitometer. By carrying out integrated microdensitometry on Feulgen stained 5 u thick paraffin sections at 480 nm wavelength the amount of DNA in 50 hepatocytes per section from the right posterior lobe, was quantified and recorded. Duplicate readings were taken for each nucleus and expressed in arbitrary machine units (A.U.) and from these the mean integrated density obtained (A.U.). These units can be converted into absolute optical densities by multiplying by 3927.5 which was the correction factor for the mask size (0.02 mm), machine and objective.

In addition the machine measured the area of the field of Feulgen stained image within the hepatocytes, the area scanned having an optical density greater than that set by the "threshold control". This was achieved by setting the area threshold control at zero.

Using the arbitrary units for integrated density (A.U.), the mean integrated density for each animal was collated into 0.5 arbitrary units from 0.0 - 18.0. The group mean for each integrated density value was

then expressed as a percentage of the total number of nuclei examined per group (Tables 3.6 and 6.6). Histograms for each group were prepared which demonstrated the mean percentage of nuclei with given values compared with another experimental group, changes in integrated density being indicative of changes in ploidy classes (Figs. 3.2 and 6.2).

For each group peaks of nuclear integrated density were identified. The first peak correlating with 2n ploidy hepatocytes occurred between integrated density 3 - 4 (A.U.). Thus in a similar manner to that employed in the analysis of the Quantimet 720 image analysing computer (Cambridge Instruments Ltd., Royston, Herts) data in order to keep analysis uniform throughout the groups and to allow for experimental error such as minute alterations in thickness of sections which would alter density, a cut off point at integrated density 5 (A.U.) was made so that nuclei with values on the downward curve from the first peak on the histogram were included in 2n ploidy classes and those beginning the upward curve towards a second curve were included in the 4n ploidy and above classes. Thus using the cut off point at integrated density 5 (A.U.), nuclei showing integrated densities up to this arbitrary unit were considered to be 2n ploidy classes and 4n and above ploidy classes had values beyond this arbitrary unit.

The number of nuclei per animal with values for the 4n and above ploidy classes were ranked and compared between the groups using the Wilcoxon 2 sample test. Where statistical analysis was made between two groups, where the sample size was too small to compare at the 5% level, statistical analysis was carried out at the 10% level.

2.7.1.2.1 Animals included in this small study

Fifteen hypophysectomised animals from four of the hypophysectomised groups in Sections III and IV (described in detail in subsections 2.8.3 and 2.8.4) were included in this investigation. These included animals from:-

2.7.1.2.1.1 Section III

B1 - Hypophysectomy, laparotomy and saline treatment (Group 1 - controls, n = 5).

B2 - Hypophysectomy, laparotomy and PB treatment (Group 2, n = 4).

B5 - Hypophysectomy, 2/3 partial hepatectomy and saline treatment (Group 5, n = 3).

B6 - Hypophysectomy, 2/3 partial hepatectomy and PB treatment (Group 6, n = 3).

Comparisons were drawn between results from different groups as indicated in the test in subsections 3.1.2.1, 5.1.1, 3.1.2.3 and 5.2.4.

2.7.1.2.1.2 Section IV

Fifteen hypophysectomised animals were included in this small investigation, from four of the hypophysectomised groups. These included animals from:-

B1 - Hypophysectomy, laparotomy and saline treatment (Group 1 - controls, n = 3).

B2 - Hypophysectomy, laparotomy and PB treatment (Group 2, n = 4).

B5 - Hypophysectomy, 2/3 portal vein ligation and saline treatment (Group 5, n = 3).

B6 - Hypophysectomy, 2/3 portal vein ligation and PB treatment (Group 6, n = 5).

In this section comparisons were drawn between results from these different groups which appear in subsections 6.1.2.1, 6.2.2.1.1, 6.1.2.3 and 6.2.2.4.2.

2.7.1.3 Determination of mitotic index

Slides were selected at random and read blind using a Carl Zeiss research microscope and an optic graticule with an area of 0.361 mm^2. Mitoses were counted for 100 fields at a magnification of x 400 (x 40 objective x 10 optic) and expressed in terms of number per 1,000 cells. Mitotic figures in prophase, metaphase, anaphase and telophase were counted. In order to standardise the counting only cells and mitoses which lay within squares on the line to the right or on the line above each square of the graticule were counted. Two further samples of the histological slides were read blind on two independent occasions and the results compared with the first reading. As these results were comparable, the first reading was considered valid and these results are recorded.

2.7.2 Determination of cell volume

H&E sections of the right posterior lobe from the liver of animals from all groups were examined.

Using an optic graticule with an area of 0.361 mm^2 the number of cells per 10 fields were recorded at a magnification of x 400 (x 40 objective x 10 optic). Mean cell volume was estimated using the formula:

$$\text{Cell volume} \sqrt{\frac{1}{(\text{no cells / unit area})^3}} \times 10^{-5}\ mm^3$$

This estimates the mean individual cell volume per 10 fields for each animal and later in the text when discussing changes in this volume

per animal group where means of the groups are compared, this will be referred to as mean estimated cell volume.

2.8 Arrangement of experimental groups

Permutations of 40 groups of rats were used involving

a) Hypophysectomy and sham hypophysectomy

b) One-third (33%) and two-thirds (67%) partial hepatectomy

c) One-third portal vein ligation (1/3 PV) and two-thirds portal vein ligation (2/3 PV)

d) Laparotomy

e) Phenobarbitone by gavage (PB)

f) Control gavage

These animals were placed into 2 small pilot studies which checked experimental procedures and 2 major sections. The latter constituted the major part of the study which examined the response to partial hepatectomy and portal vein ligation in PB treated hypophysectomised rats.

2.8.1 Pilot Study I

Experimental procedures included:

a) Phenobarbitone to sham hypophysectomised and hypophysectomised rats.

b) Control gavage to sham hypophysectomised and hypophysectomised rats.

These groups served as a pilot study to establish that (i) hypophysectomy was successful using standard criteria of body, testicular and adrenal weight and dissection of the sella turcica, (ii) phenobarbitone (PB) could be administered in doses compatible with

animal survival and good health, (iii) the administration of PB to sham hypophysectomised and hypophysectomised rats induced liver growth with or without cell division, and (iv) to establish whether the number of days for drug administration were critical either to stimulate liver changes or in the degree of response elicited. Details of the results appear in Appendix II; results verifying hypophysectomy are included with those from other sections under the heading "Verification of hypophysectomy" (AII.1) and the remaining results under the general subsection AII.2.

2.8.2 Pilot Study II

Four small groups of animals were included in this study to check the response by the liver to partial hepatectomy in both sham hypophysectomised and hypophysectomised animals compared with laparotomy control animals. Details of the study appear in Appendix II, subsection AII.3.

As Pilot Studies I and II were only preliminary investigations prior to continuing with the major part of the study simple methods were used to check the response by the liver to hypophysectomy, PB and partial hepatectomy. These included (i) relative liver weight, (ii) changes in mitotic index and (iii) changes in cell volume.

Sections III and IV constituted the major part of the experiment.

2.8.3 Section III

Seventy-eight animals were included in this section. There were two major groups (A and B), with 36 animals in the sham hypophysectomised group (Group A) and 42 in the hypophysectomised group (Group B). Within these two large groups these animals were allocated

to six smaller groups. These were either sham hypophysectomised or hypophysectomised animals which received either a laparotomy, or one-third partial hepatectomy or two-third partial hepatectomy and were gavaged with either normal saline or phenobarbitone (PB), the methods for each procedure already described.

Thus the two groups were as follows:

2.8.3.1 Sham hypophysectomy group (Group A)

A(1) Sham hypophysectomy, laparotomy and saline treatment (controls - Group 1).

A(2) Sham hypophysectomy, laparotomy and PB treatment (Group 2).

A(3) Sham hypophysectomy, 1/3 partial hepatectomy and saline treatment (Group 3).

A(4) Sham hypophysectomy, 1/3 partial hepatectomy and PB treatment (Group 4).

A(5) Sham hypophysectomy, 2/3 partial hepatectomy and saline treatment (Group 5).

A(6) Sham hypophysectomy, 2/3 partial hepatectomy and PB treatment (Group 6).

2.8.3.2 Hypophysectomy group (Group B)

B(1) Hypophysectomy, laparotomy and saline treatment (controls - Group 1).

B(2) Hypophysectomy, laparotomy and PB treatment (Group 2).

B(3) Hypophysectomy, 1/3 partial hepatectomy and saline treatment (Group 3).

B(4) Hypophysectomy, 1/3 partial hepatectomy and PB treatment (Group 4).

B(5) Hypophysectomy, 2/3 partial hepatectomy and saline treatment (Group 5).

B(6) Hypophysectomy, 2/3 partial hepatectomy and PB treatment (Group 6).

2.8.4 Section IV

Seventy-five animals were included in this section and similarly to Section III divided into two major groups (A and B). Thirty-eight animals were sham hypophysectomised (Group A) and 37 were hypophysectomised (Group B). These animals were further divided into six smaller groups with 1/3 or 2/3 portal vein ligation replacing partial hepatectomy, the method for their surgical procedure as already described. Thus the groups were as follows:

2.8.4.1 Sham hypophysectomy group (Group A)

A(1) Sham hypophysectomy, laparotomy and saline treatment (controls - Group 1).

A(2) Sham hypophysectomy, laparotomy and PB treatment (Group 2).

A(3) Sham hypophysectomy, 1/3 portal vein ligation and saline treatment (Group 3).

A(4) Sham hypophysectomy, 1/3 portal vein ligation and PB treatment (Group 4).

A(5) Sham hypophysectomy, 2/3 portal vein ligation and saline treatment (Group 5).

A(6) Sham hypophysectomy, 2/3 portal vein ligation and PB treatment (Group 6).

2.8.4.2 Hypophysectomy group (Group B)

B(1) Hypophysectomy, laparotomy and saline treatment (controls - Group 1).

B(2) Hypophysectomy, laparotomy and PB treatment (Group 2).

B(3) Hypophysectomy, 1/3 portal vein ligation and saline treatment (Group 3).

B(4) Hypophysectomy, 1/3 portal vein ligation and PB treatment (Group 4).

B(5) Hypophysectomy, 2/3 portal vein ligation and saline treatment (Group 5).

B(6) Hypophysectomy, 2/3 portal vein ligation and PB treatment (Group 6).

For each animal group the results from macroscopic and microscopic investigations were recorded and presented under the general headings Effect on liver (i) Relative liver weight, (ii) Right posterior lobe, Investigations of the right posterior lobe: Measurements used to reflect DNA synthesis and cell proliferation a) Mean nuclear area, b) Nuclear ploidy classes, c) Integrated microdensitometry, d) Mitoses, Determination of cell volume, number of nuclei/10 fields.

Taking the appropriate test and control group from Sections III and IV the results of a) the response by the liver to 1/3 and 2/3 partial hepatectomy in intact and hypophysectomised rats are presented in Chapter 3, b) the response by the liver to PB treatment in intact rats following laparotomy, 1/3 or 2/3 partial hepatectomy is presented in Chapter 4, c) the response by the liver to PB in hypophysectomised rats following laparotomy, 1/3 or 2/3 partial hepatectomy is presented in Chapter 5 and d) the response by the liver to 1/3 and 2/3 portal vein ligation in intact and hypophysectomised rats and the response to PB treatment following 1/3 or 2/3 portal vein ligation in intact and hypophysectomised rats is presented in Chapter 6.

2.9 Statistical methods

Statistical analysis of the data was by means of non-parametric methods. Non-parametric analysis was selected because the data did not meet the assumptions implicit in the use of parametric statistical techniques. These assumptions are that:

a) The data are continuous. Some of the present data are of a discontinuous nature, e.g. mitotic index.

b) The data are normally distributed. Some of the present data consist of ratios, e.g. relative liver weight = liver weight/body weight. Data such as these cannot be assumed to be normally distributed.

The test employed was the Wilcoxon 2 sample test (synonymous with the Wilcoxon rank sum test) (Sokal and Rohlf, 1981; Pearson and Hartley, 1976).

The tests were carried out as two tailed tests. Critical values for the Wilcoxon 2 sample test were taken from Table 22 in Pearson and Hartley (1976) and the data tested at 1% and 5% levels of significance. Where the numbers of the animals in the groups to be compared were small, e.g. less than 6 compared with 4 animals, the groups were analysed at the 5% level but where groups to be compared included 3 or 4 animals these could only be analysed at the 10% level.

2.10 Validity of surgical procedures used in the study

2.10.1 Verification of hypophysectomy

The effect of hypophysectomy was checked for each animal which underwent the procedure throughout the entire study. The results are therefore published in Appendix II, subsection AII.1, with details of body, adrenal and testicular weights described for Pilot Study I, II and the major part of the study Sections III and IV. Details of weights also appear for each animal per group and for the mean group weights in Tables A1-A8.

In summary the effect of hypophysectomy was as follows:

(i) Body weight

In essence body weight increased in 101 of 111 sham hypophysectomised rats and reduced in 131 of 133 hypophysectomised animals, the alteration within the majority of animal groups being significant ($p < 0.05$) by the end of the study (28 days). This confirmed other studies (Schulte-Hermann et al., 1977).

The majority of groups were homogenous in that most groups showed similar initial and final body weights and gained or lost similar amounts of weight during the study period.

(ii) Adrenal weight

All 133 hypophysectomised rats showed a reduction in adrenal weight compared with the 111 sham hypophysectomised animals indicating the expected atrophy following hypophysectomy.

(iii) Testicular weight

At the end of the study period testicular weight was reduced in all 133 hypophysectomised animals compared with the 111 sham hypophysectomised animals confirming that removal of the pituitary leads to testicular atrophy (Smith, 1930).

Phenobarbitone treatment, partial hepatectomy or portal vein ligation did not effect any of these responses by the animals to hypophysectomy, when statistically analysed using the Wilcoxon 2 sample test.

2.10.2 Verification of response by the liver to PB treatment in intact and hypophysectomised rats - Pilot Study I

Details of results appear in Appendix II, subsection AII.2, but in summary:

2.10.2.1 Sham hypophysectomised animals

PB treatment in intact animals induced a significant increase in mean relative liver weight, mean estimated cell volume and number of mitoses per 1,000 cells compared with the control animals. This indicated that liver enlargement is the result of a contribution by both hypertrophy and hyperplasia which confirmed previous observations (Schulte-Hermann, 1974).

2.10.2.2 Hypophysectomised animals

In contrast following hypophysectomy relative liver weight and mean estimated cell volume are significantly reduced compared with sham hypophysectomy controls, which indicates that hypophysectomy leads to liver atrophy. However following PB treatment there is a significant increase in mean relative liver weight and mean estimated cell volume but no increase in mitoses in contrast with the hypophysectomy controls. This suggested that the induction of liver enlargement by PB in hypophysectomised animals is the result of cell hypertrophy alone. These findings are consistent with those of Schulte-Hermann et al. (1977).

Examination of the right posterior lobe showed that the effect of treatment with PB for 12 days was similar to that after 8 days as estimated by changes in the mean estimated cell volume and number of mitoses per 1,000 cells either in sham hypophysectomised or hypophysectomised animals. Therefore the 8 day PB treatment regimen

described by Schulte-Hermann et al. (1977) was followed in all subsequent experiments.

2.10.3 The effect of partial hepatectomy - Pilot Study II

Details of results appear in Appendix II, subsection AII.3, but in summary:

2.10.3.1 Sham hypophysectomised animals

By 21 days post-hepatectomy there was no significant difference in the mean relative liver weight in Group 2 compared with Group 1 controls, indicating that the compensatory hyperplasia which had occurred in the liver remnant had restored the liver to control values.

Following one-third partial hepatectomy there was a significant increase in the relative weight of the right posterior lobe by 39.52% compared with controls indicating some compensatory growth has taken place following hepatectomy. The mean estimated cell volumes and mitoses per 1,000 cells were not significantly different compared with controls.

It would appear therefore from this small preliminary study that in the sham hypophysectomy animals, following partial hepatectomy the cells have reverted to the size of the controls, this being similar to previous descriptions (Bucher, 1963). Following partial hepatectomy the peak of mitotic activity occurs between 28 hours and 3 days, falling away during the next few days (Brues and Marble, 1937; Johnson and Albert, 1952; Harkness, 1957; Weinbren, 1959; Bucher, 1963). In Group 2, the mitotic index by 21 days post-hepatectomy was similar to control animals. This suggested that the mitotic activity had faded, consistent with previous findings.

The response by the liver to partial hepatectomy is consistent and entirely independent of any other effects such as anaesthetics as these changes did not occur in sham hypophysectomised animals in which a laparotomy was performed. Food did not influence the response either as all animals were fed ad libitum prior to all operations.

2.10.3.2 Hypophysectomised animals

Hypophysectomy resulted in liver atrophy confirmed by a significant reduction in the relative liver weight, relative weight of the right posterior lobe and reduction in cell volumes compared with intact animals. There was no alteration in the number of mitoses compared with intact controls. These results again were consistent with other authors (Schulte-Hermann et al., 1977).

Hypophysectomy in this study did not alter the contribution made by each lobe to the whole liver weight. Hypophysectomy did not prevent response by the liver to a stimulus such as partial hepatectomy and compensatory growth occurred as indicated by the increase in relative and absolute weights of the right posterior lobes in Group 2 compared with controls. However by 21 days restoration was incomplete, the mean relative liver weight being only 74.87% of control value. There was a significant increase in mean estimated cell volume by 43.35%, indicating hypertrophy of the cells of the right posterior lobe but there was no detectable hyperplasia compared with hypophysectomy controls. However the mean estimated cell volumes in hypophysectomised Group 2 were still significantly smaller in comparison with sham hypophysectomised controls (A Group 1) which indicated that atrophy had been induced following hypophysectomy and was still detectable even following partial hepatectomy in comparison to the intact controls.

The incomplete restoration of the liver compared with controls was similar to that found by Schulte-Hermann and his colleagues (1977) when following 1/3 or 2/3 partial hepatectomy they found that only 70-80% of the liver was restored in hypophysectomised rats by 20 days post-hepatectomy. This they associated to a reduced functional load in the liver, in part due to cessation of body growth and diminished food requirements (Schulte-Hermann et al., 1977).

CHAPTER 3

MICROSCOPIC EVALUATION OF DNA SYNTHESIS

3.1 The response by the liver to partial hepatectomy

The results presented here are to confirm that the methods used in this study reflect changes in DNA synthesis known to occur following partial hepatectomy both in intact and hypophysectomised rats (Harkness, 1957; Weinbren, 1959; Bucher, 1963; Geschwind et al., 1958; Schulte-Hermann et al., 1977). These results also confirm that changes in mean nuclear area and distribution of nuclear size on the histograms (Fig. 3.1) reflect a shift in ploidy and that these are suitable methods to detect increases in DNA content in subsequent experimental procedures. Measurements used to reflect the adaptive growth response by the liver and cellular enlargement are also included and the results are discussed in Chapter 7. The relevant groups investigated and reported here, form part of Section III described in the Material and Methods subsection 2.8.3.

For details of the results for individual animals from each group see Tables 3.1 and 3.2, and for a summary of the group means Table 3.3. The mean percentage contribution each liver lobe made to the liver as a whole following 1/3 or 2/3 partial hepatectomy in intact and hypophysectomised rats is expressed in Figure 3.3 Details of the mean nuclear area (um^2) and mean number of nuclei/10 fields for each animal investigated per group using the Quantimet 720 image analyser are given in Table 3.4 and a summary of the mean percentage distribution of nuclei into ploidy classes per group is given in Table 3.5. Histograms demonstrating the mean percentage distribution of nuclei into 5 um^2 size ranges from 10-100 which separate the nuclei into ploidy classes for

each group are presented in Figure 3.1. Results of the integrated microdensitometry investigations are also presented in Table 3.6 and histograms of percentage distribution of nuclei for given integrated density (A.U.) shown in Figure 3.2.

3.1.1 Sham hypophysectomy group (Group A)

3.1.1.1 Sham hypophysectomy, laparatomy, saline treatment (Controls - Group 1)

Effect on liver

(i) Relative liver weight

The mean relative liver weight in the sham hypophysectomy laparotomy saline treated animals was 3.35% (SD ± 0.09, n = 6).

The left lateral lobe contributed by a mean 32.46% (SD ±2.20, n = 6) the median lobe by a mean 31.80% (SD ±0.87, n = 6) the right posterior by a mean 25.36% (SD ±2.37,n = 6) and the caudate lobe by a mean of 10.38% (SD ±1.62, n = 6).

(ii) Right posterior lobe

The mean relative weight of the right posterior lobe was 0.85% (SD ±0.07, n = 6) and by comparison of this lobe with the R.P. lobe in other study groups indicated whether there was a response by the liver following hypophysectomy, hepatectomy, PB treatment or combinations of these procedures. Adaptive liver growth detected in this lobe may be the result of cell hypertrophy, cell replication or a mixture of both.

Investigation of the right posterior lobe

Measurements used to reflect DNA synthesis and cell proliferation

a) Mean nuclear area

The mean nuclear area was 42.13 um^2 (SD ± 3.13, n = 5).

b) Nuclear ploidy classes

57.8% of nuclei were in the 2n ploidy classes and 42.2% in the 4n and above ploidy classes.

c) Mitoses

The mean number of mitoses per 1,000 cells in the right posterior lobes was also calculated and within this control group these were barely detected, the mean number being 0.1081 (SD ±0.12, n = 6).

Determination of cell volume

The mean estimated cell volume in the sham hypophysectomised laparotomy saline treated animals was 6.60×10^{-5} mm^3 (SD ±0.27, n = 6). Increases within the study groups compared with the mean controls indicated cell hypertrophy while a decrease indicated cellular atrophy.

Number of nuclei/10 fields

The mean number of nuclei per 10 fields was 418.0 (SD ±11.24, n = 5).

3.1.1.2 Sham hypophysectomy 1/3 partial hepatectomy and saline treatment (Group 3) compared with Group 1

Following 1/3 partial hepatectomy by 21 days the mass of the liver remnant was similar to controls (3.44%, SD ±0.35 compared with 3.35%, SD ±0.09) indicating that liver growth had replaced the liver tissue removed during hepatectomy. There was clear enlargement in the right posterior lobe (R.P. 42.98% instead of 25.36%, p <0.01) with a relative weight of the R.P. 1.48% vs. 0.85% (p <0.01), a 74.59% increase compared with controls.

There was an increase in the number of 4n and above ploidy class nuclei (59.2% vs. 42.2%, p <0.01) but the mean nuclear area was similar

to controls (43.72 um^2, SD $\pm$2.57 vs. 42.13 um^2, SD $\pm$3.13). Various possible reasons for this discrepancy are discussed in detail in Chapter 7. The most likely explanation however is that there was some variability noted in the mean nuclear area of individual animals within the control group. This would result in a "group mean" value for Group 1 close to that obtained following partial hepatectomy, instead of being smaller in value. This would mask any real change in nuclear size which may have taken place. This possible explanation is discussed further in Chapter 7. There was no significant difference in the number of mitoses compared with controls (0.2380, SD $\pm$0.08 vs. 0.1081, SD $\pm$0.12) as expected by 21 days post-hepatectomy (Bucher, 1963).

Estimated cell volume was similar to controls (6.33 x 10^{-5} mm^3, SD $\pm$0.21 vs. 6.60 x 10^{-5} mm^3, SD $\pm$0.27) but there were fewer nuclei per 10 fields (376.2 vs. 418.0, $p < 0.01$) compared with Group 1. This latter observation is most likely the result of a reduction in the number of binucleate cells, which is known to occur following partial hepatectomy (Sulkin, 1943; Bucher, 1963).

3.1.1.3 Sham hypophysectomy 2/3 partial hepactectomy and saline treatment (Group 5) compared with Group 1

After 2/3 partial hepatectomy there was obvious enlargement of the right posterior lobe (R.P. 75.28% vs. 25.36%, $p < 0.01$) with a relative weight of the R.P. 1.80% instead of 0.85% ($p < 0.01$), a 112.5% increase compared with controls. The total liver only reached 77.91% of controls (2.4% vs. 3.35%, $p > 0.01$), 21 days post-hepatectomy.

There was an increase in the number of 4n and above ploidy classes (63.2% vs. 42.2%, $p < 0.01$) but no significant difference in the mean nuclear area compared with controls (44.64 um^2, SD $\pm$2.71 vs. 42.13 mm^2,

SD $\pm$3.13). This discrepancy is similar to that described following 1/3 partial hepatectomy (subsection 3.1.1.2) and is discussed in more detail in Chapter 7. Mitoses were similar to controls (0.1194, SD $\pm$0.13 vs. 0.1081, SD $\pm$0.12).

Estimated cell volume was enlarged (7.50 $um^3 \times 10^{-5}$ mm^3 vs. 6.60 $\times 10^{-5}$ mm^3, $p < 0.01$), a 13.66% increase compared with controls. The mean number of nuclei per 10 fields were significantly reduced (345.6 vs. 418.0 controls, $p < 0.01$), this effect being most likely seen because the cells were enlarged.

3.1.1.4 Summary: Effect of partial hepatectomy in sham hypophysectomised animals

These results confirm the response by the liver following 1/3 or 2/3 partial hepatectomy reported in other studies (Harkness, 1957; Leong et al., 1959, Weinbren, 1959; Bucher, 1963). The shift in ploidy from 2n to 4n and above classes compared with controls demonstrated here, confirmed that following partial hepatectomy DNA synthesis is induced (Sulkin, 1943; Alfert and Geschwind, 1958; Bucher, 1963; Schulte-Hermann et al., 1977). Neither sham hypophysectomy nor laparotomy affected this response.

3.1.2 Hypophysectomy group (Group B)

Following hypophysectomy the body weights, adrenal and testicular weights were reduced compared with the sham hypophysectomised laparotomy saline treated controls (as already described), verifying hypophysectomy.

3.1.2.1 Hypophysectomy, laparotomy and saline treatment (Controls - Group 1)

Effect on liver

(i) Relative liver weight

At the end of the 28 day study the mean relative liver weight in Group 1 was 2.97% (SD ±0.01, n = 6). This was significantly reduced (p <0.01) by 11.46% compared with a mean relative liver weight of 3.35% (SD ±0.09, n = 6) in the sham hypophysectomised controls.

The mean percentage contributed by the left lateral lobe was 30.85% (SD ±1.07, n = 6), the median lobe 32.75% (SD ±1.99, n = 6), the right posterior lobe 27.22% (SD ±2.73, n = 6) and the caudate lobe 9.18% (SD ±1.64, n = 6). This distribution was similar to the distribution in the sham hypophysectomised controls.

(ii) Right posterior lobe

Following hypophysectomy the mean relative weight of the right posterior lobe was 0.81% (SD ±0.10, n = 6). This was not significantly different from the mean relative weight of the right posterior lobe in the sham hypophysectomised controls (0.85% (SD ±0.07, n = 6)). This discrepancy may be related to differences in the amount of handling of the liver lobes during the laparotomy and is discussed further in Chapter 7. However there was a significant reduction (p <0.01) in mean absolute weight of the right posterior lobe in the Group 1 compared with the sham hypophysectomised controls, the mean values being 1.28 grams (SD ±0.11, n = 6) and 2.10 grams (SD ±0.17, n = 6) respectively, indicating that following hypophysectomy cellular atrophy occurs within the hepatocytes of the right posterior lobe.

Investigation of the right posterior lobe

Measurements used to reflect DNA synthesis and cell proliferation

a) Mean nuclear area

The mean nuclear area for Group 1 was 31.43 um^2 (SD $\pm$ 2.40, n = 6). This was significantly smaller ($p < 0.01$) compared with sham hypophysectomy controls (42.13 um^2, SD $\pm$ 3.13, n = 5).

b) Nuclear ploidy classes

There were 84.33% of nuclei in the 2n ploidy classes and 15.67% in the 4n and above ploidy classes in Group 1. The distribution in ploidy classes was significantly different ($p < 0.01$) compared with sham hypophysectomised controls (57.8% vs 42.2%).

c) Integrated microdensitometry

In Group 1 (controls) 92.4% of hepatocyte nuclei had values of integrated density ranging from 0-5 (A.U.) with a peak occurring between values 3-4 (A.U.). These values correlated with nuclei of 2n ploidy classes. 7.6% of hepatocytes showed integrated density values of 5.0-7.5 (A.U.). These values corresponded to nuclei of 4n and above ploidy classes.

d) Mitoses

The mean number of mitoses per 1,000 cells in the right posterior lobes in the hyophysectomised laparotomy saline treated controls at 28 days was 0.0151 (SD $\pm$ 0.03, n = 6). This was similar to that found in the sham hypophysectomy controls (0.1081 SD $\pm$ 0.12, n = 6).

Determination of cell volume

At the end of the 28 day study the mean estimated cell volume in the right posterior lobes of Group 1 was 2.78 x 10^{-5} mm^3 (SD $\pm$0.16,

n = 6) which was significantly reduced ($p < 0.01$) compared with 6.60 x 10^{-5} mm^3 (SD ± 0.27, n = 6) in the sham hypophysectomy controls. This was a reduction by 57.8% and confirms that cellular atrophy occurs following hypophysectomy.

Mean number of nuclei/10 fields

The mean number of nuclei per 10 fields was 656.8 (SD $\pm$ 19.5, n = 6). There were significantly more ($p < 0.01$) compared with 418.0 (SD $\pm$ 11.2, n = 5) in the sham hypophysectomy controls.

3.1.2.2 Hypophysectomy 1/3 partial hepatectomy and saline treatment (Group 3) compared with Group 1

After 1/3 partial hepatectomy by 21 days the liver remnant achieved control values (2.94% SD ± 0.19 vs. 2.97% SD ± 0.01 control) with clear enlargement in the right posterior lobe (R.P. 42.41% instead of 27.22%, $p < 0.01$) with the relative weight of the R.P. 1.25% compared with 0.81% (controls, $p < 0.01$), an increase by 54.7%.

In the R.P. lobe nuclei showed an increased mean nuclear area (38.63 um^2 vs. 31.44 um^2 controls, $p < 0.01$), increased incidence of 4n and above ploidy class nuclei (42.5% vs. 15.67% control, $p < 0.01$) but no significant difference in mitotic index (0.0183 SD ± 0.04 vs. 0.0151 SD ± 0.03, control).

Estimated cell volume was also enlarged (3.63 x 10^{-5} mm^3 vs. 2.78 x 10^{-5} mm^2, $p < 0.01$, an increase by 30.51%) and the mean number of nuclei/10 fields were significantly fewer (533.3 vs. 656.8, $p < 0.01$) compared with controls. However when compared with sham hypophysectomised controls, cell volumes were significantly smaller ($p < 0.01$).

3.1.2.3 Hypophysectomy 2/3 partial hepatectomy and saline treatment (Group 5) compared with Group 1

After 2/3 partial hepatectomy the remnant increased in mass (R.P. 1.67% relative weight vs. 0.81% ($p < 0.01$) a 106% increase compared with controls) with obvious enlargement in the right posterior lobe (R.P. 75.5% instead of 27.2% controls, $p < 0.01$). However by 21 days the compensatory growth did not achieve the relative weight of the intact liver in hypophysectomised rats (2.22% instead of 2.97%, $p < 0.01$) total liver being equivalent to 74.84% of that of controls.

The mean nuclear area was increased (38.17 um^2 instead of 31.44 um^2 controls, $p < 0.01$) and a larger percentage of nuclei fell in the 4n and above ploidy classes (39.13% vs. 15.67% control, $p < 0.01$). Integrated microdensitometry demonstrated that 81.34% of nuclei exhibited integrated density values corresponding to 4n and above ploidy classes, the histograms demonstrating peaks at 5.5-6.0 (A.U.) and 8.5-9.0 (A.U.), this representing a 970.3% increase ($p < 0.05$) compared with controls. Mitotic index was similar to controls (0.0612 SD$\pm$0.07 vs. 0.0151 SD $\pm$0.04).

The cells of the R.P. lobe were 60.4% larger than controls (mean estimated volume 5.10×10^{-5} mm^3 vs. 2.78×10^{-5} mm^3, $p < 0.01$) but 22.6% smaller ($p < 0.01$) compared with sham hypophysectomy controls (6.60×10^{-5} mm^3). Fewer nuclei/10 fields (440.1 vs. 656.8 control) reflected this increase in cell size.

3.1.2.4 Summary: Effect of partial hepatectomy in hypophysectomised animals

The effect of hypophysectomy is a significant reduction in relative liver weight, absolute right posterior weight and reduction in cell

size, indicating liver atrophy and a reduced incidence of higher ploidy nuclei along with reduced mean nuclear area compared with intact controls. The number of mitoses were barely perceptible. These changes confirm other studies (Doljanski and Novogrotzky, 1959; Alfert and Geschwind, 1958; Brues and Marble, 1937).

Following 1/3 and 2/3 partial hepatectomy an increase in DNA synthesis in these hypophysectomised rats was confirmed by an increased mean nuclear area and a shift towards polyploidy, the latter confirming other studies (Geschwind et al., 1958; Doljanski and Novogrotzky, 1959; Schulte-Hermann et al., 1977).

The results confirm that the Quantimet 720 image analysing computer (Cambridge Instruments Ltd., Royston, Herts.) and the M85 scanning microdensitometer (both of which detect shifts in nuclear ploidy) may be used to detect the induction of DNA synthesis following partial hepatectomy both in intact and hypophysectomised rats. Changes in nuclear area and shifts in ploidy are appropriate measurements to use in this study to reflect DNA synthesis.

Table 3.1.

Table 3.1 Section III - Sham hypophysectomy group

Rat no.	At start total body wt(grams)	At end total body wt(grams)	Liver wt(grams)	% liver/ body wt	% Rt post lobe/ body wt	Liver LL	Liver ML	Liver RP	Liver CL	Adrenals (grams)	Testes (grams)	No.mitoses /1000 cells	No.cells / 10 F	Cell vol $x10^{-5} mm^3$
A1 - Sham hypophysectomy, laparotomy and saline														
H10	205	235	7.85	3.34	0.8510	2.57	2.50	2.0	0.78	0.04	2.68	0.0	617	6.52
						32.74%	31.85%	25.48%	9.94%					
H11	200	250	8.14	3.26	0.952	2.42	2.50	2.38	0.84	0.04	3.05	0.0	604	6.73
						29.73%	30.71%	29.24%	10.32%					
H19	160	240	8.31	3.46	0.900	2.50	2.75	2.16	0.90	0.04	2.92	0.1569	637	6.22
						30.08%	33.09%	25.99%	10.83%					
H20	210	270	9.29	3.44	0.829	3.29	3.0	2.24	0.76	0.04	2.80	0.1715	583	7.10
						35.41%	32.29%	24.11%	8.18%					
H21	210	265	8.59	3.24	0.713	2.91	2.66	1.89	1.13	0.04	2.84	0.3205	624	6.41
						33.88%	30.97%	22.0%	13.15%					
H22	175	230	7.69	3.34	0.847	2.53	2.45	1.95	0.76	0.04	2.58	0.0	612	6.60
						32.9%	31.86%	25.36%	9.88%					
A2 - Sham hypophysectomy, laparotomy and PB														
H12	210	235	11.30	4.81	1.28	3.81	3.67	3.01	0.81	0.045	2.82	0.9333	560	7.54
						33.72%	32.48%	26.64%	7.17%					
H18	205	250	10.65	4.26	1.09	3.28	3.79	2.74	0.84	0.04	4.0	0.7380	542	7.92
						30.8%	35.59%	25.73%	7.89%					
H23	150	185	8.44	4.56	1.14	2.85	2.90	2.10	0.59	0.04	3.29	0.5586	537	8.03
						33.77%	34.36%	34.88%	6.99%					
H24	160	230	9.29	4.04	1.16	2.93	2.83	2.67	0.86	0.04	2.82	0.5555	540	7.96
						31.54%	30.46%	28.74%	9.26%					
H25	175	225	10.12	4.50	1.05	3.25	3.46	2.36	1.05	0.04	3.03	0.7590	527	8.26
						32.11%	34.19%	23.32%	10.38%					
H28	200	235	10.88	4.63	1.25	3.65	3.48	2.95	0.8	0.04	3.00	0.5555	540	7.96
						33.55%	31.99%	27.11%	7.35%					
A3 - Sham hypophysectomy, 1/3 hepatectomy and saline														
H0	150	260	9.71	3.73	1.61	4.25		4.19	1.27	0.04	3.72	0.1658	603	6.75
						43.76%		43.15%	13.09%					
H4	150	220	8.06	3.66	1.64	3.78		3.61	0.67	0.03	3.17	0.3194	626	6.38
						46.89%		44.78%	8.31%					
H5	160	240	9.27	3.86	1.75	4.14		4.20	0.93	0.03	2.78	0.3164	632	6.29
						44.66%		45.3%	10.03%					
H3	220	260	8.20	3.15	1.50	3.25		3.92	1.03	0.03	4.09	0.3149	635	6.24
						39.63%		47.8%	12.56%					
H4	190	220	6.87	3.12	1.17	3.44		2.59	0.84	0.03	3.08	0.1567	638	6.20
						50.0%		37.7%	12.22%					
H5	220	260	8.12	3.12	1.22	3.51		3.18	1.43	0.04	2.94	0.1552	644	6.11
						43.2%		39.16%	17.6%					

Table 3.1 (contd)

Rat no.	At start total body wt(grams)	At end total body wt(grams)	Liver wt(grams)	% liver/ body wt	% Rt post lobe/ body wt	Liver LL	ML	RP	CL	Adrenals (grams)	Testes (grams)	No.mitoses /1000 cells	No.cells / 10 F	Cell vol $x10^{-5}mm^3$
A4 - Sham hypophysectomy, 1/3 hepatectomy and PB														
H1	150	230	10.30	4.47	1.80	4.77		4.14	1.39	0.035	2.98	1.677	596	6.87
						46.31%		40.19%	13.49%					
H2	160	240	9.66	4.02	2.02	3.90		4.86	0.90	0.04	2.91	0.5263	570	7.34
						40.37%		50.31%	9.3%					
H3	150	230	10.12	4.4	1.83	4.30		4.23	1.59	0.045	2.80	0.5217	575	7.25
						42.49%		41.79%	15.71%					
H0	210	240	9.48	3.95	1.783	4.03		4.28	1.17	0.04	3.08	1.1532	607	6.68
						42.51%		45.14%	12.34%					
H1	190	220	8.42	3.8	1.65	3.68		3.65	1.09	0.04	2.69	0.5119	586	7.04
						43.7%		43.34%	12.94%					
H2	210	250	9.81	3.92	1.84	3.40		4.62	1.79	0.04	3.08	0.6711	592	6.94
						34.65%		47.09%	18.24%					
A5 - Sham hypophysectomy, 2/3 hepatectomy and saline														
H2	275	275	6.35	2.30	1.96			5.40	0.95	0.04	3.70	0.1782	561	7.52
								85.03%	14.96%					
H3	210	225	5.94	2.64	1.95			4.39	1.55	0.04	3.25	0.3590	557	7.60
								73.90%	26.09%					
H5	280	275	6.54	2.37	1.75			4.83	1.71	0.04	3.00	0.1792	558	7.58
								73.85%	26.14%					
H7	315	325	7.06	2.17	1.65			5.38	1.68	0.04	4.65	0.0	553	7.68
								76.20%	23.79%					
H1	280	295	7.24	2.45	1.81			5.36	1.88 5	0.04	3.10	0.0	575	7.25
								74.03%	25.96%					
H30	250	310	7.70	2.48	1.70			5.29	2.41	0.04	3.18	0.0	569	7.36
								68.70%	31.29%					
A6 - Sham hypophysectomy, 2/3 hepatectomy and PB														
H0	295	290	7.54	2.6	1.86			5.40	2.14	0.04	3.01	0.9469	528	8.24
								71.61%	28.38%					
H4	215	260	8.6	3.3	2.33			6.06	2.54	0.04	2.92	1.6363	550	7.74
								70.46%	29.53%					
H6	215	245	8.44	3.44	2.53			6.20	2.24	0.04	2.87	0.5376	558	7.58
								73.45%	26.54%					
H9	220	230	7.3	3.17	2.426			5.58	1.72	0.04	3.07	2.5641	546	7.83
								76.43%	23.56%					
H11	215	245	9.22	3.76	2.65			6.50	2.72	0.04	3.03	1.8315	540	7.96
								70.49%	29.50%					
H1	275	295	7.24	2.45	1.81			5.36	1.88	0.04	3.0	1.5094	530	8.19
								74.03%	25.96%					

Table 3.2.

