HYPERGLOBULINAEMIA IN THE PORTACAVAL SHUNTED RAT :

AN EXPERIMENTAL STUDY

Thesis for the Degree of

DOCTOR OF PHILOSOPHY

Submitted in the Faculty of Science,

University of Cape Town

by

Mogamat Mustapha Keraan

September 1975

This thesis is dedicated to :

Aziza, Thabiet, Mishal and Tauhir.

C O N T E N T S

ACKNOWLEDGEMENTS

It has been a privilege to work under the guidance of Professor O.L. Meyers, whose interest in the immunological aspects of portacaval shunting initiated this study. He has taught me a great deal and many of his ideas are encorporated in this thesis. Without his experience and expert knowledge, this study would not have been possible. It is indeed a great pleasure for me to thank him.

The interest and advice of Professor A.C. Brown of the Zoology Department are greatly appreciated and I am indebted to him for his encouragement to further my post-graduate education.

I thank Professor S.J. Saunders for granting me the opportunity to undertake this project in the Department of Medicine and for placing the facilities of the Liver Laboratory at my disposal.

I am greatly indebted to Dr Rosemary Hickman for introducing me to the field of microvascular surgery and for her sustained interest in this project.

The advice and assistance of Dr R.E. Kirsch in the preparation and purification of the rat immunoglobulins and antisera are greatly appreciated.

I would also like to acknowledge the following people : Professor J.H. Louw and Professor J. Terblanche for allowing me to use the facilities in the Department of Surgery; the valuable assistance rendered by Mr Gert Engelbrecht in particular, and Mr Stan Abbot, Mr Patrick Wheeler and Mrs C. Rose-Innes. I would

like to thank Professors A.A. Kipps and W. Du T. Naude of the Department of Bacteriology, and also to acknowledge the willing assistance of Dr P. Botha and Mrs Beyers. Dr A. Polson very kindly qualitated the rat IgM and IgA preparations on the analytical ultra-centrifuge.

Professor B.J. Cremin of the Department of Radiology, and Dr John Latham for doing the splenoportograms.

Dr H. Bazin of the University of Louvain, Belgium, who very generously donated the serum for the preparation of rat IgM and IgA.

Professor Sun Lee of La Jolla, California, for teaching me his P.C.S. technique.

Mr Mick Wells of the Department of Medicine for his expert technical advice.

I wish to thank Miss Francine Chaussée who typed the innumerable drafts, Miss Jeannie Waskansky who very expertly typed the final draft, and Mrs Phillips who typed the Tables. I am indebted to Mrs Lee Behm who drew all the graphs and to Miss Rosemary Page for the handdrawn illustrations. The assistance of Mrs Angela Solomon and Mrs Meg Meyers is gratefully acknowledged. To the Librarian and staff of the Medical Library go my sincere thanks. I am also indebted to Mr Francis Cleophas for his assistance during the course of this project.

The assistance of Mr B. Pfuhl and Mr J. Wilkenson of the Old Mutual in reproducing and binding this thesis is gratefully acknowledged.

Financial assistance was received from the Medical Research Council of South Africa, the Joseph Stone Foundation, the Harry Crossley Foundation and the U.C.T. Staff Research Fund.

SUMMARY

The construction of a portacaval shunt in the rat is followed by a series of characteristic phenomena : weight loss, hypo-albuminaemia, hyperglobulinaemia and an increase in the circulating antibacterial antibody levels to endogenous bowel micro-organisms. The weight loss is characterised by several weeks of decreasing weight followed by a slow progressive reversal to normality and it has its origins in postoperative anorexia. The hypo-albuminaemia to some extent parallels the weight loss but never reaches the same magnitude and it too is likely to be closely associated with anorexia. The hyperglobulinaemia which is polyclonal embraces a rise in all three major immunoglobulin classes of the rat. Evidence is presented that links this polyclonal hyperglobulinaemia to enhanced antibacterial antibodies which increase following the portacaval shunt. The shunt is believed to effectively separate the reticulo-endothelial system of the liver from the portal circulation thus allowing unlimited and constant access of antigenic material from the bowel to immunocompetent cells. Evidence is also advanced that such material from micro-organisms may also stimulate other immune reactions.

Similar studies in the pig are also presented and the close parallel with chronic human liver disease led to the development of an hypothesis that portal shunting of blood is the cause of hyper-globulinaemia in man. The rat is suggested as a suitable model for the experimental reproduction of human hyperglobulinaemia.

KEY TO LEGENDS

Points or graphs represent the means of observations at any given time. Variation about each point describes $\pm$ one standard error of the mean and is given, together with the number of observations (n) in relevant tables.

ABBREVIATIONS

Millilitre(s)	ml
Microlitre(s)	µl
Litre(s)	L
Gram(s)	gm
Milligram(s)	mg
Microgram(s)	µg
Volt(s)	V
Milliamp(s)	mA
Micron(s)	µ
Gravitational acceleration	g
Number of observations	n
Standard error of the mean	S.E.
Standard deviation	S.D.
Sheep red blood cells	S.R.B.C.
Phosphate buffered saline	P.B.S.
Lipopolysaccharide	L.P.S.
Immunoglobulin	Ig
Inferior vena cava	I.V.C.
Portacaval shunt(ed)	P.C.V.
Sham operated	S.O.
Sham operated treated with neomycin	S.N.
Normal control	N.C.
Human gamma-globulin	H.G.G.
Packed cell volume	P.C.V.
Aspartate aminotransferase	A.A.T.

Chapter 1

INTRODUCTION

INTRODUCTION

Hyperglobulinaemia is a characteristic feature of chronic liver disease in humans (Eisenmenger and Slater, 1953; Havens et al., 1954; Leevy, 1957; Glasgov et al., 1959; Sherlock, 1970; Lo Grippo et al., 1971; Bjørneboe et al., 1972; Finlayson, 1972; Triger et al., 1972; Triger and Wright, 1973; Prytz et al., 1974) and many hypotheses have been advanced to explain its aetiology.

The term hyperglobulinaemia, although commonly used in clinical medicine, is somewhat misleading in that evidence points to an increase in all classes of immunoglobulins to a varying extent (Finlayson, 1972). In this thesis, the term hyperglobulinaemia has been retained but it will be used throughout as pertaining to elevated levels of immunoglobulins only.

The theory that the hyperglobulinaemia of active liver disease could be explained on the basis of a reciprocal rise in gamma-globulin level consequent upon a fall in albumin concentration (Wostmann and Gordon, 1960), has been questioned as a result of evidence in experimental hypo-albuminaemia (Hoffenberg, et al., 1966; Kirsch, 1968). This has resulted in renewed interest in possible immunological mechanisms in the genesis of hyperglobulinaemia (Sherlock, 1970). It has been postulated that the immune system in the human cirrhotic is in a state of hyperreactivity due to a putative adjuvant effect of hepatocyte breakdown products (Triger and Wright, 1973). That liver cell damage can result in the production of auto-antibodies, as suggested by McKay (McKay et al., 1956), is supported by evidence of increased auto-antibodies in patients with chronic liver disease (Asheron, 1959;

Bouchier et al., 1964; Paronetto et al., 1964; Doniach et al., 1966; Finlayson, 1972; Triger et al., 1974).

Kennan in 1964, investigating the effect of diverting insulin from the liver in portacaval shunted (P.C.S.) rats, first reported an increase in gamma-globulin level in such animals and suggested that the biological effect of insulin on the liver might have been a significant factor in the production of hyperglobulinaemia (Kennan, 1964).

Therapeutic portacaval anastomosis has become an accepted mode of treatment of cirrhotic patients with portal hypertension and haemorrhage from oesophageal varices (Conn and Lindemuth, 1968; Callow et al., 1965). Recent reports have documented the effectiveness of P.C.S. on the treatment of hyperlipoproteinaemia (Starzl et al., 1973) and in juvenile glycogen storage disease (Starzl et al., 1973A)

The development of portasystemic shunts at both intra- and extra-hepatic levels in the cirrhotic liver (Bjørneboe 1972) in conjunction with the failure of the Kupffer cells to sequester antigen (Triger et al., 1972; Triger and Wright, 1973) could result in a reduction of the filtering capacity of the liver (Thomas, 1973). This would allow bowel micro-organisms or their breakdown products to enter the systemic circulation and stimulate immunocompetent tissue elsewhere in the body.

Havens and co-workers reported that patients with chronic liver disease produce better antibodies to purified diphtheria toxoid than controls (Havens et al., 1951). Bjørneboe (Bjørneboe et al., 1972) and Protell (Protell et al., 1971) reported the presence of increased titres of antibacterial antibodies in human cirrhotics. Triger (Triger et al., 1972; Triger et al.,

detected raised antibody titres to measles and rubella viruses in patients with chronic liver disease.

The concept of portacaval anastomosis was initiated in 1877 when Nicolai von Eck performed a side-to-side P.C.S. in mongrel dogs and then formed a fistula between the two vessels. The portal vein was finally ligated and divided to transform the shunt to an end-to-side portacaval anastomosis. In 1903 the first successful portasystemic shunt in man was documented (Vidal, 1903). In animals, Dragstedt (1931) described a technique of gradual obliteration of the portal vein as a substitute for Eck fistula and claimed virtually no mortality in experiments on dogs, cats, goats, rats and rabbits. A modified and more refined version of this technique was subsequently reported (Whittaker, 1946). A 2-stage Eck fistula was performed in rabbits by causing adhesion between the portal vein and the inferior vena cava (I.V.C.) and subsequently opening a 'window' through the adherent area (Klages and Kurten, 1938). In 1952 Burnett and his co-workers (Burnett et al., 1952), using a non-suture technique (Blakemore et al., 1942) performed a P.C.S. in the rat using vitallium tubing. In the early 1960s Lee and Fisher reported their findings with side-to-side and end-to-side P.C.S. in rats using a continuous suture technique (Lee and Fisher, 1961).