Table 3.2 Section III - Hypophysectomy group

Rat no.	At start total body wt(grams)	At end total body wt(grams)	Liver wt(grams)	% liver/ body wt	% Rt post lobe/ body wt	Liver LL	Liver ML	Liver RP	Liver CL	Adrenals (grams)	Testes (grams)	No.mitoses /1000 cells	No.cells / 10 F	Cell vol $x10^{-5}mm^3$
B1 - Hypophysectomy, laparotomy and saline														
H12	215	175	5.0	2.86	0.714	1.59	1.62	1.25	0.54	0.01	0.60	0.0	1062	2.88
						31.8%	32.4%	25.0%	10.8%					
H18	200	150	4.68	3.12	0.78	1.45	1.63	0.17	0.43	0.01	0.53	0.0909	1100	2.74
						30.98%	34.82%	25.0%	9.18%					
H4	220	145	4.46	3.08	0.993	1.45	1.30	1.44	0.27	0.01	0.395	0.0	1064	2.88
						32.51%	29.15%	32.29%	6.05%					
H7	230	160	4.72	2.95	0.806	1.44	1.52	1.29	0.47	0.01	0.46	0.0	1042	2.97
						30.51%	32.2%	27.33%	9.96%					
H8	235	165	4.57	2.77	0.684	1.34	1.61	1.13	0.49	0.01	0.44	0.0	1182	2.46
						29.32%	35.23%	24.73%	10.72%					
H9	230	160	4.83	3.02	0.875	1.45	1.58	1.40	0.40	0.01	0.4	0.0	1096	2.75
						30.02%	32.71%	28.99%	8.28%					
B2 - Hypophysectomy, laparotomy and PB														
H10	225	175	6.69	3.82	1.017	2.17	2.06	1.78	0.68	0.01	0.45	0.0	650	6.03
						32.43%	30.79%	26.6%	10.16%					
H15	205	150	5.77	3.85	1.00	1.90	1.73	1.50	0.64	0.01	0.53	0.0	655	5.96
						32.92%	29.98%	25.99%	11.0%					
H19	220	155	5.76	3.72	0.909	1.79	2.05	1.41	0.51	0.01	0.46	0.0	675	5.76
						31.07%	35.59%	24.47%	8.85%					
H3	230	160	6.26	3.91	1.05	1.71	2.17	1.68	0.70	0.01	0.52	0.0	660	5.89
						27.32%	34.66%	26.84%	11.18%					
H5	225	160	6.11	3.82	0.725	2.22	2.12	1.16	0.61	0.015	0.76	0.0	660	5.89
						36.33%	34.70%	18.99%	9.88%					
H6	235	165	6.95	4.21	1.248	1.74	2.36	2.06	0.79	0.015	0.52	0.0	630	6.32
						25.04%	33.96%	29.64%	11.37%					
B3 - Hypophysectomy, 1/3 hepatectomy and saline														
H0	240	170	5.10	3.0	1.088	2.48		1.85	0.77	0.01	0.66	0.0	912	3.63
						48.62%		36.29%	15.09%					
H3	240	170	5.10	3.0	1.317	2.36		2.24	0.50	0.01	0.56	0.0	963	3.34
						46.27%		43.92%	9.8%					
H4	260	190	5.50	2.89	1.073	2.78		2.04	0.68	0.01	0.50	0.0	927	3.54
						50.54%		37.09%	12.36%					
H0	190	170	5.03	2.95	1.34	2.139		2.28	0.624	0.009	0.615	0.0	890	3.76
						42.34%		45.32%	12.32%					
H4	220	170	4.47	2.62	1.170	1.94		1.99	0.54	0.01	1.08	0.0	874	3.87
						43.4%		44.51%	12.08%					
H7	210	160	5.13	3.20	1.518	1.84		2.435	0.864	0.01	0.395	0.110	908	3.65
						35.86%		47.36%	16.76%					

Table 3.2 (contd)

Rat no.	At start total body wt(grams)	At end total body wt(grams)	Liver wt(grams)	% liver/ body wt	% Rt post lobe/ body wt	Liver LL	ML	RP	CL	Adrenals (grams)	Testes (grams)	No.mitoses /1000 cells	No.cells / 10 F	Cell vol $x10^{-5}mm^3$
B4 - Hypophysectomy, 1/3 hepatectomy and PB														
H0	220	150	5.53	3.68	1.54	2.38		2.31	0.84	0.01	0.46	0.1197	835	4.14
						43.03%		41.77%	15.18%					
H3	220	160	5.10	3.18	1.412	2.02		2.26	0.82	0.01	0.48	0.2528	791	4.49
						39.60%		44.3%	16.07%					
H4	210	160	5.19	3.24	1.275	2.40		2.04	0.75	0.01	1.02	0.5221	766	4.71
						46.24%		39.3%	14.45%					
H1	210	160	5.63	3.51	1.543	2.47		2.47	0.69	0.01	0.54	0.7692	780	4.59
						43.7%		43.87%	12.25%					
H3	200	170	6.40	3.76	1.752	2.07		2.98	1.35	0.01	0.49	0.1250	800	4.41
						32.34%		46.56%	21.0%					
H4	210	160	6.15	3.84	1.518	2.83		2.43	0.89	0.01	0.49	0.2509	797	4.44
						46.0%		39.51%	14.47%					
B5 - Hypophysectomy, 2/3 hepatectomy and saline														
H3	225	175	4.33	2.47	1.862			3.26	1.07	0.01	0.52	0.0	740	4.96
								75.28%	24.71%					
H4	200	155	2.44	1.57	1.483			2.30	0.14	0.015	0.48	0.1396	716	5.21
								94.26%	5.73%					
H5	215	165	3.90	2.36	1.757			2.90	1.0	0.015	0.52	0.0	725	5.12
								74.35%	25.64%					
H7	190	165	3.44	2.08	1.539			2.54	0.90	0.015	0.50	0.0	720	5.17
								73.83%	26.16%					
H4	165	130	3.19	2.45	1.861			2.42	0.77	0.01	0.40	0.00	704	5.16
								75.86%	24.13%					
H8	190	150	3.48	2.32	1.653			2.48	1.0	0.01	0.44	0.1366	732	5.04
								71.26%	28.73%					
H9	160	110	2.64	2.4	1.70			1.87	0.77	0.01	0.40	0.1386	721	5.16
								70.83%	29.16%					
H12	210	170	3.82	2.24	1.69			2.88	0.94	0.01	0.465	0.00	700	5.07
								75.39%	24.66%					
H14	210	150	3.18	2.12	1.45			2.18	1.0	0.01	0.46	0.1360	735	5.01
								68.55%	31.44%					
B6 - Hypophysectomy, 2/3 hepatectomy and PB														
H0	195	155	3.97	2.56	1.87			2.90	1.07	0.015	0.46	0.0	712	5.26
								73.04%	26.95%					
H1	195	145	4.1	2.82	2.13			3.09	1.01	0.015	0.50	0.417	718	5.19
								75.36%	24.63%					
H2	180	140	4.15	2.96	2.213			3.10	1.05	0.01	0.40	0.7092	705	5.34
								74.69%	25.30%					
H8	170	140	3.79	2.70	1.973			2.76	1.03	0.01	0.54	1.134	705	5.34
								72.82%	27.17%					
H0	195	155	4.46	2.87	2.21			3.44	1.02	0.01	0.32	1.4224	703	5.36
								77.13%	22.86%					
H3	175	155	4.15	2.67	1.93			3.0	1.15	0.015	0.41	0.2773	721	5.35
								72.28%	27.71%					
H5	185	160	3.67	2.29	1.73			2.78	0.89	0.01	0.32	2.40	708	5.30
								75.74%	24.28%					
H6	195	160	3.62	2.49	1.68			2.69	0.93	0.015	0.39	0.2739	730	5.39
								74.3%	25.69%					
H2	195	155	3.85	2.48	1.74			2.70	1.15	0.015	0.45	0.9957	703	5.36
								70.12%	29.87%					

Table 3.3a Section III - Sham hypophysectomy group - Group means

Experiment	No. animals	Body wt. (av)grams	Body wt. end exp. (av)grams	Av.liver (av)grams	Av.% liver/ body wt.	Av.% rt.post. body wt.	Liver LL	ML	RP	CL	Adrenals av(grams)	Testes (av)grams	Av.no. mitoses /1000 cells	No.cells /10F	Mean cell vol. $\times 10^{-5} mm^3$
A1 - Group 1 Sham hypophysectomy, laparotomy and saline	6	193.33 ±20.90	248.33 ±16.33 28.45%	8.31 ±0.58	3.35 ±0.09	0.8486 ±0.0731	2.70 ±0.3 32.46% ±2.20	2.64 ±0.21 31.80% ±0.87	2.10 ±0.19 25.36% ±2.37	0.86 ±0.14 10.38% +1.62	0.04 +0.00	2.81 ±0.17	0.1081 ±0.1201	612.833 ±16.80	6.5966 ±0.2749
A2 - Group 2 Sham hypophysectomy, laparotomy and PB	6	183.33 ±25.23	226.67 ±22.06 45.46%	10.11 ±1.07	4.47 ±0.28	1.1616 ±0.0815	3.30 ±0.38 32.58% ±1.27	3.36 ±0.40 33.18% ±1.87	2.64 ±0.35 27.74% ±3.93	0.83 ±0.15 8.17% ±1.36	0.04 ±2.04 x10^{-3}	3.16 ±0.45	0.6833 ±0.1410	541.00 ±9.7979	7.945 ±0.2127
A3 - Group 3 Sham hypophysectomy, 1/3 hepatectomy and saline	6	181.67 ±33.12	243.33 ±19.66	8.37 ±1.00	3.44 ±0.35	1.4816 ±0.2158	3.73 ±0.40 44.70% ±3.53		3.62 ±0.63 42.98% ±4.31	1.03 ±0.28 12.30% +3.16	0.03 ±0.01	3.30 ±0.50	0.2380 ±0.0789	629.66 ±13.123	6.3283 ±0.2057
A4 - Group 4 Sham hypophysectomy, 1/3 hepatectomy and PB	6	178.33 ±28.58	235.0 ±10.49	9.63 ±0.66	4.10 ±0.27	1.820 ±0.1090	4.01 ±0.48 41.67% ±3.95		4.30 ±0.42 44.62% ±3.69	1.32 ±0.33 13.67% ±3.05	0.04 ±3.16 x10^{-3}	2.92 ±0.16	0.8435 ±0.4348	587.6 ±12.49	7.0206 ±0.2
A5 - Group 5 Sham hypophysectomy, 2/3 hepatectomy and saline	6	268.33 ±32.23	284.16 ±31.93	6.805 ±0.588	2.40 ±0.147	1.8033 ±0.1177			5.10 ±0.376 75.28% +4.911	1.69 ±0.432 24.705% ±4.91	0.04 ±0.0	3.48 ±0.56	0.1194 ±0.1336	562.166 ±7.536	7.4983 ±0.1479
A6 - Group 6 Sham hypophysectomy, 2/3 hepatectomy and PB	6	239.17 ±36.11	260.83 ±26.35	8.06 ±0.81	3.12 ±0.50	2.2676 ±0.3213			5.85 +0.47 72.75% ±2.34	2.21 ±0.38 27.25% +2.34	0.04 ±0.0	2.98 ±0.07	1.5043 ±0.6445	542.0 ±10.6458	7.926 ±0.2345

Table 3.3b Section III - Hypophysectomy group - Group means

Experiment	No. animals	Body wt. (av)grams	Body wt. end exp. (av)grams	Av.liver (av)grams	Av.% liver/ body wt.	Av.% rt.post. body wt.	Liver LL	Liver ML	Liver RP	Liver CL	Adrenals av(grams)	Testes (av)grams	Av.no. mitoses /1000 cells	No.cells /10F	Mean cell vol. $x10^{-5}mm^3$
B1 - Group 1 Hypophysectomy, laparotomy and saline	6	221.67 ±12.91	159.17 ±10.68	4.71 ±0.1739	2.966 ±0.0122	0.8086 ±0.1030	1.45 ±0.08 30.85% ±1.066	1.54 ±0.13 32.75% ±1.99	1.28 ±0.02 27.22% ±2.73	0.43 ±0.09 9.165% ±1.64	0.01 ±2.04 $x10^{-3}$	0.47 ±0.08	0.0151 ±0.0338	1091.00 ±45.38	2.7826 ±0.1649
B2 - Group 2 Hypophysectomy, laparotomy and PB	6	223.33 ±10.33	160.83 ±8.61	6.26 ±0.48	3.89 ±0.17	0.9915 ±0.1569	1.92 ±0.22 30.85% ±4.08	2.08 ±0.21 33.28% ±2.32	1.60 ±0.31 25.42% ±3.57	0.66 ±0.09 10.41% ±0.96	0.01 ±2.58 $x10^{-3}$	0.54 ±0.11	0.00 ±0.00	654.166 ±12.388	5.9803 ±0.1733
B3 - Group 3 Hypophysectomy, 1/3 hepatectomy and saline	6	226.67 ±25.03	171.67 ±9.83 24.25%	5.06 ±0.33	2.94 ±0.19	1.251 ±0.1572	2.26 ±0.35 44.51% ±5.24		2.14 ±0.2 42.41% ±4.6	0.66 ±0.14 13.07% ±2.47	0.01 ±4.08 $x10^{-4}$	0.64 ±0.24	0.0183 ±0.0409	912.33 ±28.193	3.6316 ±0.1668
B4 - Group 4 Hypophysectomy, 1/3 hepatectomy and PB	6	211.67 ±7.53	160.0 ±6.32	5.67 ±0.52	3.54 ±0.28	1.5066 ±0.1445	2.36 ±0.29 41.85% ±5.24		2.42 ±0.32 42.55% ±2.88	0.89 ±0.24 15.57% ±2.95	0.01 ±0.00	0.58 ±0.21	0.3399 ±0.2336	794.83 ±21.25	4.4633 ±0.1758
B5 - Group 5 Hypophysectomy, 2/3 hepatectomy and saline	9	196.11 ±22.19	152.22 ±20.78	3.38 ±0.60	2.22 ±0.28	1.6661 ±0.1425			2.54 ±0.42 75.51% ±7.45	0.84 ±0.28 24.48% ±7.45	0.01 ±2.50 $x10^{-3}$	0.47 ±0.04	0.0612 ±0.0684	721.44 ±12.685	5.10 ±0.0794
B6 - Group 6 Hypophysectomy, 2/3 hepatectomy and PB	9	187.22 ±10.03	150.0 ±7.50	3.97 ±0.27	2.76 ±0.21	1.9411 ±0.1942			2.94 ±0.25 73.94% ±2.11	1.03 ±0.09 26.05% ±2.11	0.01 ±2.64 $x10^{-3}$	0.42 ±0.07	0.8477 ±0.7009	711.666 ±8.9193	5.32 ±0.0583

Table 3.4.

Table 3.4 Mean nuclear area (um^2) and mean number of nuclei per 10 fields - Section III

Animals	Mean nuclear area (um^2)	Mean no.nuclei/10 fields
A(1) Sham hypophysectomy, laparotomy and saline treatment (Group 1)		
H11	37.280232 (SD ± 9.120918697)	436
H19	41.1119865 (SD ± 11.36145846)	412
H10	42.287886 (SD ± 11.27861452)	426
H20	46.9789695 (SD ± 7.04477089)	410
H21	42.988698 (SD ± 29.18715933)	406

Mean nuclear area = 42.129554 um^2 (SD ± 3.1251045)
Mean no.nuclei/10 fields = 418.0 (SD ± 11.242775)

Animals	Mean nuclear area (um^2)	Mean no.nuclei/10 fields
A(2) Sham hypophysectomy, laparotomy and PB treatment (Group 2)		
H18	40.5520785 (SD ± 10.5520785)	310
H23	43.919406 (SD ± 10.28009636)	322
H24	43.52265 (SD ± 11.0925992)	289
H12	44.343972 (SD ± 11.80109002)	318
H25	42.406542 (SD ± 12.33708059)	282

Mean nuclear area = 42.94893 um^2 (SD ± 1.3606031)
Mean no.nuclei/10 fields = 304.2 (SD ± 15.904716)

Animals	Mean nuclear area (um^2)	Mean no.nuclei/10 fields
A(3) Sham hypophysectomy, 1/3 partial hepatectomy and saline treatment (Group 3)		
H4	40.876992 (SD ± 7.710828282)	375
H0	42.093216 (SD ± 9.593321820)	369
H5	41.850342 (SD ± 8.327936124)	373
H5	48.37086 (SD ± 8.132257536)	377
H3	43.45776 (SD ± 8.526852823)	379
H4	45.660312 (SD ± 9.552270178)	384

Mean nuclear area = 43.718247 um^2 (SD ± 2.5719254)
Mean no.nuclei/10 fields = 376.16667 (SD ± 4.7051984

Animals	Mean nuclear area (um^2)	Mean no.nuclei/10 fields
A(4) Sham hypophysectomy, 1/3 partial hepatectomy and PB treatment (Group 4)		
H1	47.099016 (SD ± 7.783853019)	349
H3	45.035514 (SD ± 6.328452497)	347
H2	46.89693 (SD ± 11.43715594)	343
H1	50.471442 (SD ± 10.01644901)	345
H2	49.388706 (SD ± 9.008161418)	350
H0	46.783836 (SD ± 8.48825439)	249

Mean nuclear area = 47.612574 um^2 (SD ± 1.7996158)
Mean no.nuclei/10 fields = 330.5 (SD ± 36.522824)

Animals	Mean nuclear area (um^2)	Mean no.nuclei/10 fields
A(5) Sham hypophysectomy, 2/3 partial hepatectomy and saline treatment (Group 5)		
H7	40.660074 (SD ± 10.09341239)	350
H2	42.2712 (SD ± 9.10912256)	339
H5	45.67329 (SD ± 10.21798275)	358
H7	47.015586 (SD ± 12.1388591)	350
H1	47.566224 (SD ± 11.26753367)	331

Mean nuclear area = 44.637275 um^2 (SD ± 2.7101613)
Mean no.nuclei/10 fields = 345.6 (SD ± 9.47839650)

Animals	Mean nuclear area (um^2)	Mean no.nuclei/10 fields
A(6) Sham hypophysectomy, 2/3 partial hepatectomy and PB treatment (Group 6)		
H11	46.65591 (SD ± 10.34572927)	343
H9	50.667966 (SD ± 10.08961402)	328
H0	48.980826 (SD ± 9.172468226)	340
H6	47.8512765 (SD ± 11.90308476)	313
H4	48.543282 (SD ± 11.38121229)	329

Mean nuclear area = 48.539852 um^2 (SD ± 1.3226272)
Mean no.nuclei/10 fields = 330.6 (SD ± 10.594338)

Table 3.4 (continued)

Animals	Mean nuclear area (um^2)	Mean no.nuclei/10 fields
B(1) Hypophysectomy, laparotomy and saline treatment (Group 1)		
H12	29.854962 (SD ± 6.731350898)	635
H7	32.40792 (SD ± 7.611464954)	668
H4	32.828778 (SD ± 7.380767792)	685
H8	27.398412 (SD ± 7.435991572)	657
H18	31.106412 (SD ± 6.581605308)	629
H9	35.033184 (SD ± 8.557009625)	667

Mean nuclear area = 31.438278 um^2 (SD ± 2.4060544)
Mean no.nuclei/10 fields = 656.8333 (SD ± 19.462928)

Animals	Mean nuclear area (um^2)	Mean no.nuclei/10 fields
B(2) Hypophysectomy, laparotomy and PB treatment (Group 2)		
H10	29.3859 (SD ± 6.020434304)	418
H19	32.747202 (SD ± 8.272628046)	405
H15	31.36968 (SD ± 6.080944144)	391
H6	30.140478 (SD ± 7.45164853)	341
H5	37.213488 (SD ± 9.198715092)	395
H3	32.354154 (SD ± 7.817083530)	391

Mean nuclear area = 32.201817 um^2 (SD ± 2.5268048)
Mean no.nuclei/10 fields = 390.16667 (SD ± 23.9333357)

Animals	Mean nuclear area (um^2)	Mean no.nuclei/10 fields
B(3) Hypophysectomy, 1/3 partial hepatectomy and saline treatment (Group 3)		
H0	42.534468 (SD ± 8.538172481)	517
H3	38.42415 (SD ± 8.68589851)	525
H4	40.342113 (SD ± 8.842483336)	499
H0	36.77409 (SD ± 7.496575282)	559
H4	38.687418 (SD ± 7.752034802)	572
H7	35.010936 (SD ± 9.248127708)	528

Mean nuclear area = 38.628863 um^2 (SD ± 2.4070691)
Mean no.nuclei/10 fields = 533.3333 (SD ± 24.823824)

Animals	Mean nuclear area (um^2)	Mean no.nuclei/10 fields
B(4) Hypophysectomy, 1/3 partial hepatectomy and PB treatment (Group 4)		
H4	41.477688 (SD + 11.2485285)	428
H1	45.875376 (SD ± 11.40255508)	439
H3	48.947454 (SD ± 11.39543297)	426
H3	39.725658 (SD ± 6.949277165)	482
H0	42.29901 (SD ± 8.33945667)	485
H4	46.181286 (SD ± 9.702871779)	404

Mean nuclear area = 44.084412 um^2 (SD ± 3.1684648)
Mean no.nuclei/10 fields = 444.0 (SD ± 29.804921)

Animals	Mean nuclear area (um^2)	Mean no.nuclei/10 fields
B(5) Hypophysectomy, 2/3 partial hepatectomy and saline treatment (Group 5)		
H9	38.344428 (SD ± 9.141022425)	475
H8	42.541884 (SD ± 10.34005737)	425
H4	34.658676 (SD ± 8.37893391)	423
H14	39.17502 (SD ± 10.0841132)	439
H7	36.75833 (SD ± 10.211591)	436
H3	38.583594 (SD ± 9.405711756)	425
H4	38.474208 (SD ± 9.265447405)	460
H5	36.8570565 (SD ± 9.235786967)	438

Mean nuclear area = 38.17415 um^2 (SD ± 2.1349284)
Mean no.nuclei/10 fields = 440.125 (SD ± 17.265844)

Animals	Mean nuclear area (um^2)	Mean no.nuclei/10 fields
B(6) Hypophysectomy, 2/3 partial hepatectomy and PB treatment (Group 6)		
H6	38.0782817 (SD + 11.86398719)	417
H3	46.592874 (SD ± 12.74564666)	407
H0	40.286493 (SD ± 11.68217173)	400
H5	47.347452 (SD ± 12.64300033)	411
H1	39.645009 (SD ± 10.99202323)	420
H0	42.76251 (SD ± 11.28825449)	411
H2	41.3984295 (SD ± 10.18752141)	405
H1	39.3048 (SD ± 9.150195281)	430
H8	39.197268 (SD ± 9.265885828)	415

Mean nuclear area = 41.623072 um^2 (SD ± 3.1336638)
Mean no.nuclei/10 fields = 414.0 (SD ± 9.8092926)

Table 3.5 Mean distribution of nuclei* into ploidy classes: partial hepatectomy group - Section III

Group	2n classes %	4n and above classes %
A. Sham hypophysectomy group		
A(1) Sham hypophysectomy, laparotomy and saline treatment (Group 1)	57.8	42.2
A(2) Sham hypophysectomy, laparotomy and PB treatment (Group 2)	44.0	56.0
A(3) Sham hypophysectomy, 1/3 partial hepatectomy and saline treatment (Group 3)	40.84	59.16
A(4) Sham hypophysectomy, 1/3 partial hepatectomy and PB treatment (Group 4)	17.84	82.16
A(5) Sham hypophysectomy, 2/3 partial hepatectomy and saline treatment (Group 5)	36.8	63.2
A(6) Sham hypophysectomy, 2/3 partial hepatectomy and PB treatment (Group 6)	20.6	79.4
B. Hypophysectomy group		
B(1) Hypophysectomy, laparotomy and saline treatment (Group 1)	84.34	15.66
B(2) Hypophysectomy, laparotomy and PB treatment (Group 2)	84.67	15.33
B(3) Hypophysectomy, 1/3 partial hepatectomy and saline treatment (Group 3)	57.5	42.5
B(4) Hypophysectomy, 1/3 partial hepatectomy and PB treatment (Group 4)	36.17	63.83
B(5) Hypophysectomy, 2/3 partial hepatectomy and saline treatment (Group 5)	60.875	39.125
B(6) Hypophysectomy, 2/3 partial hepatectomy and PB treatment (Group 6)	52.23	47.77

* As determined by the Quantimet 720 image analyser described in the Material and Methods Section 2.7.1.1

Table 3.6 Percentage of nuclei per group exhibiting given integrated nuclear density in arbitrary units (A.U.) as determined by integrated microdensitometry for Section III

Density	Group B(1) n=5 % nuclei	Group B(2) n=4 % nuclei	Group B(5) n=3 % nuclei	Group B(6) n=3 % nuclei
0.5 - 1.0	0.4			
1.0 - 1.5	2.0	1.1		
1.5 - 2.0	8.0	4.3		
2.0 - 2.5	13.2	8.6	0.67	
2.5 - 3.0	15.6	12.9		
3.0 - 3.5	17.2	19.9		1.33
3.5 - 4.0	19.2	19.9	2.0	
4.0 - 4.5	11.6	9.14	6.67	0.67
4.5 - 5.0	5.2	4.84	9.33	0.67
5.0 - 5.5	4.8	4.3	6.67	7.33
5.5 - 6.0	2.0	2.67	10.0	20.66
6.0 - 6.5		3.23	8.0	22.66
6.5 - 7.0	0.4	2.69	6.0	18.66
7.0 - 7.5	0.4	2.69	6.67	6.6
7.5 - 8.0		2.69	6.0	1.33
8.0 - 8.5		0.5	9.33	4.66
8.5 - 9.0		0.5	10.0	1.33
9.0 - 9.5			9.33	1.33
9.5 - 10.0			4.67	2.0
10.0 - 10.5			2.0	1.33
10.5 - 11.0				2.0
11.0 - 11.5				2.66
11.5 - 12.0				0.66
12.0 - 12.5				1.33
12.5 - 13.0				2.0
13.0 - 13.5			1.33	0.67
13.5 - 14.0				
16.5 - 17.0			0.67	
17.0 - 17.5				
17.5 - 18.0			0.67	

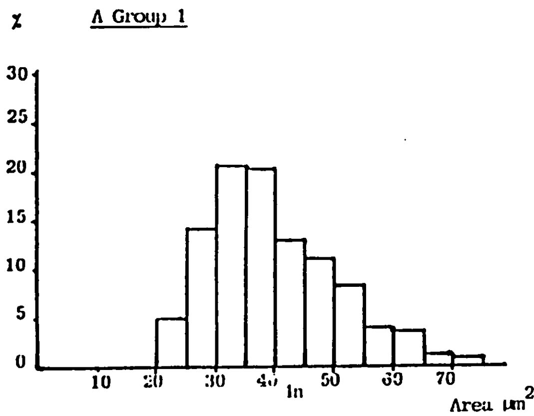
B(1) = Hypophysectomy, laparotomy and saline treatment
B(2) = Hypophysectomy, laparotomy and PB treatment
B(5) = Hypophysectomy, 2/3 partial hepatectomy and saline treatment
B(6) = Hypophysectomy, 2/3 partial hepatectomy and PB treatment

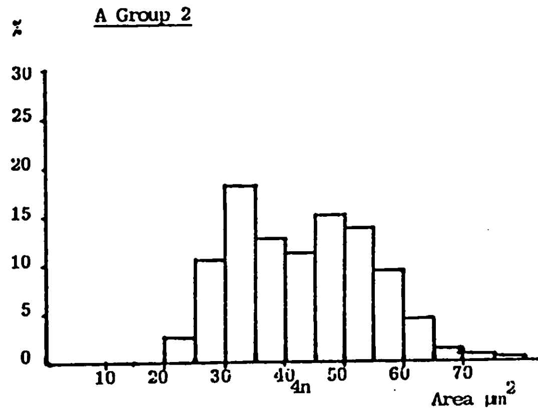
n = Number of animals investigated per group

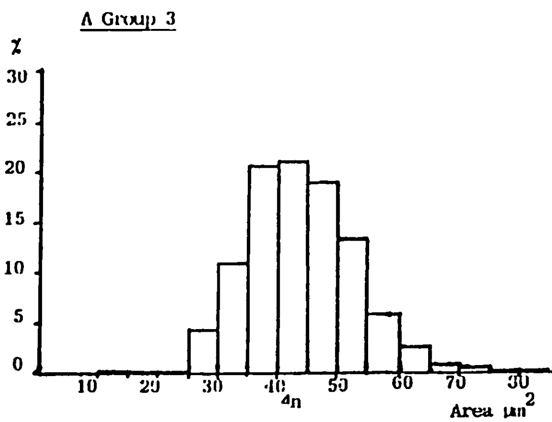
Fig 3.1.

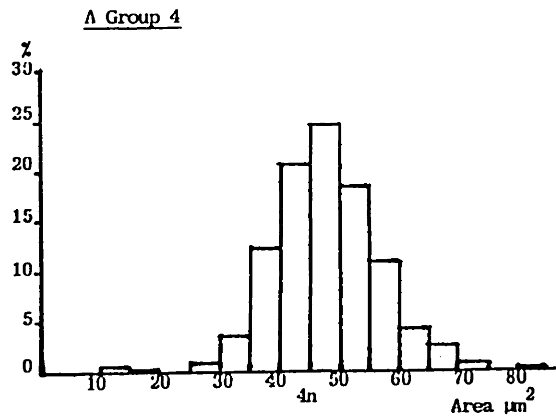
Figure 3.1 The mean percentage distribution of nuclei in size ranges of 5 um^2 classes from 10 - 100

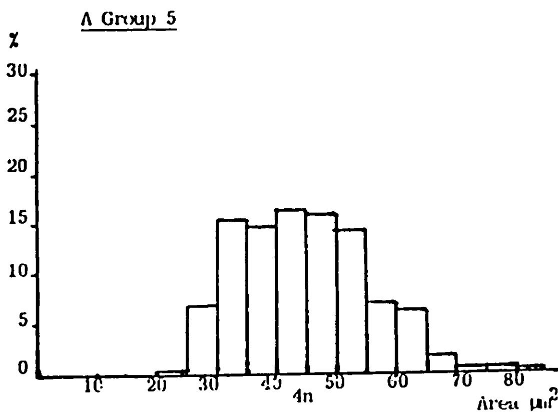
A. Group 1 = Sham hypophysectomy, laparotomy and saline treatment (controls) (n=5)
A. Group 2 = Sham hypophysectomy, laparotomy and PB treatment (n=5)
A. Group 3 = Sham hypophysectomy, 1/3 partial hepatectomy and saline treatment (n=6)
A. Group 4 = Sham hypophysectomy, 1/3 partial hepatectomy and PB treatment (n=6)
A. Group 5 = Sham hypophysectomy, 2/3 partial hepatectomy and saline treatment (n=5)
A. Group 6 = Sham hypophysectomy, 2/3 partial hepatectomy and PB treatment (n=5)











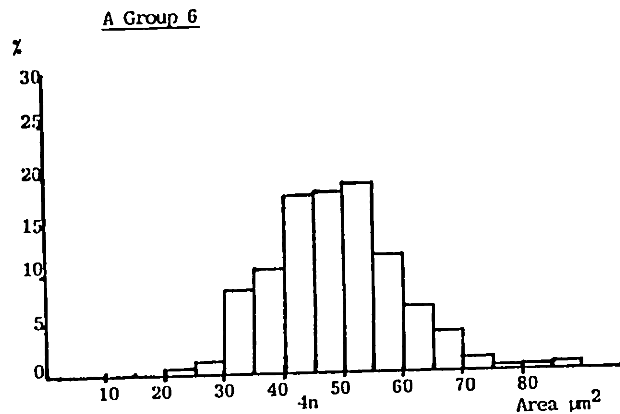
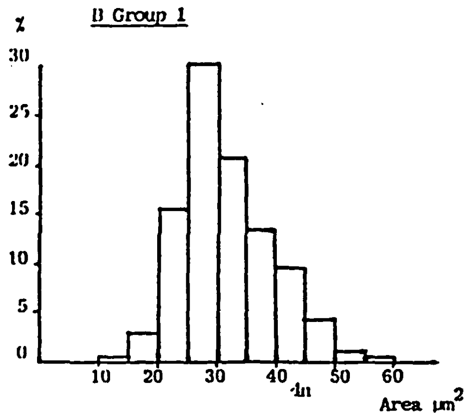
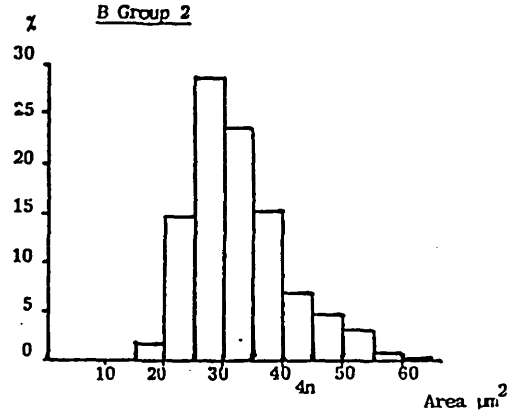


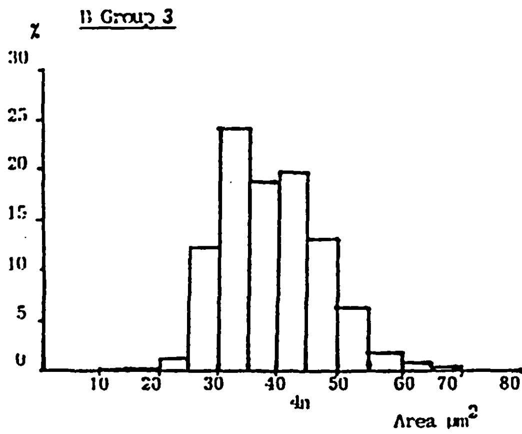
Figure 3.1 (contd)

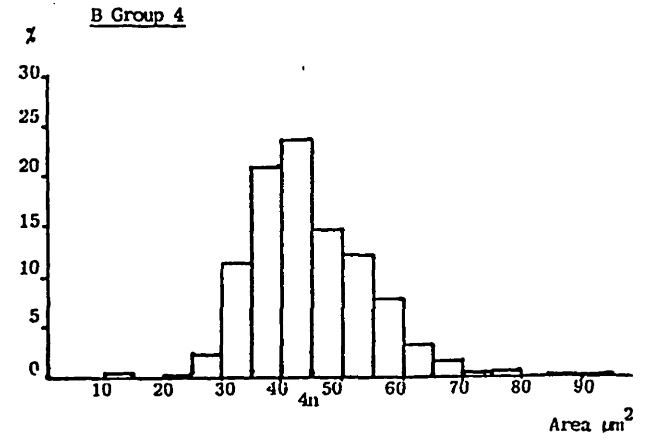
B. Group 1 = Hypophysectomy, laparotomy and saline treatment (controls) (n=6)
B. Group 2 = Hhypophysectomy, laparotomy and PB treatment (n=6)
B. Group 3 = Hypophysectomy, 1/3 partial hepatectomy and saline treatment (n=6)
B. Group 4 = Hypophysectomy, 1/3 partial hepatectomy and PB treatment (n=6)
B. Group 5 = Hypophysectomy, 2/3 partial hepatectomy and saline treatment (n=8)
B. Group 6 = Hypophysectomy, 2/3 partial hepatectomy and PB treatment (n=9)

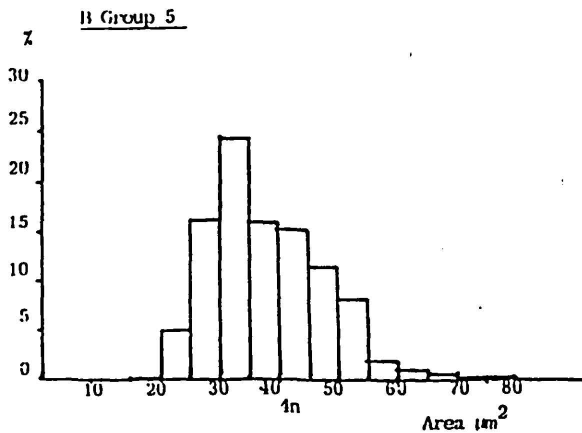
4n = Nuclei of 4n and above ploidy classes at 40 um^2 and above diameter
n = Number of animals investigated per group

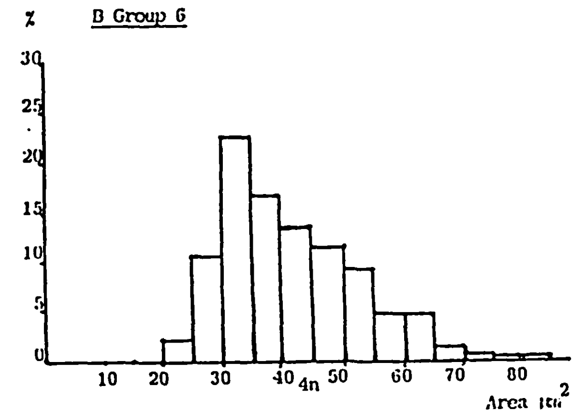












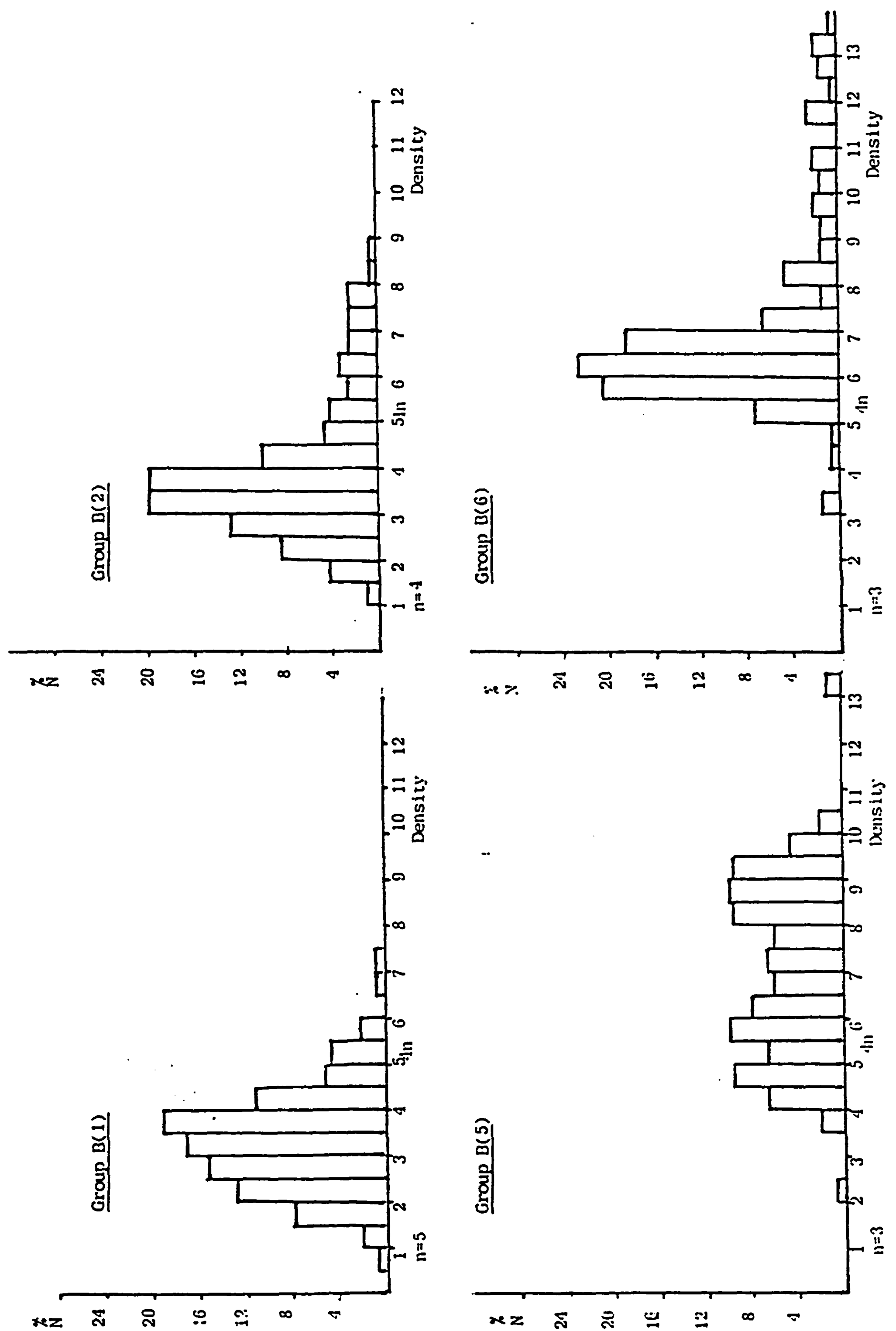


Figure 3.2 Histograms of nuclear integrated density in arbitrary units (A.U.) for partial hepatectomy groups

n = number of animals investigated per group
%N = percentage of nuclei per group with arbitrary density
Groups:
B(1) = Hypophysectomy, laparotomy and saline treatment (controls - Group 1)
B(2) = Hypophysectomy, laparotomy and PB treatment (Group 2)
B(5) = Hypophyectomy, 2/3 partial hepatectomy and saline treatment (Group 5)
B(6) = Hypophyectomy, 2/3 partial hepatectomy and PB treatment (Group 5)

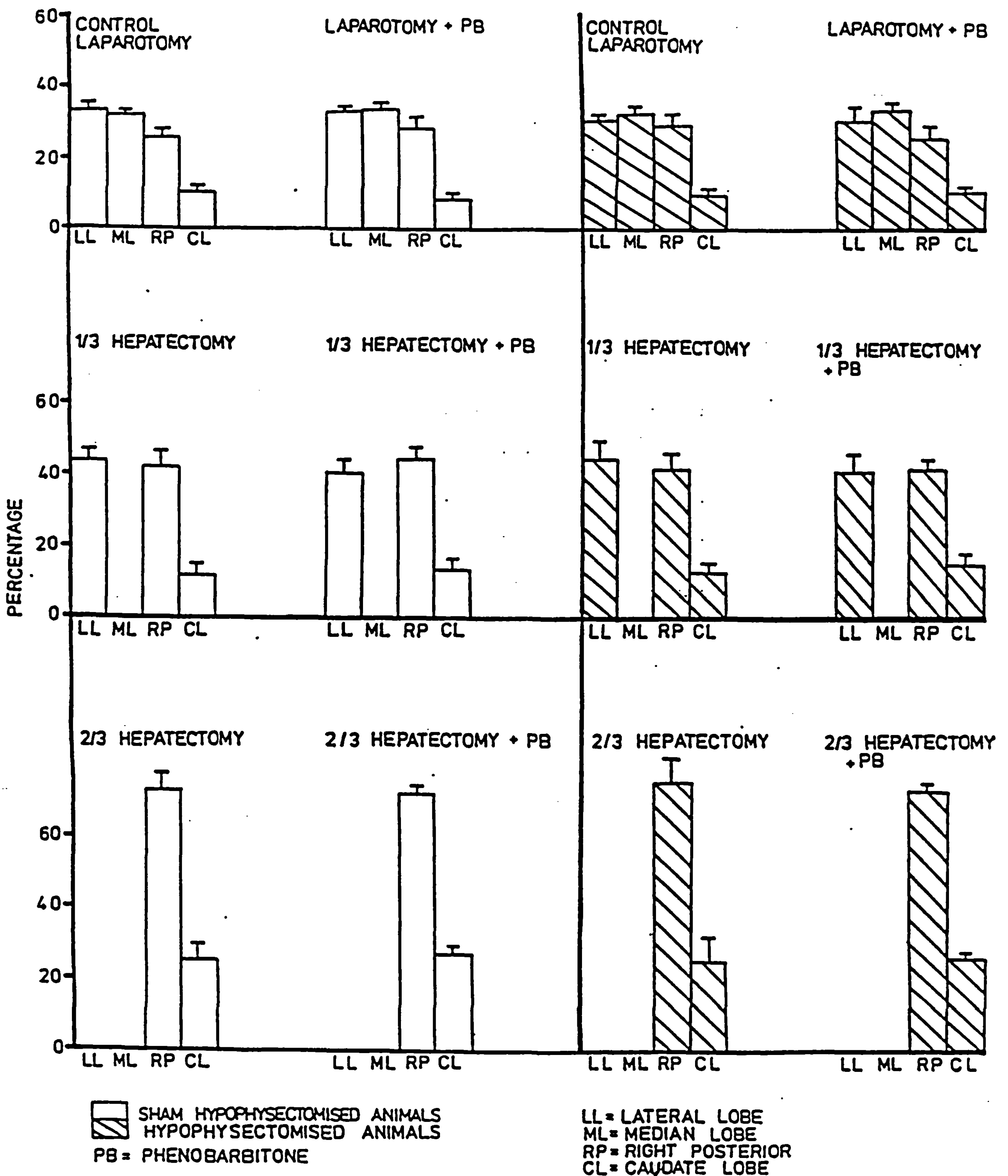


Figure 3.3 The effect of 1/3 and 2/3 partial hepatectomy with or without PB treatment on the percentage contribution by individual liver lobes to the total liver weight in intact and hypophysectomised rats

CHAPTER 4

THE RESPONSE BY THE LIVER TO PB IN INTACT RATS

Results presented in this chapter indicate the response by the liver to PB treatment in intact animals and the response to PB in intact animals following partial hepatectomy.