Following the interesting observation by Kennan and the reports of increased antibacterial antibody titres in humans, an investigation into the effect of portal shunting in the rat seemed pertinent and opportune, and it offered the prospect of developing a rat model to study experimental hyperglobulinaemia. Initial attempts at constructing P.C.S. in rats using the two-stage technique of gradual obliteration of the portal vein (Dragstedt, 1931; Whittaker, 1946) were abandoned in the face of a high mortality rate and doubts about the patency of the shunt in survivors.

Survival, in any event, appeared to depend on the formation of collaterals and it would have been difficult to gauge the effect of P.C.S. on the rat considering that portal venous blood diversion from the liver was incomplete. Direct end-to-side anastomosis of portal vein to inferior vena cava (Lee and Fisher, 1961) was chosen as the most suitable for experimental purposes and the technique was mastered and perfected following Lee's demonstration of this technique at a Microvascular Surgery Workshop held at the University of Cape Town in 1972.

The sudden diversion of portal blood, exclusion of the reticulo-endothelial system of the liver, altering the haemodynamics of the portasystemic circulation and the complete arterialization of the hepatic blood supply are bound to result in physiological, metabolic and immunological sequelae.

Weight loss is one of the most consistent consequences of end-to-side P.C.S. in the normal rat. The severity of body wastage varied in the hands of different investigators but the majority agree on a post-operative weight loss of between 20-40% (Lee and Fisher, 1961; Henzel et al., 1963; Rubin et al., 1965; Baldessarini and Fischer, 1967; Kyu and Cavanagh, 1970; Chen, 1972; Keraan et al., 1974). Assal reported a weight loss of 5-8% (Assal et al., 1971) and Reichle, using the non-suture P.C.S. technique (Burnett et al., 1952) reported no significant post-operative weight loss (Reichle et al., 1972 A).

Body wastage consequent upon the construction of a portacaval anastomosis in the dog is also well documented (Castro et al., 1963; Kirsch et al., 1965; Turney et al., 1966; Nance et al., 1971). Weight loss ranged from 20% (Smith and Mouzas, 1970; Nance et al., 1971) to 30-40% (Bollman, 1961; Doberneck et al., 1963) of initial body weight. Portacaval-shunted monkeys also experienced a weight loss of 15-40% (Kline and Nance, 1969; Kline et al., 1971) and in the normal pig, P.C.S. has been shown

to be associated with failure to thrive (Cuschieri et al., 1974; Hickman et al., 1974).

Restricted food intake, possibly due to a suppressed appetite, has been advanced as the major contributing factor to post-operative weight loss in P.C.S. animals. Data on food uptake in P.C.S. rats are conflicting and this is not surprising in the light of differing surgical techniques and post-operative care. A number of investigators are in agreement that food uptake is restricted in P.C.S. rats (Assal et al., 1971; Rubin et al., 1965; Kyu and Cavanagh, 1970) whereas others claim that food intake is 'adequate' (Doyle, 1969) or even greater than in normal animals (Kennan, 1964). Evidence in P.C.S. dogs reflects the view that post-operative food intake is insufficient to sustain the nutritional demands of shunted animals (Bollman, 1961; Kirsch, et al., 1965).

The evidence of hepatic artery compensation for diverted portal blood is conflicting. The consensus of opinion appears to favour the view that, at any given time, blood flow through the liver is reduced in rats (Flynn and Kennan, 1968; Herz et al., 1972; Ossenberg et al., 1972) and in dogs (Hallet et al., 1952; Bernstein et al., 1968; Smith and Mouzas, 1970) with varying degrees of hepatic arterial compensation. In the human cirrhotic, treated by P.C.S., hepatic arterial compensation can amount to $\pm$ 66% of total hepatic blood flow (Fauvert, 1967). Chauvand and his co-workers measured blood flow through the rat liver 24 hours after P.C.S. They detected no decrease in hepatic blood flow and inferred that hepatic arterial blood flow was able to compensate totally for portal blood diversion (Chauvand et al., 1973). Experimental evidence in rats (Silen et al., 1957; Fisher et al., 1967) supports the view that the volume, and not the quality of portal blood is necessary for the prevention of liver atrophy (Burnett et al., 1952; Fisher, B. et al., 1962).

Total diversion of portal venous blood was shown to be fatal to dogs (Covelli et al., 1967; Condon, 1971; Doberneck, 1971). A steady physical deterioration and a decrease of hepatic function, leading to hepatic coma and death (Condon, 1971) have been observed in P.C.S. dogs. It has also been suggested that the post-operative increase in serum ammonia concentration may lead to neurological symptoms of encephalopathy (Macbeth et al., 1965) which, in turn, may ultimately manifest in hepatic coma and death (Orloff et al., 1963; James et al., 1972). The significantly elevated levels of peripheral ammonia which were detected in P.C.S. dogs (Bollman, 1961; McDermott et al., 1962; Turney et al., 1966; Covelli et al., 1967; James et al., 1972) were usually associated with symptoms of encephalopathy in dogs (Kline et al., 1965; Kline and Nance, 1969; Nance and Kline, 1971), in pigs (Hickman et al., 1974; Cuschieri et al., 1974) and in man (Orloff et al., 1963; Callow et al., 1965; Grace et al., 1966; Jackson et al., 1971; Mutchnick et al., 1974). Elevated serum ammonia levels were also detected in P.C.S. rats (Lee and Fisher, 1961; Cavanagh and Kyu, 1969; Doyle, 1969; Kyu and Cavanagh, 1970; Herz et al., 1972) but these animals developed subtle mild encephalopathy (Herz et al., 1972) which seems to have escaped widespread attention (Lee et al., 1974). Although elevated ammonia levels were recorded in P.C.S. monkeys, no neurological symptoms of encephalopathy were detected (Kline et al., 1965; Kline and Nance, 1969) which seems to suggest that hyperammonaemia may not be the only biochemical abnormality in the genesis of portal-systemic encephalopathy.

The colon is generally regarded as the principal site for ammonia production which has been considered to originate from bacterial deamination of amino acids (Silen et al., 1955; McDermott et al., 1962; Orloff et al., 1963; Macbeth et al., 1965;

Kline and Nance, 1969; Nance and Kline, 1971; Grange et al., 1974). While experimental evidence on the effects of neomycin on ammonia concentration (Dawson et al., 1957; Macbeth et al., 1965; Kyu and Cavanagh, 1970) supports the role of bowel micro-organisms in the pathogenesis of hyperammonaemia, it has nevertheless been shown that elevated ammonia levels do occur in germ-free dogs (Kline and Nance, 1969; Nance and Kline, 1971) following P.C.S. It has been suggested that in the absence of resident bowel flora, non-bacterial metabolic pathways for substantial ammonia production must be operative (Nance et al., 1971).

Ammonia absorbed from the gastro-intestinal tract is detoxified in the liver by enzymatic transformation to urea (McDermott and Adams, 1954; Orloff et al., 1963). One of the major consequences of P.C.S. is to divert ammonia-rich splanchnic blood into the systemic circulation and this may account for increased serum ammonia levels. On the other hand, it has been shown that hepatic ammonia clearance in P.C.S. dogs was significantly reduced by 78% (Smith and Mouzas, 1970). Hepatocellular damage consequent upon P.C.S. may also interfere with normal conversion of ammonia to urea in the liver (McDermott and Adams, 1954), and it has also been demonstrated that as the function of the liver undergoes progressive deterioration, ammonia clearance capacity is reduced (Turney et al., 1966). This seems to suggest hyperammonaemia in P.C.S. animals may be more closely related to parenchymal deterioration than the hepatic bypass of ammonia-rich splanchnic blood.

Various experimental studies have been conducted to correlate serum ammonia levels with the development of encephalopathy and hepatic coma. The effects of feeding high protein diets and ammonia substrates have been studied in P.C.S. dogs and good correlation has been reported between elevated ammonia levels

and the onset of encephalopathy (Orloff et al., 1963; Bernstein et al., 1968). Feeding a high protein diet to P.C.S. rats did not significantly change the serum ammonia level (Kyu and Cavanagh, 1970) which seems to suggest that the rat liver can handle additional enteral ammonia loads through recirculation. It has been suggested that this phenomenon may explain the mild symptoms of encephalopathy in P.C.S. rats and it may also account for the relative tolerance to sudden diversion of splanchnic blood in rats (Lee et al., 1974).

Since McDermott and Adams presented the first well-documented case of episodic stupor (McDermott and Adams, 1954) in humans with P.C.S., portasystemic encephalopathy has been the subject of intense investigation and a substantial literature exists on the subject. The incidence of encephalopathy, which is generally considered the most common and the most serious complication of P.C.S., varies from 8.5 - 26% (Orloff et al., 1963; Callow et al., 1965; Grace et al., 1966; Conn and Lindemuth, 1968; Bernstein et al., 1968; Jackson et al., 1971; Mutchnick et al., 1974). The effects of collaterals (Aldrete et al., 1967) and side-to-side P.C.S. (Smith and Mouzas, 1970) on encephalopathy have also been investigated. In an experiment with small and large P.C.S. in dogs, Orloff reported that, following the intragastric instillation of ammonium sulphate and whole blood, significantly higher ammonia levels were detected in dogs with large shunts (2 - 4.2 cm) than in animals with small shunts (0.35 - 1.8 cm). They also reported that the lowest incidence of encephalopathy (5%) was found amongst humans with spleno-renal shunts where the volume of diverted portal blood is much lower than in conventional P.C.S. (Orloff et al., 1963). Orloff and his co-workers are of the opinion that the accepted practice of constructing the largest possible anastomosis to assure maximal portal decompression in humans may account for the increased incidence of portal-systemic encephalopathy (Orloff et al., 1963). It has been suggested that cerebellar damage and the development of neurological abnormalities

consequent upon P.C.S. in the normal rat (Cavanagh and Kyu, 1969; Cavanagh et al., 1972) may be associated with sustained elevated levels of circulating ammonia (Cavanagh and Kyu, 1971).

The following complications following P.C.S. in human patients have been documented :

Demyelination of the spinal chord (Zieve et al., 1960; Bechar et al., 1970),

Diabetes (Hearn and Paton, 1963; Conn and Lindemuth, 1968),

Ulcers (Clarke et al., 1958; Grace et al., 1966; Orloff et al., 1969),

Unilateral oedema (Conn, 1972),

Jaundice (Conn, 1972; Da Silva et al., 1963),

Massive hepatic necrosis (Koeneman and Ceballos, 1966), and

Gangrene of the ileum (Saadi, 1967).

The sudden diversion of portal blood from the liver is apparently not compatible with good hepatic function and there is no question that the normal rat liver undergoes rapid and significant atrophy (Weinbren, 1955; Henzel et al., 1963; Masland, 1964; Fisher et al., 1967; Kyu and Cavanagh, 1970; Reichle et al., 1970;Assal et al., 1971; Fischer et al., 1971; Herz et al., 1972). This loss in liver weight consequent upon P.C.S. exceeds the overall percentage of loss of body weight and suggests that the rat liver undergoes specific atrophy. Further evidence in support of this statement was provided by Reichle who reported consistent liver atrophy despite the absence of significant weight loss (Reichle et al., 1970). Experiments conducted by Fisher and his co-workers on the effect of P.C.S. on liver D.N.A. and R.N.A. in normal and

cirrhotic rats and their findings of a 25% loss in D.N.A. and 43% loss in R.N.A. in P.C.S. rats, suggest that cell loss in addition to cell atrophy, contribute to the changes in liver weight and volume (Fisher et al., 1967). Hepatic atrophy was also observed in dogs (Castro et al., 1963; Smith and Mouzas, 1970) concomitant with impairment of hepatic function (Kirsch et al., 1965) and in man (Starzl et al., 1973).

Data on the acute liver-associated metabolic abnormalities seen in P.C.S. animals (Day et al., 1963; Ossenberg et al., 1972; Bekoe et al., 1972) implicate the altered liver status with its modified blood supply or the impaired access of the liver to substances that are predominantly present in splanchnic venous blood. Significantly elevated histamine levels were detected in P.C.S. rats (Day et al., 1963; Fischer and Snyder, 1965; Kowalewski et al., 1971). Raised levels of histamine in rats were explained on the basis of failure of the liver to detoxify (Day et al., 1963) and the altered histamine metabolism following P.C.S. surgery (Fischer and Snyder, 1965). Increased histamine levels and gastric hypersecretion were implicated in severe ulceration in normal rats following P.C.S. (Day et al., 1963). Elevated histamine levels were also implicated in increased blood flow to the stomach (Delaney et al., 1964) and gastric hypersecretion (Villar-Valdes et al., 1969; Wilkens et al., 1969) in P.C.S. dogs. Marked hepatic haemosiderosis was reported in P.C.S. rats (Rubin et al., 1964; Kaplan and Peskin, 1966) and P.C.S. dogs (Doberneck et al., 1963). Canine haemopoiesis was generally depressed due to restricted availability of iron needed for haemoglobin synthesis (Doberneck et al., 1965) and this may possibly explain the significant reduction in packed cell volume in P.C.S. rats (Chen, 1972). Portal blood diversion was implicated as the cause for reduced levels of drug metabolising enzymes (Rubin et al., 1967) and thus of

cytochrome P-450 (Ossenberg et al., 1972), glutamine synthetase activity (Kennan, 1964A), hepatic S-adenosylmethionine (Baldessarini and Fischer, 1967), acetylcholinesterase (Chen, 1972), ornithine decaboxylase (Fischer et al., 1971 and serine (Reichle et al., 1970) in P.C.S. rats. Reichle and his co-workers noted the systemic accumulation of oestrogenic compounds and its effect on experimental mammary carcinoma in the P.C.S. rat (Reichle et al., 1969) and investigated the effect of P.C.S. on established experimentally induced carcinoma (Reichle et al., 1972A.). The effect of P.C.S. on intrasplenic ovarian implantation was also investigated in rats (Lee et al., 1972; Reichle et al., 1972 A; Seeger et al., 1972). Altered pancreatic secretory function (Ashikari and Dreyling, 1968) and elevated carbamyl phosphate synthetase activity (Brown and Brown, 1965) have been studied in P.C.S. dogs.

The liver is the body's primary site of synthesis for serum proteins except gamma-globulin (Owen, 1967; Song and Kappas, 1970). Rat serum albumin is most sensitive to nutritional influences (Metcoff et al., 1948; Jeffay and Winzler, 1958; Weimer, 1961), post-operative surgical trauma (Morgan and Brackenridge, 1962) and injury (Gjessing and Chanutin, 1947; Neuhaus et al., 1961) and it is not surprising that reduced serum albumin levels have been detected in P.C.S. rats (Kennan, 1964; Meyers and Keraan, 1973; Keraan et al., 1974). Likewise in dogs, albumin synthesis was shown to be sensitive to protein depletion (Weech et al., 1934; Zeldis, 1945) and decreased serum albumin levels were also detected in P.C.S. dogs (Bollman, 1961; Doberneck et al., 1963; Kirsch et al., 1965). The effect of experimental P.C.S. on serum protein fractions, other than albumin and gamma-globulin is poorly documented with the exception of a report on P.C.S. rats (Kennan, 1964).

Experimental evidence supports the view that under conditions of hypo-albuminaemia there is no reciprocal rise in

gamma-globulin concentration (Weech et al., 1934; Cannon et al., 1944; Zeldis et al., 1945; Metcoff et al., 1948; Jeffay and Winzler, 1958; Freeman and Gordon, 1962; Hoffenberg et al., 1966; Kirsch et al., 1968). The hyperglobulinaemia of human liver disease has been shown to be associated with increased gamma-globulin turnover (Finlayson, 1972) and Ramsoe has shown that increased synthesis as well as breakdown of gamma-globulins occur in cryptogenic cirrhosis (Ramsoe et al., 1970). The hyperglobulinaemia in P.C.S. rats (Kennan, 1964; Meyers and Keraan, 1973), dogs (Doberneck et al., 1963; Mazzoni, 1974) and human cirrhotics (Prytz et al., 1974), has been considered to be associated with increased antibody production and not to decreased gamma-globulin catabolism (Havens et al., 1954). Gamma-globulin levels to a large extent reflect antibody levels (Sherlock, 1970) and therefore the hyperglobulinaemia associated with P.C.S. could presumably be part of an immune response. If this is indeed the case, then the immunogen(s) involved in antibody response could be identified and it should be possible to determine whether the diversion of portal blood is in any way instrumental in stimulating the immune mechanism.

One of the major effects of a P.C.S. is to exclude the R.E.S. from contact with the portal circulation and thus allow bowel micro-organisms, or their antigenic breakdown products, access to the immune mechanism. The detection of elevated titres of antibacterial antibodies in conjunction with significantly elevated levels of immunoglobulins in human cirrhotics (Prytz et al., 1974) and rats (Meyers and Keraan, 1973; Keraan, et al., 1974) consequent upon P.C.S. supports the hypothesis that antigenic stimulation is provided by the resident bowel flora and that antibacterial antibodies may contribute significantly to the hyperglobulinaemia. The reticulo-endothelial system (R.E.S.) in the germ-free rat is underdeveloped and the gamma-globulin level is lower than in normal animals (Gustafson and Laurel, 1958; Wostmann and Gordon, 1960;

Fahey and Robinson, 1963). It has been established that the gamma-globulin level in the normal mouse is 35 times greater than its germ-free counterpart (Sell and Fahey, 1964), and 6 times greater in normal guinea pigs than in germ-free guinea pigs (Sell, 1964). It has been postulated that the gamma-globulin level in animals is dependent on antigenic stimulation by intestinal micro-organisms (Gustafson and Laurel, 1958; Sell and Fahey, 1964). Evidence in support of this hypothesis was provided by the elegant study conducted by Wostmann and Gordon who exposed germ-free rats to conventional bacterial flora by introducing a suspension prepared from the caecal contents of normal rats into the stomach and colon of germ-free animals. They reported a subsequent rise in gamma-globulin as well as alpha-2 and beta-globulin concentrations on serum protein electrophoresis and concluded that these 3 fractions could possibly harbour antibacterial antibodies (Wostmann and Gordon, 1960).

The liver is known to filter bacteria (Beeson et al., 1945) and it has been suggested that the hyperglobulinaemia in chronic liver disease is due to the reduction in the capacity of the liver to trap antigen (Triger et al., 1972; Triger and Wright, 1973; Thomas et al., 1973. The use of colloidal carbon to block the R.E.S. of the liver is well documented (Biozzi et al., 1953; Nicol et al., 1965; Souhami, 1972) and an extensive literature exists on the subject. It has also been demonstrated that R.E.S. blockage with lead acetate increases the sensitivity of rats to various L.P.S. preparations by 100,000 times above normal (Selye et al., 1966; Trejo et al., 1972). This phenomenon was explained on the basis that lead acetate induced endotoxin hypersensitivity by impairing phagocytic as well as endotoxin detoxifying properties of Kupffer cells (Trejo et al., 1972). Experimental Kupffer cell blockade with high molecular weight dextran sulphate resulted in enhanced antibody response to an immunising dose of sheep red blood cells (S.R.B.C.) in rats (Souhami et al., 1975) and R.E.S. blockage with thorium dioxide resulted in overwhelming pneumococcal septicaemia in man (Bensinger et al. 1971).