4.1 Response by the liver to PB following laparotomy

Results from two separate groups of intact rats administered PB will be presented in this chapter as one group was included in Section III together with groups undergoing partial hepatectomy and the second group was included on a subsequent occasion with those groups undergoing portal vein ligation (Section IV). The effect of PB on the liver was compared with sham hypophysectomised controls and the purpose of this part of the study was to confirm that DNA synthesis is induced in intact laparotomised animals following PB treatment.

4.1.1 Section III

Details of results for individual animals are given in Table 3.1 and for group means in Table 3.3. The mean percentage contribution by each liver lobe to the whole liver weight per group following PB treatment is found in Figure 3.3. Details of the mean nuclear area (um^2) and the number of nuclei/10 fields for each animal investigated per group using the Quantimet 720 image analyser are presented in Table 3.4. The mean percentage distribution of nuclei into ploidy classes per group are given in Table 3.5 and histograms for each group demonstrating the percentage distribution of nuclei into size ranges, separating them into ploidy classes are presented in Figure 3.1.

4.1.1.1 Sham hypophysectomy, laparotomy and PB treatment (Group 2) compared with sham hypophysectomy, laparotomy and saline treatment (Group 1 - controls)

Effect on liver

(i) Relative liver weight

In Group 2 following 8 days PB treatment, the mean relative liver weight was significantly increased ($p < 0.01$) compared with controls (Group 1) where the mean values were 4.47% (SD ±0.28, n = 6) and 3.35% (SD ±0.09) respectively. This was an increase by 33%. The right posterior lobe in Group 2 contributed to the liver by a mean of 27.74% (SD ±3.93, n = 6) compared with 25.36% (SD ±2.27) in Group 1. These were not significantly different.

However the mean relative right posterior weight in Group 2 was 1.16% (SD ±0.08, n = 6) which was significantly greater ($p < 0.01$) than 0.85% (SD +0.07, n = 6) in the controls (Group 1), an increase by 36.9%.

Investigation of the right posterior lobe

Measurements used to reflect DNA synthesis and cell proliferation

a) Mean nuclear area

The mean nuclear area for Group 2 was 42.95 um^2 (SD ± 1.36, n = 5) which was not significantly different from the mean nuclear area in Group 1 (42.13 um^2, SD ±3.13, n = 5). This unexpected concordance is discussed later.

b) Nuclear ploidy classes

In Group 2 44% of the nuclei were in the 2n ploidy classes and 56% were in the 4n and above ploidy classes. This was a significant increase ($p < 0.05$) following PB treatment compared with Group 1 (57.8% / 42.2%).

c) Mitoses

At the end of the 28 day study the mean number of mitoses per 1,000 cells following PB treatment in Group 2 was 0.6833 (SD $\pm$0.14, n = 6) which was significantly higher ($p < 0.01$) than the controls (Group 1) (0.1081 SD $\pm$0.12, n = 6).

Determination of cell volume

Following PB treatment there was a significant increase in cell volume ($p < 0.01$) in Group 2 treated with PB compared with the controls (Group 1) where the mean estimated cell volumes were 7.95×10^{-5} mm^3 (SD $\pm$0.21) and 6.60×10^{-5} mm^3 (SD $\pm$0.2749, n = 6) respectively, an increase of 20.44%.

Mean number of nuclei/10 fields

There were 304.2 (SD + 15.90, n = 5) in Group 2 following PB treatment which was a significant reduction ($p < 0.01$) compared with Group 1 (418.0, SD $\pm$ 11.24, n = 5).

4.1.1.2 Summary of results

Thus, PB induced liver enlargement as identified by the increase in mean relative weight compared with controls. Adaptive growth was identified in the right posterior lobe which was significantly heavier compared with controls, the response to PB was a combination of hyperplasia and hypertrophy. There was evidence of increased DNA synthesis, identified by a significant shift in 4n and above ploidy classes compared with controls along with cell replication as identified by an increase in mitoses per 1,000 cells compared with controls. Hypertrophy was identified by an increase in mean estimated cell volume

compared with controls, this cellular enlargement resulting in fewer nuclei per 10 fields.

An increase in mean nuclear area is also known to occur as the result of an increase in DNA synthesis reflecting a shift in nuclear ploidy. The small but statistically insignificant difference found in the mean nuclear area between these two groups may be due to experimental error as described previously (Ingram and Grasso, 1985). If nuclei with ill-defined margins were included in the recording, (e.g. in Group 1), this may have led to inaccuracy as these nuclei are more likely to be tangentially cut (Ingram and Grasso, 1985). This would result in the mean nuclear area being larger than it actually was. Another likely explanation is that there was variability of the mean nuclear area for individual animals within the sham hypophysectomy laparotomy control group (Group 1). When looking at the "mean nuclear area" of the individual animals, one animal had an obviously larger mean nuclear area than the rest of the group which might account for the statistical similarity between the two group means although the ploidy differences are obvious.

4.1.2 Section IV

4.1.2.1 Sham hypophysectomy, laparotomy and PB treatment (Group 2) compared with sham hypophysectomy, laparotomy and saline treatment (Group 1 - controls)

These two groups were included with the portal vein ligation groups, forming part of Section IV. These experiments were not concurrent with those described in subsection 4.1.1.2 and are therefore reported separately.

Changes similar to those already described in 4.1.1.2 occurred following PB treatment in intact animals compared with controls. Liver enlargement was again the result of DNA synthesis, cell multiplication and cell enlargement.

Details for individual animals are given in Table 6.1, group means in Table 6.3 and the mean percentage contribution by individual liver lobes to the whole liver weight following PB treatment compared with controls demonstrated in Figure 6.3. The mean nuclear area (um^2) and nuclei/10 fields for each animal per group examined using the Quantimet 720 image analyser is given in Table 6.4 and the summary of distribution of nuclei into ploidy classes per group for Section IV is given in Table 6.5. Figure 6.1 demonstrates the mean percentage of nuclei in 5 um^2 size ranges from 10-100 per group, thus separating the nuclei into ploidy classes.

The total liver was 22.7% larger following PB treatment (4.48% vs. 3.65%, controls, $p < 0.01$). Obvious adaptive growth occurred within the right posterior lobe (R.P. relative weight 1.12% vs. 0.92%, controls ($p < 0.05$) an increase of 21.2^%), with each liver lobe contributing to the whole liver weight with a similar distribution to controls (R.P. 25.46% $\pm$ SD 1.68 vs. 25.34% SD 1.68, controls).

The mean nuclear area was enlarged by 16.87% compared with controls (36.66 um^2 vs. 31.37 um^2, $p < 0.05$) with an obvious increase in the number of 4n and above ploidy class nuclei (35.33% vs. 13.83% controls, $p < 0.05$). Following 8 days PB treatment the mitotic index was increased (0.6314 vs. 0.1180 controls, $p < 0.01$).

Estimated cell volume was increased (10.58×10^{-5} mm^3 vs. 7.21×10^{-5} mm^3 controls, $p < 0.01$) by 46.73% and there were fewer nuclei/10 fields (312.0 vs. 466.5 controls, $p < 0.01$), most likely the result of the cell enlargement.

The interesting difference in this group is that additionally the mean nuclear area was significantly enlarged ($p < 0.05$) compared with controls whereas in Group 2 in Section III this was not detectable. A possible reason for this is that unlike Group 1 in Section III the mean nuclear area for individual animals in the control group in Section IV showed less variability. Their values were all smaller than those obtained following PB treatment (Group 2) and therefore the mean nuclear area for the control group was significantly smaller ($p < 0.05$) than the mean value obtained for Group 2.

Another difference between the PB treated animals from Sections III and IV is that while 56% of nuclei were in the 4n and above ploidy classes in Group 2 from Section III and 35.33% in Group 2 from Section IV, the shift towards polyploidy was greater in this latter group when compared with respective controls. The shift was from 13.83% of nuclei in the 4n and above classes in Section IV (155.45% increase) compared with 42.2% in the control group from Section III (33.3% increase). This disparity in 2n:4n distribution in these control animals resulting in a smaller shift towards polyploidy in Group 2 in Section III may be an alternative reason to that already suggested for the mean nuclear area only being marginally increased compared with controls and therefore statistically insignificant. However the ability to detect changes in mean nuclear area in PB treated animals in this

study using a relatively low dose PB is clearly demonstrated and along with the shifts in nuclear ploidy reflects changes in DNA synthesis.

PB induced DNA synthesis and cell multiplication in animals in which sham hypophysectomy and laparotomy had been performed.

The response to PB by the liver was similar in some respects to that detected following partial hepatectomy (Bucher, 1963) in that DNA synthesis could be detected by changes in ploidy.

In comparison however, DNA synthesis could also be detected by an increased mean nuclear area and cell replication by increased mitoses following 8 days PB treatment whereas these changes reflecting an increase in DNA content were not detectable 21 days post-hepatectomy. Again the degree of increase in 4n and above ploidy classes (40.28% and 49.76% increase following 1/3 and 2/3 hepatectomy, respectively) may account for the mean nuclear area being similar to control values despite the obvious shift in ploidy.

Cellular enlargement was detected after 8 days PB treatment and 21 days post-2/3 partial hepatectomy suggesting hypertrophy had been induced and fewer nuclei/10 fields reflected cell enlargement.

4.2 Response by the liver to PB following 1/3 or 2/3 partial hepatectomy

The purpose of presenting these results is to demonstrate that partial hepatectomy does not influence the way in which PB induces liver enlargement.

4.2.1 Sham hypophysectomy, 1/3 partial hepatectomy and PB treatment (Group 4) compared with sham hypophysectomy 1/3 partial hepatectomy and saline treatment (Group 3)

After 8 days PB treatment the liver remnant was enlarged (4.10% vs. 3.44% in Group 3, $p < 0.01$) with obvious adaptive growth in the right posterior lobe (R.P. relative weight 1.82% vs. 1.48% Group 3, $p < 0.01$).

DNA synthesis was detected by an increased mean nuclear area (47.6 um^2 vs. 43.72 um^2 Group 3, $p < 0.05$) an increase in number of 4n and above ploidy classes (82.16% vs. 59.2% Group 3, $p < 0.01$) and cell replication by an increased mitotic index (0.8435 vs. 0.2380 Group 3, $p < 0.01$).

Estimated cell volume was increased (7.02×10^{-5} mm^3 vs. 6.33×10^{-5} mm^3 Group 3, $p < 0.01$) and there were fewer nuclei/10 fields (330.5 vs. 376.2 Group 3, $p < 0.01$), most likely reflecting the cell enlargement.

4.2.2 Sham hypophysectomy, 2/3 partial hepatectomy and PB treatment (Group 6) compared with sham hypophysectomy, 2/3 partial hepatectomy and saline treatment (Group 5)

Similar changes occurred after PB treatment (Group 6) as compared with saline treatment with the whole of the liver remnant enlarging (3.12% vs. 2.61% Group 5, $p < 0.01$) and R.P. relative weight (2.27% vs. 1.80% Group 5, $p < 0.01$).

The mean nuclear area increased (48.54 um^2 vs. 44.64 um^2 Group 5, $p < 0.05$), more nuclei were in the 4n and above ploidy classes (79.4% vs. 63.2% Group 5, $p < 0.01$) and mitoses were detected (1.5043 vs. 0.1194 Group 5, $p < 0.01$).

Cell volumes were slightly larger (7.93×10^{-5} mm^3 vs. 7.50×10^{-5} mm^3 Group 5, $p < 0.05$) reflected by fewer nuclei per 10 fields (330.6 vs. 345.6 Group 5, $p < 0.01$).

4.3 Summary

Thus the effect of PB in the liver of rats following 1/3 and 2/3 partial hepatectomy is similar to that following laparotomy, the drug inducing further adaptive growth compared with saline treatment. These results confirm other studies that the liver enlargement is a combination of hyperplasia and hypertrophy (Schulte-Hermann, 1974; Schulte-Hermann et al., 1977). DNA synthesis was identified by (i) an increase in mean nuclear area, (ii) a shift in 4n and above ploidy classes and (iii) an increase in mitoses per 1,000 cells compared with saline treated animals.

Hypertrophy is the result of cellular enlargement and was identified by an increase in mean estimated cell volume, with fewer nuclei per 10 fields most probably reflecting this change in cell size.

Again these results confirm that the measurements chosen are clearly able to demonstrate changes in DNA synthesis.

CHAPTER 5

THE RESPONSE BY THE LIVER TO PB IN HYPOPHYSECTOMISED RATS

The results presented in this chapter indicate the response by the liver in hypophysectomised rats to PB, the effect that partial hepatectomy may have on this response and to examine the findings of Schulte-Hermann et al. (1977). They suggested that in hypophysectomised rats PB does not induce DNA synthesis but this ability is restored following partial hepatectomy.

Details of the results for individual animals and group means are given in Tables 3.2 and 3.3. The percentage contribution made by individual liver lobes to the whole liver weight is presented by histograms in Figure 3.3. For details of mean nuclear area (um^2) and nuclei/10 fields for each animal investigated using the Quantimet 720 image analyser and group means see Table 3.4, the distribution of nuclear ploidy per group is summarised in Table 3.5 and demonstrated by the mean percentage distribution of nuclei size ranges 10-100 in Figure 3.1. Results of integrated microdensitometry are presented in Table 3.6 and Figure 3.2.

5.1 Response by the liver to PB in hypophysectomised rats following laparotomy

Results for the hypophysectomy control group have been presented previously in Chapter 3, subsection 3.1.2.1.

5.1.1 Hypophysectomy, laparotomy and PB treatment (Group 2) compared with hypophysectomy, laparotomy and saline treatment (Group 1 - controls)

Effect on liver

(i) Relative liver weight

Following 8 days PB treatment the mean relative liver weight was significantly increased ($p < 0.01$) in Group 2 compared with Group 1 where the mean relative liver weights were 3.89% (SD +0.17, n = 6) and 2.97% (SD +0.0122, n = 6) respectively, PB thus induced an increase in relative liver weight of 31.15%.

(ii) Right posterior lobe

The contribution made by the right posterior lobe in these PB treated animals was a mean of 25.42% (SD ±3.57, n = 6) which was not significantly different compared with Group 1 where the mean was 27.22% (SD ±2.73, n = 6).

There was a significant increase ($p < 0.01$) in the mean relative weight of the right posterior lobe (0.99% SD ±0.16, n = 6) in Group 2 compared with 0.81% (SD ±0.10, n = 6) in Group 1. This is an increase by 22.61%.

Investigation of the right posterior lobe

Measurements used to reflect DNA synthesis and cell proliferation

a) Mean nuclear area

The mean nuclear area was 32.20 um^2 (SD ± 2.53, n = 6) in Group 2. This was not significantly different compared with Group 1 controls (31.44 um^2, SD ± 2.41, n = 6).

b) Nuclear ploidy classes

84.67% of nuclei were in the 2n ploidy classes and 15.3% in the 4n and above ploidy classes. There was no significant difference compared with Group 1.

c) Integrated microdensitometry

80.65% of nuclei had values of integrated density ranging from 0-5 (A.U.), again with a peak occurring between integrated density 3-4 (A.U.), correlating with the peak for the 2n ploidy classes. 19.35% of nuclei had integrated density values of 5.0 (A.U.) and above ranging from 5.0-9.0 (A.U.), these falling into the 4n and above ploidy classes.

There was no significant difference between the ploidy distribution compared with hypophysectomised controls confirming that following PB treatment there was no detectable increase in DNA content. These results confirm the results of the data using the Quantimet 720 image analysing computer in which no increased DNA synthesis following PB treatment in hypophysectomised animals was recorded, in contrast to intact animals.

d) Mitoses

Following 8 days PB treatment mitoses were not detected in the right posterior lobe. This was not significantly different compared with Group 1 where the mean number of mitoses per 1,000 cells for both groups were 0.00 (SD $\pm$0.00, n = 6) and 0.0151 (SD $\pm$0.03, n = 6) respectively.

Determination of cell volume

At the end of the 8 day PB treatment there was a significant increase in mean estimated cell volume ($p < 0.01$) in Group 2 in comparison to Group 1 in which the mean estimated volumes were

5.98×10^{-5} mm^3 (SD ± 0.17, n = 6) and 2.78×10^{-5} mm^3 (SD ± 0.16) respectively, this being an increase by 114.90%. This mean cell volume was however still significantly smaller ($p < 0.01$) than the sham hypophysectomy controls (6.60×10^{-5} mm^3 SD ± 0.27, n = 6) indicating that the atrophic effects of the hypophysectomy can still be detected despite PB treatment.

Mean number of nuclei/10 fields

The mean number of nuclei per 10 fields was 390.2 (SD + 23.9, n = 6). This is significantly fewer ($p < 0.01$) than Group 1 (656.8, SD + 19.5, n = 6).

5.1.2 Summary

Following PB treatment to hypophysectomised animals adaptive response occurred in the liver confirmed by an increase in relative weight of the liver and right posterior lobe compared with hypophysectomy controls (Group 1).

However there was no evidence of DNA synthesis as there was no change in the mean nuclear area or shift in ploidy classes and no evidence for cell replication with no mitoses detected in the right posterior lobe. Cell enlargement was detected. These results indicated that the DNA content remained the same as the controls (Group 1) and adaptive growth was the result of hypertrophy alone consistent with other studies (Schulte-Hermann et al., 1977). An increase in mean estimated cell volume would account for the fewer number of nuclei in the PB treated group compared with controls.

5.2 Response by the liver to PB in hypophysectomised rats following partial hepatectomy

In order to demonstrate the effect of PB on the liver in hypophysectomised animals following 1/3 or 2/3 partial hepatectomy the results from these animal groups were compared with the response to PB in hypophysectomised animals in which laparotomy had been performed (Group 2), just described.

5.2.1 Hypophysectomy, laparotomy and PB treatment (Group 2) compared with hypophysectomy, 1/3 partial hepatectomy and PB treatment (Group 4)

Following 1/3 partial hepatectomy there was clear enlargement of the right posterior lobe (R.P. 42.55% vs. 25.42% Group 2, $p < 0.01$) with a relative weight of the R.P. 1.51% instead of 0.99% in Group 2 ($p < 0.01$).

DNA synthesis was detected by an increased mean nuclear area ($44.08\ um^2$ vs. $32.20\ um^2$ Group 2, $p < 0.01$) and an increase in number of nuclei in 4n and above ploidy classes (63.83% vs. 15.33% Group 2, $p < 0.01$). Cell replication was confirmed by an increased mitotic index (0.3399 vs. 0.0 Group 2, $p < 0.01$).

Estimated cell volume was smaller than Group 2 ($4.46 \times 10^{-5}\ mm^3$ vs. $5.98 \times 10^{-5}\ mm^3$ $p < 0.01$) and there were more nuclei/10 fields (444.0 vs. 390.2 Group 2, $p < 0.01$), this effect most probably the result of cell replication.

5.2.2 Hypophysectomy, laparotomy and PB treatment (Group 2) compared with hypophysectomy, 2/3 partial hepatectomy and PB treatment (Group 6)

After 2/3 partial hepatectomy obvious enlargement occurred in the right posterior lobe (R.P. 73.94% vs. 25.42% Group 2, $p < 0.01$) with a relative liver weight of the R.P. 1.94% instead of 0.99% ($p < 0.01$).

An increased mean nuclear area was detected (41.62 um^2 instead of 32.20 um^2 Group 2, $p < 0.01$) and there were more nuclei in the 4n and above ploidy classes (47.8% vs. 15.33% Group 2, $p < 0.01$).

Integrated microdensitometry demonstrated that in Group 6 there was a large number of nuclei with higher values of integrated density compared with Group 2, ranging from 3.0-13.5 (A.U.) with a peak occurring at 6.0-6.5 (A.U.) and a second at 8.0-8.5 (A.U.). 2.67% of the nuclei had integrated density values in the 2n ploidy classes and 97.33% in the 4n and above ploidy classes in contrast to 80.65% and 19.35% respectively in Group 2. In Group 6 the number of nuclei with integrated densities in 4n and above ploidy classes was significantly increased ($p < 0.05$) compared with hypophysectomy controls (Group 1) and at the 10% level significantly increased ($p < 0.1$) by 403% compared with Group 2.

As there was no significant difference in the distribution of integrated nuclear density between Group 1 and 2 this data confirms statistically that following 2/3 partial hepatectomy in hypophysectomised animals the ability of PB to induce DNA synthesis is restored and confirms the Quantimet 720 data.

There was an increased mitotic index compared with Group 2 (0.8477 vs. 0.0 Group 2, $p < 0.01$) indicating cell replication.

The mean estimated cell volume was smaller (5.32×10^{-5} mm^3 vs. 5.98×10^{-5} mm^3 Group 2, $p < 0.01$) and there were more nuclei/10 fields (414 vs. 390 Group 2, $p < 0.01$), this most probably the effect of cell multiplication.

In order to confirm that in hypophysectomised animals this ability by PB to induce DNA synthesis following partial hepatectomy is independent of other factors and is the result of the action of the drug on the liver, results were compared between Groups 3 and 4, and Groups 5 and 6.

5.2.3 Hypophysectomy, 1/3 partial hepatectomy and PB treatment (Group 4) compared with hypophysectomy, 1/3 partial hepatectomy and saline treatment (Group 3)

The whole of the liver remnant was 20.4% larger following PB treatment (3.54% vs. 2.94% Group 3, $p < 0.01$) with a relative weight of the R.P. lobe 1.51% instead of 1.25% in Group 3 ($p < 0.01$).

The mean nuclear area was enlarged (44.08 um^2 vs. 38.63 um^2 Group 3, $p < 0.05$), there was a greater number of nuclei in the 4n and above ploidy classes (63.8% vs. 42.5% Group 3, $p < 0.05$) and the mitotic index was increased (0.3399 vs. 0.0183 Group 3, $p < 0.01$).

The mean estimated volume was increased (4.46×10^{-5} mm^3 vs. 3.63×10^{-5} mm^3 Group 3, $p < 0.01$) and there were fewer nuclei/10 fields (444.0 vs. 533.3 Group 3, $p < 0.01$).

5.2.4 Hypophysectomy, 2/3 partial hepatectomy and PB treatment (Group 6) compared with hypophysectomy, 2/3 partial hepatectomy and saline treatment (Group 5)

Similarly in Group 6 the liver remnant enlarged by 21% following PB treatment (2.76% instead of 2.22% Group 5, $p < 0.05$) with an increased

relative weight of the R.P. lobe 1.94% compared with 1.66% (Group 5), $p < 0.01$).

Evidence of increased DNA synthesis was based on an increased mean nuclear area (41.62 um^2 compared with 38.17 um^2 Group, $p < 0.01$) and an increase in nuclei in 4n and above ploidy classes (47.8% compared with 39.13% Group 5, $p < 0.05$), confirmed by integrated microdensitometry.

Although it was not possible to analyse statistically at the 5% level, using the Wilcoxon 2 sample test, it appears that PB did induce DNA synthesis in Group 6 and this was not just the effect of the 2/3 partial hepatectomy (as in Group 5). There was a 19.66% increase in the number of nuclei with values of integrated densities (A.U.) in the 4n and above ploidy classes compared with Group 5. The values ranged from 5.0-13.5 (A.U.) with an obvious peak between 6.0-6.5 (A.U.). This was a significant increase ($p < 0.1$) at the 10% level.

The mitotic index was increased (0.8477 vs. 0.0612 Group 5, $p < 0.01$) demonstrating cell replication.

The mean estimated cell volumes were similar (5.32×10^{-5} mm^3 SD ± 0.06 vs. 5.10×10^{-5} mm^3 SD ± 0.08 Group 5) but there were fewer nuclei/10 fields in Group 6 (414 vs. 440.1 Group 5, $p < 0.01$) which cannot readily be explained.

5.2.5 <u>Summary</u>

These results therefore confirm the investigations by Schulte-Hermann et al. (1977) that PB fails to induce DNA synthesis in hypophysectomised rats but its ability is restored following partial hepatectomy.

In addition, while following 1/3 partial hepatectomy (Group 3) the mass was restored to the control value (Group 1), following PB treatment (Group 4) further adaptive growth occurred in the liver which was detected by increased mean relative weights of the liver and right posterior lobe. After 2/3 partial hepatectomy (Group 5) while the liver mass reached 77.84% of control values by 21 days, it reached 93.01% of control values after PB treatment (Group 6). This adaptive growth was a combination of cell hyperplasia and hypertrophy. Hyperplasia was detected by the evidence of DNA synthesis (a larger mean nuclear area, a larger shift towards polyploidy and cell multiplication).

PB induced hypertrophy in Group 4 as detected by larger cell volumes compared with Group 3. In Group 6 cell size was the same as in Group 5 but they were both significantly enlarged compared with controls.

The response by the liver to PB treatment in hypophysectomised animals following partial hepatectomy is similar to that following laparotomy or partial hepatectomy in intact animals.

CHAPTER 6

THE RESPONSE BY THE LIVER TO 1/3 AND 2/3 PORTAL VEIN LIGATION WITH OR WITHOUT PB TREATMENT

For details of the results from each group see Tables 6.1 and 6.2 and for a summary of the group means Table 6.3. Histograms demonstrating the percentage contribution made by the individual liver lobes to the whole liver weight following hypophysectomy, PB treatment and/or portal vein ligation both in intact and hypophysectomised rats are presented in Figure 6.3.

Details of the mean nuclear area (um^2) for each animal investigated per group using the Quantimet 720 image analyser are given in Table 6.4 and a summary of percentage distribution of nuclei into ploidy classes per group is given in Table 6.5. Histograms demonstrating the mean percentage distribution of nuclei of a given area separating the nuclei into ploidy classes per group are given in Figure 6.1.

The results and histograms of the integrated microdensitometry investigation are also presented in Table 6.6 and Figure 6.2.

The purpose of this chapter is to report results of this part of the study which examined whether the ability of PB to induce DNA synthesis in hypophysectomised rats could be restored following portal vein ligation, in a manner similar to that which occurs following partial hepatectomy.

However it is first necessary to report the response by the liver to portal vein ligation alone, both in intact and hypophysectomised rats and to demonstrate that portal vein ligation induces DNA synthesis in

the surgically unmanipulated lobes in the liver, the response being similar to that following partial hepatectomy.

Separate control animals were included in this section in order to compare the response by the liver to PB and portal vein ligation both in intact and hypophysectomised rats.

6.1 The response by the liver to 1/3 and 2/3 portal vein ligation in intact and hypophysectomised animals

6.1.1 Sham hypophysectomised animals (Group A)

6.1.1.1 Sham hypophysectomy, laparotomy and saline treatment (Controls - Group 1)

Effect on liver

(i) Relative liver weight

The mean relative liver weight in these controls animals was 3.65% (SD ± 0.21, n = 6).

At the end of the 28 day study the mean percentage of the left lateral lobe was 31.75% (SD ± 2.27, n = 6), the median lobe 32.89% (SD ± 1.83, n = 6), the right posterior 25.34% (SD ± 2.72, n = 6) and the caudate lobe 9.95% (SD ± 0.71, n = 6).

(ii) Right posterior lobe

The mean relative weight of the right posterior lobe was 0.93% (SD ±0.10, n = 6) and comparison with the study groups indicated whether there was a response by the right posterior lobe as a result of the hypophysectomy, portal vein ligation, PB treatment or combinations of these variables.

Investigation of the right posterior lobe

Measurements to reflect DNA synthesis and cell replication

a) Mean nuclear area

The mean nuclear area for Group 1 was 31.37 um^2 (SD $\pm$ 1.18, n = 6).

b) Nuclear ploidy classes

86.17% of nuclei were in the 2n ploidy classes and 13.83% were in the 4n and above ploidy classes.

c) Mitoses

The mean number of mitoses per 1,000 cells was 0.1180 (SD $\pm$0.13, n = 6) which is within normal resting values for rat liver.

Determination of cell volume

The mean estimated cell volume was 7.21×10^{-5} mm^3 (SD $\pm$ 0.27, n = 6).

Mean number of nuclei/10 fields

The mean number of nuclei per 10 fields was 466.5 (SD $\pm$ 50.34, n = 6).

6.1.1.2 Sham hypophysectomy 1/3 portal vein ligation and saline treatment (Group 3) compared with Group 1

Following 1/3 portal vein ligation there was obvious atrophy of the median lobe (7.06% instead of 32.89% controls, $p < 0.01$) and enlargement of the right posterior lobe (R.P. 37.97% instead of 25.34% controls, $p < 0.01$) with a 25.2% increase in relative weight of the R.P. (1.16% vs. 0.92% controls, $p < 0.01$). Compensatory growth in the unligated lobes was however, insufficient to restore the liver to the control value as total liver weight was only 83.01% of controls (3.03% vs. 3.65% controls, $p < 0.05$).

DNA synthesis was detected by an increased mean nuclear area (38.63 um^2 vs. 31.37 um^2 controls, $p < 0.01$) and an increase in number of 4n and above ploidy class nuclei (41.75% vs. 13.83% controls, $p < 0.05$). No change in mitotic index was detected compared with controls as expected by 21 days post-portal vein ligation (0.1234 SD $\pm$0.18 vs. 0.1180 SD $\pm$0.13).

Estimated cell volume was similar to controls (7.29×10^{-5} mm^3 SD $\pm$0.18 vs. 7.21×10^{-5} mm^3 SD $\pm$0.27) but the mean number of nuclei/10 fields were fewer compared with controls 406.25 vs. 466.5 controls, $p < 0.01$). This latter effect was most probably due to a reduction in the number of binucleate cells which is known to occur in associatin with a shift towards a higher ploidy (Sulkin, 1943; Bucher, 1963).

6.1.1.3 Sham hypophysectomy 2/3 portal vein ligation and saline treatment (Group 5) compared with Group 1

Following 2/3 portal vein ligation atrophy occurred in the anterior lobes (15.7% and 24.32% instead of 31.72% and 32.89% controls, $p < 0.01$) while in the unligated right posterior lobe clear enlargement occurred (R.P. 48.51% instead of 25.34% controls, $p < 0.01$) with a relative weight of the R.P. 1.54% as compared with 0.92% of controls ($p < 0.01$), an increase by 89.48%. However by 28 days compensatory growth in the unmanipulated lobes had not restored the liver to control values as total liver reached 3.14% compared with 3.65% (equivalent to 85.94% of controls).

Mean nuclear area was increased (45.93 um^2 vs. 31.37 um^2 controls, $p < 0.01$) and the number of nuclei in 4n and above ploidy classes increased (68.2% instead of 13.83%, controls, $p < 0.01$). No significant

changes in mitotic index were detected (0.1183 SD $\pm$0.13 vs. 0.1180 SD $\pm$0.13).

Mean estimated cell volume was slightly enlarged compared with controls (7.60 x 10^{-5} mm^3 vs. 7.20 x 10^{-5} mm^3 controls, $p < 0.05$) by 5.48% and there were fewer nuclei/10 fields (354.8 compared with 466.5, $p < 0.01$) compared with controls, most probably reflecting the slight cell enlargement.

Thus in summary following both 1/3 and 2/3 portal vein ligation atrophy occurred in ligated lobes accompanied by compensatory growth in the remaining surgically unmanipulated lobes, involving hyperplasia, detected by an increased nuclear area and a shift towards polyploidy.

Mitotic activity which occurs early in the response was undetected by 21 days post-portal vein ligation, and similarly following 1/3 portal vein ligation cell enlargement was not detected by 21 days although it was just detectable following 2/3 portal vein ligation.

6.1.1.4 Summary

These responses are consistent with other studies (Weinbren and Tarsh, 1964; Weinbren, 1978) and very similar to those reported in this study and by others, following 1/3 or 2/3 partial hepatectomy (Bucher, 1963; Weinbren, 1959), DNA synthesis being detected following both procedures by a shift in polyploidy.

6.1.2 Hypophysectomised animals (Group B)

As already described following hypophysectomy in all groups total body weight, adrenal and testicular weight were reduced compared with the sham hypophysectomised laparotomy saline treated group.

6.1.2.1 Hypophysectomy, laparotomy and saline treatment (Controls - Group 1)

At the end of the 28 day study the mean percentage of the left lateral lobe was 34.64% (SD±1.19), the median lobe 31.5^% (SD±2.16), the right posterior lobe 23.75% (SD±1.70) and the caudate lobe 9.63% (SD±2.40).

Following hypophysectomy atrophy occurred in the liver detected by a 14.8% reduction in relative liver weight (3.11% vs. 3.65% sham hypophysectomy controls, $p < 0.01$) and a 19.67% reduction in relative weight of the R.P. lobe (0.74% instead of 0.92% sham hypophysectomy controls, $p < 0.05$). The response was confirmed by a reduced mean estimated cell volume (2.40×10^{-5} mm^3 vs. 7.21×10^{-5} mm^3 sham hypophysectomy controls ($p < 0.01$) equivalent to 66.7% reduction) and increased nuclei/10 fields (715.3 vs. 466.5 sham hypophysectomy controls, $p < 0.01$), this latter effect reflecting the reduction in cell size. There were no changes in mitoses (0.0152 SD ±0.03 vs. 0.1180 SD ±0.1326). The majority of nuclei were in the 2n ploidy classes (92.83%) similar to hypophysectomy controls in Section III. Integrated microdensitometry confirmed this 2n:4n distribution with 80.64% of nuclei with integrated density ranging from 1.5-5.0 (A.U.) with a peak at integrated density 3.0-3.5 (A.U.). These values and distribution correspond to 2n ploidy classes and were similar to those reported for hypophysectomised animals (Group 1) in Section III, indicating that hypophysectomy arrests progression of polyploidy. The mean nuclear area was smaller than sham hypophysectomy controls, again suggesting arrest of development of polyploidy (28.45 um^2 instead of 31.37 um^2, $p < 0.05$).

The percentage of nuclei in the 2n ploidy classes (92.83%) were however not significantly different from the distribution in the sham hypophysectomy controls (86.17%) as opposed to the significant difference found in hypophysectomy controls in Section III compared with intact controls (84.34% vs. 57.8% intact controls, $p < 0.01$). This may be influenced by the somewhat lower development in polyploidy for the age and weight of the sham hypophysectomy controls in this section and will be discussed later.

6.1.2.2 Hypophysectomy 1/3 portal vein ligation and saline treatment (Group 3) compared with Group 1

Following 1/3 portal vein ligation, by 21 days there was atrophy in the median lobe (7.58% vs. 31.85% controls, $p < 0.01$) with enlargement in the unmanipulated right posterior lobe (R.P. 36.35% instead of 23.75% controls, $p < 0.01$) with the mean relative weight of the R.P. increased by 79.27% (1.33% vs. 0.74% controls, $p < 0.01$). The total liver weight exceeded control levels (3.66% instead of 3.11%, $p < 0.01$) by 17.68%.

DNA synthesis was detected by an increased mean nuclear area (33.88 um^2 vs. 28.45 um^2 controls, $p < 0.01$) and an increase in nuclei in 4n and above ploidy classes (15.67% vs. 7.17% controls, $p < 0.05$). Mitotic index was similar to controls (0.0191 SD $\pm$0.04 vs. 0.0152 SD $\pm$0.03).

Cells were enlarged by 66.17% compared with controls (3.99×10^{-5} mm^3 vs. 2.40×10^{-5} mm^3) with fewer nuclei/10 fields (554.3 vs. 715.3 controls, $p < 0.01$) but were atrophied compared with sham hypophysectomy controls (3.99×10^{-5} mm^3 vs. 7.21×10^{-5} mm^3, $p < 0.01$).

6.1.2.3 Hypophysectomy, 2/3 portal vein ligation and saline treatment (Group 5) compared with Group 1

Similarly following 2/3 portal vein ligation there was atrophy of the anterior lobes (31.51% vs. 66% controls, $p < 0.01$) but the R.P. lobe was much increased (51.25% vs. 23.75% controls, $p < 0.01$) the relative weight increasing by 59.22% from 0.74% (controls) to 1.18% ($p < 0.01$). Compensatory growth in the unmanipulated lobes did not restore liver weight to control values as total liver weight was 2.37% instead of 3.11% ($p < 0.01$) (equivalent of 76.21% of controls).

Mean nuclear area was increased (37.38 um^2 vs. 28.45 um^2 controls, $p < 0.01$) and the number of nuclei in the 4n and above ploidy classes increased (39.3% vs. 7.17% controls, $p < 0.01$). Integrated microdensitometry confirmed this shift in ploidy with higher integrated density values compared with controls ranging from 4.0-17.0 (A.U.). Peaks occurred at 6.0-6.5 (A.U.), 8.5-9.0 (A.U.) and 11.5-12.0 (A.U.), 88.5% of nuclei in the 4n and above ploidy ($p < 0.01$) an increase by 357.64% compared with controls, these measurements detecting DNA synthesis. Mitotic index was similar to controls (0.0423 SD $\pm$0.06 vs. 0.0152 SD $\pm$0.03).

Estimated cell volume was increased by 82.7% compared with controls (4.39×10^{-5} mm^3 vs. 2.40×10^{-5} mm^3, $p < 0.01$) with fewer nuclei/10 fields (496.5 vs. 715.3 controls, $p < 0.01$) but compared with sham hypophysectomy controls atrophy was detected (4.39×10^{-5} mm^3 vs. 7.21×10^{-5} mm^3, $p < 0.01$).

6.1.2.4 Summary

Thus in summary compensatory growth was detected in the surgically unmanipulated unligated right posterior lobe in hypophysectomised

animals following 1/3 and 2/3 portal vein ligation, the response consisting of both hyperplasia and hypertrophy.

These results confirm that following portal vein ligation in hypophysectomised animals, DNA synthesis can be detected by a shift in polyploidy as well as in intact animals and the response is similar to that seen following partial hepatectomy reported both in this study and by other authors (Harkness, 1957; Weinbren, 1959; Weinbren and Tarsh, 1964; Leong et al., 1959; Bucher, 1963; Schulte-Hermann et al., 1977).

6.2 The response by the liver to PB in portal vein ligated animals both in intact and hypophysectomised animals

6.2.1 Sham hypophysectomised animals (Group A)

6.2.1.1 Sham hypophysectomy, laparotomy and PB treatment (Group 2) compared with sham hypophysectomy, laparotomy and saline treatment (controls)

In the intact animal in which a laparotomy has been performed PB induces adaptive growth in the liver with liver enlargement. Details of the results of this group have already been reported in Chapter 4, subsection 4.1.2.1 but in summary the adaptive response was detected by an increase in the mean relative weight of the liver and right posterior lobe compared with controls.

The adaptive growth consisted of both hyperplasia and hypertrophy.

In this study when PB was administered to animals in which portal vein ligation had been performed, that response to PB was compared with saline treated animals. The results are as follows.

6.2.1.2 Sham hypophysectomy, 1/3 portal vein ligation and PB treatment (Group 4) compared with sham hypophysectomy, 1/3 portal vein ligation and saline treatment (Group 3)

The whole liver increased by 40.59% following PB treatment in 1/3 portal vein ligated animals (4.26% vs. 3.03% Group 3, $p < 0.01$) compared with saline treatment, with a 59.03% increase in relative weight of the R.P. lobe (1.75% instead of 1.16% Group 3, $p < 0.01$). In contrast to the response in Group 3 in which total liver only reached 83.01% of controls, total liver increased by 16.71% compared with controls (4.26% vs. 3.65%, $p < 0.01$) following PB treatment.

DNA synthesis was detected by an increased mean nuclear area (41.63 um^2 as compared with 38.63 um^2 Group 3, $p < 0.01$) and an increased number of nuclei in 4n and above classes (57.17% vs. 41.75% Group 3, $p < 0.01$). The mitotic index was increased (1.7392 vs. 0.1234 Group 3, $p < 0.01$) and cells were enlarged (12.36×10^{-5} mm^3 instead of 7.29×10^{-5} mm^3 Group 3, $p < 0.01$) with fewer nuclei/10 fields (363 vs. 406.25, $p < 0.01$) following PB treatment in contrast to saline treatment.

6.2.1.3 Sham hypophysectomy, 2/3 portal vein ligation and PB treatment (Group 6) compared with sham hypophysectomy, 2/3 portal vein ligation and saline treatment (Group 5)

Following PB treatment in 2/3 portal vein ligated animals total liver reached 3.72% compared with 3.14% ($p < 0.01$) in Group 5, and while in the latter only 85.94% of the control value was reached, total liver was increased by 2% compared with controls in Group 6. PB obviously induced adaptive growth in the right posterior lobe (R.P. 2.31% vs. 1.54% Group 5, $p < 0.01$).

Mean nuclear area was enlarged (56.23 um^2 vs. 45.93 um^2 Group 5, $p < 0.05$) and a greater number of nuclei in the 4n and above ploidy

classes (90.8% compared with 68.2%, $p < 0.01$) following PB in contrast to saline treatment. Mitotic index was increased (2.1543 vs. 0.1183 Group 5, $p < 0.01$) and cells were enlarged (13.58×10^{-5} mm^3 vs. 7.60×10^{-5} mm^3 Group 5, $p < 0.01$) with fewer nuclei/10 fields (336.8 instead of 354.8 Group 5, $p < 0.001$).

6.2.1.4 Summary

Thus PB induces an adaptive response in the liver already subjected to both 1/3 and 2/3 portal vein ligation, detected by greater mean relative weights of the liver and right posterior lobes compared with saline treated animals (Groups 3 and 5).

Both hyperplasia and hypertrophy were detected in the enlargement. The response to PB is similar to that found in this study on rats previously subjected to laparotomy (Chapter 4) and to changes reported by other authors (Schulte-Hermann, 1974). This indicates that the response by the liver to the drug is not affected by portal vein ligation. These results are also similar to the responses to PB in rats subjected previously to partial hepatectomy as already described (Chapter 3).

6.2.2 Hypophysectomised animals (Group B)

The remaining results are reported in order to establish (i) whether the ability of PB to induce DNA synthesis as demonstrated here in intact animals, occurs in hypophysectomised animals and (ii) whether PB induces DNA synthesis in hypophysectomised animals in which portal vein ligation had been carried out.

6.2.2.1 The response by the liver to PB following laparatomy

6.2.2.1.1 Hypophysectomy, laparotomy and PB treatment (Group 2) compared with Group 1 (Controls)

The whole of the liver enlarged by 33.44% following PB treatment (4.15% vs. 3.11% controls, $p < 0.01$) with obvious adaptive growth in the R.P. lobe (relative weight 1.1% compared with 0.74% controls ($p < 0.01$), an increase of 42.15%).