An extensive literature exists on experimental portal shunting in animals and prophylactic portacaval anastomosis in humans. In rats, in particular, the metabolic changes of portacaval shunting have been subjected to intensive investigation. Relatively little documented data, however, exist on the changes in serum proteins after P.C.S., and the immunological effects of portal blood diversion have hardly been studied. Of great theoretical and perhaps practical interest is the effect of R.E.S. exclusion from the splanchnic blood.

Hyperglobulinaemia in the P.C.S. rat, and in the cirrhotic man, is an interesting phenomenon which, despite the many explanations offered for its occurrence in man, needs closer study;in particular the complex relationships which exist between a putative large antigenic load from the bowel, the R.E.S of the liver and the link supplied by the splanchnic venous system.

A portal shunt in the rat may be viewed as a caricature of the many portal-systemic shunts which occur at both intra- and extrahepatic levels in humans with chronic liver disease. Implicit in both the experimental animals and in man is the diversion of portal venous blood, the exclusion of the R.E.S. of the liver and the possibility that antigenic material, previously destined to be trapped in the liver, would gain access to the systemic circulation and the immunocompetent cells. Uncontrolled or prolonged immunological stimulation may be expected to lead to hyperglobulinaemia. The parallels between the experimental situation and the human disease offer an exciting possibility of developing the P.C.S. rat as a model for hyperglobulinaemia in humans.

It is the aim of this thesis to :-

(i) Characterize the changes in serum protein fractions consequent upon the construction of an end-to-side P.C.S. in the normal rat.

(ii) Investigate the aetiology of the reported post-shunt hyperglobulinaemia in rats.

(iii) Identify the immunoglobulin classes involved.

(iv) Examine the feasibility of using the P.C.S. rat as an animal model for studying hyperglobulinaemia of chronic liver disease in humans.

THE FOLLOWING HYPOTHESIS WAS POSTULATED :

HYPERGLOBULINAEMIA IN THE P.C.S. RAT IS A DIRECT CONSEQUENCE OF THE DIVERSION OF SPLANCHNIC BLOOD FROM THE LIVER. THE EFFECT OF SUCH PORTAL DIVERSION COULD BE EXPECTED TO MANIFEST AS HYPERGLOBULINAEMIA IN A NUMBER OF WAYS :

(i) THE CHANGED HEPATIC CELL METABOLISM AND POSSIBLY HEPATIC CELL DEATH MAY ACCOUNT FOR THE OBSERVED HYPERGLOBULINAEMIA.

(ii) EXCLUSION OF A MAJOR PORTION OF THE RETICULO-ENDOTHELIAL SYSTEM OF THE LIVER MIGHT BE EXPECTED TO LEAD TO THE DIVERSION OF ANTIGENIC MATERIAL FROM THE BOWEL TO THE IMMUNE SYSTEM. SUCH PUTATIVE ANTIGENIC MATERIAL COULD EITHER BE DERIVED FROM INGESTED FOOD OR RESIDENT MICROFLORA OF THE BOWEL.

(iii) THE ACCESS OF BACTERIA OR THEIR PRODUCTS INTO THE CIRCULATION WOULD BE EXPECTED, UNDER SOME CIRCUM-STANCES, TO ACT AS ADJUVANTS.

Chapter 2

MATERIALS and METHODS

MATERIALS AND METHODS

The study of P.C.S. in rats extended over a period of 3 years during which time various problems were investigated. Some experiments were planned to answer one or a few questions whereas others were large and comprehensive studies in which many facets of portal shunting were studied. The following is a schematic representation of all the experimental protocols setting out in summary the object of the experiment and the parameters which were measured.

Experiment 1 :

The aim of this experiment was to document the acute phase changes after the construction of a portacaval shunt in the normal rat. The animals were sacrificed at 6, 24, 72 and 96 hours following shunt or sham surgery and the following parameters were investigated :

i) changes in body and liver weight

ii) changes in serum protein fractions

iii) antibacterial L.P.S. antibodies

iv) aspartate aminotransferase (A.A.T.) activity

v) packed cell volume

vi) liver histology

Experiment 2 : (zero time to 12 weeks - intermediate phase study)

The aim of the experiment was to document changes in P.C.S., S.O. and N.C. rats in the following parameters :

i) changes in body weight

ii) total serum protein levels

iii) serum protein fractions (on both agar slides and cellulose acetate electrophoresis)

iv) serum IgG, IgM and IgA levels

Experiment 3 : (zero time to 36 weeks - long-term study)

The aim of this experiment was to verify the changes observed in Experiment 2 and to determine whether certain trends were maintained over a 36-week period. All the animals were bled at the start of the experiment and then at 3, 6, 12, 18, 24, 27 and 36 weeks post-operatively. Changes, as well as the effect of neomycin, were documented on the following parameters :

i) body, liver and spleen weights

ii) serum protein fractions

iii) antibacterial L.P.S. antibodies

iv) A.A.T. levels

v) liver histology

vi) splenoportograms

Experiment 4 : (zero time to 6 weeks - splenectomy study)

The aim of this experiment was to determine the effect of splenectomy on serum protein fractions and in gamma-globulin levels in particular, in P.C.S. and S.O. rats. The following parameters were measured :

i) body weight

ii) serum protein fractions

Experiment 5 :

The aim of this experiment was to document changes in body weight in small rats (180 - 250 g) after P.C.S.

Experiment 6 : (Experiments with single antibody-forming cells in the spleen)

The aim of this experiment was to determine whether the spleen was the site of antibacterial L.P.S. antibody production and to correlate plaque-forming cells to L.P.S. with antibacterial L.P.S. antibody titres. Antibacterial L.P.S. antibody titres and the number of individual L.P.S. plaque-forming cells were determined.

Experiments 7A and 7B :

The aim of these experiments was to study the immune response to H.G.G. in P.C.S., S.O. and N.C. rats. In addition,

antibacterial L.P.S. antibodies were also measured in these sera.

Experiments 8 and 9 :

The aim of these experiments was to study the effect of restricted food intake on serum albumin and gamma-globulin levels 2 and 7 weeks after pair-feeding, in P.C.S., S.O. and N.C. rats.

Experiment 10 :

The aim of this experiment was to document changes in serum protein fractions consequent upon the construction of a P.C.S. in the pig.

Experiment 11 :

The aim of this experiment was to detect antibodies to purified L.P.S. preparations in rat sera.

Experiment 12 :

The aim of this experiment was to compare antibacterial L.P.S. antibody levels in normal rats repeatedly stimulated with a commercial L.P.S. preparation with that of P.C.S. rats.

Experiment 13 : (Gel filtration of P.C.S. rat serum)

The aim of this experiment was to determine whether L.P.S. antibody activity resided in IgG or IgM fraction of rat serum.

Experiment 14 :

The aim of this experiment was to determine the effect of 2-Mercaptoethanol (2-Me) on antibacterial L.P.S. antibody activity.

Experiment 15 :

The aim of this experiment was to demonstrate immune inhibition with bacterial L.P.S.

Experiment 16 :

The aim of this experiment was to determine the effect of a given dose of neomycin on resident bowel flora in the rat.

Experiment 17 :

The aim of this experiment was to demonstrate immune inhibition with H.G.G. in rat serum samples with known titres of anti-H.G.G. antibodies.

Experiment 18 :

The aim of this experiment was to detect antibodies to rat 7S globulin in animals immunized with H.G.G. and to determine whether there is cross-reactivity between rat IgG and H.G.G.

Experiment 19 :

The aim of this experiment was to detect antibodies to denatured rat 7S globulins in animals immunized with H.G.G.

Experiment 20 :

The aim of this experiment was to detect fluorescent auto-antibodies in P.C.S. rat serum samples.

Male albino rats of Wistar-derived strain, which have been bred locally for a period of fifteen years, and weighing between 250- 350 grams were used in all the experiments. Depending on the numerical requirements of a particular experiment, a pool of rats was divided randomly into experimental, sham and normal control groups. Five to six rats were housed in cages of the following dimensions : 45 cm long, 30 cm wide and 24 cm deep. This avoided overcrowding but allowed sufficient body contact for elective warmth. The animal room was fully air-conditioned and was also equipped with a small extractor fan. The temperature was maintained at $\pm$ 22°C and the relative humidity at about 50%. The animal room was lighted for 12 hours each day by artificial light. Vermiculite was used as bedding and was replaced three times per week.

The animals were allowed to become acclimatised for 2 - 3 days before experimental procedures were commenced. Rat cubes (Vereeniging Consolidated Mills) and water were allowed ad libitum. Cages were washed at regular intervals and were soaked overnight in a strong antiseptic solution before being used to house new batches of animals; water bottles were washed frequently.

Surgical Procedures

The rat because of its small size poses technical operative problems to the uninitiated operator. Such difficulties can readily be overcome by using appropriate instruments and by regular practice to maintain average manual dexterity. The major advantage of using the rat as an experimental animal is its almost legendary resistance which enables it to survive considerable surgical operative trauma with a minimum of post-operative care.

Construction of an End-to-Side Portacaval Anastomosis

Rats were denied access to food on the day of the operation. A clean but not sterile surgical technique was used

as described by Lee and Fisher (1961) and which Lee demonstrated at a Microvascular Surgery Workshop held in the Department of Surgery at the University of Cape Town in 1972.

Standard dissecting instruments suitable for microvascular surgery (Fisher and Lee, 1965) were used and a binocular magnifier with 2.25 magnification and a focal length of approximately 10 cm proved adequate as an optical aid. Lighting was provided by a set of Attralux Cool spot-lamps (Phillips, 240 V, 150W) positioned about 45 cm from the operating area.

Under light anaesthesia with ether or halothane, the abdomen was shaved and swabbed with absolute alcohol. After making a midline incision, the wound edges were separated by retractors and the intestines were displaced to the animal's left in order to expose the portal vein and the inferior vena cava (Fig. 1). Drapes were fashioned from gauze onto which the exposed viscera were placed to avoid contact with the animal's fur. The exposed intestines were covered with gauze moistened with Ringer's lactate solution because the use of isotonic saline often resulted in oedema of the portal vein and mesentery (Henzel, Turcotte and Child, 1963).

The pyloric vein was carefully dissected free and divided between ligatures (6-0 silk, Ethicon) close to its junction with the portal vein. This pyloric venous tributary was sacrificed to facilitate mobility of the portal vein. The portal vein was then dissected free from the surrounding connective tissue and was mobilised as close as possible to the hepatic hilum. The hepatic artery was gently separated from the portal vein by cautiously dissecting the connective tissue with a moist cotton-wool bud. Initial dissection of the inferior vena cava (I.V.C.) was accomplished by splitting the overlying parietal peritoneum using two gauze swabs to exert divergent lateral traction. The vena cava was carefully dissected

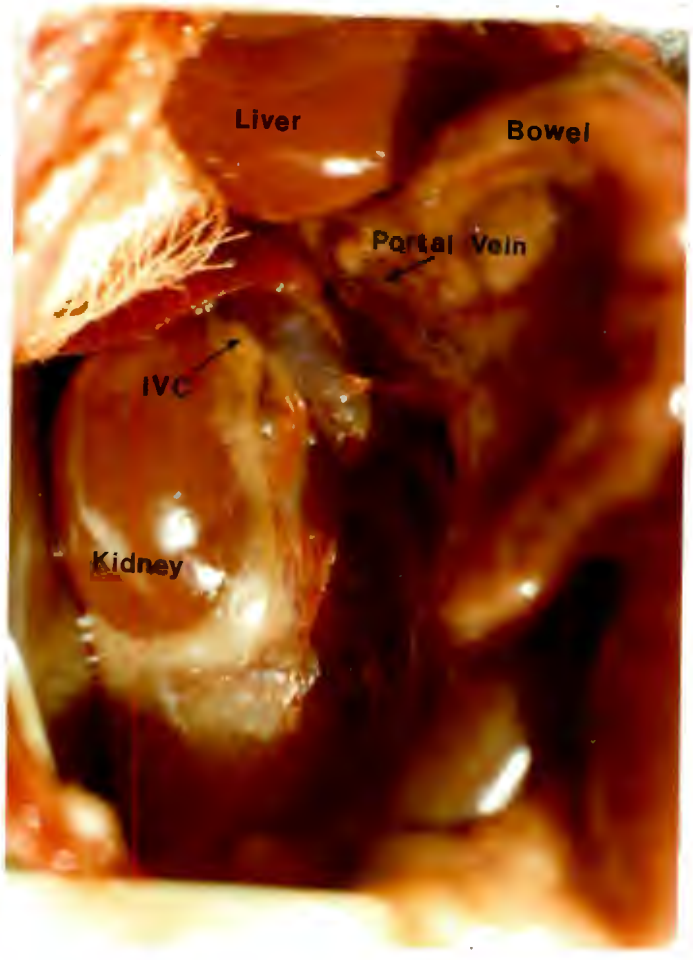

Fig. 1. The relative anatomy of the rat following laparotomy and the displacement of the intestines to the animal's left.

free in the area of its confluence with the right renal vein and was clamped parallel to its flow with a small curved haemostat (3.7 cm in length) which was fashioned from a paper clip and shod with silk. The size of this haemostat facilitated the ease with which the anastomosis could be executed because, unlike most of the bigger clamps in general use, it produced minimal interference in a rather restricted area. To facilitate the application of the vena-caval occlusion clamp, the anterior wall of the I.V.C. was grasped and 'tented' with a non-toothed iris forceps held in the left hand while the haemostat was applied with the right hand. An elliptical opening approximately 3 mm in length was made in the isolated part of the vena cava to accommodate the end of the portal vein. The aperture in the I.V.C. was flushed with Ringer's lactate solution. The portal vein was ligated with 6-0 silk at the liver hilum, clamped with a shod miniature bulldog clamp (2.8 cm in length) as far from the hilum as possible and divided proximal to the ligature. The lumen of the portal vein was also flushed with Ringer's solution (Fig. 2).

After the insertion of two corner stay sutures, the two vessels were anastomosed posteriorly with 7-0 braided silk (Ethicon). Five to six continuous stitches were inserted, care being taken to place the first and the last stitches as close to the stay sutures as possible (Fig. 3). Anterior anastomosis was similarly achieved. Knots on both ends of the suture line were tied on the outside of the vessels. Oozing, following the release of the clamps, was readily controlled by applying slight pressure with a pledget of moist gauze until haemostasis was effected. During the entire procedure the I.V.C. and portal vein were clamped for 8-12 minutes. This period was well within the accepted 15 minutes (Fisher and Lee, 1965) beyond which the animal's chances of survival are reduced. The anastomosis was inspected visually for evidence of venous obstruction. Whenever doubt existed about shunt

Fig. 2. The curved haemostat (on the left) positioned on the I.V.C. and the miniature bulldog clamp on the portal vein.

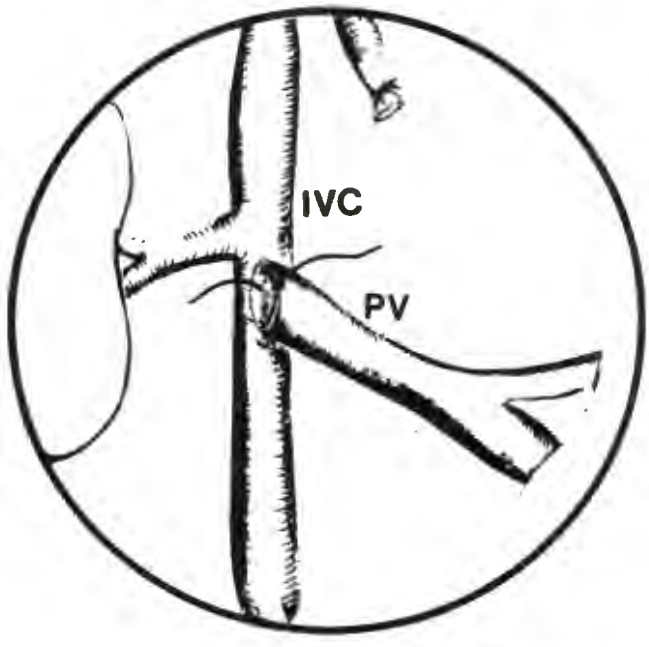

Fig. 3. A diagrammatic representation of the I.V.C. and the portal vein being anastomosed posteriorly.

patency, the animal concerned was not used in the experiments (Figs 4 and 5).

At this stage of the operation the administration of halothane or ether was stopped and the animal was either given oxygen (with 5% CO_2 as a respiratory analeptic) or allowed to breathe room air. Five ml of warmed Ringer's lactate solution were administered intravenously via the dorsal vein of the penis to compensate for blood loss and dehydration. The intestines, and other organs exposed during the course of the operation, were well moistened with Ringer's lactate solution prior to their being replaced in the abdominal cavity. This was done in an attempt to eliminate or minimise the possibility of adhesions forming.

The skin and body wall was stitched with medium chromic 0-0 catgut (Davis and Geck) and the abdominal suture line was sprayed with transparent antiseptic aerosol dressing ('Polyflex - Pentagon Pharmaceutical Corporation). The abdomen was closed as one layer, stitching successively through the skin, muscle and peritoneal layers with a continuous suture.

The majority of portacaval shunted (P.C.S.) rats recovered within 30 minutes provided the state of narcosis was not too deep. Immediately post-operatively the animals were housed in individual cages near an artificial source of heat until they had recovered fully from the effects of the anaesthetic. They were then transferred to the animal room and housed as previously described.

Sham Surgery

The sham-operated (S.O.) rat was similarly anaesthetised. Following laparotomy the intestines were displaced to the animal's

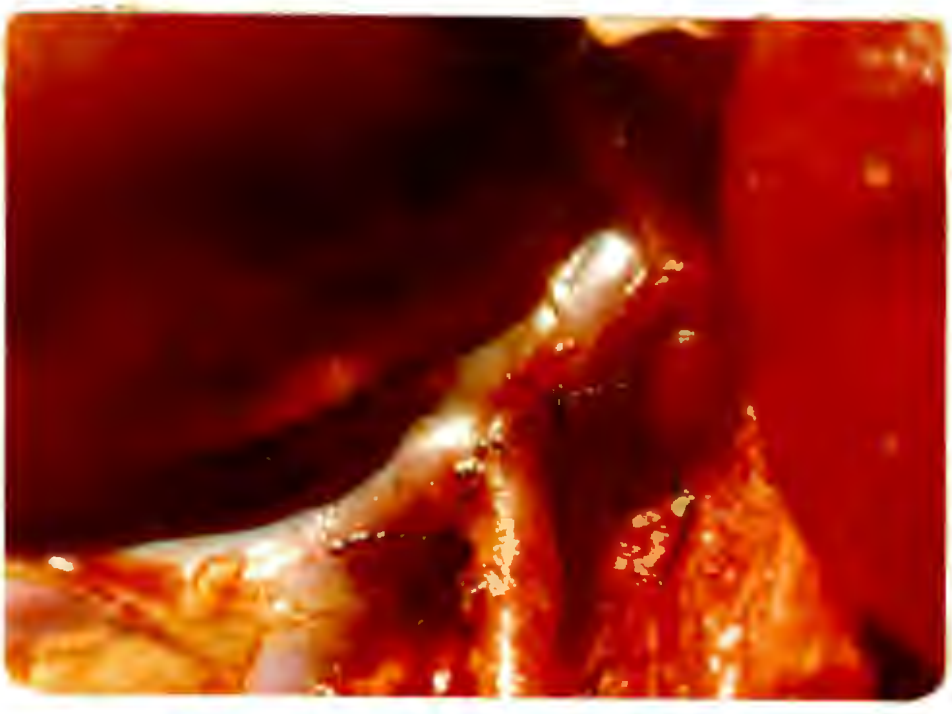

Fig. 4. The completed portacaval anastomosis.