There was no increase in mean nuclear area (29.46 um^2 SD $\pm$1.85 vs. 28.45 um^2 SD $\pm$1.83, $p < 0.01$), in number of nuclei in 4n and above ploidy classes (10.5% instead of 7.15%) or mitotic index (0.0260 SD $\pm$0.06 vs. 0.0152 SD $\pm$0.03) following PB treatment compared with saline (controls). Integrated microdensitometry confirmed that PB did not induce a shift towards polyploidy as integrated density values ranged from 1.5-7.5 (A.U) with the integrated density peak at 3.0-3.5 (A.U.), 77.5% of the nuclei with integrated densities correlating with 2n ploidy classes and 22.5% with 4n and above ploidy classes. While statistical analysis could not be made at the 5% level using the Wilcoxon 2 sample test, the distribution of 2n/4n ploidy nuclei was not significantly different to Group 1 at the 10% level. This confirms previous findings in Section III and the Quantimet 720 studies that following PB treatment in hypophysectomised animals PB does not induce DNA synthesis and the DNA content remains the same.

Estimated cell volumes were increased by 157.5% (6.18×10^{-5} mm^3 instead of 2.40×10^{-5} mm^3 controls, $p < 0.01$) with fewer nuclei/10 fields (435.3 vs. 715.3 controls, $p < 0.01$) reflecting the cell enlargement.

Thus liver enlargement in hypophysectomised animals detected by an increase in mean relative weights of the liver and right posterior lobe following PB treatment compared with saline is the result of cellular enlargement alone; no evidence of DNA synthesis and cell multiplication were observed.

Fewer nuclei per 10 fields in Group 2 may be the result of PB inducing cell enlargement.

These results were consistent with those in Section III and previous studies (Schulte-Hermann et al., 1977).

6.2.2.2 The response by the liver to PB treatment in animals following portal vein ligation compared with laparotomy

6.2.2.2.1 Hypophysectomy, laparotomy and PB treatment **(Group 2)** compared with hypophysectomy, 1/3 portal vein ligation and PB treatment **(Group 4)**

Following 1/3 portal vein ligation there was clear enlargement of the right posterior lobe (R.P. 36.53% vs. 25.39% Group 2, $p < 0.01$) with a relative weight of R.P. 1.58% instead of 1.1% in Group 2 ($p < 0.01$).

DNA synthesis was detected by an increased mean nuclear area (35.44 um^2 vs. 29.46 um^2 Group 2, $p < 0.01$), increase in number of nuclei in 4n and above ploidy classes (26.33% instead of 10.5% Group 2, $p < 0.01$) and cell replication by an increased mitotic index (0.2664 instead of 0.0260 Group 2, $p < 0.01$).

Cells were smaller (4.14×10^{-5} mm^3 vs. 6.18×10^{-5} mm^3 Group 2, $p < 0.01$) and there were more nuclei/10 fields (464 instead of 435.33 Group 2, $p < 0.01$), most probably the result of the smaller cells.

6.2.2.2.2 Hypophysectomy, laparotomy and PB treatment (Group 2) compared with hypophysectomy, 2/3 portal vein ligation and PB treatment (Group 6)

Similarly following 2/3 portal vein ligation enlargement of the right posterior lobe occurred (R.P. 58.25% instead of 25.39% Group 2, $p < 0.01$) with a 78.3% increase in relative weight of R.P. (1.89% vs. 1.06% Group 2, $p < 0.01$). The total liver however was larger in Group 2 (4.15% instead of 3.26% Group 6, $p < 0.01$).

Mean nuclear area was increased (44.35 um^2 instead of 29.46 um^2 Group 2, $p < 0.01$) and 59.75% of nuclei were in 4n and above ploidy classes compared with 10.5% (Group 2, $p < 0.01$). This increase towards higher ploidy levels was confirmed by integrated microdensitometry in which the nuclei demonstrated integrated density values ranging from 3.0-15.5 (A.U.) with peaks occurring at 5.5-6.0 (A.U.), 7.5-8.0 and 15-15.5 (A.U.), 92% of nuclei in 4n and above ploidy classes compared with 10.5% in Group 2 ($p < 0.05$). Mitotic index was increased (0.8966 instead of 0.0260 Group 2, $p < 0.01$) indicating cell replication.

Mean estimated cell volume was reduced (4.85×10^{-5} mm^3 compared with 6.18×10^{-5} mm^3 Group 2, $p < 0.01$) with an increased number of nuclei/10 fields (448.5 vs. 435.3 Group 2, $p < 0.05$), most probably the result of the smaller cells.

6.2.2.3 Summary

Following PB treatment (Group 4) the liver mass was greater than with 1/3 portal vein ligation alone (Group 3) so that the relative liver weight was similar to Group 2, and following 2/3 portal vein ligation (Group 6) PB increased the liver mass to control levels. However

despite this response the mean relative liver weight was less than that following PB treatment in laparotomised animals (Group 2).

However the mean relative weight of the unligated right posterior lobe was greater both in Group 4 and Group 6 compared with Group 2, the adaptive response a result of hyperplasia including both DNA synthesis and cell replication, demonstrating that following 1/3 and 2/3 portal vein ligation the ability for DNA synthesis to be induced by PB had been restored.

Hypertrophy was induced in both groups but the cells in Group 2 were 40% larger than Group 4 and 27.5% larger than Group 6.

It was confirmed that following portal vein ligation in hypophysectomised animals the observed ability by PB to induce DNA synthesis compared with its absence in laparotomised animals was the effect of PB alone because its effect in Groups 4 and 6 were greater than that of the portal vein ligation alone (Groups 3 and 5). These results are presented below.

6.2.2.4 The response by the liver to PB treatment in portal vein ligated animals compared with saline

6.2.2.4.1 Hypophysectomy, 1/3 portal vein ligation and saline treatment (Group 3) compared with hypophysectomy, 1/3 portal vein ligation and PB treatment (Group 4)

The whole liver increased by 18.3% following PB treatment (4.33% vs. 3.66% Group 3, $p < 0.01$) with obvious adaptive growth in the right posterior lobe (R.P. 1.58% instead of 1.33% Group 3, an increase of 18.7%, $p < 0.01$), the R.P. showing similar contribution to the whole liver as Group 3 (36.53% SD ± 2.76 and 36.35% SD ± 1.57 respectively).

Mean nuclear area was enlarged (35.44 um^2 vs. 33.88 um^2 Group 3, $p < 0.01$), there were more nuclei in 4n and above ploidy classes (26.33% instead of 15.6% Group 3, $p < 0.05$) and an increased mitotic index (0.2664 vs. 0.0191 Group 3, $p < 0.01$).

Estimated cell volume was increased (4.41×10^{-5} mm^3 compared with 3.99×10^{-5} mm^3 Group 3, $p < 0.01$) with fewer nuclei/10 fields (464.0 vs. 554.3 Group 3, $p < 0.01$).

6.2.2.4.2 Hypophysectomy, 2/3 portal vein ligation and saline treatment (Group 5) compared with hypophysectomy, 2/3 portal vein ligation and PB treatment (Group 6)

Similarly following PB treatment in Group 6, significant adaptive growth was detected in the right posterior lobe (R.P. relative weight 1.89% instead of 1.18% Group 5, $p < 0.01$), the whole liver being 37.55% larger by 28 days compared with Group 5 (3.26% instead of 2.37%, $p < 0.01$).

Mean nuclear area was considerably enlarged (44.35 um^2 vs. 37.38 um^2 Group 5, $p < 0.01$), 59.75% of nuclei were in the 4n and above ploidy classes compared with 39.38% in Group 5 ($p < 0.01$), the increased ploidy confirmed by integrated microdensitometry. This demonstrated that PB did induce an increase in DNA synthesis in Group 6 rather than the portal vein ligation alone as there was a 3.84% increase ($p < 0.05$) in the number of nuclei with integrated density values in the 4n and above ploidy classes compared with Group 5 (92% vs. 88.6% Group 5). Integrated density values ranged from 3.0-15.5 (A.U.) with peaks occurring at 5.5-6.0 (A.U.), 7.5-8.0 (A.U.) and 15-15.5 (A.U.) in contrast to Group 5 where integrated density values ranged from 4.0-17.0

(A.U.) with peaks occurring at 6.0-6.5 (A.U.), 8.5-9.0 (A.U.) and 11.5-12.0 (A.U.).

Mitotic index was also increased (0.8966 vs. 0.0423 Group 5, $p < 0.01$).

Mean estimated cell volume was enlarged by 10.5% (4.85×10^{-5} mm^3 vs. 4.39×10^{-5} mm^3 Group 5, $p < 0.05$) with fewer nuclei/10 fields (448.5 compared with 496.5 Group 5, $p < 0.01$).

Thus the inductive ability by PB to increase liver mass was seen in Groups 4 and 6, the mean relative weight of the liver and right posterior lobes being heavier compared with Groups 3 and 5. The adaptive growth was a combination of both hyperplasia and hypertrophy. Hyperplasia was detected by an increased mean nuclear area and a greater shift towards polyploidy and an increase in mitoses per 1,000 cells following 8 days PB treatment prior to sacrifice 24 hours later.

PB further induced hypertrophy in Groups 4 and 6 the cell volumes being enlarged compared with Groups 3 and 5. This effect by PB on the cells following 1/3 and 2/3 portal vein ligation was however smaller than the effect of PB on cells in liver in rats which had undergone laparotomy (Group 2) where PB induced liver enlargement by 157.45% compared with controls (Group 1).

6.2.2.5 Summary

These results confirm that the response by the liver to PB in hypophysectomised animals previously subjected to portal vein ligation is similar to that seen following administration of PB to intact animals in which laparotomy has been performed (Schulte-Hermann, 1974; Schulte-

Hermann et al., 1977). The response is similar to that found in the liver of intact animals following 1/3 and 2/3 portal vein ligation and similar also to that seen in the hypophysectomised animals subjected to 1/3 or 2/3 partial hepatectomy before being given PB (Schulte-Hermann et al., 1977).

Table 6.1.

Table 6.1 Section IV - Sham hypophysectomy group

Rat no.	At start total body wt(grams)	At end total body wt(grams)	Liver wt(grams)	% liver/ body wt	% Rt post lobe/ body wt	Liver LL	Liver ML	Liver RP	Liver CL	Adrenals (grams)	Testes (grams)	No.mitoses /1000 cells	No.cells / 10 F	Cell vol $x10^{-5}mm^3$
A1 - Sham hypophysectomy, laparotomy and saline														
H0	195	250	9.69	3.88	0.968	3.01	3.38	2.42	0.88	0.035	2.95	0.1748	572	7.30
						31.06%	34.8%	24.9%	9.0%					
H1	195	240	8.65	3.60	1.0958	2.42	2.63	2.63	0.97	0.04	3.01	0.1763	567	7.406
						27.97%	30.4%	30.4%	11.2%					
H2	185	240	8.97	3.74	0.8916	3.09	2.85	2.14	0.89	0.035	2.88	0.3571	569	7.546
						34.4%	31.77%	23.85%	9.9%					
H7	185	170	5.65	3.32	0.8588	1.84	1.79	1.46	0.56	0.03	2.76	0.00	588	7.013
						32.74%	31.68%	25.8%	9.9%					
H10	210	210	7.04	3.52	0.7900	2.35	2.42	1.58	0.69	0.04	2.96	0.00	605	6.7199
						33.3%	34.3%	22.44%	9.8%					
H11	195	240	9.16	3.82	0.9416	2.84	3.15	2.26	0.91	0.03	2.95	0.00	574	7.2716
						31.0%	34.38%	24.67%	9.9%					
A2 - Sham hypophysectomy, laparotomy and PB														
H3	180	200	8.63	4.32	0.98	2.75	3.11	1.96	0.81	0.04	2.90	0.6849	438	10.909
						31.86%	36.0%	22.71%	9.38%					
H4	210	210	9.04	4.52	1.255	2.91	2.88	2.51	0.74	0.04	2.90	0.4842	413	11.914
						32.19%	31.85%	27.76%	8.1%					
H5	175	190	8.04	4.73	1.0789	2.38	2.63	2.05	0.98	0.04	2.35	0.8264	484	9.3914
						29.60%	32.71%	25.49%	12.18%					
H6	205	200	8.92	4.46	1.1300	2.99	2.99	2.26	0.68	0.04	3.6	0.3929	509	8.7080
						33.52%	33.52	25.33%	7.6%					
H8	185	210	8.63	4.11	1.0285	2.88	2.92	2.16	0.67	0.04	2.62	0.7009	428	11.293
						33.37%	33.83%	25.0%	7.6%					
H9	170	185	8.76	4.74	1.2540	2.64	3.01	2.32	0.79	0.04	2.69	0.6993	429	11.254
						30.13%	34.36%	26.48%	9.0%					
A3 - Sham hypophysectomy, 1/3 portal vein ligation and saline														
H6	180	180	4.57	2.54	0.9333	1.87	0.32	1.68	0.70	0.04	2.38	0.0	571	7.3290
						40.91%	7.0%	36.76%	15.81%					
H8	190	225	7.88	3.50	1.3911	3.14	0.47	3.13	1.14	0.05	2.77	0.1680	595	6.8900
						39.84%	5.76%	39.72%	14.46%					
H9	195	165	4.18	2.53	1.0363	1.650	0.27	1.71	0.55	0.04	0.80	0.0	565	7.4460
						39.47%	6.4%	40.9%	13.15%					
H10	175	220	7.36	3.35	1.600	2.75	0.39	3.52	0.70	0.04	2.83	0.0	566	7.4263
						37.36%	5.29%	47.82%	9.51%					
H11	155	200	5.53	2.77	0.500	2.95	0.55	1.00	1.03	0.05	2.86	0.5217	575	7.2526
						53.34%	9.9%	18.0%	18.62%					
H12	160	135	3.7	2.74	1.2593	1.30	0.28	1.70	0.42	0.04	0.41	0.1745	573	7.2906
						35.18%	7.56%	45.94%	11.35%					
H14	160	210	7.91	3.77	1.3809	3.54	0.60	2.90	0.87	0.04	2.73	0.0	566	7.4263
						44.75%	7.5%	36.66%	10.99%					

Table 6.1 (contd)

Rat no.	At start total body wt(grams)	At end total body wt(grams)	Liver wt(grams)	% liver/ body wt	% Rt post lobe/ body wt	Liver LL	ML	RP	CL	Adrenals (grams)	Testes (grams)	No.mitoses /1000 cells	No.cells / 10 F	Cell vol $x10^{-5}mm^3$
A4 - Sham hypophysectomy, 1/3 portal vein ligation and PB														
H0	185	220	9.32	4.24	1.377	3.33	1.72	3.03	1.24	0.048	2.88	1.699	412	11.957
						35.72%	18.45%	32.51%	13.3%					
H1	155	190	8.13	4.28	1.7157	3.48	0.18	3.26	1.21	0.05	2.45	2.2900	393	12.835
						42.8%	2.2%	40.0%	14.88%					
H2	175	220	9.82	4.46	2.000	3.19	0.94	4.40	1.29	0.04	3.36	2.2784	395	12.738
						32.48%	9.5%	44.8%	13.1%					
H3	165	200	8.61	4.31	1.700	2.82	1.10	3.40	1.29	0.04	2.73	1.9559	409	12.089
						32.75%	12.77%	39.48%	14.98%					
H4	150	200	8.02	4.01	1.925	3.63	0.54	3.85	1.07	0.04	2.67	1.7326	404	12.314
						45.2%	6.7%	48.0%	13.34%					
H5	175	180	7.61	4.23	1.9111	2.29	0.92	3.44	0.96	0.05	2.71	1.2135	412	11.957
						30.0%	12.0%	45.2%	12.61%					
H7	150	190	8.14	4.28	1.6052	3.75	0.21	3.05	1.13	0.05	2.91	1.0050	398	12.594
						46.0%	2.58%	37.46%	13.88%					
A5 - Sham hypophysectomy, 2/3 portal vein ligation and saline														
H0	185	235	7.44	3.17	2.234	0.58	0.87	5.25	0.74	0.04	2.866	0.0	532	8.149
						7.79%	11.69%	70.56%	9.9%					
H1	195	230	7.20	3.13	1.1130	2.01	1.71	2.56	0.92	0.04	2.68	0.3502	571	7.3290
						27.91%	23.75%	35.55%	12.7%					
H5	175	250	7.97	3.19	1.088	1.75	2.40	2.72	1.10	0.04	2.78	0.1808	553	7.6897
						21.95%	30.1%	34.12%	13.8%					
H8	185	230	6.47	2.81	1.1826	0.92	2.05	2.72	0.78	0.05	3.715	0.1788	559	7.5662
						14.2%	31.68%	42.04%	12.05%					
H6	190	230	8.46	3.68	2.300	0.54	2.11	5.29	0.53	0.05	3.23	0.00	561	7.5258
						6.38%	24.94%	62.53%	6.15%					
H7	175	225	6.4	2.84	1.3155	1.02	1.52	2.96	0.90	0.04	2.83	0.00	569	7.3676
						15.94%	23.75%	46.25%	14.06%					
A6 - Sham hypophysectomy, 2/3 portal vein ligation and PB														
H2	185	210	7.38	3.51	2.4190	0.60	1.05	5.08	0.65	0.04	2.92	2.3316	386	13.186
						8.13%	14.22%	68.8%	8.60%					
H7	175	225	8.66	3.85	2.5911	0.75	0.80	5.83	1.28	0.04	2.83	2.5641	390	12.983
						8.66%	9.23%	67.32%	14.78%					
H9	160	250	8.22	3.29	1.852	1.28	1.32	4.63	0.99	0.05	2.50	1.8324	382	13.393
						15.57%	16.0%	56.32%	12.0%					
H10	185	220	8.46	3.85	2.4681	0.48	0.92	5.43	1.63	0.05	3.02	2.1621	370	14.050
						5.67%	10.87%	64.15%	19.26%					
H11	215	260	9.38	3.61	1.9192	0.95	0.99	4.99	2.45	0.06	2.75	2.1739	368	14.165
						10.12%	10.55%	53.19%	26.11%					
H4	180	200	8.37	4.19	2.600	0.75	1.01	5.20	1.41	0.04	2.50	1.8617	376	13.715
						8.96%	12.07%	62.13%	16.85%					

Table 6.2.

Table 6.2 Section IV - Hypophysectomy group

Rat no.	At start total body wt(grams)	At end total body wt(grams)	Liver wt(grams)	% liver/ body wt	% Rt post lobe/ body wt	Liver LL	ML	RP	CL	Adrenals (grams)	Testes (grams)	No.mitoses /1000 cells	No.cells / 10 F	Cell vol $x10^{-5} mm^3$
B1 - Hypophysectomy, laparotomy and saline														
H0	215	170	5.36	3.15	0.7586	1.87	1.66	1.29	0.54	0.015	0.52	0.0913	1095	2.7598
						34.8%	30.97%	24.0%	10.0%					
H3	210	155	5.34	3.45	0.8322	1.75	1.89	1.29	0.41	0.01	0.48	0.00	1302	2.1285
						32.77%	35.39%	24.1%	7.6%					
H7	210	140	3.58	2.56	0.5214	1.23	1.11	0.73	0.51	0.01	0.47	0.00	1250	2.2627
						34.35%	31.0%	20.39%	14.2%					
H14	200	150	5.28	3.52	0.8800	1.83	1.68	1.32	0.45	0.015	0.46	0.00	1190	2.4360
						34.6%	31.8%	25.0%	3.5%					
H10	185	130	3.61	2.78	0.6923	1.32	1.05	0.90	0.34	0.01	0.43	0.00	1133	2.6221
						36.5%	29.0%	24.9%	9.4%					
H7	175	135	4.31	3.19	0.7703	1.50	1.42	1.04	0.35	0.01	0.47	0.00	1275	2.1965
						34.8%	32.94%	24.12%	3.1%					
B2 - Hypophysectomy, laparotomy and PB														
H2	185	125	4.85	3.88	1.0320	1.47	1.64	1.29	0.45	0.01	0.38	0.0	628	6.3541
						30.31%	33.81%	26.60%	9.28%					
H3	185	125	4.29	3.43	0.816	1.22	1.70	1.02	0.35	0.01	0.41	0.0	645	6.1046
						28.43%	39.62%	23.77%	8.1%					
H4	200	135	5.96	4.41	0.9851	1.96	2.07	1.33	0.60	0.01	0.49	0.1564	639	6.1908
						32.8%	34.73%	22.31%	10.0%					
H5	190	155	6.60	4.26	1.1419	2.12	2.03	1.77	0.68	0.015	0.43	0.00	620	6.4775
						32.12%	30.75%	26.81%	10.30%					
H6	195	140	6.27	4.48	1.1928	2.09	2.03	1.67	0.48	0.01	0.52	0.00	660	5.8977
						33.3%	32.3%	26.66%	7.6%					
H6	195	145	6.45	4.45	1.1655	2.10	2.03	1.69	0.63	0.01	0.41	0.00	648	6.0623
						32.56%	31.47%	26.20%	9.77%					
B3 - Hypophysectomy, 1/3 portal vein ligation and saline														
H0	170	120	4.91	4.09	1.4750	2.19	0.43	1.77	0.52	0.01	0.34	0.0	848	4.0495
						44.6%	8.75%	36.0%	10.5%					
H2	195	140	5.01	3.58	1.3428	2.17	0.38	1.88	0.58	0.01	0.45	0.0	840	4.1075
						43.31%	7.5%	37.52%	11.57%					
H5	190	140	4.89	3.49	1.2571	2.16	0.42	1.76	0.55	0.01	0.49	0.1150	860	3.9650
						44.17%	8.5%	35.99%	11.24%					
H18	150	110	3.65	3.32	1.1272	1.85	0.31	1.24	0.25	0.01	0.24	0.0	915	3.613
						50.68%	8.4%	33.97%	6.8%					
H19	180	130	5.14	3.95	1.4076	2.28	0.30	1.83	0.73	0.01	0.40	0.0	850	4.0352
						44.35%	5.8%	35.6%	14.2%					
H23	170	130	4.59	3.53	1.3769	1.97	0.30	1.79	0.53	0.01	0.38	0.0	832	4.1669
						42.9%	6.5%	38.99%	11.54%					

Table 6.2 (contd)

Rat no.	At start total body wt(grams)	At end total body wt(grams)	Liver wt(grams)	% liver/ body wt	% Rt post lobe/ body wt	Liver LL	ML	RP	CL	Adrenals (grams)	Testes (grams)	No.mitoses /1000 cells	No.cells / 10 F	Cell vol $x10^{-5}mm^3$
B4 - Hypophysectomy, 1/3 portal vein ligation and PB														
H9	210	140	5.43	3.88	1.5357	2.36	0.26	2.15	0.66	0.015	0.45	0.2544	786	4.5380
						43.46%	4.78%	39.59%	12.15%					
H12	180	130	6.51	5.01	1.9769	2.70	0.44	2.57	0.80	0.01	0.42	0.2564	780	4.5904
						41.47%	6.7%	39.47%	12.28%					
H14	170	115	5.15	4.48	1.7565	2.20	0.31	2.02	0.62	0.01	0.42	0.3640	824	4.2277
						42.71%	6.0%	39.2%	12.0%					
H15	195	140	7.42	5.3	1.7642	32.74	0.51	2.47	0.70	0.01	0.42	0.1291	774	4.6439
						50.4%	6.8%	33.78%	9.43%					
H1	225	180	6.95	3.86	1.2777	2.94	0.69	2.30	1.02	0.015	0.59	0.3764	797	4.4443
						42.3%	9.9%	33.0%	14.6%					
H4	210	160	6.12	3.83	1.4187	2.35	0.64	2.27	0.80	0.01	0.44	0.2409	830	4.1819
						38.39%	10.45%	37.0%	14.0%					
H6	215	145	5.76	3.97	1.3379	2.55	0.58	1.94	0.69	0.01	0.53	0.2439	820	4.2587
						44.27%	10.07%	33.68%	11.98%					
B5 - Hypophysectomy, 2/3 portal vein ligation and saline														
H2	220	160	3.77	2.36	1.275	0.51	0.49	2.04	0.73	0.015	0.50	0.0	772	4.6620
						13.52%	12.99%	54.11%	19.36%					
H4	210	145	4.31	2.97	1.0896	1.14	1.06	1.58	0.53	0.015	0.50	0.0	804	4.3864
						26.45%	24.59%	36.6%	12.29%					
H0	230	160	4.14	2.59	0.975	1.14	0.94	1.56	0.50	0.015	0.43	0.0	905	3.6730
						29.53%	22.7%	37.68%	12.0%					
H41	175	145	3.02	2.08	1.2344	0.28	0.301	1.79	0.65	0.01	0.52	0.0	788	4.5207
						9.27%	9.97%	59.27%	21.52%					
H19	165	165	3.29	1.99	1.2121	0.36	0.31	2.0	0.62	0.015	0.47	0.1315	760	4.7728
						10.94%	9.42%	60.79%	18.84%					
H16	165	140	3.10	2.21	1.3071	0.29	0.32	1.83	0.66	0.015	0.49	0.1228	814	4.3058
						9.35%	10.32%	59.03%	21.29%					
B6 - Hypophysectomy, 2/3 portal vein ligation and PB														
H8	230	185	6.21	3.36	2.048	0.49	0.64	3.79	1.29	0.015	0.51	1.0389	770	4.6801
						7.89%	10.3%	61.0%	20.77%					
H11	230	125	4.2	3.36	1.968	0.35	0.50	2.46	0.89	0.01	0.5	0.9090	770	4.6801
						8.33%	11.9%	58.57%	21.19%					
H3	215	155	5.09	3.28	1.870	0.52	0.66	2.90	1.01	0.015	0.44	0.7905	759	4.7823
						10.21%	12.96%	56.97%	19.84%					
H16	165	150	5.49	3.66	2.06	0.25	0.78	3.09	1.37	0.01	0.38	0.4347	690	5.5172
						4.5%	14.2%	56.2%	24.95%					
H20	215	150	4.41	2.94	1.6466	0.42	0.51	2.47	1.01	0.01	0.44	1.3962	795	4.4611
						9.5%	11.56%	56.01%	22.90%					
H15	176	175	5.12	2.93	1.7771	0.51	0.56	3.11	0.94	0.05	0.51	0.8108	740	4.9676
						9.96%	10.94%	60.74%	18.36%					

Table 6.3a Section IV - Sham hypophysectomy group - Group means

Experiment	No. animals	Body wt. (av)grams	Body wt. end exp. (av)grams	Av.liver (av)grams	Av.% liver/ body wt.	Av.% rt.post. body wt.	Liver LL	ML	RP	CL	Adrenals av(grams)	Testes (av)grams	Av.no. mitoses /1000 cells	No.cells /10F	Mean cell vol. $x10^{-5}mm^3$
A1 - Group 1 Sham hypophysectomy, laparotomy and saline	6	194.17 ±9.17	223.33 ±31.43	8.19 ±1.54	3.65 ±0.21	0.9243 ±0.0957	2.59 ±0.48 31.75% ±2.27	2.70 ±0.57 32.89% ±1.83	2.08 ±0.47 25.34% ±2.72	0.82 ±0.16 9.95% ±0.71	0.04 ±0.0045	2.92 ±0.09	0.1180 ±0.1326	577.66 ±14.862	7.2094 ±0.2714
A2 - Group 2 Sham hypophysectomy, laparotomy and PB	6	187.5 ±16.36	194.17 ±14.29	8.67 ±0.35	4.48 ±0.24	1.1210 ±0.1048	2.76 ±0.22 31.78% ±1.63	2.92 ±0.16 33.71% ±1.43	2.21 ±0.20 25.46% ±1.68	0.78 ±0.11 8.99% ±1.72	0.04 ±0.0	2.85 ±0.44	0.6314 ±0.1465	450.166 ±34.338	10.5784 ±1.1379
A3 - Group 3 Sham hypophysectomy, 1/3 PV and saline	7	173.15 ±15.74	187.86 ±34.50	5.88 ±1.82	3.03 ±0.50	1.1572 ±0.3397	2.46 ±0.85 41.55% ±5.99	0.41 ±0.13 7.06% ±1.51	2.23 ±0.94 37.97% ±9.78	0.77 ±0.26 13.41% ±3.15	0.040 ±0.00488	1.75 ±1.19	0.1234 ±0.1789	573.0 ±9.665	7.2944 ±0.1789
A4 - Group 4 Sham hypophysectomy, 1/3 PV and PB	7	167.86 ±17.76	200.0 ±15.28	8.52 ±0.79	4.26 ±0.13	1.7477 ±0.2009	3.21 ±0.51 37.85% ±6.6	0.80 ±0.54 9.17% ±5.85	3.49 ±0.49 41.06% ±5.31	1.17 ±0.12 13.73% ±0.90	0.05 ±0.01	2.82 ±0.28	1.7392 ±0.4564	403.285 ±7.439	12.3553 ±0.3427
A5 - Group 5 Sham hypophysectomy, 2/3 PV and saline	6	184.17 ±8.01	233.33 ±8.76 26.69%	7.32 ±0.7438	3.14 ±0.29	1.5389 ±0.5203	1.136 ±0.56 15.695% ±7.53	1.776 ±0.49 24.32% ±6.43	3.58 ±1.198 48.51% ±13.56	0.71 ±0.285 11.44% ±2.73	0.04 ±0.01	3.02 ±0.39	0.1183 ±0.1312	557.7 ±12.9067	7.6045 ±0.2718
A6 - Group 6 Sham hypophysectomy, 2/3 PV and PB	6	183.33 ±18.07	227.50 ±23.18	8.41 ±0.65	3.72 ±0.31	2.3082 ±0.3061	0.80 ±0.28 9.52% ±3.31	1.02 ±0.17 12.16% ±2.52	5.19 ±0.41 61.99% ±6.15	1.40 ±0.62 16.27% ±6.09	0.05 ±0.01	2.72 ±0.22	2.1543 ±0.2546	378.66 ±8.0583	13.5822 +0.4337

Table 6.3b Section IV – Hypophysectomy group – Group means

Experiment	No. animals	Body wt. (av)grams	Body wt. end exp. (av)grams	Av.liver (av)grams	Av.% liver/ body wt.	Av.% rt.post. body wt.	Liver LL	ML	RP	CL	Adrenals av(grams)	Testes (av)grams	Av.no. mitoses /1000 cells	No.cells /10F	Mean cell vol. x10^{-5}mm^3
B1 – Group 1 Hypophysectomy, laparotomy and saline	6	199.17 ±5.94	146.67 ±14.72	4.58 ±0.86	3.11 ±0.37	0.7425 ±0.150	1.58 ±0.27 34.64% ±1.19	1.47 ±0.34 31.58% ±2.16	1.10 ±0.25 23.75% ±1.70	0.43 ±0.08 9.63% ±2.40	0.01 ±0.0258	0.47 ±0.03	0.0152 ±0.0340	1207.5 ±75.0616	2.4009 +0.2287
B2 – Group 2 Hypophysectomy, laparotomy and PB	6	191.67 ±6.06	137.5 ±11.73	5.74 ±0.86	4.15 ±0.38	1.0555 ±0.1299	1.83 ±0.39 31.58% ±1.69	1.92 ±0.19 33.78% ±2.94	1.46 ±0.26 25.39% ±1.72	0.53 ±0.13 9.18% ±0.10	0.01 ±0.0204	0.44 ±0.05	0.0260 ±0.0582	640.0 ±13.1275	6.1811 ±0.1906
B3 – Group 3 Hypophysectomy, 1/3 PV and saline	6	175.83 ±14.83	128.33 ±10.67	4.69 ±0.50	3.66 ±0.27	1.3311 ±0.1124	2.10 ±0.15 45.0% ±2.61	0.36 ±0.06 7.58% ±1.10	1.71 ±0.21 36.35% ±1.57	0.52 ±0.14 10.98% ±2.19	0.01 ±0.0	0.38 ±0.08	0.0191 ±0.0428	875.5 ±0.0428	3.9895 ±27.13
B4 – Group 4 Hypophysectomy, 1/3 PV and PB	7	200.71 ±19.88	144.29 +20.90	6.19 ±0.82	4.33 ±0.61	1.5810 ±0.2392	2.69 ±0.52 43.29% ±3.66	0.49 ±0.16 7.81% ±2.28	2.25 ±0.23 36.53% ±2.99	0.76 ±0.13 12.35% ±1.66	0.01 ±0.0244	0.47 ±0.07	0.2664 ±0.0733	801.571 ±21.178	4.4121 ±0.1744
B5 – Group 5 Hypophysectomy, 2/3 PV and saline	6	194.17 ±27.90	152.50 ±10.37	3.61 ±0.55	2.37 ±0.36	1.1822 ±0.1149	0.62 ±0.41 16.51% +9.08	0.57 ±0.34 15.0% ±6.84	1.80 ±0.20 51.25% ±11.16	0.62 ±0.09 17.55% ±4.32	0.01 ±0.0209	0.49 ±0.03	0.0423 ±0.0599	807.166 ±47.358	4.3867 ±0.3556
B6 – Group 6 Hypophysectomy, 2/3 PV and PB	6	205.17 ±27.90	156.67 ±21.13	5.09 ±0.73	3.26 ±0.28	1.8949 ±0.1484	0.42 ±0.11 8.40% +2.12	0.61 ±0.11 11.98% ±1.41	2.97 ±0.49 58.25% ±2.22	1.09 ±0.20 21.34% ±2.32	0.01 ±0.00274	0.46 ±0.05	0.8966 ±0.2892	754.00 ±32.939	4.8480 ±0.3347

Table 6.4.

Table 6.4 Mean nuclear area (um^2) and mean number of nuclei per 10 fields - Section IV

Animals	Mean nuclear area (um^2)	Mean no.nuclei/10 fields
A(1) Sham hypophysectomy, laparotomy and saline treatment (Group 1)		
H10	29.79378 (SD ± 7.536537008)	573
H11	28.692504 (SD ± 7.7036072)	438
H2	31.614408 (SD ± 6.903692959)	438
H7	32.75091 (SD ± 6.305438035)	480
H1	34.082082 (SD ± 7.415112977)	441
H0	31.28625 (SD ± 6.628640930)	429

Mean nuclear area = 31.369989 um^2 (SD ± 1.7786936)
Mean no.nuclei/10 fields = 466.5 (SD ± 50.341535)

Animals	Mean nuclear area (um^2)	Mean no.nuclei/10 fields
A(2) Sham hypophysectomy, laparotomy and PB treatment (Group 2)		
H3	42.369462 (SD ± 12.36020942)	305
H6	37.582434 (SD ± 8.843086874)	324
H8	37.977336 (SD ± 7.893515144)	263
H5	30.197952 (SD ± 7.210297062)	333
H9	36.7170795 (SD ± 11.18493678)	331
H4	35.125884 (SD ± 9.137604834)	316

Mean nuclear area = 36.661691 um^2 (SD ± 3.6354512)
Mean no.nuclei/10 fields = 312 (SD ± 23.846783)

Animals	Mean nuclear area (um^2)	Mean no.nuclei/10 fields
A(3) Sham hypophysectomy, 1/3 portal vein ligation and saline treatment (Group 3)		
H14	38.91546 (SD ± 8.404407544)	402
H12	35.322408 (SD ± 7.130118102)	405
H11	37.523106 (SD ± 7.69606363)	416
H10	42.775488 (SD ± 7.408040383)	402

Mean nuclear area = 38.634116 um^2 (SD ± 2.7125573)
Mean no.nuclei/10 fields = 406.25 (SD ± 5.7608593)

Animals	Mean nuclear area (um^2)	Mean no.nuclei/10 fields
A(4) Sham hypophysectomy, 1/3 portal vein ligation and PB treatment (Group 4)		
H4	42.176646 (SD ± 6.810272057)	366
H5	39.444777 (SD ± 6.598283010)	365
H7	41.472126 (SD ± 7.793694602)	383
H3	39.94443 (SD ± 6.739233908)	366
H2	43.654284 (SD ± 10.40843238)	353
H1	42.319404 (SD ± 9.073896982)	349
H0	42.399126 (SD ± 8.666033027)	359

Mean nuclear area = 41.630113 um^2 (SD ± 1.3682444)
Mean no.nuclei/10 fields = 363 (SD ± 10.239978)

Animals	Mean nuclear area (um^2)	Mean no.nuclei/10 fields
A(5) Sham hypophysectomy, 2/3 portal vein ligation and saline treatment (Group 5)		
H1	42.18777 (SD ± 8.459128009)	356
H5	46.524276 (SD ± 10.21422544)	350
H8	43.900866 (SD ± 10.80069773)	351
H0	49.475844 (SD ± 12.64396806)	348
H6	47.579202 (SD ± 10.95306931)	369

Mean nuclear area = 45.933592 um^2 (SD ± 2.5987553)
Mean no.nuclei/10 fields = 354.8 (SD ± 7.5736385)

Animals	Mean nuclear area (um^2)	Mean no.nuclei/10 fields
A(6) Sham hypophysectomy, 2/3 portal vein ligation and PB treatment (Group 6)		
H11	49.234824 (SD ± 11.7446704)	314
H9	50.47515 (SD ± 10.64876864)	339
H10	53.495316 (SD ± 12.75228424)	342
H2	64.597068 (SD ± 13.35660543)	346
H7	63.371574 (SD ± 11.57680764)	340

Mean nuclear area = 56.234786 um^2 (SD ± 6.4890534)
Mean no.nuclei/10 fields = 336.8333 (SD ± 10.462898)

Table 6.4 (continued)

Animals	Mean nuclear area (um^2)	Mean no.nuclei/10 fields
B(1) Hypophysectomy, laparotomy and saline treatment (Group 1)		
H0	30.144186 (SD ± 6.938889179)	669
H7	25.294122 (SD ± 5.200305174)	780
H3	30.750444 (SD ± 7.377086570)	684
H10	27.0684 (SD ± 6.538173423)	760
H14	28.629468 (SD ± 6.829805205)	673
H7	28.800036 (SD ± 7.092021287)	726

Mean nuclear area = 28.447776 um^2 (SD ± 1.8341845, n = 6)
Mean no.nuclei/10 fields = 715.3333 (SD ± 43.226792)

Animals	Mean nuclear area (um^2)	Mean no.nuclei/10 fields
B(2) Hypophysectomy, laparotomy and PB treatment (Group 2)		
H3	29.050326 (SD ± 6.663555121)	435
H4	29.33028 (SD ± 7.030745459)	432
H5	28.753685 (SD ± 8.49213427)	434
H2	27.196326 (SD ± 5.570962830)	443
H16	33.283008 (SD ± 7.399477247)	439
H6	29.15415 (SD ± 7.663851294)	429

Mean nuclear area = 29.461296 um^2 (SD ± 1.8491748)
Mean no.nuclei/10 fields = 435.3333 (SD ± 4.5704376)

Animals	Mean nuclear area (um^2)	Mean no.nuclei/10 fields
B(3) Hypophysectomy, 1/3 portal vein ligation and saline treatment (Group 3)		
H23	32.75091 (SD ± 5.919170495)	546
H19	33.535152 (SD ± 6.003905059)	520
H18	33.114294 (SD ± 5.493529571)	599
H6	35.070264 (SD ± 6.022868676)	547
H2	35.289036 (SD ± 7.246798712)	547
H0	33.524028 (SD ± 5.670764524)	567

Mean nuclear area = 33.880614 um^2 (SD ± 0.9582009, n = 6)
Mean no.nuclei/10 fields = 554.3333 (SD ± 24.191367)

Animals	Mean nuclear area (um^2)	Mean no.nuclei/10 fields
B(4) Hypophysectomy, 1/3 portal vein ligation and PB treatment (Group 4)		
H6	35.91198 (SD ± 6.806498572)	455
H4	36.388458 (SD ± 7.689201373)	458
H14	35.56899 (SD ± 7.625974080)	449
H15	37.586142 (SD ± 7.371994012)	459
H12	33.898536 (SD ± 6.344103648)	449
H9	33.301548 (SD ± 6.375171409)	514

Mean nuclear area = 35.442609 um^2 (SD ± 1.4545302, n = 6)
Mean no.nuclei/10 fields = 464.0 (SD ± 22.700954)

Animals	Mean nuclear area (um^2)	Mean no.nuclei/10 fields
B(5) Hypophysectomy, 2/3 portal vein ligation and saline treatment (Group 5)		
H41	37.651032 (SD ± 9.059960478)	467
H16	38.626236 (SD ± 9.428398581)	459
H19	36.933534 (SD ± 9.793099269)	463
H2	40.459842 (SD ± 9.911423076)	478
H0	39.53655 (SD ± 10.38716015)	544
H4	31.102704 (SD ± 7.929519093)	568

Mean nuclear area = 37.384983 um^2 (SD ± 3.0377164)
Mean no.nuclei/10 fields = 496.5 (SD ± 43.030028)

Animals	Mean nuclear area (um^2)	Mean no.nuclei/10 fields
B(6) Hypophysectomy, 2/3 portal vein ligation and PB treatment (Group 6)		
H3	48.832506 (SD ± 11.96327897)	455
H11	43.704342 (SD ± 10.60938979)	447
H15	40.016736 (SD ± 8.557934694)	443
H16	44.835282 (SD ± 11.33539045)	449

Mean nuclear area = 44.347217 um^2 (SD ± 3.1433413)
Mean no.nuclei/10 fields = 448.5 (SD ± 4.330127)

Table 6.5 Mean distribution of nuclei* into ploidy classes: portal vein ligation group - Section IV

Group	2n classes %	4n and above classes %
A. Sham hypophysectomy group		
A(1) Sham hypophysectomy, laparotomy and saline treatment (Group 1)	86.17	13.83
A(2) Sham hypophysectomy, 'aparotomy and PB treatment (Group 2)	64.67	35.33
A(3) Sham hypophysectomy, 1/3 portal vein ligation and saline treatment (Group 3)	58.25	41.75
A(4) Sham hypophysectomy, 1/3 portal vein ligation and PB treatment (Group 4)	42.84	57.16
A(5) Sham hypophysectomy, 2/3 portal vein ligation and saline treatment (Group 5)	31.8	68.2
A(6) Sham hypophysectomy, 2/3 portal vein ligation and PB treatment (Group 6)	9.2	90.8
B. Hypophysectomy group		
B(1) Hypophysectomy, laparotomy and saline treatment (Group 1)	92.83	7.17
B(2) Hypophysectomy laparotomy and PB treatment (Group 2)	89.5	10.5
B(3) Hypophysectomy, 1/3 portal vein ligation and saline treatment (Group 3)	84.34	15.66
B(4) Hypophysectomy, 1/3 portal vein ligation and PB treatment (Group 4)	73.67	26.33
B(5) Hypophysectomy, 2/3 portal vein ligation and saline treatment (Group 5)	60.67	39.33
B(6) Hypophysectomy, 2/3 portal vein ligation and PB treatment (Group 6)	40.25	59.75

* As determined by the Quantimet 720 image analyser described in the Material and Methods Section 2.7.1.1

Table 6.6 Percentage of nuclei per group exhibiting given integrated nuclear density in arbitrary units (A.U.) as determined by integrated microdensitometry for Section IV

Density	Group B(1) n=3 % nuclei	Group B(2) n=4 % nuclei	Group B(5) n=3 % nuclei	Group B(6) n=5 % nuclei
1.5 - 2.0	2.67	1.0		
2.0 - 2.5	9.33	6.5		
2.5 - 3.0	17.33	19.0		
3.0 - 3.5	20.67	22.0		0.8
3.5 - 4.0	18.0	13.0		0.4
4.0 - 4.5	6.67	9.5	2.0	2.8
4.5 - 5.0	6.0	6.5	5.33	4.0
5.0 - 5.5	6.67	7.0	10.67	6.4
5.5 - 6.0	5.33	7.5	16.0	10.8
6.0 - 6.5	4.67	4.0	22.0	5.6
6.5 - 7.0	2.0	3.0	20.0	4.8
7.0 - 7.5	0.66	1.0	8.0	10.4
7.5 - 8.0			2.67	14.0
8.0 - 8.5			1.33	8.4
8.5 - 9.0			2.67	10.0
9.0 - 9.5			1.33	4.4
9.5 - 10.0			1.33	3.6
10.0 - 10.5				2.4
10.5 - 11.0			1.33	1.6
11.0 - 11.5			0.66	1.2
11.5 - 12.0			2.0	0.4
12.0 - 12.5			1.33	1.2
12.5 - 13.0				1.6
13.0 - 13.5				1.6
13.5 - 14.0				0.8
14.0 - 14.5				
14.5 - 15.0				
15.0 - 15.5			0.66	2.4
15.5 - 16.0				
16.0 - 16.5				
16.5 - 17.0			0.66	

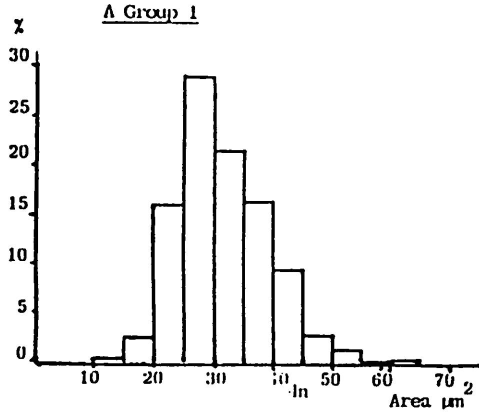
B(1) = Hypophysectomy, laparotomy and saline treatment
B(2) = Hypophysectomy, laparotomy and PB treatment
B(5) = Hypophysectomy, 2/3 portal vein ligation and saline treatment
B(6) = Hypophysectomy, 2/3 portal vein ligation and PB treatment

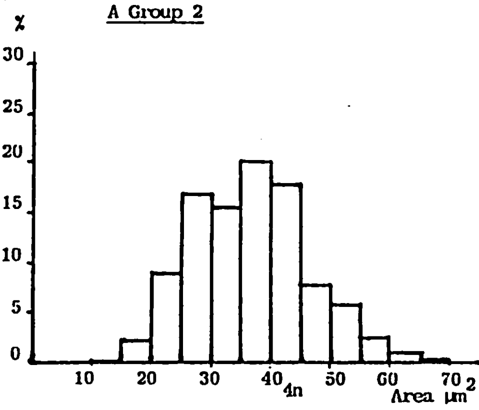
n = Number of animals investigated per group

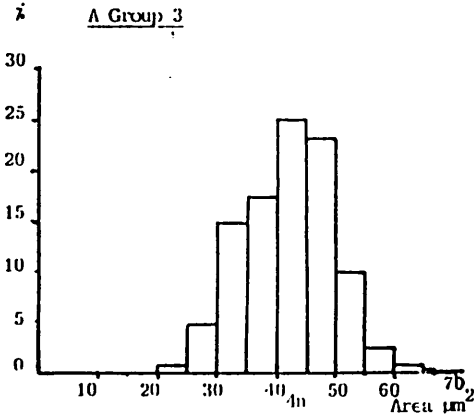
Fig 6.1.

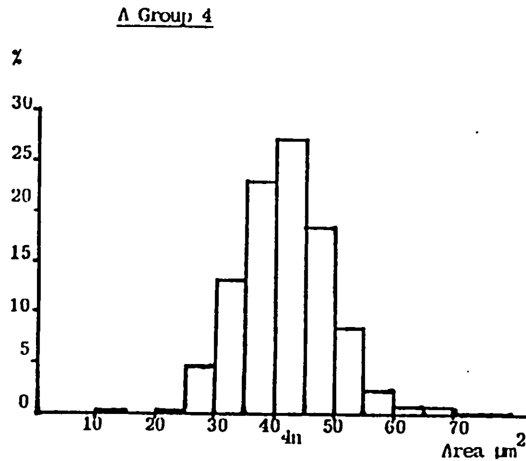
Figure 6.1 The mean percentage distribution of nuclei in size ranges of 5 um^2 classes from 10 - 120

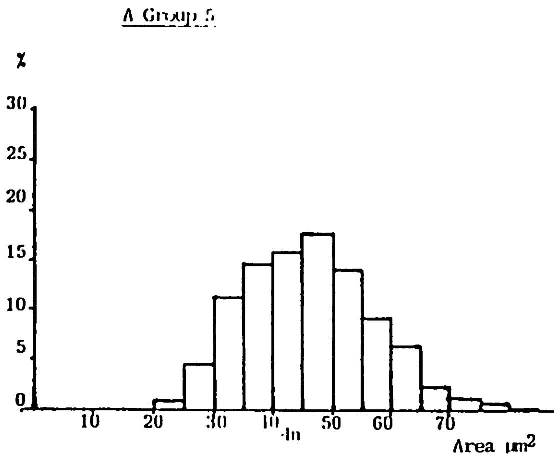
A. Group 1 = Sham hypophysectomy, laparotomy and saline treatment (n=6)
A. Group 2 = Sham hypophysectomy, laparotomy and PB treatment (n=6)
A. Group 3 = Sham hypophysectomy, 1/3 portal vein ligation and saline treatment (n=4)
A. Group 4 = Sham hypophysectomy, 1/3 portal vein ligation and PB treatment (n=7)
A. Group 5 = Sham hypophysectomy, 2/3 portal vein ligation and saline treatment (n=5)
A. Group 6 = Sham hypophysectomy, 2/3 portal vein ligation and PB treatment (n=5)











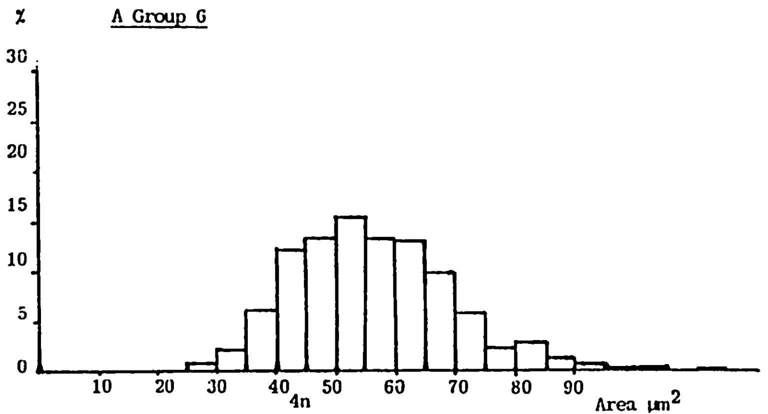
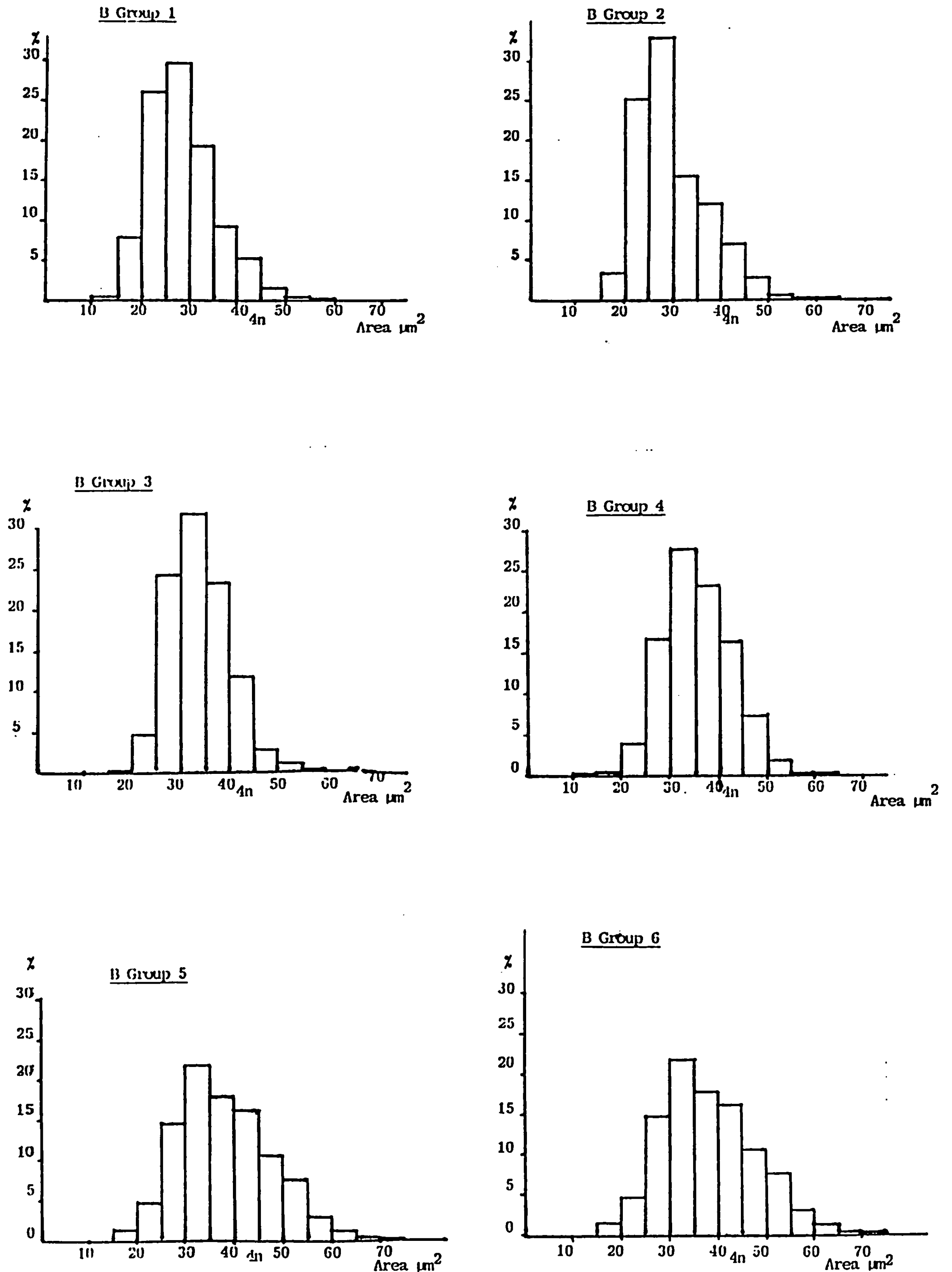


Figure 6.1 (contd)

B. Group 1 = Hypophysectomy, laparotomy and saline treatment (n=6)
B. Group 2 = Hypophysectomy, laparotomy and PB treatment (n=6)
B. Group 3 = Hypophysectomy, 1/3 portal vein ligation and saline treatment (n=6)
B. Group 4 = Hypophysectomy, 1/3 portal vein ligation and PB treatment (n=6)
B. Group 5 = Hypophysectomy, 2/3 portal vein ligation and saline treatment (n=6)
B. Group 6 = Hypophysectomy, 2/3 portal vein ligation and PB treatment (n=4)

4n = Nuclei of 4n and above ploidy classes at 40 um^2 and above diameter
n = Number of animals investigated per group



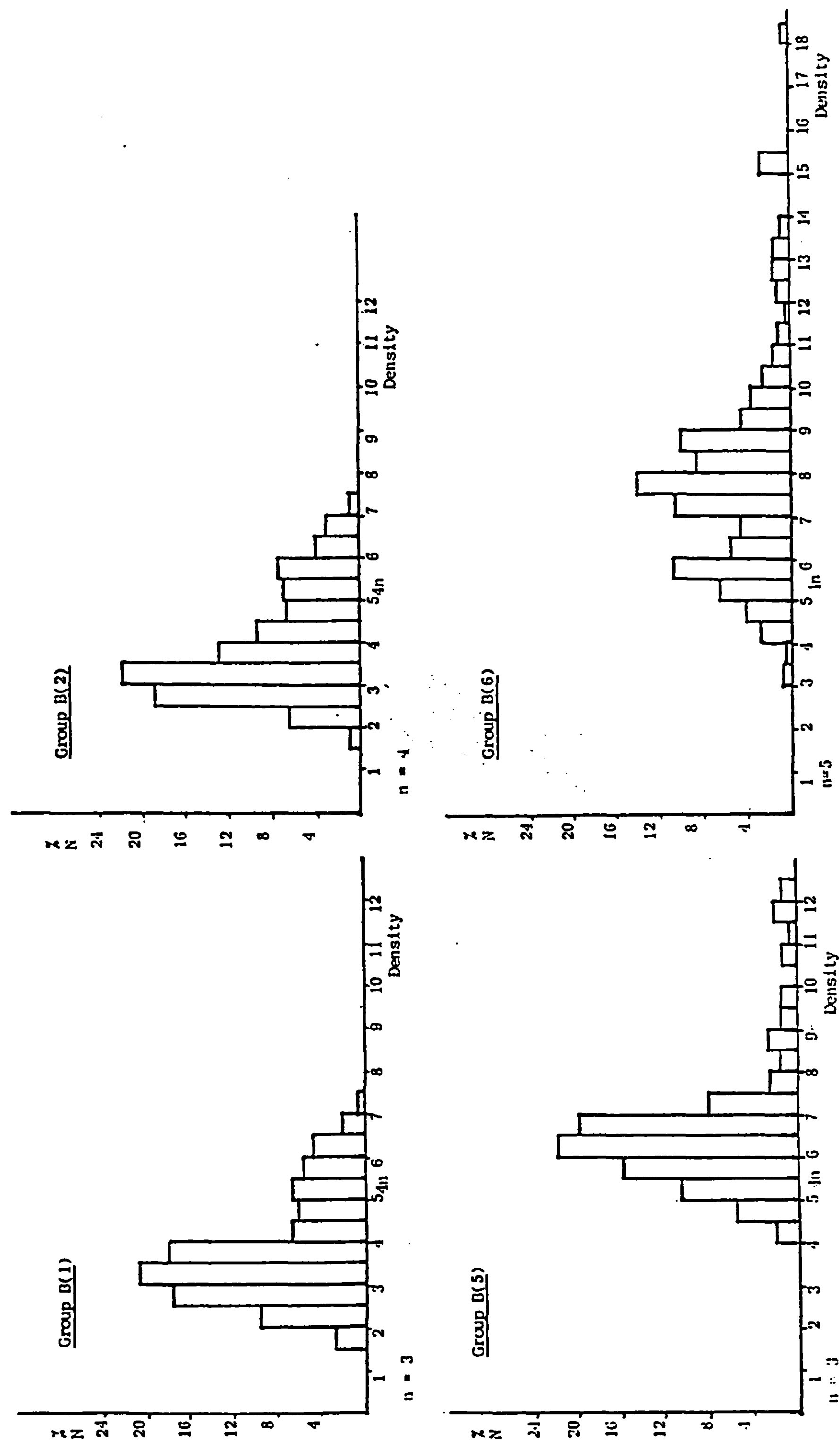


Figure 6.2 Histograms of nuclear integrated density in arbitrary units (A.U.) for portal vein ligation groups

n = number of animals investigated per group
%N = percentage of nuclei per group with arbitrary density
Groups:
B(1) = Hypophysectomy, laparotomy and saline treatment (controls - Group 1)
B(2) = Hypophysectomy, laparotomy and PB treatment (Group 2)
B(5) = Hypophysectomy, 2/3 portal vein ligation and saline treatment (Group 5)
B(6) = Hypophysectomy, 2/3 portal vein ligation and PB treatment (Group 6)

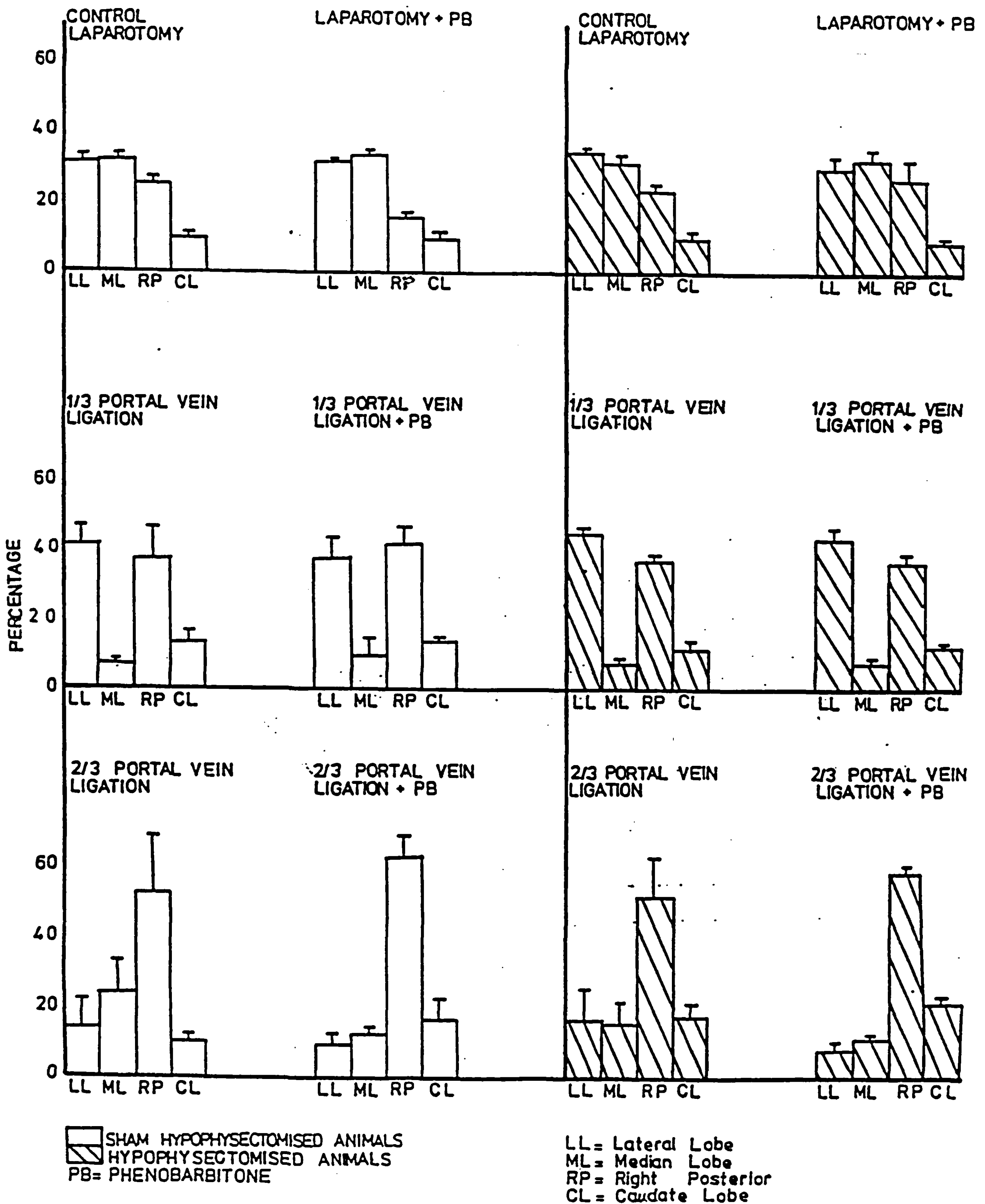


Figure 6.3 The effect of 1/3 and 2/3 portal vein ligation with or without PB treatment on the percentage contribution by individual liver lobes to the total liver weight in intact and hypophysectomised rats

CHAPTER 7

DISCUSSION

In order to establish whether the ability of PB to induce liver DNA synthesis could be restored in hypophysectomised rats in which the "DNA surplus" is retained (Schulte-Hermann et al., 1977), such a situation was experimentally engineered by performing 1/3 and 2/3 portal vein ligations. These surgical manoeuvres result in atrophy in the ligated lobe(s) with the DNA content remaining the same, while compensatory growth occurs in the unligated lobe(s) to such a degree that the original liver mass is restored (Weinbren, 1978). This growth is usually a combination of hypertrophy and hyperplasia and the present study was designed to investigate these aspects. The terms hypertrophy and hyperplasia have been used here as defined by other authors. "Hypertrophy" has been restricted to "an increase in cell size without an increase in ploidy" (Barka and Popper, 1967; Schulte-Hermann, 1974; Schlicht et al., 1968; Staubli et al., 1969). However parameters correlating with an increased cell size (e.g. increased cellular protein or RNA content) were not measured. "Hyperplasia" refers to "an increase in DNA content resulting from DNA synthesis which may lead to an increase in nuclear ploidy and/or an increase in cell numbers".

7.1 Pilot Study I

Initially animal survival was low, both in intact and hypophysectomised rats. Despite the dose regimen being that used by Schulte-Hermann et al. (1977), in this study the dose of PB appeared too high. An amount equivalent to 50% of their dose was subsequently employed as it was compatible with animal health and survival. The discrepancy in tolerance to the dose of PB may be due to a difference in

strain and sex of the rats, as male Wistar rats were used in this study as opposed to female CFHB rats in their work (Schulte-Hermann et al., 1977).

7.1.1 Sham hypophysectomised animals (Group A)

7.1.1.1 Response by the liver to PB treatment

Body weight increased in intact animals over the study period. The increase was consistent with the normal growth pattern for animals of 160-220 g and approximately 6-7.5 weeks of age at the start of the experiment (personal communication from the animal breeding facility, University of Nottingham). The response by the liver to PB resulted in increases in relative liver weight ranging from 23% - 40.79% and in cell size ranging from 20.97% - 22.79%. The mitotic index was also increased. These results confirm studies by other authors, (Conney, 1957; Herdson, 1964; Argyris, 1974; Schulte-Hermann et al., 1968; Schulte-Hermann, 1974) which show that PB induces liver growth and that this is attributable to a combination of hypertrophy and hyperplasia. As prolonging PB treatment to 12 days instead of 8 days failed to induce any further adaptive growth (as estimated by changes in cell volume and mitotic index), in all subsequent groups PB was given for the 8 day period. This is consistent with the study by Schulte-Hermann et al. (1977).

7.1.2 Hypophysectomised animals (Group B)

Hypophysectomy was verified by reductions in body, adrenal and testicular weight compared with sham hypophysectomy control animals (A - Group 1). These observations were consistent with those of previous authors (Di Stefano et al., 1955; Weinbren, 1959; Schulte-Hermann et al., 1977). These changes were not influenced by PB treatment.

7.1.2.1 Response by the liver to hypophysectomy

At the end of the study periods all hypophysectomised animals showed a significant reduction ($p < 0.05$) in relative liver weight ranging from 15.17% - 13.1%. Cell volumes were reduced by 61.98% - 63.7%. Together these observations suggested cell atrophy had occurred compared with sham hypophysectomy controls. The changes are possibly related to cessation of body growth and diminished food requirements and are consistent with those described by previous authors. Their studies demonstrated that following hypophysectomy body growth ceases, relative liver weight is reduced due to liver atrophy and the DNA content remains unchanged (Brues and Marble, 1937; Di Stefano et al., 1955; Alfert and Geschwind, 1958; Doljanski and Novogrotzky, 1959; Weinbren, 1959; Christensson et al., 1975; Leblond and Walker, 1965; Christensson et al., 1975; Schulte-Hermann et al., 1977).

7.1.2.2 Response by the liver to PB in hypophysectomised animals

Following PB treatment, in all groups there was a significant increase in mean relative liver weight (range 24.6% - 31.8%) and cells enlarged by 111.4% - 138.55% compared with hypophysectomised controls. There was no change in mitotic index. This suggested that PB induced an increase in liver mass by cell hypertrophy alone, in agreement with findings of Schulte-Hermann et al. (1977).

Thus in this small pilot study the response by the liver to hypophysectomy and to PB treatment both in intact and hypophysectomised animals was consistent with previous studies. The simple methods used before proceeding to the major part of the experiment gave adequate indication of responses by the liver.

7.2 Pilot Study II

7.2.1 Sham hypophysectomised animals (Group A)

7.2.1.1 Response by the liver to partial hepatectomy

Compared with controls (Group 1), a 34.13% increase in mean relative weight of the right posterior lobe following partial hepatectomy (Group 2) confirmed that compensatory growth had occurred. The liver mass was restored to control levels by 21 days. Cell volumes and the mitotic index were also similar to Group 1. Thus the response to partial hepatectomy was consistent with the findings of other authors (Harkness, 1957; Weinbren, 1959; Bucher, 1963).

7.2.2 Hypophysectomy group (Group B)

7.2.2.1 Response by the liver to hypophysectomy

The effect of hypophysectomy was seen by a reduction in body, adrenal and testicular weights compared with intact animals. A 4.3% reduction in the mean relative weight of the liver (B Group 1) compared with sham hypophysectomy controls (A Group 1) indicated liver atrophy. This was confirmed by the 57.94% reduction ($p < 0.01$) in cell volumes seen in the right posterior lobe. These features were consistent with Pilot Study I and other studies (Di Stefano et al., 1955; Doljanski and Novogrotsky, 1959; Weinbren, 1959; Bucher, 1963; Schulte-Hermann et al., 1977).

7.2.2.2 Response by the liver to partial hepatectomy in hypophysectomised animals

In this study hypophysectomy did not prevent the liver's response to partial hepatectomy (group 2), in agreement with the findings of other authors (Harkness, 1957; Weinbren, 1959). There was a significant increase (by 20.40%) in the mean relative weight of the right posterior

lobe compared with hypophysectomised controls (group 1). This indicated that compensatory growth had occurred. Despite the mean estimated cell volume following partial hepatectomy being 43.35% larger than in hypophysectomy controls (B group 1), atrophy was still apparent as the cell volumes were 39.71% smaller compared with sham hypophysectomy controls (A group 1). There was no difference in the mitotic index compared with controls, at 21 days post-hepatectomy.

Liver mass was restored only to 77% of hypophysectomy control values. This was consistent with the reports of other workers. In their studies the liver was restored by between 70-80% of hypophysectomy controls (Weinbren, 1959; Bucher, 1963; Schulte-Hermann et al., 1977).

Thus Pilot Studies I and II established that the responses to hypophysectomy, PB or partial hepatectomy in intact or hypophysectomised animals were consistent with previous studies. Simple measurements including changes in relative weight of the liver and right posterior lobe were appropriate methods to use for this study as they detected atrophy following hypophysectomy, adaptive growth following PB treatment and compensatory hyperplasia following partial hepatectomy. Similarly changes in mitotic index reflected cell replication following PB treatment in intact rats. Changes in mean estimated cell volume compared with appropriate control groups reflected cellular enlargement following PB treatment in intact or hypophysectomised rats and cell atrophy in hypophysectomised animal groups.

7.3 Measurements used to reflect DNA synthesis, cell proliferation and cell enlargement in Sections III and IV

Quantitative morphometric analysis undertaken using the Quantimet 720 image-analysing computer clearly demonstrated that at the

end of the experimental period shifts in ploidy had occurred following partial hepatectomy, portal vein ligation and PB treatment. These shifts reflected DNA synthesis. The persistence of this polyploidy was consistent with other studies (Bucher, 1963; Schulte-Hermann et al., 1971). Integrated microdensitometry showed a positive correlation between increased integrated nuclear density and shifts in ploidy. Microdensitometry results clearly confirmed that the increases in nuclear area detected by the Quantimet 720 image analysing computer reflected this change in ploidy, thereby confirming an increase in DNA synthesis. Similarly the mitotic index, which was increased after 8 days PB treatment (in the appropriate groups) but undetected 21 days after partial hepatectomy or portal vein ligation alone paralleled other reports (Schulte-Hermann et al., 1968). This indicated that cell proliferation had occurred (Schulte-Hermann, 1974; Bucher, 1963; Weinbren and Tarsh, 1964).

Frequently throughout this experiment the increase in mitotic index observed in some groups, compared with their respective controls, was considerable. If the change was expressed in terms of a percentage increase there would be enormous increases (often over 1000%). This may be misleading as the actual figures obtained by counting the number of mitoses per 100 fields were low, e.g. 2-3 per 100 fields. In determining the incidence of a rare phenomenon, in this case mitosis, the chance inclusion or exclusion or an observation could bias the resulting mitotic index. Such chance events could be due to sampling artifacts or to observational error. For example, if one mitosis per 100 fields was missed, then with these low absolute values percentage differences could be considerable. The methods used as described in

subsection 2.7.1.3 were deliberately intended to minimise the biasing effects of such factors. In view of these considerations it was decided not to express alteration in mitotic index in the following discussion in terms of percentage change but to just indicate when the increase occurred. Numerical values expressed in terms of mitoses per 1,000 cells have already been cited in the appropriate results chapters.

The measurement of cell volume in Sections III and IV also confirmed previous studies. These have shown that following PB treatment cell enlargement contributes to liver enlargement (Kunz et al., 1966; Staubli et al., 1969; Schulte-Hermann, 1977). Cell enlargement regresses after withdrawal of xenobiotics, at times varying from a few days with PB to 2 weeks using HCH (Kunz et al., 1966; Schlicht et al., 1968; Argyris, 1968; Schulte-Hermann, 1974). Thus in this study the measurement of cell size at 24 hours following the final dose of PB was clearly an appropriate time to detect changes in cell volume.

7.3.1 Response to partial hepatectomy - Section III

7.3.1.1 Sham hypophysectomised animals (Group A)

Neonatal rat liver consists principally of diploid cells and as the animals mature polyploidy increases with age, the timing of which is strain-dependent (Alfert and Geschwind, 1958; James et al., 1979; van Zwieten and Hollander, 1985; Van Bejooijen, 1984; Engelmann et al., 1981). For example, in Long Evans rats by 7.5 weeks 30% of nuclei are tetraploid, increasing to 43% by 70 days (Alfert and Geschwind, 1958). In six week old WAG/Rij rats the number of mononuclear tetraploids is approximately 53% (van Bezooijen et al., 1984) and in male Wistar rats by 6.5 weeks 48% of the nuclei are mononuclear tetraploids. The latter

increase to 60% by 65 days, with binuclear diploid cells increasing between 30-99 days (James et al., 1979). Thus the distribution of ploidy classes in this study would appear consistent with these other results. Male Wistar sham hypophysectomy control animals (group 1) aged approximately 70 days showed 57.8% of nuclei in the 2n ploidy classes and 42.2% in 4n and above ploidy classes.

Following partial hepatectomy in young growing rats there is a shift to a higher ploidy state, whereas in older rats the ploidy state remains fairly constant (Nadal and Zajdela, 1966). Following partial hepatectomy the ploidy shift persists indefinitely and there is a reduction in the number of binucleate cells (Sulkin, 1943). In male Wistar rats by 56 days post-hepatectomy there is a shift in the proportion of 4n ploidy nuclei from approximately 60% to 75% and in the 8n ploidy class from approximately 2.5% to 5% (James et al., 1966). In normal adult parenchymal cells approximately 75-80% of the nuclei are tetraploid and 1-2% octaploid. After restoration is complete following partial hepatectomy tetraploid cells are increased by 10% and octaploid by 50% (Alfert and Geschwind, 1958; Bucher, 1963). However, in James' study the animals were young growing 7 week old rats which may account for the larger shift in ploidy. Shifts in ploidy in this study were similar to the findings of James et al. (1966) as by 21 days following 1/3 or 2/3 partial hepatectomy (Groups 3 and 5) there were shifts of 40.20% and 49.76% respectively in the 4n and above ploidy classes compared with controls.

Changes in nuclear size correspond to shifts in ploidy levels (Sulkin, 1943; Christie and LePage, 1961; Ingram and Grasso, 1985;

1987). However in Groups 3 and 5 there was no statistically significant difference in the mean nuclear area compared with controls although from the histograms a shift in ploidy was evident (Fig. 3.1) as they showed peaks corresponding to 2n and 4n above ploidy classes. This disparity may have been due to technical limitations. For example, if cells with an irregular outline were inadvertently included when measuring nuclear area using the Quantimet 720 image analyser, these may have been tangentially cut leading to inaccurate measurements (Ingram and Grasso, 1985). Alternatively, if sections were not cut exactly through the diameter of the nucleus the true area would not be recorded which again would lead to inaccuracies. Another possibility is that changes in nuclear area following partial hepatectomy are less perceptible using this method than those following drug treatment. Another reason may have been that the magnitude of the shift towards polyploidy in these groups compared with controls, although significant, was not sufficiently great to result in an overall increase in mean nuclear area. However the most likely explanation is due to the variation in mean nuclear area found in Group 1 as suggested in subsection 3.1.1.2. In Group 1 there was one animal in which the mean nuclear area was considerably larger than the rest of the group. If this animal had been excluded from the study the mean nuclear area for the whole group would be significantly smaller compared with Group 3 ($p < 0.01$) and Group 5 ($p < 0.05$).

By 21 days following 1/3 and 2/3 partial hepatectomy the mitotic index in this study was low (being 0.2380 (SD $\pm$ 0.0789, n = 6) and 0.1194 (SD $\pm$ 0.1336, n = 6) respectively per 1,000 cells). This was not significantly different to controls. Again, this is in keeping with

other authors in which an increase in mitoses (20/1,000 cells) was found by 24 hours following partial hepatectomy, with mitotic activity exhibiting a distinct diurnal rhythm after 29 hours before declining during the next few days (Harkness, 1957; Weinbren, 1959; Bucher, 1963; Weinbren and Tarsh, 1964; Fabrikant, 1968). Occasional cells in division have been observed 1 and 2 weeks following partial hepatectomy (Fabrikant, 1968). In this study an occasional mitosis was seen 21 days post-hepatectomy when the usual waves of mitoses would have been complete. The mean number observed were not significant compared with controls.

Twenty-one days following 1/3 partial hepatectomy while compensatory hyperplasia was evident in the liver remnant and with the mean relative right posterior weight increased by 74.59% compared with controls, the relative liver weight was similar to controls. This indicates that the liver mass had been restored to control values. These responses are consistent with other studies (Harkness, 1957; Weinbren, 1959; Bucher, 1963) and reports that following both 1/3 and 2/3 partial hepatectomy the liver mass and liver DNA content are restored almost completely by one week (Schulte-Hermann et al., 1977).

Cell volumes are known to increase by 153% within 48 hours after partial hepatectomy, thereafter returning to normal size within a few days (Bucher, 1963; Murray et al., 1980). In this study 21 days after 1/3 partial hepatectomy cell size was consistent with these reports, as there was no difference in the mean estimated cell volume compared with controls. The finding of fewer nuclei/10 fields compared with controls reported here appears consistent with previous studies in which the number of binucleate cells were shown to fall rapidly following partial

hepatectomy, from the normal adult level of 25-30% to 8-10% (Sulkin, 1943; Bucher, 1963).

Following 2/3 partial hepatectomy while compensatory hyperplasia was evident in the right posterior lobe only 77.91% of total liver weight was achieved by 21 days. This differs from some workers but agrees with others, the reasons for these differences being unknown (Harkness, 1957; Weinbren, 1959; Leong et al., 1959).

Although following 2/3 partial hepatectomy cell volumes are reputed to enlarge within 48 hours, reverting to normal within a few days (Bucher, 1963; Murray, 1980) in this study cell volumes were still slightly enlarged (by 13.6%) compared with controls, 21 days post-hepatectomy. This could possibly be the result of the whole restorative process proceeding at a slightly slower rate. This would also account for the incomplete restoration of liver mass compared with controls. The larger cells in Group 5 would also contribute to fewer nuclei per 10 fields compared with controls.

7.3.1.2 <u>Hypophysectomy animals (Group B)</u>

Following hypophysectomy Di Stefano reported that the progression towards polyploidy was arrested at 10 days with 74% of nuclei in the 2n ploidy class and 26% in the 4n ploidy class. This contrasted with 47% (2n) and 53% (4n) in control animals (Di Stefano et al., 1955). Similarly 7 days post-hepatectomy Geschwind et al. (1958) found that in 28 day old animals, the distribution of 2n, 2n bi, 4n and 4n bi was 72.2%, 21.9%, 4.5% and 0.3% respectively. However in 49 day old animals ploidy distribution was 36.6%, 21.9%, 37.4% and 2.2% respectively, the development of polyploidy having been arrested by the hypophysectomy

at a more advanced stage (Geschwind et al., 1958). In this study 28 days after hypophysectomy the ploidy distribution was consistent with these authors, as in hypophysectomy control rats (group 1) aged approximately 70 days, 84.34% of the nuclei were in the 2n ploidy classes and 15.66% in the 4n and above classes.

Following hypophysectomy in Section III, despite the obvious reduction in relative liver weight in Group 1 compared with sham hypophysectomy control animals, there was no obvious difference in the mean relative weight of the right posterior lobe compared with the intact control group (see subsection 3.1.2.1). While it is possible that the hypophysectomised animals ate more than is usual following hypophysectomy (they usually eat less than intact animals), the most reasonable explanation for this discrepancy is that the anterior lobes of the liver in hypophysectomy control animals were handled more roughly compared with those in sham hypophysectomy controls. It is known that if this occurs the capsules surrounding the anterior lobes thicken, cause increased intra-hepatic pressure within these lobes resulting in compensatory hyperplasia in the posterior lobes (Weinbren, 1959). Evidence that this may have occurred in this study is given by a larger than expected contribution by the R.P. lobe to the whole liver weight (27.22%) compared with the sham hypophysectomy controls (25.36%).

All other responses by the animal and liver to hypophysectomy were consistent with other studies (Schulte-Hermann et al., 1977).

Following partial hepatectomy in hypophysectomised animals the ability of the liver to synthesise DNA is retained as measured by biochemical assays and a shift in ploidy, the latter resulting

predominantly from an increase in tetraploid cells (Doljanski and Novogrotzky, 1959; Geschwind et al., 1958; Schulte-Hermann et al., 1977). In Geschwind's study following Feulgen staining the nuclear size was measured microscopically and this showed an increase in polyploidy with the distribution of 2n, binucleate cells and 4n nuclei being 59.7%, 9.6% and 28.4% respectively, compared with 81.2%, 13.5% and 3.8% in hypophysectomised controls. 1.2% of nuclei in their study group were 4n bi and 8n combined. The shift in ploidy was not significantly different to that seen in normal unoperated controls at a similar age (Geschwind et al., 1958).

In this study following 1/3 and 2/3 partial hepatectomy in hypophysectomised rats (Groups 3 and 5) a shift towards higher ploidy was detected. The mean nuclear area was significantly increased by 22.87% and 21.43% respectively compared with controls. 42.5% of nuclei in Group 3 and 39.125% in Group 5 were in the 4n and above ploidy classes compared with 15.67% in the hypophysectomised controls (Group 1). The difference in the percentage distribution of ploidy levels in this study compared with Geschwind's study (1958) is most probably due to the differences in animal strain and age. In Geschwind's study the animals were younger and of Long-Evans strain as opposed to Wistar used in this study. The changes seen in the present study are consistent with these other authors (Geschwind et al., 1958; Bucher, 1963). The reduction in the number of nuclei/10 fields reported in this study compared with controls is most probably related to the changes in cell size.

It is well known that hypophysectomy delays the events following partial hepatectomy which culminate in DNA synthesis and cell replication (Bucher, 1963). This results in a lag in the increased

mitotic index compared with intact rats. However despite this phenomenon there was no significant difference in the mitotic index at 21 days in this study following 1/3 or 2/3 partial hepatectomy, compared with hypophysectomy controls. No difference would be expected as the increased mitotic index however delayed would have regressed by 21 days 'post-hepatectomy'.

Several authors have observed that following partial hepatectomy in hypophysectomised animals the liver is incompletely restored. Franseen et al. (1938) reported that the liver was restored to between 2/3 and 3/4 of control values, Doljanski and Novogrotzky (1959) reported 74% liver restoration and Schulte-Hermann et al. (1977) between 70-80% compared with controls. This is thought to be the result of a reduced food intake.

In this study the liver mass was restored completely following 1/3 partial hepatectomy in contrast to 70-80% restoration in Schulte-Hermann's study (Schulte-Hermann et al., 1977). Similar reduction in body weight occurred following hypophysectomy in this group compared with controls (Bl - Group 1) and so unless the food intake was significantly larger than the usual reduced intake which occurs following hypophysectomy, this response by the liver is unexplained. However in this study the mean estimated cell volume in this group was 30.5% larger than hypophysectomy controls (Bl - Group 1) but smaller than intact controls (Al - Group 1) (the latter difference reflecting liver atrophy induced by hypophysectomy). This may indicate that following partial hepatectomy protein synthesis in the first few days was larger than usually detected after hepatectomy (Bucher, 1963). A

hypertrophic effect such as this could possibly account for the liver being apparently completely restored.

However following 2/3 partial hepatectomy 74.84% of the liver was restored compared with hypophysectomised controls. This is in keeping with other studies (Franseen et al., 1938; Doljanski and Novogrotzky, 1958; Schulte-Hermann et al., 1977). Compensatory growth was evident by a 106% increase in the mean relative weight of the right posterior lobe compared with hypophysectomy controls. The mean estimated cell volume in this study was 60.40% larger than in hypophysectomised controls (B - group 1) although smaller than seen in intact controls (A1 - group 1). This latter difference is consistent with hypophysectomy.

7.3.2 Response by the liver to PB treatment - Section III

7.3.2.1 Sham hypophysectomised group (Group A)

7.3.2.1.1 Response by the liver to PB following laparotomy

Following 8 days PB treatment there was a 32.70% increase in 4n and above ploidy classes in sham hypophysectomy rats (group 2) compared with sham hypophysectomy controls (group 1). Fifty-six percent of nuclei were in the 4n and above ploidy classes compared with 42.2% in Group 1 controls. This is in agreement with reports by Schulte-Hermann in which PB was shown to induce an increase in nuclear tetraploidy by 20-40% (Schulte-Hermann et al., 1968; Schulte-Hermann, 1974).