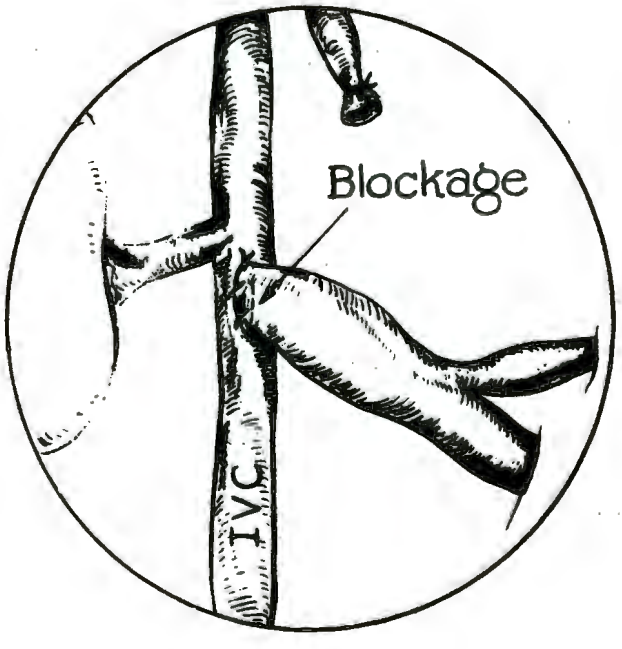

Fig. 5. A diagrammatic representation of an obstructed shunt.

left and the pyloric vein was doubly ligated and divided as in the experimental animal. Both the I.V.C. and the portal vein were dissected free from the surrounding connective tissue and clamped for exactly the same duration of time as in P.C.S. surgery, viz. 10-12 minutes. With the exception of the construction of an actual anastomosis, sham-operated rats were thus subjected to the same surgical procedures as the experimental animal. All the sham-operated controls recovered within 15 minutes.

P.C.S., S.O. and unoperated normal controls (N.C.) were housed under identical environmental conditions at all times.

Splenectomy in P.C.S. and S.O. Rats

Adult male rats were subjected to end-to-side P.C.S. or sham operation as described. Animals with apparently patent shunts, and control animals were splenectomised immediately after P.C.S. or sham surgery. The spleen was carefully excised after all the tributaries of the splenic vein and artery were doubly ligated and divided proximal to the spleen. All the animals were bled prior to P.C.S. or sham surgery and then post-operatively at weekly intervals.

Anaesthesia

A. Halothane (I.C.I.)

The advantages of using halothane as an anaesthetic agent are twofold:

i) minimal attention to the anaesthesia of the animal is required once an optimum state of narcosis is induced and the operator can concentrate on the operation without the possibility of the rat recovering consciousness at a critical stage of the anastamosis.

ii) Anaesthetic collapse, characteristic of excessive ether administration, is seldom encountered.

A mixture of 95% O_2/5% CO_2 at a flow rate of 4 litres/minute was bubbled over halothane in a glass dispenser. The halothane-enriched oxygen/carbon dioxide mixture was led into a bell-jar in which the experimental animal was placed. The rat was removed from the bell-jar as soon as anaesthesia was induced, and immobilised on the dissecting board. The animal's head was suitably positioned, with the aid of a rubber flange, in a metal canister into which the halothane/oxygen/carbon dioxide mixture was fed (Fig. 6). The canister was also equipped with an exhaust lead to conduct away excess halothane. Anaesthesia was controlled by adjusting the gas flow rate or by the manipulation of the halothane enrichment of the gas mixture. At the completion of the operation, the administration of halothane was stopped and the animal was allowed to breathe the oxygen/carbon dioxide mixture. The stimulatory effect of the carbon dioxide was used to facilitate the animal's recovery from anaesthesia. The hepatotoxic effect of halothane (Perez et al., 1972) mitigated against its continued use and it was abandoned in favour of ether.

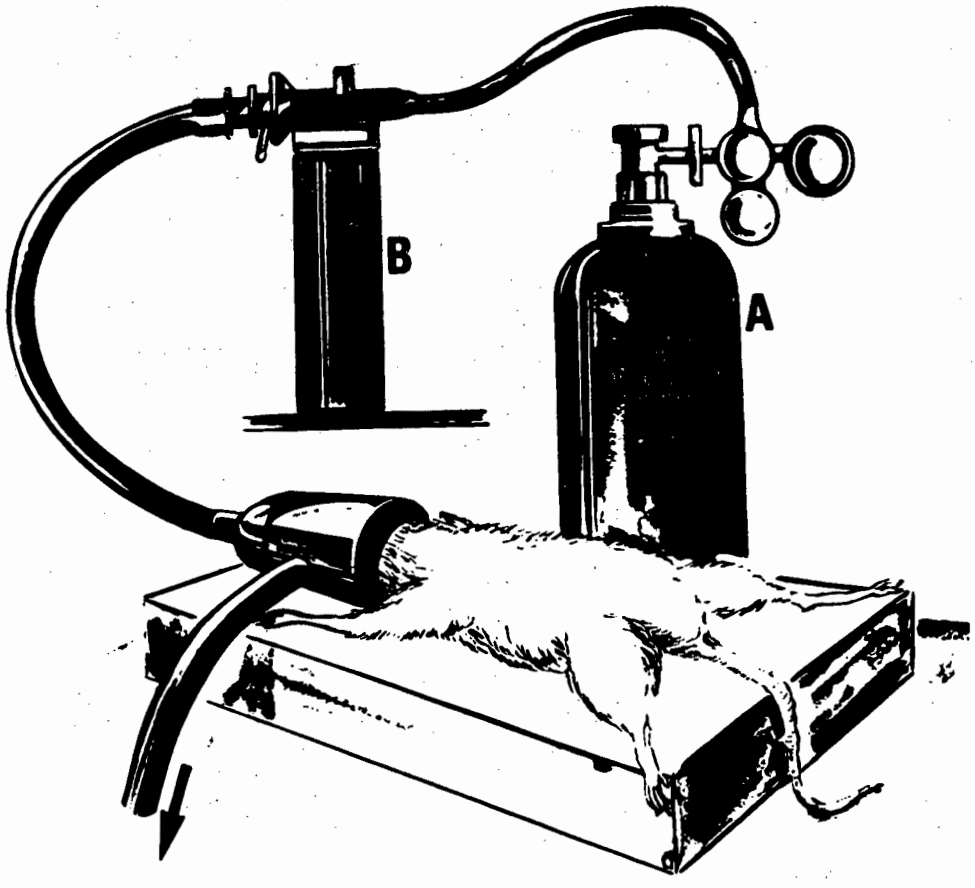

Fig. 6. A diagrammatic representation of the apparatus used in the administration of halothane to the rat; showing the cylinder containing O_2/CO_2 (A) and the halothane dispenser (B).

B. Ether

Ether is in many ways the narcotic agent of choice since no sophisticated dispensing apparatus is required for its use and also :

i) it is not necessary to calculate the dose according to the animal's body weight

ii) the length and depth of anaesthesia is easily controlled

iii) recovery following anaesthesia is quick

The rat was placed in a bell-jar containing a wad of cotton-wool soaked in ether (containing 0.001% propyl gallate as preservative - The Natal Cane Byproducts Ltd).

The anaesthetised animal was immobilised with its snout in a large plastic centrifuge tube (3 cm diameter and 12 cm length) containing a ball of cotton-wool soaked with ether and the depth of anaesthesia was controlled by approaching and withdrawing the ether container. The ether source was usually completely removed once both the I.V.C. and portal vein were clamped and it was only necessary on very rare occasions to re-establish mild anaesthesia before the completion of the operation.

Blood Collection and Storage of Sera

All rats were bled prior to surgery and as demanded by the experimental protocol, at weekly or three-weekly intervals post-operatively. During the course of the experiments blood was obtained:

a) from the tail vein, and terminally

b) by cardiac puncture

a) The rat was positioned in a restraining cage and its protruding tail was immersed in warm water (40-50°C) for 1-2 minutes. Approximately 1 mm of the tip of the tail was severed with a scalpel blade and approximately 1 ml of blood was collected into a small glass test tube by gentle 'milking' of the tail.

b) Cardiac puncture was resorted to when large volumes of blood were required. Approximately 10 ml of blood could be drawn from a fully grown animal using a standard cardiac puncture technique (Herbert, 1974).

Weighing of Animals

Rats in all experiments were weighed pre-operatively and then at weekly intervals on a triple beam balance (Ohaus). The animals were weighed at a set time, viz. before replenishing their daily food supply.