These authors also reported that 60-80% of the total increase in DNA content following PB treatment was due to nuclear multiplication. In this study the numbers of mitoses were also increased by PB treatment, being 0.6833 per 1,000 cells compared with 0.1081 per 1,000 cells in sham hypophysectomy controls. This is a considerable

increase (over six times larger) compared with controls. The dose used in this study was 50% of the dose used by Schulte-Hermann et al. (1977). In an earlier investigation these workers reported 1.25 mitoses per 1,000 cells compared with 0.36 per 1,000 cells in control animals after 15 days PB treatment, an increase of over twofold (Schlicht et al., 1967; Schulte-Hermann et al., 1968). The mitotic index in this study therefore seems consistent with these earlier studies, although somewhat larger.

Following PB treatment the mean estimated cell volume was increased by 20.44% compared with controls. This is consistent with the 21% increase in hepatocyte volume reported by Staubli et al. (1969) following 5 days PB treatment at a dose of 100 mg/kg body weight (Staubli et al., 1969). Thus in this study the hyperplastic and hypertrophic response to PB reflected by an overall increase in relative liver weight and weight of the right posterior lobe is consistent with other studies (Schulte-Hermann, 1974).

7.3.2.1.2 Response by the liver to PB following partial hepatectomy

If PB is administered to intact rats following partial hepatectomy its capacity to induce both hypertrophy and hyperplasia is retained. In this study following 1/3 or 2/3 partial hepatectomy the effect of PB was studied by comparing Group 4 with Group 3 and Group 6 with Group 5. By 21 days after 1/3 partial hepatectomy (Group 3) the liver mass was restored to control values but following 8 days PB treatment (Group 4) the mean relative weight was increased above control values by 19.19%. Compensatory hyperplasia was detected in Group 4 by a 22.84% increase in the mean relative weight of the right posterior lobe compared with saline treated animals (Group 3). Similarly while the liver mass was

only restored to 77.91% of control value after 2/3 partial hepatectomy (Group 5), following PB treatment (Group 6) liver mass was restored to control values. The mean relative weight of the liver was increased by 19.54%, and the mean relative weight of the right posterior lobe increased by 25.75% compared with Group 5. This further adaptive growth induced by PB following both 1/3 and 2/3 partial hepatectomy was a combination of hyperplasia and hypertrophy.

DNA synthesis was detected by a significant shift in ploidy. The mean nuclear area was greater in Groups 4 and 6 compared with Groups 3 and 5. The number of nuclei in the 4n and above ploidy classes in Groups 4 and 6 were increased by 38.87% and 25.63% respectively compared with non-PB treated animals. This is consistent with the 20%-40% shift in ploidy levels in rat livers following PB treatment described by Schulte-Hermann and colleagues (Schulte-Hermann et al., 1968; Schulte-Hermann, 1974). In addition these increases are over and above the 40.2% and 49.76% shift in ploidy in Groups 3 and 5 compared with controls, thus indicating the independent hyperplastic effect induced by PB.

Following 1/3 and 2/3 partial hepatectomy an increase in the number of mitoses per 1,000 cells was detected after PB treatment. A large increase was observed (over double the number per 1,000 cells) in Group 4 compared with Group 3 and considerably increased (over six times the number per 1,000 cells) in Group 6 in contrast to Group 5. This is consistent with the reports by previous workers following 15 days PB treatment to non-hepatectomised animals (Schlicht et al., 1967; Schulte-Hermann et al., 1968). These present results again suggest that PB

shows an additive hyperplastic effect over and above the hyperplastic response following partial hepatectomy.

In Groups 4 and 6 the mean estimated cell volumes were enlarged by 10.94% and 5.71% compared with their respective saline treated groups (Groups 3 and 5). This indicates that PB had induced a hypertrophic effect on the cells possibly due to an increase in protein synthesis. The enlargement was smaller than the 20.44% increase in Group 2. This indicates that PB had less of an hypertrophic effect on the hepatocytes following partial hepatectomy than following laparotomy.

In a study by Aletti et al. (1981) the capacity of the liver to respond to PB was studied in male Wistar rats at 48 and 96 hours after 2/3 partial hepatectomy. At 48 hours the capacity of the liver to respond to PB was partially impaired, with PB having no influence on the relative liver weight, DNA and protein content. In contrast by 96 hours the relative liver weight was significantly increased compared with 2/3 hepatectomised animals. In these animals relative liver weight was equal to control values at 4 days compared with at 10 days in non-PB treated animals. Their investigations suggested that following 2/3 partial hepatectomy the liver response is below its functional capacity and that like normal non-hepatectomised liver, it has a functional reserve which can be utilised when demanded as, for example, by PB administration (Ragnotti and Aletti, 1974; Aletti et al., 1981). Thus the response to PB in Groups 4 and 6 is consistent with the liver's capacity to respond to an increased functional demand by a combination of hyperplasia and hypertrophy (Ragnotti and Aletti, 1974).

7.3.2.2 Hypophysectomy group (Group B)

7.3.2.2.1 Response by the liver to PB following laparotomy

Using biochemical assays to estimate changes in DNA and protein synthesis, it has been shown that following PB treatment DNA content in the hypophysectomised animal remains constant, hypophysectomy blocking DNA synthesis (Schulte-Hermann et al., 1977). Using nuclear area and ploidy levels as indicators of change in DNA synthesis and content, there was no significant difference in the mean nuclear area or distribution of nuclear ploidy classes in Group 2 following PB treatment compared with hypophysectomy controls (Group 1) (84.67% vs. 15.33%). This suggested arrest in the development of polyploidy consistent with other studies (Alfert and Geschwind, 1958). In contrast with intact animals, the ability of PB to induce mitoses in hypophysectomised animals also appeared to be blocked. There was no significant difference in the number of mitoses per 1,000 cells between these two groups, the mean number per 1,000 cells being 0.00 (SD $\pm$ 0.0, n = 6) (Group 2) and 0.0151 (SD $\pm$ 0.0338, n = 6 - Group 1) respectively.

However in this study following PB treatment the mean relative weights of the liver and right posterior lobe in hypophysectomised animals (group 2) were increased by 31.15% and 22.61% respectively compared with controls. This increased liver mass was due to a hypertrophic response demonstrated by a 114.90% increase in mean estimated cell volume. Thus the overall response to PB following hypophysectomy in this study is consistent with the findings of Schulte-Hermann et al. (1977).

7.3.2.2.2 Response by the liver to PB following partial hepatectomy

The study undertaken by Schulte-Hermann and his colleagues (1977) demonstrated that in hypophysectomised rats following 1/3 or 2/3 partial hepatectomy, PB could induce liver enlargement together with DNA synthesis. This was in contrast to an increase in liver mass without any increase in liver DNA content in non-hepatectomised animals. It was suggested that it was the relative DNA surplus resulting from hypophysectomy which prevented PB from inducing DNA synthesis in these rats. They concluded that it was the removal of this "surplus" during 1/3 or 2/3 partial hepatectomy which restored the ability of PB to induce DNA synthesis.

In this study the ability of PB to induce DNA synthesis following 1/3 and 2/3 partial hepatectomy was seen by comparing changes induced by PB in hypophysectomised laporotomy animals (group 2) with those following 1/3 and 2/3 partial hepatectomy (Groups 4 and 6). Hyperplasia was detected in Groups 4 and 6 respectively by a 36.9% or 29.26% increase in mean nuclear area. This correlated with a 316.37% or 211.61% increase in the number of nuclei in the 4n and above ploidy classes. The increase in mitotic index was large (more than doubled) in Group 4 compared with Group 2, and considerably increased (by more than six times) in Group 6 in contrast to Group 2, these changes indicating an increase in cell numbers. The mean estimated cell volume was reduced by 33.98% and 12.41% in Groups 4 and 6 respectively compared with Group 2 and there were 13.8% and 6.11% more nuclei per 10 fields which reflected this reduction in cell size. These results therefore confirm the responses in hypophysectomised rats to PB following partial hepatectomy described by Schulte-Hermann et al. (1977).

While the cell volumes in Groups 4 and 6 were smaller than those in Group 2, the mean estimated cell volumes were 60.39% and 91.18% larger than control animals, thus confirming that hypertrophy had been induced. The smaller cell volumes in Groups 4 and 6 compared with Group 2, along with an increased mitotic index indicate that the hyperplastic component of the response to PB following 1/3 and 2/3 partial hepatectomy, was a combination of both cell multiplication and a significant shift towards higher nuclear ploidy.

In Group 2 the mean relative liver weight was 9.89% heavier than Group 4 and 40.94% heavier than Group 6. This is despite significant increases in relative weight of the right posterior lobe in these two groups (51.95% Group 4, 95.77% Group 6) with obvious hyperplasia demonstrated in the lobes compared with Group 2 (where DNA synthesis has been blocked). As however the cell volumes in Group 2 are larger compared with the other two groups this may indicate that PB induces a larger hypertrophic effect in the liver of laparotomised animals and could account for the heavier liver. The larger hypertrophic response in Group 2 would also account for fewer nuclei being observed per 10 fields compared with Groups 4 and 6.

Cell volumes in Group 6 were similar to Group 5 following 2/3 partial hepatectomy alone. It appears therefore that the adaptive response to PB in Group 6 was predominantly the result of hyperplasia.

It was confirmed that the response to PB following 1/3 or 2/3 partial hepatectomy compared with laparotomised animals (Group 2) in hypophysectomised rats was entirely independent of other factors. This

resulted from comparing Groups 4 and 6 with saline treated animals following hepatectomy (Groups 3 and 5) respectively.

Adaptive growth induced by PB over and above the compensatory hyperplasia which followed 1/3 and 2/3 partial hepatectomy was confirmed by a 20.43% and 16.86% increase in the mean relative weight of the right posterior lobe in Groups 4 and 6 compared with Group 3 and 5. The mean relative liver weight was also increased by 20.41% and 21.05% in Groups 4 and 6 respectively compared with the saline treated groups.

DNA synthesis was detected by a significant increase in mean nuclear area in Groups 4 and 6 due to a 50.19% and 22.1% shift in 4n and above ploidy classes compared with Groups 3 and 5. This is consistent with the ploidy changes induced by PB described by Schulte-Hermann and his colleagues (Schulte-Hermann et al., 1968; Schulte-Hermann, 1974). The considerable increase (over six times) in mitotic index in Groups 4 and 6 compared with Groups 3 and 5 reflects cell replication.

The 22.90% increase in mean estimated cell volume in Group 4 compared with Group 3 was also consistent with the 21% increase following 5 days PB treatment described by Staubli et al. (1969). Thus the 20.41% increase in mean relative liver weight induced by PB in Group 4 over and above 1/3 partial hepatectomy (Group 3) was the result of both hyperplasia and hypertrophy, this increase again similar to the 28.5% increase in relative liver weight following PB treatment described by these authors (Staubli et al., 1969).

The mean estimated cell volume in Group 6 however was not significantly different from that in Group 5 although the cell volumes were 91.18% larger than control animals (Group 1). This apparent lack

of hypertrophic effect may be partly due to the larger increase in number of cells in the right posterior lobe in comparison with Group 4, indicated by a higher mitotic index and smaller shift in ploidy.

To conclude, these results appear consistent with the study undertaken by Schulte-Hermann et al. (1977). They confirm that following partial hepatectomy PB can induce DNA synthesis in hypophysectomised rats (Schulte-Hermann et al., 1977).

7.3.3 Response by the liver to portal vein ligation - Section IV

7.3.3.1 Sham hypophysectomised animals (Group A)

Following portal vein ligation atrophy occurs in the ligated lobes while the remaining unligated tissue shows cellular hypertrophy and DNA synthesis (Weinbren, 1978). This response continues for 14-18 days after which no further atrophy occurs and the posterior lobes no longer increase in size (Duchen, 1961; Weinbren and Tarsh, 1964). Following ligation the unligated lobes become enlarged and paler while their cells and nuclei rapidly increase in size during the first few days. Mitoses are visible by 24 hours and their frequency increases for 3-12 days after which their incidence decreases until few mitoses are present by 18 days and they are rare by 28 days. This response is comparable to that following 2/3 partial hepatectomy (Duchen, 1961; Weinbren and Tarsh, 1964). Both cell and nuclear size become more uniform by 28 days although the liver lobules remain enlarged (Duchen, 1961). Whilst these changes are taking place, it has been demonstrated by biochemical analysis that the DNA level in the liver is unaltered in the ligated lobes deprived of portal blood flow (Duchen, 1961).

In this study, following 1/3 or 2/3 portal vein ligation (Groups 3 and 5) compensatory hyperplasia occurred in the unmanipulated lobes. This was detected by a 25.20% and 89.48% increase in mean relative weight in the right posterior lobe in Groups 3 and 5 respectively, compared with controls. However by 21 days, following 1/3 or 2/3 portal vein ligation the enlargement of the unligated lobes, combined with any proliferative response which occurred in the atrophied cells of the ligated lobes, failed to restore the liver mass to control values. The mean relative weights were 83.01% and 85.94% respectively compared with controls. This is consistent with some reports (Lawrence et al., 1959; Weinbren, 1959) although in other studies the relative liver weight was fully restored to control values (Steiner and Martinez, 1961; Rozga et al., 1985).

DNA synthesis was detected by 21.16% and 46.33% increases in nuclear area in Groups 3 and 5 with 201.81% and 393.13% increases in nuclei in the 4n and above ploidy classes compared with controls (group 1). Twenty-one days following 1/3 or 2/3 portal vein ligation, the number of mitoses per 1,000 cells were not significantly different from control values. As mitoses occur early in the reactive growth phase following portal vein ligation, it would not be expected that they would be detected (Weinbren and Tarsh, 1964; Rozga et al., 1985).

Following 1/3 portal vein ligation cell volumes were similar to controls indicating that any earlier cell enlargement had by 21 days returned to normal, as shown in other studies (Duchen, 1961; Weinbren and Tarsh, 1964; Weinbren et al., 1972). However following 2/3 portal vein ligation a small but significant increase by 5.48% in the mean estimated cell volume was seen on day 28 compared with controls.

The stimulation of DNA synthesis following 1/3 or 2/3 portal vein ligation in this study was comparable to the responses reported in this study following 1/3 or 2/3 partial hepatectomy. These results clearly confirm other studies which have demonstrated that, following ligation of a liver lobe, DNA synthesis is stimulated in the unligated lobe in a manner comparable to the response following hepatectomy. In addition DNA synthesis stimulated by portal vein ligation occurs during a situation where DNA content remains unaltered (Duchen, 1961; Weinbren and Tarsh, 1964; Weinbren et al., 1972).

7.3.3.2 Hypophysectomised animals (Group B)

As in Section III following hypophysectomy liver atrophy was indicated by the 14.8% and 19.67% decrease in relative weight of the liver and right posterior lobe compared with intact controls, consistent with the findings of other authors (Doljanski and Novogrotzky, 1959; Schulte-Hermann et al., 1977). This was confirmed by a 66.67% reduction in mean estimated cell volume compared with sham hypophysectomised animals. This again was similar to the response in Section III. The larger number of nuclei per 10 fields in the hypophysectomy controls compared with sham hypophysectomy controls also indicated cell atrophy. Ploidy levels were similar compared with hypophysectomy controls in Section III, again representing a block in development towards polyploidy known to occur following hypophysectomy (Geschwind et al., 1958).

7.3.3.2.1 Response by the liver to portal vein ligation in hypophysectomised animals

Hypophysectomy does not prevent DNA synthesis in the liver following partial hepatectomy (Bucher, 1963; Section III this study).

The same applies after 1/3 and 2/3 portal vein ligation in the hypophysectomised animals (Groups 3 and 5). Compensatory hyperplasia was identified by a 79.27% and 59.22% increase in mean relative weight of the right posterior lobe in Groups 3 and 5 and a 17.68% increase beyond the control value in relative liver weight following 1/3 portal vein ligation. Only 76.21% of liver mass was restored compared with controls following 2/3 portal vein ligation although compensatory hyperplasia was clearly evident. DNA synthesis was reflected by a 118.61% and 448.84% increase in the 4n and above ploidy classes and a 19.10% and 31.42% increase in mean nuclear area in Groups 3 and 5 compared with controls. By 21 days post-portal vein ligation, no mitoses were detected in either group, the waves of mitosis being over by this stage (Weinbren and Tarsh, 1964). A 66.17% and 82.71% increase in mean estimated cell volume, indicating hypertrophy with an associated decrease in nuclei per 10 fields, was detected in both Groups 3 and 5 unlike other reports (Duchen, 1961) in which cells by this time had returned towards control values.

7.3.4 Response by the liver to PB treatment following portal vein ligation

7.3.4.1 Sham hypophysectomised group (Group A)

7.3.4.1.1 Response by the liver to PB following laparotomy

In Group 2 adaptive growth was reflected by a 21.28% increase in the mean relative weight of the right posterior lobe and a 22.7% increase in mean relative liver weight, consistent with other studies (Staubli et al., 1969). These changes were smaller than those reported for Group 2 in Section III, which may reflect a seasonal variation in response to PB.

DNA synthesis was detected in Group 2 by a shift towards polyploidy (an increase in frequency of 4n and above nuclei by 155.46%). This was also demonstrated by a 16.87% increase in mean nuclear area. This ploidy shift was larger than reported by Schulte-Hermann et al. (1968). They described a 5-15% shift following HCH treatment and a 5% shift with PB compared with controls. There was a large increase in the number of mitoses per 1,000 cells compared with controls which was about double the increase reported by Schulte-Hermann et al. (1968). This reflected a large increase in cell numbers. There was a 46.73% increase in cell volume in this group compared with 21% reported by Staubli and colleagues (1969). Thus with a similar strain and weight of animal compared with these other reports, in this study a 50% lower dose of PB induced a greater hypertrophic and hyperplastic response.

7.3.4.1.2 Response by the liver to PB following portal vein ligation

To detect changes induced by PB following 1/3 or 2/3 portal vein ligation Groups 4 and 6 were compared with saline treated controls, Groups 3 and 5.

In Groups 4 and 6 the response to PB was similar to that already described following laparotomy. Adaptive growth was detected by a 59.03% and 49.98% increase in mean relative weight of the right posterior lobes and a 40.59% and 18.47% increase in relative liver weight in Groups 4 and 6 compared with Groups 3 and 5. This was consistent with other studies (Schlicht et al., 1968) but greater than in Group 2.

DNA synthesis was apparent from a 36.92% and 33.13% shift towards polyploidy and a 7.75% and 22.43% increase in mean nuclear area compared

with Groups 3 and 5 respectively. Cell replication was reflected by a considerable increase in the number of mitoses per 1,000 cells (by more than six times) compared with Groups 3 and 5. This is consistent with an increased mitotic index after 8 days PB treatment, described by Schulte-Hermann and colleagues (1968), although considerably greater than in Group 2. The shift in ploidy was greater than in these other studies (Schulte-Hermann et al., 1968), but smaller compared with Group 2.

Cellular enlargement suggesting hypertrophy was also detected following PB treatment with a 69.38% and 78.68% increase in the mean estimated cell volume in Groups 4 and 6 compared with Groups 3 and 5. This is considerably greater than the 21% enlargement described by Staubli and colleagues (1969) and the 46.73% increase in Group 2 (A2).

Overall there was a greater hypertrophic response and more cell multiplication following PB treatment after 1/3 or 2/3 portal vein ligation compared with following laparotomy (Group 2).

7.3.4.2 Hypophysectomy group (Group B)

7.3.4.2.1 Response by the liver to PB following laparotomy

Following hypophysectomy PB did not induce an increase in mean nuclear area, a shift in ploidy or change in mitotic index. An adaptive response was reflected by a 42.15% and 33.4% increase in the relative weight of the liver and right posterior lobe. This was associated with a 157.45% increase in cell volume along with fewer nuclei/10 fields compared with controls. This confirms that following hypophysectomy PB induces an increase in liver mass by hypertrophy alone. This was

consistent with Section III and other studies (Schulte-Hermann et al., 1977).

7.3.4.2.2 Response by the liver to PB following portal vein ligation

Adaptive growth was detected by a 49.79% and 79.48% increase in the mean relative weight of the right posterior lobe in Groups 4 and 6 compared with Group 2. The mean relative weight in Group 4 was 39.22% heavier compared with controls whereas following 2/3 portal vein ligation the liver mass was restored to control values.

Following portal vein ligation the ability of PB to induce DNA synthesis is restored in hypophysectomised animals. This is apparent by the response to PB in Groups 4 and 6 compared with laparotomy animals (Group 2), where DNA synthesis and mitotic activity were blocked. In Groups 4 and 6 there were increases of 150.76% and 469% respectively in the number of nuclei in the 4n and above ploidy classes, indicating a significant shift in polyploidy compared with Group 2, in which any shift towards polyploidy was blocked. These ploidy shifts are reflected by a 20.30% and 50.53% increase in mean nuclear area in comparison with Group 2 where the mean nuclear area was similar to controls. Cell replication was also detected by a considerable increase (approximately over 10 times) in the number of mitoses per 1,000 cells compared with Group, although the actual figures were small.

The mean estimated cell volume however was 40.1% and 27.5% smaller in Groups 4 and 6 compared with Group 2 although they were significantly enlarged compared with controls. This, along with more nuclei/10 fields associated with the smaller cells, again suggested that cell

multiplication had taken place. Thus the ability for PB to induce both hypertrophy and hyperplasia was restored.

The extent to which PB alone was responsible for this response is seen by comparing hypophysectomised 1/3 or 2/3 portal vein ligated animals treated with saline (Groups 3 and 5) with Groups 4 and 6. Following 1/3 or 2/3 portal vein ligation PB induced an 18.30% and 37.55% increase in mean relative liver weight. Adaptive growth was induced in the right posterior lobes in which there were increases of 18.77% and 60.29% in the mean relative weight in Groups 4 and 6 compared with Groups 3 and 5. This was the result of DNA synthesis detected, by a 68.14% and 51.92% shift in polyploidy, as shown by a 4.61% and 18.62% increase in mean nuclear area in Groups 4 and 6 compared with Groups 3 and 5. This latter increase was not statistically significant in Group 4 compared with Group 3. This may have been due to inter-animal variation as one animal in Group 4 showed a slightly smaller mean nuclear area compared with the remaining five. If this animal is excluded from the data the remaining five show a significant difference ($p < 0.05$) in the mean nuclear area compared with Group 3 (Wilcoxon 2 sample test). PB induced considerable increases in the number of mitoses per 1,000 cells (over 10 times) compared with Groups 3 and 5 although the actual figures are small. Hypertrophy was also induced by PB as the mean estimated cell volume in Groups 4 and 6 increased by 10.6% and 10.51% compared with Groups 3 and 5.

7.4 Conclusion

In this study it has been shown that in hypophysectomised animals the response by the liver to PB is similar following portal vein ligation or partial hepatectomy, in terms of the ability to induce DNA

synthesis. This is in contrast to the situation following laparotomy in hypophysectomised animals in which PB fails to stimulate an increase in DNA content. However, following 1/3 portal vein ligation PB induced less of a shift in ploidy but greater cell replication compared with following 1/3 partial hepatectomy. After 2/3 portal vein ligation, PB induced a greater increase both in ploidy and cell replication compared with 2/3 partial hepatectomy.

As PB can induce DNA synthesis in hypophysectomised animals following portal vein ligation, in a situation where the hepatic DNA content in the animal remains constant, rather than when it has been reduced following partial hepatectomy, these results refute the concept put forward by Schulte-Hermann et al. (1977). They proposed that PB induced DNA synthesis in hypophysectomised animals following partial hepatectomy because the "relative DNA excess" was removed by the hepatectomy, thus eliminating the block on DNA synthesis. The present study clearly demonstrates that PB can induce DNA synthesis in hypophysectomised animals without prior removal of the "relative DNA excess", and in circumstances where only cytoplasm is reduced. This suggests that the compartment in which the stimulus for DNA synthesis may reside is more likely to be cytoplasmic or plasmalemmal rather than nuclear in origin.

The mechanisms by which DNA synthesis is induced in the unligated lobe of the liver following portal vein ligation remain unclear. While some studies have reported that in the ligated lobes atrophy is accompanied by hepatocyte necrosis (often referred to as apoptosis) (Steiner and Martinez, 1961; Kerr, 1971; Kerr et al., 1972; Rozga et al., 1985; Daoust and Morais, 1986) others have not reported this phenomenon (Weinbren and Tarsh, 1964; Dubuisson et al., 1982). Kerr and

his colleagues suggested that apoptosis may play a role in the mitogenic processes (Kerr, 1971; Kerr et al., 1972). However apoptosis is not observed in the liver remnant after partial hepatectomy nor is DNA synthesis observed when apoptosis is induced by subnecrogenic doses of diethyl nitrosamine (DEN) (Kerr et al., 1972; Daoust and Morais, 1986).

When considering the possibility of the apoptotic phenomenon being involved in the growth response in the unligated lobes of the liver following portal vein ligation, it must be emphasised that mitosis (which follows earlier DNA synthesis) is prominent by 24 hours in both ligated and unligated lobes (Weinbren and Tarsh, 1964; Weinbren et al., 1972) whereas apoptotic bodies are not seen before 24-48 hours (Kerr, 1971; Kerr et al., 1972; Rozga et al., 1985). Another observation is that despite the compensatory hyperplasia in the unligated lobes, the DNA content is not altered in the ligated lobes at 3 days post-portal vein ligation (Weinbren and Tarsh, 1964). Apoptotic bodies are only prominent 3 days post-ligation at which time they are ingested by hepatocytes and histiocytes and subsequently degraded by lysosome activity thereby removing DNA and cytoplasmic material. Thus the evidence for a role for apoptosis in the control of DNA synthesis remains unclear and the requirement for a reduction in the amount of DNA present in order to stimulate DNA synthesis appears unlikely.

Following portal vein ligation Weinbren and his colleagues observed that DNA synthesis and mitosis could be induced in the atrophied right posterior lobe with or without the stimulus of partial hepatectomy. This is a situation where the DNA content remained constant but the cytoplasmic compartment of the cell was reduced (Weinbren et al., 1971; 1972). It has therefore been suggested that the stimulation for

regeneration does not depend on destruction or removal of nuclear material (Weinbren and Tarsh, 1964). Their experiments also highlighted that while the portal blood supply may influence the size of hepatocytes, the ability of hepatocytes to undergo DNA synthesis is independent of it (Weinbren et al., 1971; 1972).

It is clear therefore from the present study that the initiation of reactive hyperplasia or adaptive growth following partial hepatectomy or portal vein ligation does not depend on removal of nuclear material as suggested by Schulte-Hermann et al. (1977). As atrophy is the major event following portal vein ligation and occurs prior to the formation of apoptotic bodies, this study would suggest that cytoplasmic or plasma lemmal changes are more likely to be involved in the proliferative signal(s) in the control of DNA synthesis, a concept proposed by these previous workers (Weinbren and Tarsh, 1964; Weinbren, 1982; Weinbren and Hadjis, 1988).

This study has also demonstrated that the Quantimet 720 image analyser clearly detected changes in nuclear area, indicative of altered ploidy states and hence changes in DNA content. These findings were confirmed by integrated microdensitometry of Feulgen stained sections. These results have also clearly shown that measuring nuclear area and thus investigating alteration in nuclear ploidy is a suitable method for detecting DNA synthesis associated with increased ploidy. It is therefore an appropriate technique to use in order to study adaptive cell growth in the liver.

By the use of histologically based methods this study has allowed data to be generated specifically from the examination of hepatocytes.

From the response by the liver to PB treatment in animals following hypophysectomy and laparotomy, this study has additionally demonstrated that hypertrophy can be separated from hyperplasia. This confirms the work of Schulte-Hermann et al. (1977), and the views of others who proposed that the mechanisms involving hyperplasia or hypertrophy can be separated and that these are two separate processes (Malamud, 1972; Weinbren et al., 1972; Weinbren and Washington, 1976; Schulte-Hermann et al., 1977).

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APPENDIX I

SURGICAL PROCEDURES

A.I.1 Hypophysectomy

Hypophysectomy was performed using a modified method based on those of Smith (1930) and Weinbren and Fitschen (1959). A parapharyngeal approach was adopted. The rat was weighed and anaesthetised using ether anaesthesia. The ventral surface of the neck was shaved, a median incision made through the skin and skin margins separated to expose the neck muscles. A polythene catheter was then passed perorally and laryngeal intubation achieved. Using blunt dissection, the hyoid muscles were separated and dissection continued deeper behind the pharynx and upwards until the fascia covering the basisphenoid suture was exposed. The fascia was cut using fine surgical scissors, and the fascia and muscle attachments scraped away from the suture line. Access to the sella turcica was achieved by drilling through the skull using a dental drill with a rose-head burr no.8 (Wright Dental Co.), exactly in the centre of the basisphenoid suture line. This was very precise as any deviation from the centre caused either considerable bleeding or death of the animal, or an inability to remove the pituitary intact. The dura mater was pierced with a right-angled dental probe and the pituitary sucked out by using a hypodermic syringe with catheter tubing attached to it, which fitted exactly over the hole made with the drill thus creating a vacuum. The completeness of removal of the pituitary was tested by floating the syringe contents in water and looking for the characteristic butterfly shape of the organ. Anaesthesia was discontinued, and the operation rapidly finished by closing the wound with surgical clips.

It was necessary throughout the whole operation to keep the anaesthesia as light as possible and to maintain it for as little time as possible in order to avoid anaesthetic deaths. Bleeding was kept to a minimum, especially by careful division of muscles and piercing of the dura mater, as again bleeding was another common cause of post-operative death. Controlling the environmental temperature was an important factor in post-operative care as depending on temperatures the animals became either hypothermic or hyperthermic, most often hypothermic. Increasing thirst was also a post-operative complication and water was kept freely available. Asepsis was maintained as far as possible throughout the operation.

A.I.2 Partial hepatectomy

One-third (33%) and two-third (67%) partial hepatectomy were performed under ether anesthesia using a technique based on the method of Higgins and Anderson (1931). Asepsis was maintained as strictly as possible. The weight of the animal was recorded and the abdominal surface of the rat was shaved. A median line incision reaching 3-4 cm posteriorly from the xiphoid process was made. The median and left lateral lobes of the liver were easily identified.

One-third partial hepatectomy 33%

The median lobe of the liver was delivered from the peritoneal cavity and ligated with 5/0 Ethicon Mersilk material (577), excised and weighed. The aim of this surgical procedure was to remove as accurately as possible one-third of the total liver but even with extreme care by ligating the lobe close to its origin, a small remnant of the median lobe remained.

Two-thirds partial hepatectomy 67%

The median and left lateral lobes of the liver were delivered from the peritoneal cavity, ligated with suture material, excised and both lobes weighed and recorded. During this procedure, two-thirds of the total liver was removed as accurately as possible, but as already described a small amount of the lobes may remain.

The abdomen was closed in two layers. The peritoneum and abdominal muscles were closed in the first layer with dissolvable cat-gut suture material (Ethicon) and the skin closed with surgical clips. Post-operative care included adequate water and food and a stable environmental temperature.

A.I.3 Portal vein ligation

One-third and two-third portal vein ligation were performed under ether anaesthesia employing the method of Weinbren and Tarsh (1964) using, when necessary, the aid of a Zeiss dissecting microscope. The weight of the animal was recorded and the fur from the abdominal wall of the animal shaved. A median line incision was made from the xiphoid process of the sternum, extending posteriorly 3-4 cm. The median and left lateral lobes were exposed and atrophy of the lobes induced by ligation of the relevant branch of the portal vein only, leaving the artery and duct intact.

One-third portal vein ligation

This is performed to induce atrophy of the median lobe of the liver in the rat. This was undertaken by ligation of the two branches of the left trunk of the portal vein supplying the right and left portion of the median lobe. The Ramus centralis supplies the right portion of the

median lobe and was ligated with Mersilk sutures (7/0 Ethicon) at a position along its length to avoid ligation of the ramus caudatus communis which supplies the caudate lobe and is located either proximally or distally from the origin of the ramus centralis. The ramus quadratis was ligated with silk sutures, and supplies the left portion of the median lobe (Gershbein and Elias, 1954).

Two-third portal vein ligation

This was undertaken to induce atrophy in both the median and left lateral lobes of the liver. The left trunk of the portal vein was ligated, the pars transversa trunci sinistri venae portal(TS) at a position where the origin of the ramus caudatus communis supplying the caudate lobe was not occluded. This resulted in atrophy to both the median and left lateral lobes of the liver, approximately two-thirds (67%) of the liver (Gershbein and Elias, 1954).

The peritoneum and abdominal muscles were closed by cat-gut suture material and the skin layer closed with surgical clips.

Recovery was uneventful, no special post-operative procedures were necessary except to ensure adequate food, water and warmth were available.

APPENDIX II

VERIFICATION OF HYPOPHYSECTOMY AND DETAILS OF PILOT STUDIES

A.II.1 Verification of hypophysectomy

The completeness of the hypophysectomy was verified by dissection of the sella turcica following sacrifice of the animal and identifying an empty pituitary fossa. If any remnants were found the animal was excluded from the study. In addition the effects of hypophysectomy were demonstrated in the animals by a reduction in body, adrenal and testicular weight in comparison to intact animals.

Essentially the results show that body, adrenal and testicular weights were reduced whenever a hypophysectomy was done irrespective of any other procedure.

(1) Body weight

The summary of the results are tabulated in Tables A1 and A2.

Following hypophysectomy there was a significant reduction in body weight in all groups ($p < 0.05$) by 28 days. In Group B.A the reduction was significant at the 10% level. This is in contrast to the intact animals in which all sham hypophysectomy groups showed a significant increase ($p < 0.05$) in body weight; group A.A at the 10% level.

Statistical analysis using the Wilcoxon 2 test was made to see whether treatment with PB influenced the increase or decrease in body weight over the 28 days study. In Pilot Study I, Sections III and IV in sham hypophysectomised and hypophysectomised groups the change in body weight of Group 2 was compared with Group 1 and similarly Group 4 with Group 3 and Group 6 with Group 5. There was no significant difference

in the amount of increase or decrease in body weight between the majority of groups indicating that PB did not affect body weight.

Similarly where partial hepatectomy or portal vein ligation was performed there was no significant difference in body weight increase or decrease in the majority of groups compared with controls, either in sham hypophysectomised or hypophysectomised groups. These other experimental procedures therefore have no influence on the effect of hypophysectomy.

(2) Adrenal weight

The results are tabulated in Table A3.

Following hypophysectomy in all groups there was an obvious and statistically significant reduction ($p < 0.05$) in the mean adrenal weight compared with sham hypophysectomy controls and when comparing comparative sham hypophysectomy with hypophysectomy groups.

Statistical analysis made as described for body weight confirmed that experimental procedures such as PB treatment, partial hepatectomy or portal vein ligation did not influence adrenal weight in intact or hypophysectomised animals.

(3) Testicular weight

Results are tabulated in Table A.4.

After hypophysectomy the mean testicular weight was significantly smaller ($p < 0.05$) in all groups compared with sham hypophysectomy control (Group 1) indicating testicular atrophy. In all groups when analysed statistically, neither PB, partial hepatectomy or portal vein

ligation influenced the effect of hypophysectomy as seen by testicular atrophy.

A.II.2 <u>Pilot Study I</u>

This group served as a pilot study to establish that (i) hypophysectomy was successful using standard criteria of body, testicular and adrenal weight and dissection of the sella turcica (already described), (ii) phenobarbitone (PB) could be administered in doses compatible with animal survival and good health, (iii) the administration of PB to sham hypophysectomised and hypophysectomised rats induced liver growth with or without cell division, and (iv) to establish whether the number of days for drug administration were critical either to stimulate liver changes or in the degree of response elicited.

Experimental procedures included

a) Phenobarbitone to sham hypophysectomised and hypophysectomised rats.

b) Control gavage to sham hypophysectomised and hypophysectomised rats.

<u>Experimental procedure</u>

This group was divided into 3 subgroups A, B and C.

A. Thirty-six animals were allotted to this group. Twelve animals were sham hypophysectomised and 24 hypophysectomised. Intact and half the hypophysectomised rats were fed phenobarbitone by gavage 80 mg/kg for four days and 100 kg/mg for four days. These animals were sacrificed 24 hours later. The remaining controls received saline.

Among the hypophysectomised rats fed phenobarbitone only one animal survived and, as survival of these animals were imperative for continuation of the study, it was decided to see whether survival could be improved using 3/4 of the dose recommended by Schulte-Hermann et al. (1977).

B. Five animals with a mean weight of 250 grams were fed phenobarbitone by gavage 60 mg/kg for 4 days, 75 mg/kg for 4 days and sacrificed 24 hours later.

No animal survived the complete regimen.

From these deaths it was decided to test half the dose used by Schulte-Hermann et al. (1977).

C. Sixty-six animals were included in this latter part of the pilot study. Forty-one animals were hypophysectomised and 25 sham hypophysectomised.

<u>Response by the liver to PB treatment in intact and hypophysectomised animals</u>

Hypophysectomised animals included in this section were divided into three major groups (A, B and C) and compared with sham hypophysectomised saline treated animals. The experimental study for these groups lasted for 24, 28 and 32 days respectively. The dose regimen altered after 2 days in the first group, 4 days in the second and after 6 days in the last group. 40 mg/kg of PB was administered first followed by 50 mg/kg, the animal sacrificed 24 hours after the last dose. The day of hypophysectomy or sham hypophysectomy was day 0. This was carried out in order to establish (i) whether there was a difference in response to phenobarbitone treatment using half the dose

recommended over a 4, 8 or 12 day period compared with saline treated controls, (ii) whether the 8 day drug regimen used by Schulte-Hermann et al. (1977) was a critical time period, (iii) whether animals could survive this dose of PB and finally (iv) whether changes resulting from the hypophysectomy could be detected using this dose of PB.

Completeness of hypophysectomy was verified as already described.

At the end of the study period the liver was weighed and sections from the right posterior lobe prepared as already described in the Material and Methods.

The response to hypophysectomy was assessed by (i) changes in relative liver weight, (ii) changes in cell volume, (iii) number of mitoses per 1,000 cells, compared with sham hypophysectomy controls.

The effect of PB on the response by the liver in both sham hypophysectomised and hypophysectomised animals was similarly investigated and compared with their appropriate saline treated controls, statistical analysis carried out using the Wilcoxon 2 sample test and where animal numbers were small tested at the 10% level.

For details of the results see Tables A5 and A6.

A. Sham hypophysectomy group

Group A - 4 days treatment

Sham hypophysectomy, and saline treatment (Group 1 - controls)

(i) Relative liver weight (% liver/100 grams body weight)

The mean relative liver weight of Group 1 (controls) was 3.69% (SD ±0.21, n = 3) at the end of the 24 days study.

Investigation of right posterior lobe

(i) Cell volume

The mean estimated cell volume of Group 1 was 6.84×10^{-5} mm^3 (SD ± 0.23, n = 3).

(ii) Mitoses

At the end of the 24 day study the mean number of mitoses per 1,000 cells in Group 1 was 0.0544 (SD ± 0.08, n = 3).

Sham hypophysectomy and PB treatment (Group 2) compared with sham hypophysectomy and saline treatment (Group 1 - controls)

Following 4 days PB treatment total liver weight was increased (relative weight 4.56% vs. 3.69% controls, $p < 0.01$), cell volumes were increased by 27.89% (8.75×10^{-5} mm^3 vs. 6.84×10^{-5} mm^3 controls, $p < 0.01$) and mitoses increased (0.2957 instead of 0.0544 controls, $p < 0.1$).

Group B 8 days treatment

Sham hypophysectomy and saline treatment (Group 1 - controls)

At the end of the 28 day study the mean relative weight was 4.12% (SD ± 0.38, n = 3), mean estimated cell volume 7.24×10^{-5} mm^3 (SD ± 0.26, n = 3) and number of mitoses per 1,000 cells 0.0595 (SD ± 0.08, n = 3).

Sham hypophysectomy and PB treatment (Group 2) compared with sham hypophysectomy and saline treatment (Group 1 - controls)

After 8 days PB treatment the relative liver weight was increased by 23% (5.07% vs. 4.12% controls, $p < 0.01$), cell volumes were enlarged (8.85×10^{-5} mm^3 instead of 7.24×10^{-5} mm^3 controls, $p < 0.01$) by 22.8% and an increase in mitoses observed (0.6294 vs. 0.0595 controls, $p < 0.05$).

Group C 12 days treatment

Sham hypophysectomy and saline treatment (Group 1 - controls)

After 32 days the mean relative weight ws 4.02% (SD $\pm$0.21, n = 4), the mean estimated cell volumes 7.55 x 10^{-5} mm^3 (SD $\pm$0.22, n = 4) and the number of mitoses per 1,000 cels 0.0891 (SD $\pm$0.15, n = 4).

Sham hypophysectomy and PB treatment (Group 2) compared with sham hypophysectomy and saline treatment (Group 1 - controls)

The liver was enlarged by 40.8% following 12 days PB treatment (relative weight 5.66% instead of 4.02% controls, p <0.01), mean estimated cell volumes increased by 20.97% (9.13 x 10^{-5} mm^3 vs. 7.55 x 10^{-5} mm^3 controls, p <0.01) and the number of mitoses increased (0.8533 vs. 0.0891 controls, p <0.01).

Thus PB induced liver enlargement as a result of cellular enlargement and an increase in cell numbers as detected by the mitoses. The number of days treatment did not seem to effect the response and so the 8 days regime as described by Schulte-Hermann et al. (1977) was continued throughout the experiment.

B. Hypophysectomy group

Group A 4 days treatment

Hypophysectomy and saline treatment (Group 1 - controls)

Following hypophysectomy the relative liver weight was similar compared with sham hypophysectomy controls at the 10% level (3.59% SD $\pm$0.16, n = 3, vs. 3.69% SD $\pm$0.21, n = 3) although reduction in cell volumes could be detected (3.25 x 10^{-5} mm^3 vs. 6.84 x 10^{-5} mm^3 controls, p <0.01). There was no difference in numbers of mitoses (0.00 SD $\pm$0.0, n = 3 vs. 0.0544 SD $\pm$0.08 sham hypophysectomy controls).

Hypophysectomy and PB treatment (Group 2) compared with hypophysectomy and saline treatment (Group 1 - controls)

Following PB treatment in hypophysectomised animals relative liver weight was similar at the 10% level (3.66% SD ± 0.43, n = 4 vs. 3.59% SD ± 0.16, n = 3) compared with controls as were the number of mitoses (0.0386 SD ± 0.07 vs. 0.00 SD ± 0.0) but larger cell volumes were detected (6.02×10^{-5} mm^3 instead of 3.25×10^{-5} mm^3 controls, $p < 0.01$).

Group B 8 days treatment

Hypophysectomy and saline treatment (Group 1 - controls)

Twenty-eight days after hypophysectomy the mean relative weight was reduced by 13.1% compared with sham hypophysectomy controls (relative weight 3.58% instead of 4.12%, $p < 0.05$) the result of liver atrophy seen by smaller cell volumes (2.63×10^{-5} mm^3 vs. 7.24×10^{-5} mm^3, $p < 0.01$), a reduction by 63.7%. No mitoses were detected.

Hypophysectomy and PB treatment (Group 2) compared with hypophysectomy and saline treatment (Group 1)

Total liver enlarged by 31.84% following 8 days PB treatment (relative weight 4.72% vs. 3.58% controls, $p < 0.01$) the result of an 138.5% increase in mean estimated cell volume (6.26×10^{-5} mm^3 instead of 2.63×10^{-5} mm^3). No mitoses were detected.

Group C 12 days treatment

Hypophysectomy and saline treatment (Group 1 - controls)

At the end of the 32 day study period total liver was reduced by 15.17% compared with sham hypophysectomy controls (3.41% instead of 4.02%, $p < 0.05$) the result of a 61.98% decrease in mean estimated cell volume (2.87×10^{-5} mm^3 vs. 7.55×10^{-5} mm^3, $p < 0.01$). No changes in

mitoses were detected (0.0184 SD ±0.04 vs. 0.0891 SD ±0.15 sham hypophysectomy controls).

Hypophysectomy and PB treatment (Group 2) compared with hypophysectomy and saline treatment (Group 1 - controls)

After 12 days PB treatment the liver enlarged by 24.63% compared with controls (relative liver 4.25% instead of 3.41%, $p < 0.01$) the result of considerable cellular enlargement (6.06×10^{-5} mm^3 vs. 2.87×10^{-5} mm^3 controls, $p < 0.01$, a 111.4% increase). No changes in the number of mitoses were detected.

Summary

Hypophysectomised animals survived the length of the pilot study on half the dose of PB described (Schulte-Hermann et al., 1977). Hypophysectomy results in reduction in total liver weight the result of reduction in cell size indicating liver atrophy.

Following PB treatment liver enlargement was the result of cell enlargement. In Group A the effect of hypophysectomy on the relative liver weight was not seen in the liver of hypophysectomy control animals compared with intact controls. In addition, following 4 days PB treatment the relative liver weight was not increased in hypophysectomised rats compared with hypophysectomy controls. Failure to detect these expected changes is most likely due to the small numbers of animals in the groups making statistical analysis difficult. In the other groups the response by the liver to PB in hypophysectomised animals was similar whether the animals received PB treatment for 8 or 12 days. Therefore, as in the intact animals, the regime followed by Schulte-Hermann et al. (1977) was continued throughout the study using 50% of the recommended dose of PB for 8 days.

In conclusion this pilot study confirms previous observations that PB in intact animals induces liver enlargement by a combination of both hypertrophy and hyperplasia but following hypophysectomy, which induces liver atrophy, liver enlargement following PB treatment is the result of cellular enlargement alone without a concomitant increase in DNA content (Schulte-Hermann, 1974; Schulte-Hermann et al., 1977).

A.II.3 Pilot Study II

Experimental procedures

The purpose of this pilot study was to check the response by the liver to 1/3 partial hepatectomy i.e. removal of the median lobe in intact and hypophysectomised animals compared with controls.

Twenty-three animals were included in this study, 11 were hypophysectomised, 12 underwent sham hypophysectomy and the animals divided into 4 small groups. One-third partial hepatectomy was performed in 6 hypophysectomised rats and 6 sham hypophysectomised rats. The remaining animals underwent laparotomy. The hepatectomy or laparotomy was performed 6 days after the hypophysectomy and for 8 days prior to sacrifice each animal received one measure of saline by gavage. The animals were sacrificed 28 days after hypophysectomy. Verification of hypophysectomy was undertaken as previously described.

Subdivision of groups

A. Sham hypophysectomy group

(1) Sham hypophysectomy, laparotomy and saline treatment (Group 1).

(2) Sham hypophysectomy, 1/3 partial hepatectomy and saline treatment Group 2).

B. Hypophysectomy group

(1) Hypophysectomy, laparotomy and saline treatment (Group 1).

(2) Hypophysectomy, 1/3 partial hepatectomy and saline treatment (Group 2).

At the end of the 28 day study in these and all subsequent studies individual liver lobes were weighed. As in previous experiments special attention was paid to the right posterior lobe. In each animal the right posterior lobe was weighed, cell numbers and the number of mitoses counted and recorded per highpower fields as already described.

For details of the results see Tables A.7 and A.8.

A. Sham hypophysectomy group - Effect of one-third partial hepatectomy

Sham hypophysectomy, 1/3 partial hepatectomy and saline treatment (Group 2) compared with sham hypophysectomy, laparotomy and saline treatment (Group 1 - controls)

By 21 days post-hepatectomy the whole of the liver remnant was similar to controls (3.52% SD ±0.52 vs. 3.83% SD ±0.24), this indicating that the growth response in the remaining lobes following 1/3 partial hepatectomy had resulted in the liver returning to the relative liver weight of the control group.

Each lobe was individually weighed and the percentage it contributed to the total liver noted. In Group 1 the anterior lobes contributed by approximately 62% to the total liver weight and the posterior lobes 38%. This latter contribution is a little high (usually 33%) and may be the result of inadvertent heavy handling during the laparotomy (Weinbren et al., 1969).

In Group 2 following removal of the median lobe, there was clear enlargement of the right posterior lobe (R.P. 41.48% instead of 29.33% controls, $p < 0.01$) indicating that adaptive growth had occurred in the remaining liver remnant, with a relative weight of the R.P. increased by 34.13% (relative weight 1.46% vs. 1.09% controls, $p < 0.01$).

By 21 days post-hepatectomy there was no significant difference in the mean estimated cell volume following one-third partial hepatectomy 6.75×10^{-5} mm^3, ± 0.30 vs. (6.75×10^{-5} mm^3 ± 0.29 controls) and no difference in the number of mitoses compared with controls (0.1107 SD ± 0.13 and 0.0273 SD ± 0.06 controls).

B) Hypophysectomy group - Effect of hypophysectomy and response to partial hepatectomy

Hypophysectomy, laparotomy and saline treatment (**Group 1**)

Following hypophysectomy the relative liver weight was significantly reduced ($p < 0.05$) compared with sham hypophysectomy controls (3.83% instead of 3.67%) suggesting that atrophy had occurred. While individual lobes in the hypophysectomy group were all significantly reduced compared with sham hypophysectomy controls ($p < 0.01$), the contribution by the median and left lateral lobes in the hypophysectomy group was 66.79% and posterior lobes 33.13% which is a normal distribution and confirms that while liver weight may be reduced following hypophysectomy, the weights of the individual lobes remain in correct proportion compared with intact animals.

Relative weight of the right posterior lobe was 0.89% (SD $\pm$ 0.06) compared with 1.09% (SD $\pm$ 0.17) in sham hypophysectomy controls ($p < 0.01$), a significant reduction by 18%) indicating liver atrophy. This amount of reduction is apparently large compared with the 4.2%

reduction in total relative liver weight and may be accounted for by the larger than average mean relative weight of the right posterior lobe in the intact animals possibly due to heavy handling during laparotomy (Weinbren et al., 1969).

Cell atrophy was obvious in the R.P. with smaller mean estimated cell volumes compared with sham hypophysectomy controls 2.38×10^{-5} mm^3 vs. 6.75×10^{-5} mm^3).

There was no difference in mitotic index (0.0198 SD $\pm$0.04 vs. 0.0273 SD $\pm$0.06 sham hypophysectomy controls).

Hypophysectomy, 1/3 partial hepatectomy and saline treatment (Group 2) compared with hypophysectomy, laparotomy and saline treatment (Group 1 - controls)

After 1/3 partial hepatectomy there was obvious enlargement in the right posterior lobe (R.P. 39.42% instead of 24.36% controls, $p < 0.01$) with a 27% increase in mean relative liver weight (1.13% compared with 0.89%, $p < 0.05$) but the total liver only reached 77% of controls (2.83% vs. 3.67% controls, $p < 0.01$) by 21 days.

Estimated cell volume was enlarged compared with controls (4.07×10^{-5} mm^3 vs. 2.84×10^{-5} mm^3, $p < 0.01$) but compared with sham hypophysectomy control cell volume following 1/3 partial hepatectomy was still significantly reduced ($p < 0.01$) indicating the effects of atrophy are still detected.

Mitoses were similar to controls (0.0379 SD $\pm$0.53 vs. 0.0198 SD $\pm$0.04 controls) and no significant difference to the sham hypophysectomy group (A Group 1) in which the mean number of mitoses was 0.0273 (SD $\pm$ 0.06).

Summary

Thus in summary in intact animals, the response to partial hepatectomy was consistent with other studies (Harkness, 1959; Weinbren, 1959; Bucher, 1963) with compensatory hyperplasia occurring in the liver remnant restoring the liver mass to control values.

Hypophysectomy induced liver atrophy. Hypophysectomy did not prevent a response by the liver to partial hepatectomy, the response being consistent with previous studies (Bucher, 1963; Schulte-Hermann et al., 1977) again with evidence of compensatory hyperplasia in the liver remnant.

Table A.1 Body weights - Sham hypophysectomy groups - all sections

Group		No.days PB treatment	Mean Body weight (grams) beginning	end	increase
Pilot Study I					
A.A.	Sham hypophysectomy and saline treatment (Group 1 - controls)(n=3)	4	210 (SD+10.0)	281.67 (SD+18.93)	71.66 (SD+16.49)
	Sham hypophysectomy and PB treatment (Group 2)(n=4)	4	215 (SD+28.39)	258.75 (SD+28.39)	43.75 (SD+24.84)
A.B.	Sham hypophysectomy and saline treatment (Group 1 - controls) (n=3)	8	206.67 (SD+5.77)	236.67 (SD+28.87)	30.0 (SD+20.60)
	Sham hypophysectomy and PB treatment (Group 2)(n=5)	8	160 (SD+18.71)	220.0 (SD+24.49)	60.0 (SD+10.95)
A.C.	Sham hypophysectomy and saline treatment (Group 1 - controls) (n=4)	12	202.50 (SD+45.73)	232.50 (SD+61.85)	30.0 (SD+18.7)
	Sham hypophysectomy and PB treatment (Group 2) (n=6)	12	223.33 (SD+12.11)	280.0 (SD+12.65)	56.66 (Sd+7.45)
Pilot Study II					
A(1)	Sham hypophysectomy, laparotomy and saline treatment (Group 1 - controls) (n=6)	8	175.0 (SD+10.49)	250.83 (SD+7.312)	75.83 (SD+10.172)
A(2)	Sham hypophysectomy, 1/3 partial hepatectomy and saline treatment (Group 2) (n=6)	8	176.67 (SD+8.16)	251.67 (SD+9.83)	71.66 (SD+10.67)
Section III					
A(1)	Sham hypophysectomy, laparotomy and saline treatment (Group 1 - controls) (n=6)	8	193.33 (SD+20.90)	248.3 (SD+16.33)	52.5 (SD+16.52)
A(2)	Sham hypophysectomy, laparotomy and PB treatment) (Group 2) (n=6)	8	183.33 (SD+25.25)	266.67 (SD+22.06)	43.33 (SD+14.33)
A(3)	Sham hypophysectomy, 1/3 partial hepatectomy and saline treatment (Group 3) (n=6)	8	181.67 (SD+33.12)	243.33 (SD+19.66)	61.66 (SD+27.93)
A(4)	Sham hypophysectomy, 1/3 partial hepatectomy and PB treatment (Group 4) (n=6)	8	178.33 (SD+28.58	235.00 (SD+10.49)	56.66 (SD+23.50)
A(5)	Sham hypophysectomy, 2/3 partial hepatectomy and saline treatment (Group 5) (n=6)	8	268.33 (SD+32.23)	284.16 (SD+31.94)	15.83 (SD+21.09)
A(6)	Sham hypophysectomy, 2/3 partial hepatectomy and PB treatment (Group 6) (n=6)	8	239.17 (SD+36.11)	260.83 (SD+26.35)	21.66 (SD+15.98)
Section IV					
A(1)	Sham hypophysectomy, laparotomy and saline (Group 1 - controls) (n=6)	8	194.17 (SD+9.17)	223.33 (SD+31.43)	37.5 (SD+18.2)
A(2)	Sham hypophysectomy, laparotomy and and PB treatment (Group 2) (n=6)	8	187.5 (SD+16.36)	194.17 (SD+14.29)	13.33 (SD+8.49)
A(3)	Sham hypophysectomy, 1/3 portal vein ligation and saline treatment(Group 3) (n=7)	8	173.15 (SD+15.74)	187.86 (SD+34.50)	32.85 (SD+15.97)
A(4)	Sham hypophysectomy, 1/3 portal vein ligation and PB treatment (Group 4) (n=7)	8	167.86 (SD+17.76)	200.0 (SD+15.28)	35.0 (SD+15.77)
A(5)	Sham hypophysectomy, 2/3 portal vein ligation and saline treatment (Group 5) (n=6)	8	184.17 (SD+8.01)	233.33 (SD+8.76)	49.16 (SD+12.72)
A(6)	Sham hypophysectomy, 2/3 portal vein ligation and PB treatment (Group 6) (n=6)	8	183.33 (SD+18.07)	227.5 (SD+23.18)	43.33 (SD+22.85)

Table A.2 Body weights - Hypophysectomy groups - all sections

Group		No.days PB treatment	Mean body weight (grams) Beginning	End	Decrease
Pilot Study I					
B.A	Hypophysectomy and saline treatment (Group 1 - controls) (n=3)	4	222.6 (SD±5.77)	193.33 (SD±5.77)	33.3 (SD±4.71)
	Hypophysectomy and PB treatment (Group 2)(n=4)	4	243.75 (SD±52.82)	200.0 (SD±27.08)	43.75 (SD±22.74)
B.B.	Hypophysectomy and saline treatment (Group 1 - controls) (n=14)	8	170.71 (SD±17.74)	147.86 (SD±12.97)	22.85 (SD±12.35)
	Hypophysectomy and PB treatment (Group 2)(n=8)	8	182.5 (SD±30.59)	156.25 (SD±34.64)	27.5 (SD±8.29)
B.C.	Hypophysectomy and saline treatment (Group 1 - controls) (n=5)	12	164.0 (SD±5.48)	134.0 (SD±21.91)	28.0 (SD±11.6)
	Hypophysectomy and PB treatment (Group 2) (n=7)	12	191.43 (SD±30.78)	179.29 (SD±22.07)	25.0 (SD±8.01)
Pilot Study II					
B(1)	Hypophysectomy, laparotomy and saline treatment (Group 1 - controls)(n=5)	8	194.0 (SD±39.79)	162.0 (SD±24.2)	32.0 (SD±18.05)
B(2)	Hypophysectomy and 1/3 partial hepatectomy and saline treatment (Group 2) (n=6)	8	221.67 (SD±24.83)	178.33 (SD±11.69)	43.33 (SD±17.95)
Section III					
B(1)	Hypophysectomy, laparotomy and saline treatment (Group 1 - controls) (n=6)	8	221.67 (SD±12.91)	159.17 (SD±10.68)	62.5 (SD±12.82)
B(2)	Hypophysectomy, laparotomy and PB treatment (Group 2) (n=6)	8	223.33 (SD±10.33)	160.83 (SD±8.61)	62.5 (SD±7.5)
B(3)	Hypophysectomy, 1/3 partial hepatectomy and saline treatment (Group 3) (n=6)	8	226.67 (SD±25.03)	171.67 (SD±9.83)	55.0 (SD±18.02)
B(4)	Hypophysectomy, 1/3 partial hepatectomy and PB treatment (Group 4) (n=6)	8	211.67 (SD±7.53)	160.0 (SD±6.32)	51.66 (SD±12.13)
B(5)	Hypophysectomy, 2/3 partial hepatectomy and saline treatment (Group 5) (n=9)	8	196.11 (SD±22.19)	152.2 (SD±20.78)	43.88 (SD±9.65)
B(6)	Hypophysectomy, 2/3 partial hepatectomy and PB treatment (Group 6) (n=9)	8	187.22 (SD±10.03)	150.0 (SD±7.50)	35.55 (SD±8.54)
Section IV					
B(1)	Hypophysectomy, laparotomy and saline treatment (Grop 1 - controls)(n=6)	8	199.17 (SD±5.94)	146.67 (SD±14.72)	52.5 (SD±9.45)
B(2)	Hypophysectomy, laparotomy and PB treatment (Group 2) (n=6)	8	191.67 (SD±6.06)	137.5 (SD±11.73)	54.16 (SD±9.75)
B(3)	Hypophysectomy, 1/3 portal vein ligation and saline treatment (Group 3) (n=6)	8	175.833 (SD±14.83)	128.33 (SD±10.67)	47.5 (SD±5.59)
B(4)	Hypophysectomy, 1/3 portal vein ligation and PB treatment (Group 4)(n=7)	8	200.71 (SD±19.88)	144.29 (SD±20.90)	56.42 (SD±9.14)
B(5)	Hypophysectomy, 2/3 portal vein ligation and saline treatment (Group 5) (n=6)	8	194.17 (SD±29.23)	152.50 (SD±10.37)	41.66 (SD±25.27)
B(6)	Hypophysectomy, 2/3 portal vein ligation and PB treatment (Group 6)(n=6)	8	205.17 (SD±27.90)	156.67 (SD±21.13)	48.5 (SD±34.12)

Table A.3 Adrenal weight – Sham hypophysectomy and hypophysectomy groups – all sections

A. Sham hypophysectomy group

Group	No. days PB treatment	Mean adrenal weight (mgs)
Pilot Study I		
A.A. Sham hypophysectomy and saline treatment (Group 1 - controls) (n=3)	4	39.23 (SD ± 1.084)
Sham hypophysectomy and PB treatment (Group 2)(n=4)	4	42.35 (SD ± 4.0159)
A.B. Sham hypophysectomy and saline treatment (Group 1 - controls) (n=3)	8	41.33 (SD ± 5.55)
Sham hypophysectomy and PB treatment (Group 2)(n=5)	8	41.80 (SD ± 2.227)
A.C. Sham hypophysectomy and saline treatment (Group 1 - controls) (n=4)	12	48.82 (SD ± 11.6)
Sham hypophysectomy and PB treatment (Group 2)(n=6)	12	49.91 (SD ± 16.4)
Pilot Study II		
A(1) Sham hypophysectomy, laparotomy and saline treatment (Group 1 - controls) (n=6)	8	40.0 (SD ± 16.4)
A(2) Sham hypophysectomy, 1/3 partial hepatectomy and saline treatment (Group 2) (n=6)	8	40.83 (SD ± 1.863)
Section III		
A(1) Sham hypophysectomy, laparotomy and saline treatment (Group 1 - controls) (n=6)	8	40.0 (SD±0.0)
A(2) Sham hypophysectomy, laparotomy and PB treatment (Group 2) (n=6)	8	40.83 (SD±1.86339)
A(3) Sham hypophysectomy, 1/3 partial hepatectomy and saline treatment (Group 3) (n=6)	8	33.33 (SD±4.7140)
A(4) Sham hypophysectomy, 1/3 partial hepatectomy and PB treatment (Group 4) (n=6)	8	40.00 (SD±2.8867)
A(5) Sham hypophysectomy, 2/3 partial hepatectomy and saline treatment (Group 5) (n=6)	8	40.0 (SD±0.0)
A(6) Sham hypophysectomy, 2/3 partial hepatectomy and PB treatment (Group 6) (n=6)	8	40.0 (SD±0.0)
Section IV		
A(1) Sham hypophysectomy, laparotomy and saline treatment (Group 1 - controls) (n=6)	8	35.0 (SD±4.082)
A(2) Sham hypophysectomy, laparotomy and PB treatment (Group 2) (n=6)	8	40.0 (SD±0.0)
A(3) Sham hypophysectomy, 1/3 portal vein ligation and saline treatment (Group 3) (n=7)	8	43.0 (SD±4.715)
A(4) Sham hypophysectomy, 1/3 portal vein ligation and PB treatment (Group 4) (n=7)	8	45.42 (SD±4.7466)
A(5) Sham hypophysectomy, 2/3 portal vein ligation and saline treatment (Group 5) (n=6)	8	43.0 (SD±4.715)
A(6) Sham hypophysectomy, 2/3 portal vein ligation and PB treatment (Group 6) (n=6)	8	46.66 (SD±7.453)

B. Hypophysectomy group

Group	No. days PB treatment	Mean adrenal weight (mgs)
Pilot Study I		
B.A. Hypophysectomy and saline treatment (Group 1 - controls) (n=3)	4	16.7 (SD ± 0.163)
Hypophysectomy and PB treatment (Group 2)(n=4)	4	18.1 (SD ± 4.89)
B.B. Hypophysectomy and saline treatment (Group 1 - controls) (n=14)	8	15.91 (SD ± 5.068)
Hypophysectomy and PB treatment (Group 2)(n=8)	8	14.85 (SD ± 4.008)
B.C. Hypophysectomy and saline treatment (Group 1) - controls (n=5)	12	22.3 (SD ± 4.681)
Hypophysectomy and PB treatment (Group 2)(n=7)	12	17.42 (SD ± 4.271)
Pilot Study II		
B(1) Hypophysectomy, laparotomy and saline treatment (Group 1 - controls)(n=5)	8	11.0 (SD ± 2.0)
B(2) Hypophysectomy, 1/3 partial hepatectomy and saline treatment (Group 2)(n=6)	8	10.50 (SD ± 2.95)
Section III		
B(1) Hypophysectomy, laparotomy and saline treatment (Group 1 - controls) (n=6)	8	11.6 (SD±2.357)
B(2) Hypophysectomy, laparotomy and PB treatment treatment (Group 2) (n=6)	8	11.66 (SD±2.3570)
B(3) Hypophysectomy, 1/3 partial hepatectomy and saline treatment (Group 3) (n=6)	8	9.83 (SD±0.3726)
B(4) Hypophysectomy, 1/3 partial hepatectomy and PB treatment (Group 4) (n=6)	8	10.0 (SD±0.0)
B(5) Hypophysectomy, 2/3 partial hepatectomy and saline treatment (Group 5) (n=9)	8	11.66 (SD±2.3570)
B(6) Hypophysectomy, 2/3 partial hepatectomy and PB treatment (Group 6) (n=9)	8	12.83 (SD±2.4267)
Section IV		
B(1) Hypophysectomy, laparotomy and saline treatment (Group 1 - controls) (n=6)	8	11.66 (SD±2.357)
B(2) Hypophysectomy, laparotomy and PB treatment (Group 2) (n=6)	8	10.83 (SD±1.863)
B(3) Hypophysectomy, 1/3 portal vein ligation and saline treatment (Group 3) (n=7)	8	10.0 (SD±0.0)
B(4) Hypophysectomy, 1/3 portal vein ligation and PB treatment (Group 4) (n=)	8	11.42 (SD±2.2587)
B(5) Hypophysectomy, 2/3 portal vein ligation and saline treatment (Group 5) (n=6)	8	14.16 (SD±1.863)
B(6) Hypophysectomy, 2/3 portal vein ligation and PB treatment (Group 6) (n=6)	8	12.5 (SD±2.5)

Table A.4 Testicular weight - Sham hypophysectomy and hypophysectomy groups - all sections

A. Sham hypophysectomy group

Group	No. days PB treatment	Mean testicular weight (mgs)
Pilot Study I		
A.A. Sham hypophysectomy and saline treatment (Group 1 - controls) (n=3)	4	3.10 (SD ± 0.30)
Sham hypophysectomy and PB treatment (Group 2)(n=4)	4	2.82 (SD ± 0.24)
A.B. Sham hypophysectomy and saline treatment (Group 1 - controls) (n=3)	8	2.84 (SD ± 0.19)
Sham hypophysectomy and PB treatment (Group 2)(n=5)	8	2.77 (SD ± 0.29)
A.C. Sham hypophysectomy and saline treatment (Group 1 - controls) (n=4)	12	2.67 (SD ± 0.62)
Sham hypophysectomy and PB treatment (Group 2)(n=6)	12	3.20 (SD ± 0.18)
Pilot Study II		
A(1) Sham hypophysectomy, laparotomy and saline treatment (Group 1 - controls) (n=6)	8	3.22 (SD ± 0.35)
A(2) Sham hypophysectomy, 1/3 partial hepatectomy and saline treatment (Group 2) (n=6)	8	3.08 (SD ± 0.38)
Section III		
A(1) Sham hypophysectomy, laparotomy and saline treatment (Group 1 - controls) (n=6)	8	2.8116 (SD±0.1530)
A(2) Sham hypophysectomy, laparotomy and PB treatment (Group 2) (n=6)	8	3.16 (SD±0.4075)
A(3) Sham hypophysectomy, 1/3 partial hepatectomy and saline treatment (Group 3) (n=6)	8	3.2966 (SD±0.4592)
A(4) Sham hypophysectomy, 1/3 partial hepatectomy and PB treatment (Group 4) (n=7)	8	2.9233 (SD±0.1426)
A(5) Sham hypophysectomy, 2/3 partial hepatectomy and saline treatment (Group 5) (n=6)	8	3.48 (SD±0.5678)
A(6) Sham hypophysectomy, 2/3 partial hepatectomy and PB treatment (Group 6) (n=6)	8	2.9833 (SD±0.0677)
Section IV		
A(1) Sham hypophysectomy, laparotomy and saline treatment (Group 1 - controls) (n=6)	8	2.92 (SD±0.09)
A(2) Sham hypophysectomy, laparotomy and PB treatment (Group 2) (n=6)	8	2.85 (SD±0.41)
A(3) Sham hypophysectomy, 1/3 portal vein ligation and saline treatment (Group 3) (n=7)	8	2.066 (SD±1.040)
A(4) Sham hypophysectomy, 1/3 portal vein ligation and PB treatment (Group 4) (n=7)	8	2.8157 (SD±0.2626)
A(5) Sham hypophysectomy, 2/3 portal vein ligation and saline treatment (Group 5) (n=6)	8	3.0168 (SD±0.3559)
A(6) Sham hypophysectomy, 2/3 portal vein ligation and PB treatment (Group 6) (n=6)	8	2.7533 (SD±0.1971)

B. Hypophysectomy group

Group	No. days PB treatment	Mean testicular weight (mgs)
Pilot Study I		
B.A. Hypophysectomy and saline treatment (Group 1 - controls) (n=3)	4	0.75 (SD ±0.21)
Hypophysectomy and PB treatment (Group 2)(n=4)	4	0.64 (SD ± 0.04)
B.B. Hypophysectomy and saline treatment (Group 1 - controls (n=14)	8	0.50 (SD ± 0.31)
Hypophysectomy and PB treatment (Group 2)(n=8)	8	0.40 (SD ± 0.10)
B.C. Hypophysectomy and saline treatment (Group 1) - controls (n=5)	12	0.39 (SD ± 0.05)
Hypophysectomy and PB treatment (Group 2)(n=7)	12	0.59 (SD ± 0.09)
Pilot Study II		
B(1) Hypophysectomy, laparotomy and saline treatment (Group 1 - controls)(n=5)	8	0.47 (SD ± 0.1344)
B(2) Hypophysectomy, 1/3 partial hepatectomy and saline treatment (Group 2)(n=6)	8	0.5753 (SD ± 0.1020)
Section III		
B(1) Hypophysectomy, laparotomy and saline treatment (Group 1 - controls) (n=6)	8	0.4708 (SD±0.0730)
B(2) Hypophysectomy, laparotomy and PB treatment (Group 2) (n=6)	8	0.54 (SD±0.1031)
B(3) Hypophysectomy, 1/3 partial hepatectomy and saline treatment (Group 3) (n=6)	8	0.635 (SD±0.2162)
B(4) Hypophysectomy, 1/3 partial hepatectomy and PB treatment (Group 4) (n=6)	8	0.58 (SD±0.1982)
B(5) Hypophysectomy, 2/3 partial hepatectomy and saline treatment (Group 5) (n=9)	8	0.465 (SD±0.0429)
B(6) Hypophysectomy, 2/3 partial hepatectomy and PB treatment (Group 6) (n=9)	8	0.4211 (SD±0.0704)
Section IV		
B(1) Hypophysectomy, laparotomy and saline treatment (Group 1 - controls) (n=6)	8	0.47 (SD±0.03)
B(2) Hypophysectomy, laparotomy and PB treatment (Group 2) (n=6)	8	0.44 (SD±0.0489)
B(3) Hypophysectomy, 1/3 portal vein ligation and saline treatment (Group 3) (n=6)	8	0.3833 (SD±0.0801)
B(4) Hypophysectomy, 1/3 portal vein ligation and PB treatment (Group 4) (n=7)	8	0.46 (SD±0.06)
B(5) Hypophysectomy, 2/3 portal vein ligation and saline treatment (Group 5) (n=6)	8	0.48 (SD±0.3872)
B(6) Hypophysectomy, 2/3 portal vein ligation and PB treatment (Group 6) (n=6)	8	0.46 (SD±0.04)

Table A.5 Pilot Study I - 4, 8 and 12 days PB treatment

Rat no.	At start total body wt (grams)	At end total body wt (grams)	Liver wt (grams)	% liver/ body wt	Adrenals (grams)	Testes (grams)	No. mitoses /1000 cells	No. cells 10F	Cell vol x 10^{-5} mm^3
4 days PB treatment									
A1 - Sham hypophysectomy and saline									
H0	220	295	10.5247	3.56	0.04	3.4136	0.00	603	6.753
H1	210	260	9.30	3.57	0.04	2.81	0.00	580	7.159
H2	200	290	11.41	3.93	0.0377	3.07	0.1633	612	6.605
A2 - Sham hypophysectomy and PB									
H3	210	275	11.60	4.37	0.0437	2.50	0.3824	523	8.360
H4	220	290	13.37	4.61	0.0456	3.0253	0.6048	496	9.052
H5	200	230	10.68	4.64	0.0468	2.97	0.00	501	8.957
H6	230	240	11.20	4.6	0.04	2.77	0.1956	511	8.657
B1 - Hypophysectomy and saline									
H5	230	190	6.97	3.6	0.0169	0.62	0.0	1002	3.1528
H11	230	200	7.50	3.75	0.0165	0.64	0.00	960	3.361
H8	220	190	6.52	3.43	0.0167	0.99	0.0	986	3.229
B2 - Hypophysectomy and PB									
H103	200	180	6.31	3.50	0.0251	0.5947	0.1545	647	6.076
H104	220	190	6.36	3.34	0.0122	0.68	0.0	639	6.1908
H105	320	240	8.47	3.52	0.02	0.6776	0.0	660	5.897
H8	235	190	8.16	4.29	0.0152	0.61	0.00	658	5.924
8 days PB treatment									
A1 - Sham hypophysectomy and saline									
H1	200	220	10.166	4.62	0.036	2.832	0.1765	560	7.5460
H2	210	220	8.93	4.05	0.039	2.66	0.00	594	6.9074
H5	210	270	10.76	3.70	0.049	3.04	0.00	575	7.2526
A2 - Sham hypophysectomy and PB									
H2	190	260	12.0	4.6	0.04	2.85	0.5769	520	8.4332
H4	140	200	9.33	4.66	0.0440	2.27	1.5621	512	8.6316
H5	160	200	10.6468	5.3	0.04	2.9307	0.2000	500	8.9442
H7	160	220	12.18	5.5	0.04	2.80	0.00	486	9.3335
H8	150	220	11.6849	5.3	0.0450	3.0108	0.8080	495	9.0801
B1 - Hypohysectomy and saline									
H34	190	160	6.048	3.78	0.0248	0.3447	0.00	1183	2.4576
H35	190	160	6.10	3.81	0.0280	0.4166	0.0860	1162	2.5245
H37	200	160	5.3555	3.34	0.0157	0.4230	0.00	1107	2.7150
H65	150	130	4.197	3.22	0.0115	0.4230	0.00	1150	2.5643
H66	160	130	4.762	3.66	0.0107	0.3873	0.00	1155	2.5475
H67	150	150	5.12	3.41	0.0140	0.3600	0.00	1115	2.6858
H68	160	130	5.172	3.97	0.0170	0.370	0.00	1150	2.5642
H70	190	170	6.22	3.645	0.0137	0.5345	0.00	1165	2.5148
H71	170	145	4.9924	3.44	0.0128	0.4535	0.00	1173	2.4891
H60	170	140	4.95	3.53	0.0115	0.3774	0.00	1160	2.5311
H62	160	145	4.3648	3.0	0.0122	0.3322	0.00	1094	2.7635
H63	190	150	6.10	4.06	0.0154	0.7354	0.00	1067	2.8691
H69	160	160	6.27	3.91	0.0215	0.4556	0.00	1090	2.7788
H60	150	140	4.64	3.31	0.0140	0.3390	0.00	1095	2.7598
B2 - Hypophysectomy and PB									
H41	200	180	7.9360	4.40	0.02	0.4955	0.00	650	6.034
H42	210	180	8.19	4.55	0.0140	0.4565	0.00	647	6.0763
H43	200	170	7.39	4.61	0.0135	0.3972	0.00	635	2.2494
H44	210	200	8.7748	4.61	0.0216	0.5599	0.00	620	6.4775
H45	200	170	8.0151	5.00	0.0167	0.4842	0.00	660	5.8977
H48	140	110	5.4052	4.9	0.0130	0.2785	0.00	601	6.7871
H58	160	130	6.6362	5.1	0.01	0.5067	0.00	623	6.4308
H50	140	110	5.08	4.61	0.01	0.3022	0.00	641	6.1618
12 days PB treatment									
A1 - Sham hypophysectomy and saline									
H9	150	150	6.017	4.0	0.0316	1.759	0.3565	561	7.5258
H10	210	240	10.3436	4.3	0.0453	3.0826	0.00	543	7.9031
H11	190	240	9.305	3.8	0.056	2.847	0.00	570	7.3483
H12	260	300	11.88	3.96	0.0624	3.0	0.00	567	7.4067
A2 - Sham hypophysectomy and PB									
H0	210	270	11.34	5.4	0.0435	3.04	0.8064	496	9.0526
H1	230	280	12.564	5.4	0.032	3.219	0.6109	491	9.1913
H2	240	290	13.392	5.58	0.065	3.516	0.5928	506	8.7856
H3	210	260	12.39	5.9	0.04	3.03	0.3906	512	8.6316
H4	230	290	13.18	5.7	0.04	3.25	1.2396	484	9.3914
H5	220	290	13.392	6.0	0.079	3.158	1.4799	473	9.7209
B1 - Hypophysectomy and saline									
H72	170	160	5.420	3.38	0.0207	0.448	0.0	998	3.1717
H75	170	140	5.35	3.8	0.0154	0.4230	0.0920	1086	2.7864
H76	160	140	5.0144	3.57	0.0294	0.35	0.00	1136	2.6117
H77	160	120	2.681	2.68	0.021	0.322	0.00	1109	2.7077
H78	160	130	4.684	3.6	0.025	0.391	0.0	1021	3.0652
B2 - Hypophysectomy and PB									
H18	210	190	8.8724	4.6	0.02	0.5045	0.00	655	5.9653
H19	230	190	8.53	3.15	0.0238	0.6650	0.00	671	5.7532
H22	200	170	8.17	4.0	0.0129	0.5329	0.00	631	6.3089
H24	210	190	7.59	3.99	0.0134	0.50	0.00	650	6.0343
H27	190	160	8.1538	4.28	0.0119	0.55	0.00	671	5.7532
H1	150	130	7.8886	5.2	0.02	0.59	0.00	609	6.6538
H4	150	135	6.6261	4.56	0.02	0.79	0.00	654	5.9790

Table A.6 Pilot Study I - Group means

Experiment	No. PB days	No. animals	Total wt. (av)grams	Total wt. end exp. (av)grams	Av.liver (av)grams	% liver /100 g body wt.	Adrenals (grams)	Testes (grams)	Av.no. mitoses /1000 cells	No.cells /10F	Mean cell vol. $x10^{-5} mm^3$
AA(1) Group 1 Sham hypophysectomy and saline	4	3	210.0 ±10.00	281.67 ±18.93	10.41 ±1.06	3.69 ±0.21	0.04 ±1.33 $x10^{-3}$	3.10 ±0.30	0.0544 ±0.0769	598.33 ±13.474	6.839 ±0.2342
AA(2) Group 2 Sham hypophysectomy and PB	4	4	215.00 ±12.91	258.75 ±28.39	11.71 ±1.17	4.56 ±0.12	0.04 ±2.91 $x 10^{-3}$	2.82 ±0.24	0.2957 ±0.2238	507.75 ±10.328	8.7465 ±0.2644
BA(1) Group 1 Hypophysectomy and saline	4	3	226.67 ±5.77	193.33 ±5.77	7.00 ±0.49	3.59 ±0.16	0.02 ±2.00 $x10^{-4}$	0.75 ±0.21	0.0 ±0.0	982.66 ±17.307	3.2476 ±0.0860
BA(2) Group 2 Hypophysectomy and PB	4	4	243.75 ±52.82	200.0 ±27.08	7.33 ±1.15	3.66 ±0.43	0.02 ±0.01	0.64 ±0.04	0.0386 ±0.0669	651.0 ±8.5416	6.0219 ±0.1189
AB(1) Group 1 Sham hypophysectomy and saline	8	3	206.67 ±5.77	236.67 ±28.87	9.95 ±0.93	4.12 ±0.3791	0.04 ±0.01	2.84 ±0.19	0.0595 ±0.0841	576.33 ±13.912	7.2353 ±0.2609
AB(2) Group 2 Sham hypophysectomy and PB	8	5	160.0 ±18.71	220.0 ±24.49	11.17 ±1.19	5.07 ±0.3686	0.05 ±0.01	2.77 ±0.29	0.6294 ±0.5449	502.6 ±12.0929	8.8845 ±0.3197
BB(1) Group 1 Hypophysectomy and saline	8	14	170.71 ±17.74	147.86 ±12.97	5.31 ±0.72	3.58 ±0.31	0.02 ±0.01	0.4251 ±0.1012	0.0061 ±0.0221	1133.28 ±35.78	2.6260 ±0.1263
BB(2) Group 2 Hypophysectomy and PB	8	8	182.50 ±30.59	156.25 ±34.62	7.18 ±1.35	4.72 ±0.25	0.01 ±4.28 $x10^{-3}$	0.44 ±0.10	0.00 ±0.00	634.62 ±17.86	6.2643 ±0.2692
AC(1) Group 1 Sham hypophysectomy and saline	12	4	202.5 ±45.73	232.50 ±61.85	9.39 ±2.48	4.02 ±0.21	0.05 ±0.01	2.67 ±0.62	0.0891 ±0.1543	560.25 ±10.473	7.5459 ±0.2158
AC(2) Group 2 Sham hypophysectomy and PB	12	6	223.33 ±12.11	280.0 ±12.65	12.71 ±0.79	5.66 ±0.25	0.05 ±0.02	3.20 ±0.18	0.8533 ±0.3840	493.66 ±13.046	9.1289 ±0.3638
BC(1) Group 1 Hypophysectomy and saline	12	5	164.00 ±5.48	134.00 ±21.91	4.63 ±1.13	3.41 ±0.43	0.0223 ±4.681 $x10^{-3}$	0.39 ±0.05	0.0184 ±0.0368	1070.4 ±52.507	2.8684 ±0.2141
BC(2) Group 2 Hypophysectomy and PB	12	7	191.43 ±30.79	179.29 ±22.07	7.98 ±0.73	4.25 ±0.64	0.02 ±3.96 $x10^{-3}$	0.5903 ±0.0970	0.00 ±0.00	648.71 ±20.540	6.0639 ±0.2975

Table A.7 Pilot Study II - Group results

Rat no.	At start total body wt(grams)	At end total body wt(grams)	Liver wt(grams)	% liver/ body wt	% Rt post lobe/ body wt	Liver LL	ML	RP	CL	Adrenals (grams)	Testes (grams)	No.mitoses /1000 cells	No.cells / 10 F	Cell vol x10^{-5}mm^{3}
A1 - Sham hypophysectomy, laparotomy and saline														
H3	160	250	10.75	4.30	1.408	3.03 28.0%	3.07 29.0%	3.52 33.0%	1.13 11.0%	0.04	3.90	0.3395	589	6.9956
H4	190	260	9.42	3.62	0.9076	3.02 32.0%	3.12 33.0%	2.36 25.0%	0.92 10.0%	0.04	3.28	0.0	578	7.1962
H6	180	245	8.93	3.64	1.1142	2.53 28.0%	2.89 32.0%	2.73 31.0%	0.78 9.0%	0.04	3.09	0.1672	598	6.8383
H9	180	250	9.37	3.75	0.884	2.97 32.0%	3.44 37.0%	2.21 24.0%	0.75 8.0%	0.04	2.94	0.0	624	6.4153
H10	170	240	9.48	3.95	1.145	2.93 31.0%	3.04 32.0%	2.75 29.0%	0.76 8.0%	0.04	3.0	0.0	625	6.400
H11	170	260	8.27	3.70	1.073	2.43 29.4%	2.36 28.5%	2.79 34.0%	0.69 8.3%	0.04	3.09	0.1642	609	6.6538
A2 - Sham hypophysectomy, 1/3 hepatectomy and saline														
H0	170	250	9.61	3.84	1.576	4.46 46.7%		3.94 40.99%	1.21 12.59%	0.045	2.97	0.0	620	6.4775
H1	129	260	10.37	3.98	1.500	4.80 46.28%		3.90 37.6%	1.67 16.1%	0.04	3.79	0.1686	593	6.9249
H2	180	260	8.17	3.14	1.3461	3.61 44.18%		3.40 41.61%	1.16 14.19%	0.04	3.02	0.1577	634	6.2642
H5	170	240	6.62	2.75	1.1416	3.18 48.03%		2.74 41.38%	0.70 10.57%	0.04	3.10	0.0	605	6.7199
H7	180	240	9.71	4.04	1.5125	4.70 48.4%		3.63 37.38%	1.38 14.21%	0.04	2.67	0.0	580	7.1590
H8	170	260	8.77	3.37	1.6846	3.10 35.34%		4.38 49.94%	1.29 14.7%	0.04	2.93	0.3384	591	6.9601
B1 - Hypophysectomy, laparotomy and saline														
H5	180	150	5.92	3.95	0.9200	1.89 31.92%	2.05 34.62%	1.38 23.3%	0.60 10.0%	0.01	0.34	0.0	1259	2.2365
H5	170	145	5.12	3.53	0.9310	1.64 32.03%	1.72 33.59%	1.35 26.35%	0.41 8.0%	0.01	0.34	0.0	1005	3.1387
H6	140	135	4.72	3.50	0.9037	1.63 34.53%	1.54 32.62%	1.22 25.84%	0.33 6.99%	0.01	0.42	0.0	1073	2.8451
H11	240	180	6.95	3.86	0.9444	2.25 32.37%	2.39 34.39%	1.70 24.46%	0.61 8.78%	0.015	0.57	0.09920	1008	3.1247
H4	240	200	7.04	3.52	0.7700	2.33 33.10%	2.46 34.94%	1.54 21.88%	0.71 10.09%	0.01	0.68	0.0	1034	3.0075
B2 - Hypophysectomy, 1/3 hepatectomy and saline														
H6	240	190	4.65	2.44	0.8263	2.48 53.33%		1.57 33.76%	0.60 12.9%	0.01	0.74	0.00	800	4.4194
H7	240	180	5.59	3.10	1.2944	2.66 47.58%		2.33 41.68%	0.60 10.73%	0.01	0.62	0.1135	781	4.5816
H12	230	170	4.89	2.87	1.0882	2.35 48.05%		1.85 37.83%	0.69 14.11%	0.01	0.50	0.0	894	3.7410
H13	240	190	5.93	3.12	1.3368	2.54 42.87%		2.54 42.87%	0.85 14.33%	0.015	0.63	0.0	905	3.6730
H0	190	180	4.09	2.27	0.8500	1.973 48.16%		1.538 37.46%	0.596 14.42%	0.006	0.422	0.0	837	4.1296
H4	190	160	5.08	3.16	1.3625	2.373 46.74%		2.181 42.99%	0.529 10.25%	0.012	0.54	0.1144	874	3.8701

Table A.8 Pilot Study II - Group means

Experiment	No. animals	Total wt. (av)grams	Total wt. end exp. (av)grams	Av.liver (av)grams	Av.% liver/ body wt.	Av.% rt.post. body wt.	Liver LL	Liver ML	Liver RP	Liver CL	Adrenals av(grams)	Testes (av)grams	Av.no. mitoses /1000 cells	No.cells /10F	Mean cell vol. $x10^{-5}mm^3$
A1 Group 1 (controls) Sham hypophysectomy, laparotomy and saline	6	175.00 ±10.49	250.83 ±7.312	9.37 ±0.74	3.83 ±0.24	1.0886 ±0.1735	2.82 ±0.24 30.1% ±1.70	2.48 ±1.11 31.92% ±2.81	2.72 ±0.42 29.33% ±3.77	0.84 ±0.15 9.05% ±1.12	0.04 ±0.00	3.22 ±0.35	0.1118 ±0.1259	603.83 ±18.36	6.7498 ±0.2918
A2 Group 2 Sham hypophysectomy, 1/3 hepatectomy and saline	6	176.67 ±8.16	251.67 ±9.83	8.88 ±1.35	3.52 ±0.52	1.4601 ±0.1744	3.98 ±0.77 44.77% ±4.86		3.67 ±0.56 41.48% ±4.5	1.24 ±0.32 13.73% ±1.91	0.04 ±2.04 $x10^{-3}$	3.08 ±0.38	0.1107 ±0.1252	603.83 ±17.33	6.7509 ±0.3036
B1 Group 1 (controls) Hypophysectomy, laparotomy, saline	5	190.0 ±34.64	157.85 ±22.49	5.95 ±0.9371	3.672 ±0.1926	0.8938 ±0.0633	1.948 ±0.3 32.79% ±0.96	2.032 ±0.36 33.95% ±0.98	1.44 ±0.17 24.366% ±1.64	0.53 ±0.14 8.772% ±1.18	0.011 ±2.00 $x10^{-3}$	0.47 ±0.1344	0.0198 ±0.0396	1074.71 ±83.216	2.8670 ±0.2956
B2 Group 2 Hypophysectomy, 1/3 hepatectomy and saline	6	221.67 ±24.83	178.33 ±11.69	5.04 ±0.66	2.83 ±0.38	1.1263 ±0.2221	2.40 ±0.24 47.79% ±3.35	±0.41	2.00 ±0.41 39.42% ±3.70	0.64 ±0.11 12.79% ±1.86	0.01 ±2.95 $x10^{-3}$	0.58 ±0.11	0.0379 ±0.0537	865.16 ±36.025	4.0691 ±0.3398

APPENDIX III

FURTHER DETAILS OF LABORATORY TECHNIQUES AND DISCUSSION OF MORPHOMETRIC AND GEOMETRIC PROBLEMS RELATED TO THIS STUDY

A.III.1 Details of histological sections and staining techniques

When using histological sections for morphometric analysis it is important that during the preparation and staining of the sections, sources of variability are reduced to a minimum. This will allow results to be critically analysed from standardised data.