Administration of Neomycin

Neomycin was administered to P.C.S.. S.O. and N.C. rats in an attempt to sterilise the bowel of the animals concerned.

a) from the tail vein, and terminally

b) by cardiac puncture

a) The rat was positioned in a restraining cage and its protruding tail was immersed in warm water (40-50°C) for 1-2 minutes. Approximately 1 mm of the tip of the tail was severed with a scalpel blade and approximately 1 ml of blood was collected into a small glass test tube by gentle 'milking' of the tail.

b) Cardiac puncture was resorted to when large volumes of blood were required. Approximately 10 ml of blood could be drawn from a fully grown animal using a standard cardiac puncture technique (Herbert, 1974).

Weighing of Animals

Rats in all experiments were weighed pre-operatively and then at weekly intervals on a triple beam balance (Ohaus). The animals were weighed at a set time, viz. before replenishing their daily food supply.

Administration of Neomycin

Neomycin was administered to P.C.S.. S.O. and N.C. rats in an attempt to sterilise the bowel of the animals concerned.

Neomycin sulphate ('Neopan' - Propan Pharmaceuticals) was administered daily by stomach tube starting one week prior to surgery and continuing for the duration of the experiment. The dose used was 7.5 mg/100 gm body weight which is equivalent to the universally accepted human dose of 4 g/70 kg body weight. Initially the neomycin was administered at a fixed dose, irrespective of body weight but the fact that the P.C.S. rat suffered severe body wastage whereas the S.O. and N.C. rats gained weight post-operatively, necessitated the introduction of a dosage in proportion to body weight.

The Effect of Pair-Feeding on Serum Proteins in P.C.S. Rats

Two separate groups of P.C.S., S.O. and N.C. rats were pair-fed according to the method of Kirsch (1968). In the first experiment (Experiment 8), the pair-fed rats were bled 2 weeks post-operatively and the second experiment 7 weeks post-operatively (Experiment 9). The rat sera were analysed for total serum protein concentration according to the method of Fernandez et al. (1966), with modification to be described later in the text, and by serum protein electrophoresis on cellulose acetate.

Total Serum Protein Determination

The Biuret method for the determination of total protein concentration (Fernandez et al., 1966) was used with one modification. The small volumes of rat sera available necessitated the reduction of the usual volumes of the Biuret method to a total sample volume of 1 ml as indicated in the following flow-sheet :

Blank (in duplicate)

0.83 ml 3% sodium hydroxide

0.17 ml Biuret reagent (APPENDIX I)

Standard (in quadruplicate)

0.017 ml protein standard (3% human albumin -'Pentex', Miles Laboratories)

0.813 ml 3% sodium hydroxide

0.17 ml Biuret reagent

Samples (in duplicate)

0.017 ml rat serum

0.813 ml 3% sodium hydroxide

0.17 ml Biuret reagent

Turbidity

0.017 ml rat serum

0.983 ml 3% sodium hydroxide

All serum samples were centrifuged at 1600 g for 10 minutes before serum protein determinations were done.

After the addition of the Biuret reagent the contents of the tubes were thoroughly mixed. The tubes were allowed to stand at room temperature for 15 minutes and the absorbance was read in a spectrophotometer (Beckman DB) at 540 u wavelength in a one-ml quartz microcuvette in the following order :

i) turbidities

ii) blank

iii) standard

iv) samples

v) standard

The total protein concentration of the test rat serum was calculated as follows :

$$\text{test serum} = \frac{3 \times \text{samples reading} - (\text{Bl.} + \text{Ts})}{\text{Std. reading} - (\text{Bl.} + \text{Tstd})}$$

$$= \text{gm. \%}$$

where Bl. = Blank

Ts. = Turbidity of sample

Tstd. = Turbidity of standard

Electrophoresis of Rat Serum

1. Agar slide technique

The method of Wieme (1959) was followed for the electrophoresis of rat serum on agar slides with some modifications.

One per cent Special Noble agar (Difco) in Veronal buffer pH 8.4 (APPENDIX II) was dissolved by boiling and 40 ml of warm agar was poured into a large petri dish and allowed to solidify on a horizontal surface. Four appropriately marked microscope slides were placed on the surface of the agar and 35 ml of warm agar was added. The slides were stored overnight at $4^{\circ}C$ and removed from the petri dish prior to the electrophoretic run. Agar was also poured into the central compartment of the electrophoresis cell (Fig. 7) and allowed to solidify into blocks.

A piece of Whatman's No. 3 filter paper was plunged perpendicularly into the agar layer on the microscope slide and approximately 3.5 cm from the cathode end. The filter paper was removed after 10 minutes during which time it had absorbed sufficient buffer from the agar to form a 'well'. A lambda pipette, equipped with a fine needle, was used to apply 1.5 microlitres of rat serum to the well. Two serum samples were electrophoresed concurrently per slide. The central compartment of the electrophoresis cell was filled with petroleum ether (B.pt 40-60$^{\circ}C$) and the buffer compartments were filled with the Veronal buffer.

A current of air was directed over the petroleum ether in the electrophoresis compartment and by manipulating the rate of its evaporation, a constant temperature of $10^{\circ}C$ was maintained. The powerpack (Beckman Duostat) was set to deliver a constant voltage

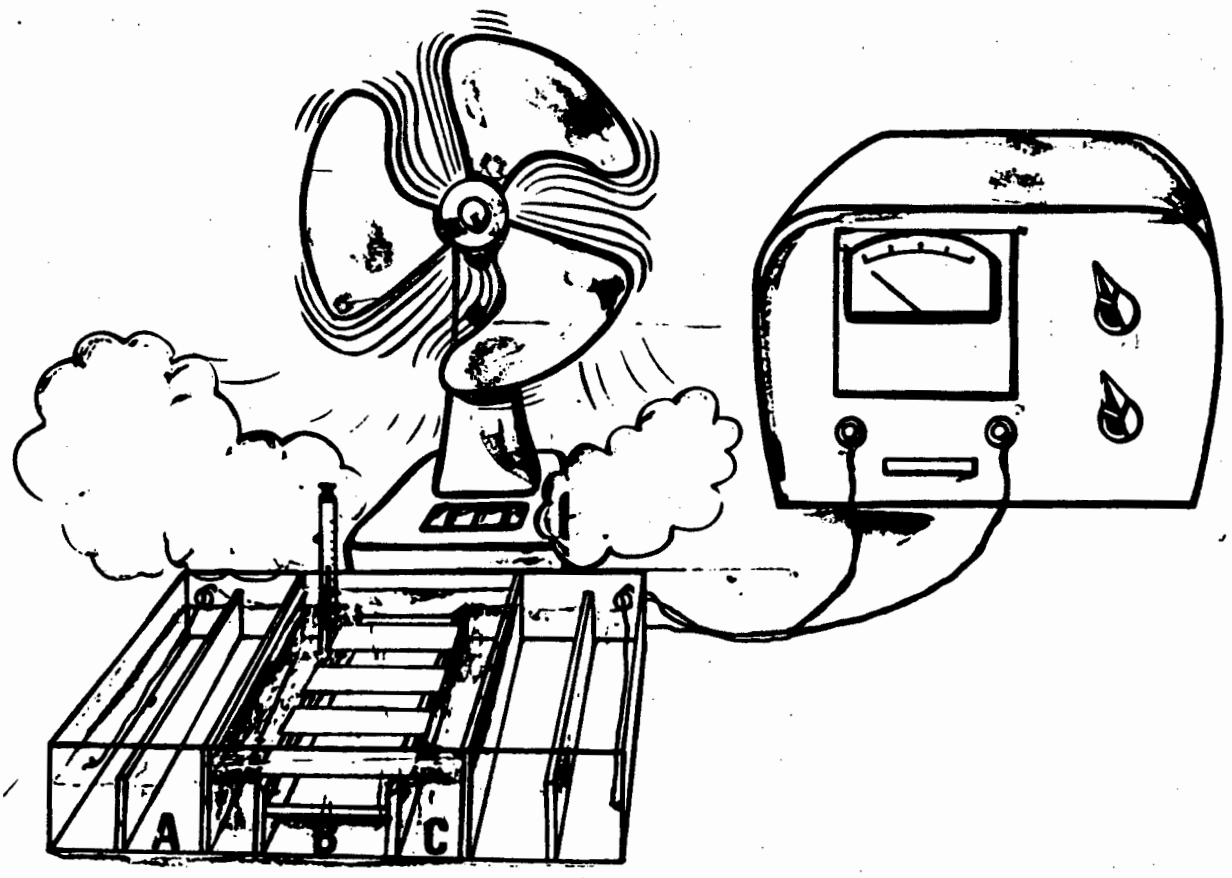

Fig. 7. A diagrammatic representation of the apparatus used in the electrophoresis of rat serum on agar slides; showing the Buffer compartment (A), the central compartment containing petroleum ether (B) and the blocks of agar (C).

of 250V with 50 mA for 45 minutes. At the completion of the electrophoretic run the slides were removed from the electrophoresis cell and fixed for 30 minutes in an ethanol-glacial acetic acid solution (APPENDIX II).

The slides were covered with strips of moist filter paper (Whatman's No. 1), dried overnight at room temperature and then stained for an hour in Amido black (APPENDIX II). They were washed in several changes of 5% acetic acid until the background became clear, allowed to dry and then scanned in a reflectance densitometer (Joyce Loebl). The areas under the curves were measured by autoplanimetry and the integrated count for each serum protein fraction was expressed as a percentage of the total count.

This electrophoretic technique produced reasonable separation and resolution of the different protein fractions and reduced 'trailing' of rat serum fractions to a minimum. The major disadvantage of this method was that the number of sera which could be processed per day was limited, and this electrophoresis method was abandoned in favour of the microzone system of electrophoresis in cellulose acetate (Beckman).