With this aim in mind certain procedures were routinely carried out during this study. These were:-

(1) All liver samples were fixed in phosphate buffered formal alcohol. This fixative was chosen as the liver lobes shrink less compared with e.g. phosphate buffered formal saline. Additionally this fixative is known to give good hepatocyte nuclear presentation (personal communications H.K. Weinbren).

(2) All sections and staining procedures were undertaken in the RPMS research laboratory by the same person. Large batches of sections were prepared at any one time to eliminate variations which may occur by samples being sectioned on different days. However, the precautions undertaken were designed to overcome this possible variation and was therefore considered to be a minor problem. With the size of the project it was not possible to section all the samples at one time.

(3) Tissue was selected from the same area of the fixed right posterior lobe.

(4) Paraffin wax was supplied by Raymond A. Lamb Laboratories, for the preparation of paraffin wax blocks. The wax has a melting point of 56°C. In the preparation of blocks and sections, the following procedures were followed in order to reduce variability in section thickness.

(a) The blocks were prepared with parallel surfaces. This was achieved by embedding the tissue in paraffin wax on its flat surface and the opposite surface of the block cut until the full surface of the tissue is exposed. If this is not done the ribbon produced will be curved and the sections of uneven thickness (Disbrey and Rack, 1970).

(b) Before mounting onto the microtome, all paraffin blocks were cooled on ice. This allows the paraffin to become more crystalline and therefore harder and makes cutting easier.

(c) Sections were cut on all occasions using the same Cambridge Rocker microtome, with corresponding knife. The knife was at all times kept sharp, clean, firmly clamped and at the same angle. This setting was predetermined and known to produce good quality sections.

(d) The blocks were firmly clamped to the microtome as any minor movement either of the knife or blocks can alter section thickness.

(e) The section thickness setting on the microtome was kept constant at 5 u.

(f) Sections were cut in a ribbon as they are easier to handle and less easily damaged.

(g) Ribbons were flattened during cutting by gently blowing on the sections. The first section was always discarded as this tends to vary in thickness.

These precautions aimed to produce sections without surface "corrugations", which result from sections being prepared with variation in thickness.

An ultimate check to ensure all sections are standardised can be undertaken by preparing a block, embedding some of the tissue from a sample at right angles to the rest of the sample, which is routinely embedded and sectioned in the normal manner. Using this block, section thickness is checked by measuring the sections prepared from it with a micrometer gauge. When interpreting results from this present study it should be remembered that this was not undertaken.

Serial sections were prepared and some were stained with Meyer's Haematoxylin and Eosin and the rest reserved for Feulgen staining.

All reagents were supplied by Raymond A. Lamb Laboratories.

Meyer's Haematoxylin and Eosin Staining Method

This technique was based on the original Meyer (1903) and modified by Lendrum and McFarlane (1940).

Standardization of staining of the sections was achieved by careful adherence to this protocol.

(1) Sections were dewaxed by immersing in

Xylene	3 mins
Absolute alcohol	2 mins
70% alcohol	2 mins
Rinsing in tap water	

(2) Sections were stained with Meyer's Haematoxylin for 10 minutes.

(3) To remove excess haematoxylin from the tissues, sections were rinsed in Scott's tap water for 10 seconds. (Ordinary tap water is replaced by Scott's water where the water in the laboratory is neutral or acid (Scott, 1912).)

(4) Sections were differentiated precisely in acid alcohol for 10 seconds. (One second for every minute in the haematoxylin.) This is in order to achieve the correct degree of staining with haematoxylin.

(5) Blue in Scott's tap water substitute for 10 minutes.

(6) Stain with eosin for 5 minutes. As eosin is a water soluble reagent care has to be taken not to introduce variability in the staining by washing out an excess amount. This is achieved by washing in tap water for 3 minutes and 50% alcohol for 2 minutes.

(7) Dehydrate by immersing sections in

70% alcohol	2 mins
90% alcohol	2 mins
100% alcohol	2 mins
Xylene	3 mins

(8) Routine mounting procedures were undertaken using DPX as mounting medium.

A.III.2 Account of the Feulgen staining method used and reference to possible sources of variability

The technique used for the identification of deoxyribonucleic acid (DNA) in tissue sections was described by Disbrey and Rack (1970) based on the original (Feulgen and Rossenbeck, 1924).

Various precautions were taken.

(1) Sections used were prepared as already described (Appendix III Section 1) in order to reduce the likelihood of variability in section thickness to a minimum, which would alter the staining by Feulgen.

(2) All sections were stained at the same time in order to reduce any variability in staining. This is particularly important as the Feulgen staining of DNA is designed to allow for quantitative measurement of DNA from the sections.

(3) All sections were stained with the same batch of any particular reagent. Schiff's reagent and the sulphurous rinse were made up fresh prior to use. Only Schiff's reagent designated by the manufacturer suitable for Feulgen staining was used and the sections were stained in a Coplin jar. This latter precaution was taken because if Schiff's reagent is exposed to air it will lead to recolourisation of the non-specific staining in the sections.

(4) Hydrolysis time. As all the material was fixed in phosphate buffered formal alcohol the hydrolysis time prior to immersing in Schiff's reagent was kept at the standard 8 minutes. This was in order to expose aldehyde groups in the DNA to which the Schiff's

reagent histochemically reacts to give the magenta staining. If however, the fixative used had been acid based the hydrolysis time would have been reduced as the fixative will cause some hydrolysis in the section itself and therefore require less hydrolysis time.

(5) Following the Schiff's reaction before sections are washed in water, they should be washed in a sulphurous rinse. This should contain sodium metabisulphite to ensure that the excess Schiff's reagent is removed so that it does not act as a dye and stain other structures in the sections.

(6) DNA is the only substance that is Feulgen-positive. To ensure that the sections have responded to the Schiff's reagent following hydrolysis (i.e. DNA aldehyde groups have been exposed) a control section should be treated with the Schiff's reagent without prior hydrolysis. Only structures that were stained magenta in the test section and unstained in the control may be described as Feulgen-positive.

(7) The timing of all reagents used and washings were precise in order to ensure even staining results.

Feulgen staining method followed

(1) Sections were dewaxed and taken to water as described for Haematoxylin and Eosin staining in Appendix III Section 1.

(2) Sections were rinsed in N/1 hydrochloric acid.

(3) Hydrolysis stage. N/1 hydrochloric acid was warmed in a water bath to 60°C and the sections placed in the acid for 8 minutes.

(4) Sections were rinsed in distilled water.

(5) Sections were placed in Schiff's reagent for 1 hour.

(6) Sections were placed in the sulphurous rinse for 2 minutes. This procedure was repeated twice.

(7) Sections placed in running tap water for 10 minutes.

(8) Rinse in alcohol.

(9) Sections were dehydrated, cleared and mounted as already described in Appendix III Section I for Haematoxylin and Eosin staining.

(10) No counterstain was used as Feulgen-positive structures can be obscured by counterstain.

Preparation of Schiff's reagent

Basic fuchsin - suitable for Feulgen	1.0 g
Sodium metabisulphite	1.9 g
N/1 hydrochloric acid	15 ml
Distilled water	85 ml
Activated charcoal	0.5 g

The basic fuchsin and metabisulphite were dissolved in acid and water, shaking frequently for 2 hours. Charcoal was added and the liquid shaken for 1-2 minutes. The liquid was filtered and stored at 4°C. The solution was only used as long as it remained colourless.

Sulphurous rinses

10% aqueous sodium metabisulphite	6 ml
N/1 hydrochloric acid	5 ml
Distilled water	to 100 ml

A.III.3 Morphometry, geometric problems and problems relating to these subjects raised in this study (see Acknowledgement page 26)

A.III.3.1 Tissue sectioning

In morphological research one of the methods of choice is the microscopic study of sections across the structure under examination, e.g. the hepatocyte. The advantage of this is that the structure can be examined without altering the relative position of internal objects. However in some biological circumstances the information offered from a two-dimensional section may be different from its three-dimensional existence, e.g. red corpuscles, endoplasmic reticulum.

Thus when adopting quantitative morphometric analysis in a study, the geometric aspects of sectioning must be considered. In this particular study for example, in which liver slices were sectioned 5 μ in thickness in order to examine the nuclei of the hepatocytes and to determine the ratio of 2n:4n ploidy classes, it was inevitable during the sectioning that some nuclei may have been missed entirely or sectioned at differing levels from the equator to its pole. This resulted in the histograms Figures 3.1 and 6.1 (main text pp.90 and 140) showing distribution of mean area in size ranges of 5 μm^2 classes from 10-100 in which a percentage of nuclei were recorded away from the 2n or 4n peaks, e.g. Figure 3.1, A Group 2. These discrepancies can be corrected using stereological methods but were not undertaken in this study. Methods for doing this are discussed later.

Another important aspect to be considered regarding sectioning is that when sections are cut not only may they vary in thickness unless good technical procedures are observed, but the thickness of the section itself will increase the number of objects observed in the higher size

bracket. In this study this would increase the number of nuclei with larger mean apparent nuclear area.

While rigorous technical procedures were observed in order to reduce to the minimum any variation in thickness between batches of sections (already described in A.III.1) correction for thickness of the section was not undertaken, a factor which should be remembered during the interpretation of the results. Methods for this correction are also discussed later.

In order to compensate for these and other deficiencies mathematical corrections can be made using stereological analysis. Stereology is defined as a body of mathematical methods relating three-dimensional parameters defining the structure to two-dimensional measurements obtainable on sections of the structure (Weibel, 1979).

Stereology employs various terms, some of which are defined as follows:-

(1) Section = a plane intersecting the structure: it hence also intersects some of the "components" of the structure.
In this study structure = liver
important component = nucleus

(2) Profile = the "image" of any spatial component (and of the structure), i.e. the sharply defined flat trace on the section plane.

(3) Phase Is the aggregate of all parts which are identical in nature, e.g. mitochondria.

(4) Objects or particles are components which are isolated units within the structure, e.g mitochondria, lysosomes, microbodies.

The quantitative description of a structure is the density of the various components within the structure where

(5) Density is defined as "the quantity per unit volume, unit area" of the phase contained in the unit volume of the structure.

A relationship exists between the density of component profiles (e.g. nuclei) within the profile of the structure (i.e. on the section including the rest of sectioned hepatocyte structures observed) and the density of components within the structure. Stereology seeks to establish these relationships by mathematical reasoning. Thus in stereological procedures all measurements are obtained as a ratio between the components and structure in order to establish the relationship between ratios measured in sections and the corresponding ratios in the spatial structure.

While some key stereological principles described in this Appendix were employed in this study thus validating the results described, others were not. These principles are discussed in relation to the present study.

3.1.1 Other aspects relating to tissue sectioning

3.1.1.1 Geometric probability

When considering the possibility of whether a particular object is included as a profile when a structure is cut at random in the preparation of a section, it is assumed that profiles are obtained with a certain well-defined probability.

In defining this probability when experiments are repeated (e.g. the preparation of several sections) the probability of obtaining the expected outcome (particular profiles of interest) is the number of "positive" outcomes divided by the number of attempts, provided the number of attempts are large.

This probability can be predicted theoretically, details of which are found in Weibel (1979), Practical Methods for Biological Morphometry, pages 13-17.

When considering the geometric probability of a nucleus being obtained in a section prepared by random slicing of hepatocytes it must be considered that the structure, i.e. the hepatocyte, is a cube and the nucleus a sphere.

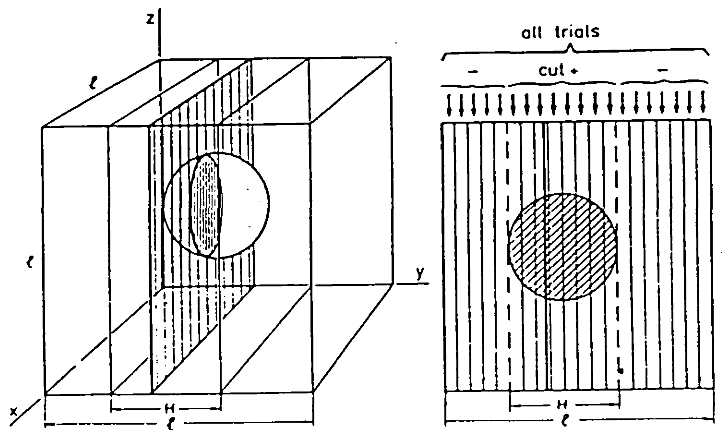

Fig. 1

FIG. 2.3: Model structure, composed of sphere contained in cube, is used to derive probability of cutting an object. At right, projection of sphere and section onto front face of cube. Probability depends on ratio of tangent (or caliper) diameter H of object to the side length of the cube.

The section will contain a profile of the object (i.e. nucleus) where slices are made anywhere in the area between the two planes which touch the object tangentially. The number of slices within this range is $n=H/dy$ where H is the distance between the two tangential planes

(caliper diameter) and dy. is the thickness of the slice. Thus the probability of cutting the object is

$$\Pr\{a\} = n \cdot dy/l = H/l =$$

the ratio of the caliper diameter of the object to the side of thecube l, where l defines the number of possible cuts, H are those "favourable".

Thus when considering whether during the preparation of sections slicing through a particular hepatocyte includes part of the nucleus or entirely misses it, from a practical point of view as there are always several nuclei obtained per section, it can be assumed that "the number of favourable outcomes" relating to the number of sections made are sufficiently high (Weibel, 1979 Stereological Methods for Biological Morphometry, p.13). It was therefore considered unnecessary in this study to undertake any probability studies.

3.1.1.2 Plane of orientation

A further fundamental aspect to consider during random sectioning is that the probability of hitting an object also depends on the orientation of the plane of section. The theoretical consideration of this is described in detail by Weibel (1979) in Stereological Methods for Biological Morphometry, pp.18-24. However, relating to this present study averaging for orientation is not required as the hepatocyte nuclei appear to be spheres. Thus the mean caliper diameter, H, of the nucleus remains constant and does not alter depending on the relative orientation of the plane of section.

3.1.1.3 Random sectioning

In deriving stereological principles it is assumed that an object can be cut at different levels and different directions. A sample must provide a representative number of profiles to include all possible

cuts. Thus in random sectioning provided the structure contains a very large number of the objects under consideration, then a section cut at random into the structure will provide sufficient profiles to represent all possible cuts. This is provided the object has no preferred orientation in the structure.

In this study 5 µ thick sections from slices of liver containing numerous nuclei per section would appear to fulfil these criteria. As nuclei are spheres there is no preferred orientation. All sections cut in this study could therefore be assumed to be "random sections".

3.2 Aspects related to the association between volume and area

3.2.1 Sectioning used in stereology

In addition to the geometric aspects of sectioning already discussed (Section 3.1), the trace of an object obtained on a section depends on the properties of the object and on the way it has been cut.

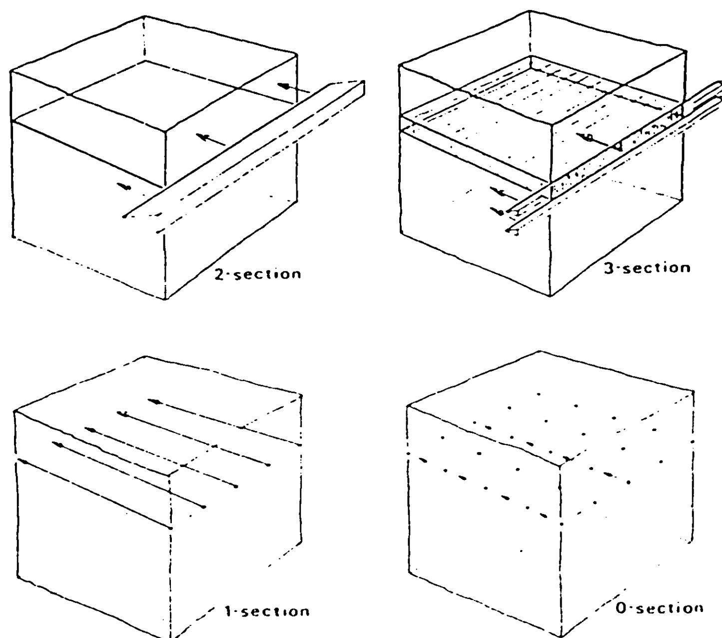

Fig. 2

FIG. 2.1: Sectioning with a microtome knife can be used to illustrate the generalized concept of "sectioning" used in stereology. (Reproduced by permission from Weibel, 1967c.)

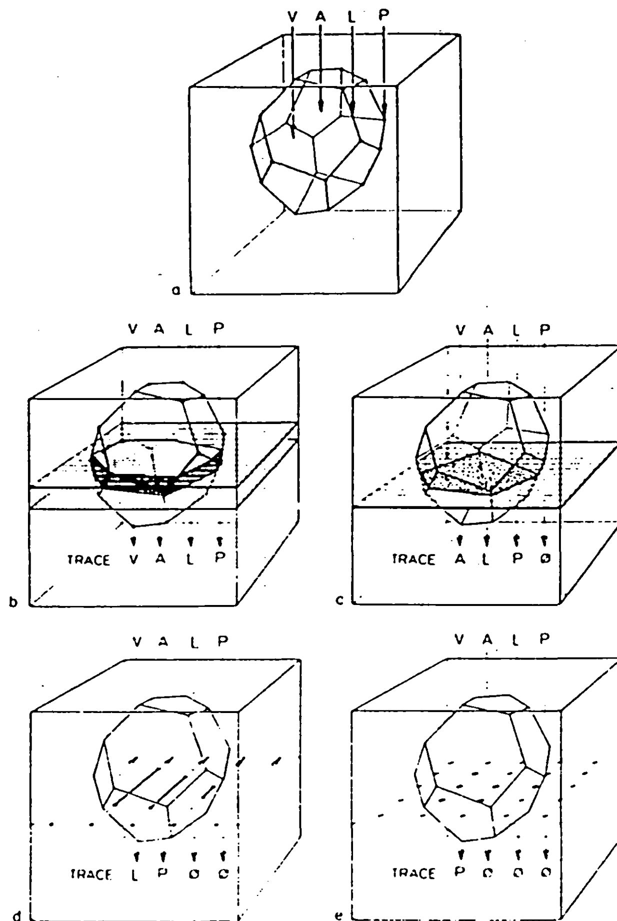

Fig. 3

FIG. 2.2: (a) A polyhedron can serve as a general model object because it can be quantitatively defined by the four basic parameters: V = volume of the solid: A = area of the faces: L = length of the edges: P = number of corner points. (b)–(e) "Sectioning" with a slice (b), a plane (c), a set of lines (d), and a set of points (e) produces traces of different dimensions.

From Figure 2 it can be seen that if the cut is three-dimensional a slice is produced, if the cut is two-dimensional the section thus is a plane, a one-dimensional cut produces a line and a zero-dimensional cut produces a set of points within the tissue.

From Figure 3 it can be seen that a slice produces four parameters; volume, face area, edge length and corner points. Following a two-dimensional cut only three parameters are represented; area A^1, boundary length L^1 and a number of corner points.

It is evident that A^1 is related to the volume V, L^1 to the surface A, and P^1 to the edge length L, with the corner points not represented on the section. Thus from a section it is correct to say that the area observed of an object is related to its volume and well known geometric formulae state that

$$r \propto \sqrt{A} \qquad A = \pi r^2$$

$$V = 2/3\pi r^3 \qquad V \propto r^3$$

$$V \propto A^{3/2}$$

r = radius
A = area
V = volume

3.2.2 Volume density measurement - the Principle of Delesse

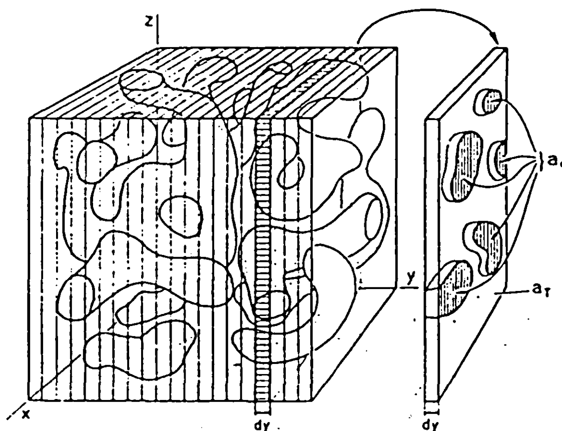

Fig. 4

FIG. 2.16: Model for deriving Delesse principle.

It has been proved that the volume density of various components making up a structure can be estimated on random sections by measuring the relative areas of their profiles (areal density of profiles on section).

By taking a model cube containing one particular object irrespective of its size or shape the cube is cut out of a layer structure so that objects may fill the cube in all parts. By slicing the cube in the X, Y, Z co-ordinate system parallel to the X-Z plane of

thickness dy, slices obtained all have an area a_T. The profiles of the object concerned are measured = a_o. The slice inevitably contains a certain volume v_o of the objects which must be equal to the profile area multiplied by the slice thickness. $vo = a.o. \times dy$.

The volume of the slice however is :-

$$v_T = a_T \times dy$$

By summing all the slice volumes and adding all the object volumes contained in them and dividing the summed object volumes by summed slice volumes - this defines volume density

$$\frac{\Sigma v_o}{\Sigma v_T} = \frac{V_o}{V_T} = Vv \qquad \text{(A)}$$

If V_o and V_T are replaced by area times thickness then:-

$$\frac{\Sigma(ao.dy)}{\Sigma(a_T.dy)} = \frac{dy\Sigma ao}{dy\Sigma a_T} = \frac{Ao}{A_T} = A_A \qquad \text{(B)}$$

Slice thickness is constant and can therefore be cancelled from sums.

The ratio of the sum of the profile area to the sum of section area is evidently the areal density on the section A_A. Thus as both (A) and (B) are equal to each other

$$V_V = A_A$$

In practice the reliability of the estimate will increase with the size of the sample.

Measurements of areal density may be estimated using several stereological methods including linear integration and point counting methods of which are described in detail in Weibel (1979) Stereological Methods for Biological Morphometry, pp.7-30.

It can therefore be assumed in this study in addition to the discussion in subsection 3.2.1 that nuclear area measured in this study using the Quantimet 720 image analyser is directly proportional to their volume.

It is commonly assumed that the volume of a 4n mononuclear nucleus is double that of a 2n mononuclear nucleus. Thus in this study in the description under the subsection 2.7.1.1 using the Quantimet 720 image analyser to measure mean nuclear area and using the nuclear size distribution histograms to determine the percentage of nuclei in 2n and 4n ploidy classes, it was a correct assumption when a definite 2n and 4n peak could not be found, to convert the area of the obvious peak into its volume, doubling or halving the volume and converting back into the area thereby identifying the other peak.

This was obtained by the following formula:-

$$\text{2n area} = \left(\sqrt[3]{\frac{(\sqrt{\text{Area 4n}})^3}{2}}\right)^2$$

$$\text{4n area} = \left(\sqrt[3]{2(\sqrt{\text{Area 2n}})^3}\right)^2$$

3.3 Aspects relating to variations in nuclear size

When considering the histograms produced in Figures 3.1 and 6.1 (from the main text) it has already been discussed that there an obvious number of nuclei with mean apparent nuclear area of "intermediate" size between 2n and 4n peaks, below and above. This relates to the level at which these nuclei were cut, knowing nuclei are spheres.

3.3.1 Profiles derived from spheres of equal size

Assuming the nuclei to be all the same size stereological methods are available described in detail in Weibel (1979) Stereological Methods

for Biological Morphometry pp.52-57 whereby the actual size of the nuclei within the whole structure can be calculated from the observed nuclei sizes in the section.

3.3.2 Methods of determining actual nuclear size when a mixture of varying sized nuclei are sectioned

In this study not only may nuclei be sectioned in different parts of their sphere but the nuclei may also be different sizes as there is more than one population of ploidy class to consider.

Thus when considering the sectioning of the nuclei it is possible to visualise that profiles of nuclei produced by sectioning will fall into size classes depending on the size of the whole nucleus.

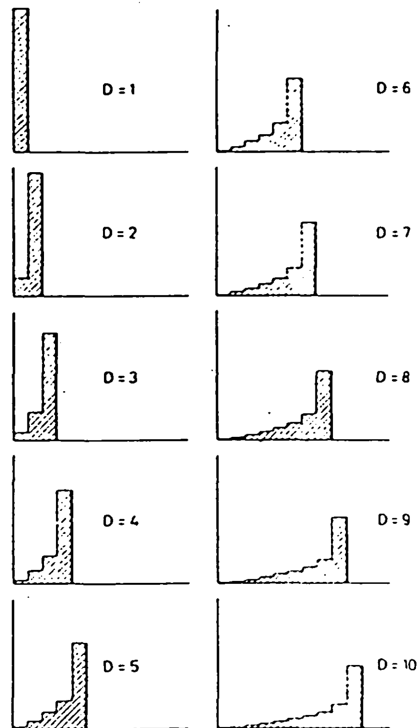

FIG. 2.41: Profile size distribution for spheres of varying size *D*.

Fig. 5

Profile size distribution if a mixture of spheres of varying size is sectioned

From figure 5 it can be seen that in general each profile class receives profiles from spheres whose diameter are larger or equal to the size of these profiles. Thus in histograms prepared in this study where a percentage of nuclei appear to have a mean nuclear area more towards the 2n ploidy class, some may have been 4n ploidy class nuclei sectioned away from the equator.

The number of profiles provided by each sphere size class depends on the probability of obtaining a profile from the spheres in each class.

When considering spheres of a particular radius it has been mathematically calculated that the numerical density of the profiles derived from such spheres is proportional to the numerical density of the spheres multipled by their diameter

$$\therefore \quad Naj = Nvj \cdot 2Rj$$

By further mathematical calculations described in detail by Weibel (1979) in Stereological Methods for Biological Morphometry, p.58, it is possible to express the profile frequency as numerical densities per unit section area as in Figure 6 as seen for three different sphere sizes and from this construct the corresponding overall profile size distribution which is shown in the last histogram.

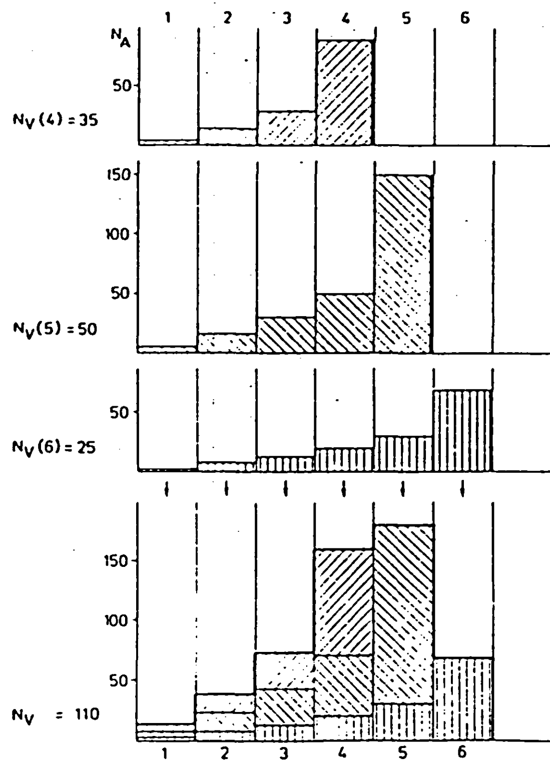

Fig. 6

FIG. 2.42: Mixture of spheres of three size classes results in compound size distribution of profiles.

This will enable calculations of what the true profile distribution should be if varying sized spheres are sectioned. However in this study it is the consideration of how to determine the true nuclear size from the observed nuclear areas on the sections which is of particular interest and this may be mathematically calculated using accepted stereological methods using the profile size distribution obtained by measurements first described, an example of which will now be described.

<u>Derivation of sphere size distribution from a measured profile size distribution</u>

By knowing the profile size distribution it is possible to calculate the sphere size distribution in the following manner.

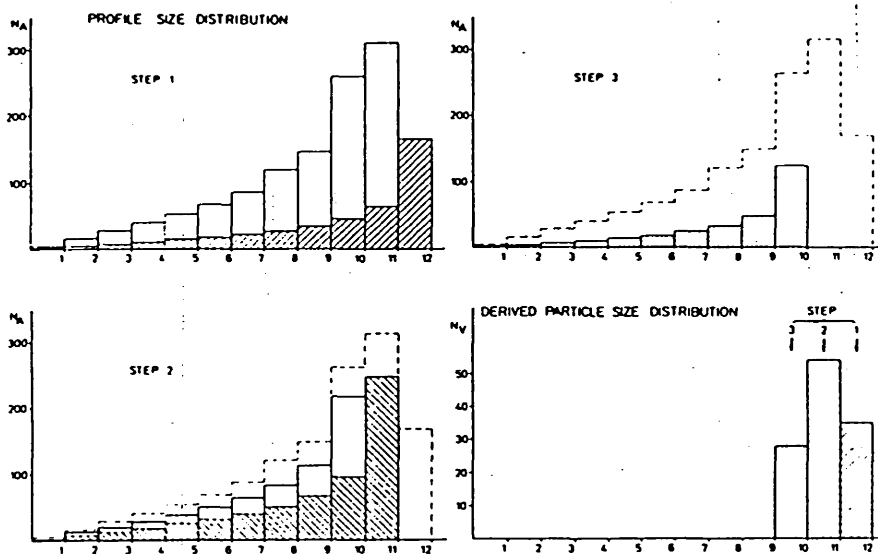

FIG. 2.43: Principle for deriving particle size distribution from profile size distribution.

Fig. 7

Taking Figure 7 it can be seen that the largest profile size (12) has received profiles from the largest sphere class only. By mathematically calculating the numerical density of the profiles in the largest class (m) using the formula

$$N_{vm} = N_{Am} \; [2\Delta r \sqrt{(2m - 1)}]$$

(for further details see Weibel (1979) Stereological Methods for Biological Morphology, pp.59-60), the profile numbers contributing to smaller size classes then then be calculated and subtracted from the histogram. This leaves the histogram with the largest profiles now in class 11 (m-1). The numerical densities are then calculated for spheres in class 11 and their contribution subtracted from the histogram until the histogram is exhausted.

This is a very cumbersome and rather inaccurate method to use as the whole method relies on one value, i.e. the largest, to base the calculations on throughout the entire method. It is now possible by computation devised by Wicksell (1925) and Saltykov (1958) to measure

profile frequencies and obtain the frequency distribution of spheres. Details of this procedure will now be discussed.

Reconstruction of the frequency distribution of particle size from that of profiles

Procedures for reconstructing a particle size distribution from a measured profile size distribution are based on what is seen in Figure 6 in that:-

(1) profiles of a certain size class (i) may be derived from all spheres of size classes $j \geqslant i$.

(2) a given sphere of size class j contributes to all profile classes $i \leqslant j$ in proportion to the probability of obtaining this profile size, which decreases as the profile becomes smaller.

Using a procedure based on Wicksell's (1925) method of reconstruction allowing for finite section thickness (t), Weibel (1979) described the material procedure involved using the computer program developed from Wicksell (1925) by Bandhuin (1968).

This is essentially

(1) Record size distribution of profiles. If using the Wicksell method the diameter class of the circle is recorded which best fits the profile. If using the Saltykov type determine between which two circles the profile fits and record the diameter of the largest. With between 12-15 classes, a few hundred profiles are measured to give adequate precision in the analysis.

(2) Calculate numerical densities of profiles, plot as a histogram and correct for missing small profiles. Amend the table.

(3) Estimate section thickness and decide if it should be considered. If so make the correction using the appropriate table of coefficients devised by Cruj-Orive (1978).

(4) Correction for missing more profiles is checked to see whether many profiles were missing.

Figure 8 is the result of a practical application to this approach to the sizing of hepatocyte nuclei.

Weibel et al. (1969) during a study correlated morphometric and biochemical studies on the liver cells. The top panel shows the original frequency distribution of observed profiles (cross-hatched) and the profile distribution after correction for missed small profiles and for section thickness. The bottom panel reports result of unfolding this profile size distribution (reconstitution according to the Wicksell procedure).

Thus while this reconstruction technique was not completed in the present study it is demonstrated by these authors that such calculations are possible, this should be remembered when interpreting the results in the present study.

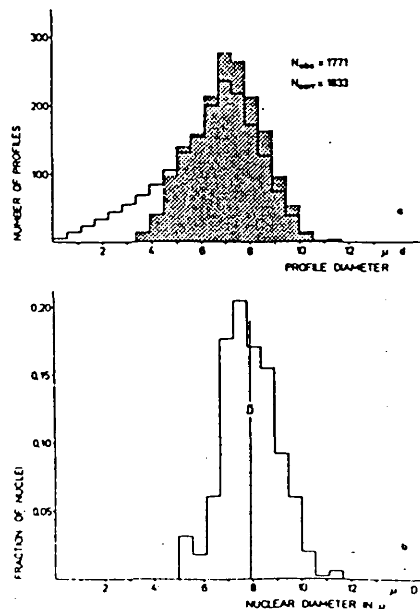

FIG. 5.17: Estimation of the size distribution of hepatocyte nuclei from a section profile distribution by Wicksell transformation according to Baudhuin (1968). (Reproduced by permission from Weibel *et al.*, 1969.)

Fig. 8

3.4 Consideration of thickness of the section

The finite section thickness (t) increases the number of profiles in the largest profile size class derived from any particular particle. From Figure 9 it can be seen that the largest profile size class will be increased in proportion to t/(D+t) as shown in Figure 10.

If several particle size classes are present then this effect will result in an excessive profile number in all size classes that correspond to the particle size distribution. Whereas most methods for creating information on particles from profile size distribution require the particles to be cut by true section planes, most real profile size distribution will be altered by section thickness effect in the larger classes.

It is possible to correct the actual over-observed profile size distribution (completed for missing small profiles) for the over-estimation of large profiles due to section thickness effect.

Using methods of Bach (1959; 1967) or Wicksell (1925) it is possible to correct for this factor and Weibel described an approximate procedure, details of which are given in pages 176-180.

As no correction was made in this study, this must be remembered in the interpretation of results.

Summary

Thus, in summary, when considering various aspects of the present study, in view of the morphometric analyses undertaken it should be noted where stereological analysis was not undertaken.

Geometric probability and random sectioning were not considered.

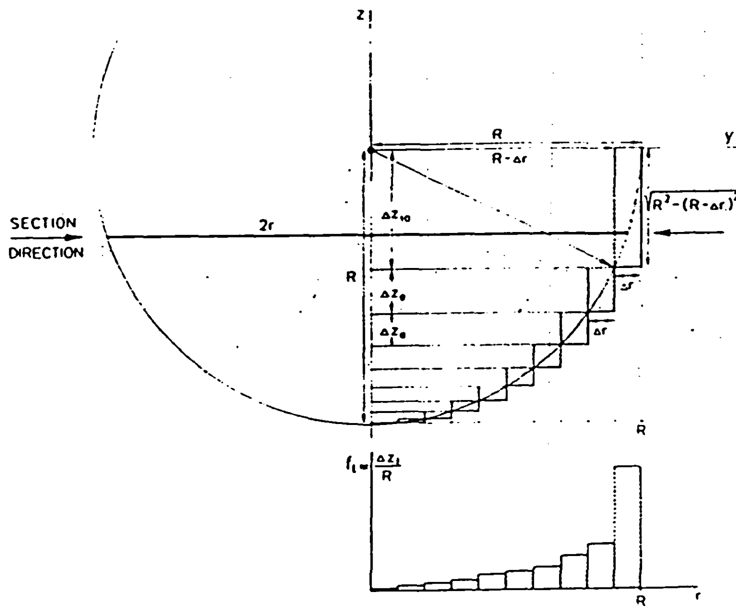

FIG. 2.40: Graphical construction of frequency distribution of profile size classes.

Fig. 9

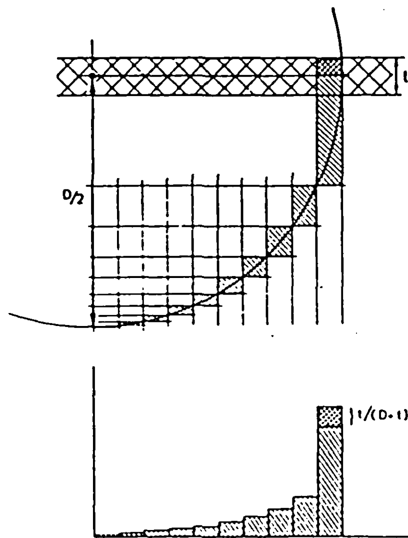

FIG. 5.10: Demonstration that the frequency of profiles in largest class alone is increased in proportion to t/D (compare with Fig. 2.40).

Fig. 10

When aspects relating to nuclear size were considered while reconstruction corrections were not made, when selecting nuclei for inclusion in the Quantimet 720 image analysis only nuclei which showed distinct nucleoli were included and where the nuclear outline was ill-defined, the nucleus was excluded. These are known to correspond: the former to a nucleus sectioned nearer its "equator" and the latter closer to the nuclear pole, the ill-definition being the result of the concentration of heterogenous chromatin at the periphery of the nucleus. This basic principle of choosing a nucleus was described by Ingram and Grasso (1985; 1987). However these correction factors available should be borne in mind when considering the results from the present study.

Correction of thickness of section was not made in this study. This has to be borne in mind in relation to the results but the reproducibility of section thickness per se was the ultimate aim of the rigorous attention to detail in the preparation of sections as already described (A.III1).

Various stereological methods have been shown to be successfully employed while characterising the basic properties of liver cells, the effects which occur in hepatocytes following phenobarbitone treatment (Weibel et al., 1969; Staubli et al., 1969), and more recently during morphometric studies of the liver cell nuclei in liver cell hepatomas using computer-aided studies which measure nuclear size and shape (Jagoe et al., 1982; 1984).

While these stereological analyses were not undertaken in this present study, it must be remembered that the basic principle employed where necessary in this study, namely that an area measured in a section

is proportional to its volume, has been well documented by stereological methods.

Thus the method used in this study when employing this concept rather than more complicated stereological methods to determine actual nuclear size from the representative nuclear profiles, has enabled the changes produced by portal vein ligation, partial hepatectomy and PB treatment in hypophysectomised animals to be demonstrated.

Acknowledgement

The discussion of stereological principles in this Appendix is based on the text by E. Weibel Practical Methods for Biological Morphometry, Volume 1, Academic Press (1979).

All figures included were taken from this text.

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