2. Cellulose acetate microzone system (Beckman)

The cellulose acetate membrane (Beckman) was floated onto the surface of the buffer (APPENDIX III) in a suitable container, and totally immersed after allowing the membrane to soak up buffer. The membrane was blotted gently and then loaded onto the bridge of the microzone cell.

The membrane was allowed to equilibrate for a minimum of 2 minutes before 0.25 μl volumes of rat sera were applied with

a special applicator. The cell was connected to the powerpack (Beckman Duostat) which was set to deliver a constant voltage of 250V and an initial current of 4-5 mA for 30 minutes. At the completion of the electrophoretic run the membrane was removed from the bridge of the electrophoresis cell and immersed in the fixative dye (APPENDIX III) for 9 minutes and then differentiated in 5% acetic acid for 2-3 minutes or until the unstained background became clear. The stained membrane was immersed in 95% alcohol for one minute and then in the clearing solution (APPENDIX III) for exactly 60 seconds. Subsequent to clearing, the membrane was placed on a glass plate and the excess liquid removed. The membrane was then placed in a preheated oven at 100°C for 10-15 minutes. The processed membrane was stored in a semi-rigid transparent envelope and scanned in the densitometer (Beckman R-110). The area under the peak of every serum protein fraction was measured and expressed as a percentage of the total protein.

Detection of IgG Levels in Rat Serum

The purity of a commercial preparation of rat 7 S globulin (Pentex, Miles Laboratories, Lot 14) was tested on immuno-electrophoresis (Ouchterlony and Nilsson, 1974) using Shandon immuno-electrophoresis equipment (Shandon Scientific Co.). The production of a single precipitin line, corresponding to the IgG component, was used as the criterion of purity.

A. The raising of rabbit anti-rat IgG antiserum

Five mg of rat IgG were dissolved in 1 ml of P.B.S. and an equal volume of Freund's complete adjuvant (Difco Laboratories)

was added. The mixture was emulsified using a standard technique (Herbert, 1974) and 1 ml of the emulsion containing 2.5 mg of rat IgG was injected into rabbits in multiple sites :

i) subcutaneously in the nape of the neck

ii) intramuscularly into both thighs

Antiserum to rat immunoglobulin components was raised in a commercial hybrid strain of white rabbit - an F_1 cross between a New Zealand white origin and a commercial strain. The rabbits were bled from the ear vein (APPENDIX IV) before and 3 weeks after immunisation and the antiserum was tested for anti-rat IgG activity. The animals were boosted with 2.5 mg of alum precipitated rat 7 S globulin, subcutaneously into multiple sites and bled again 7 days later. Immuno-electrophoresis was again utilised to demonstrate the presence and monospecificity of anti-rat 7 S globulin antibodies in consecutive bleeds of antiserum. The latter were subsequently pooled and used for the quantitation of rat IgG in P.C.S., S.O. and N.C. animals.

B. Single radial immunodiffusion technique for the quantitation of rat IgG

Melted 3% Noble agar (Difco Laboratories) in 0.05M Veronal buffer pH 8.6 and cooled to 45°C was added to an equal volume of rabbit anti-rat IgG antiserum diluted 1 : 10 with P.B.S. prewarmed to 45°C (Fahey & McKelvey, 1965). The agar-antiserum mixture was poured as a 1-2-mm thick layer onto a glass plate measuring 10 cm X 9 cm and allowed to set in a humidity chamber for 4 hours. Wells 2 mm in diameter were punched in the agar and filled with 5 ul aliquots of test sera. A series of IgG dilutions

of known concentration corresponding to 40, 20, 10 and 5 mg/ml were included on every plate. The plates were incubated in a moist atmosphere and the diameters of the precipitin rings were measured at 24, 48 and 60 hours. The diameters of the precipitin rings were plotted against the concentration of rat 7S globulin. The concentrations of rat IgG in the test were extrapolated from the standard curve and expressed as mg/ml (or gm%). The above method was also used for the quantitation of rat IgG but the diameters of the precipitin rings were read after an incubation period of 72 hours. In the quantitation of rat IgG the rabbit anti-rat IgG antiserum was diluted 1 : 40 and the diameters of the precipitin rings were read after an incubation period of 72 hours.

The Preparation of Rat IgM

Rat IgM was prepared using serum from LOU/Wal rats bearing immunocytoma IR202 according to the method of Bazin et al. (1974) and using the Pevikon block electrophoresis technique of Vriesman and Feldman (1972) as an added modification.

Fifteen ml of LOU/Wal rat serum was diluted with 2 volumes of P.B.S. pH 7.4. Saturated ammonium sulphate was added to a final concentration of 40% and the solution was stirred for 3 hours at 4°C. The precipitate was recovered by centrifugation and washed 3 times with 45% ammonium sulphate. The precipitate was dissolved in a minimum of 0.05M Tris-HCl buffer pH 8.0 and dialysed for 18 hours at 4°C against the same buffer. The euglobulin precipitate was recovered by centrifugation and resuspended in 3 ml of 2% w/v sodium chloride buffered with 0.02M Tris-HCl, pH 8 containing 0.1% w/v sodium azide.

Molecular exclusion chromatography was performed using a Sephadex G-200 column 45 cm by 2.5 cm with a pump-driven upward flow system. The eluant was 0.02 M Tris-HCl buffer pH 8.0 and 3 ml aliquots were collected in a Gilson fraction collector. A flow rate of $\pm$ 37.5 ml was maintained and the entire apparatus was housed in a refrigerated cabinet at 4°C. Optical densities of the eluted fractions were measured at 280 μ in an ultra-violet spectrophotometer (Unicam SP 1700). The fractions constituting the upward slope of the IgM component were pooled and the volume reduced by negative pressure filtration to about 4 ml. Three ml of the IgM preparation was subjected to electrophoresis on a Pevikon block.

Two hundred grams of Pevikon (Mercer Chemical Co., New York) was washed 4 times with 500 ml volume of distilled water on a coarse filter. After a final wash in 0.06 M Veronal buffer pH 8.6 (Muller - Eberhard, 1960), the Pevikon was suspended in 300 ml of the Veronal buffer. The mixture was poured into a 38 cm X 10 cm X 0.6 cm deep glass tray which had previously been lined with Parafilm and fitted across each end with wicks. Excessive buffer was allowed to drain through the wicks onto paper towel for about 30 minutes. The IgM samples were applied into a 1mm-wide slot cut approximately 10 cm from the cathodal end. The Pevikon block was transferred to a 4°C coldroom and connected, via the wicks, to 2 chambers containing the above buffer and each equipped with a platinum electrode. After allowing 30 minutes for fluid equilibration in the Pevikon block, the latter was suitably covered to prevent excessive evaporation and a constant current of 36 mA and about 300 V was supplied for approximately 18 hours to obtain protein separation.

At the conclusion of the electrophoretic run, the Pevikon block was divided into 1 cm segments and each segment was

eluted with 8 ml P.B.S. using a sintered glass funnel. The eluants were centrifuged to remove fine Pevikon particles (Fahey & McLaughlin, 1963) and their optical densities were measured at 280 μ in a spectrophotometer. Eluants with increased optical activity were first reduced in volume to 1-2 ml by negative pressure dialysis and then subjected to immuno-electrophoresis. After the purity of the IgM was established by immuno-electrophoresis as well as ultra-centrifugation, 2 rabbits were immunised as follows :

> Each of 2 rabbits were immunised with 50 μgm rat IgM in 1.5 ml Freund's complete adjuvant into the footpads and sub-cutaneously, in multiple sites, on the animals' backs.

The rabbits were boosted at 3-weekly and then bled at weekly intervals and each batch of antiserum was immuno-electrophoresed to verify the monospecificity of anti-IgM antibody subsequent to being pooled for detection of IgM activity in rat sera.

Ultracentrifugation of Rat IgM

A sample of the above IgM preparation was subjected to ultracentrifugation at 56,100 r.p.m. at 8-minute intervals in a Spinco Model E analytical ultracentrifuge at 20^{o}C. The sedimentation co-efficient was calculated and expressed in Svedberg units using a standard procedure.

Gel Filtration of P.C.S. Rat Serum

A volume of P.C.S. rat serum with known antibody titres to E. coli (sucrose positive) bacterial L.P.S. was diluted with an equal volume (1.8 ml) of P.B.S. and 3.6 ml of saturated ammonium

sulphate was added drop-wise and with constant shaking. The solution was stirred at 4°C for 2 hours. The globulin precipitate was recovered by centrifugation, resuspended in 2 ml P.B.S. and dialysed against 0.02 M Tris-HCl-NaCl starting buffer pH 8.0 for 72 hours. The dialysate was chromatographed on a Sephadex G-200 column and 2.5 ml fractions were eluted at 4°C as described above. Optical densities of the column effluents were measured at 280 μ in a spectrophotometer (Beckman). The eluted fractions constituting the upslope of the first or IgM component were pooled, as were the fractions constituting the peak of the IgG component, and their volumes reduced, by negative pressure filtration through cellulose tubing, to $\pm$ 1.5 ml.

Whole rat serum as well as its isolated IgG and IgM components were serially diluted in microtitration plates and assayed for antibacterial L.P.S. antibody activity.

Preparation of Rat IgA

IgA was prepared using serum from LOU/Wal rats bearing immunocytoma IR 22 (Basin et al., 1972).

Serum from LOU/Wal rats bearing immunocytoma IR 22 was diluted with P.B.S. and fractionated with ammonium sulphate as described earlier. The euglobulin precipitate, after prior washing with 45% ammonium sulphate was dissolved in P.B.S. and dialysed against P.B.S. for 24 hours. The precipitate was recovered by centrifugation, dissolved in 7 ml P.B.S. and then subjected to electrophoresis on a Pevikon block. At the completion of the electrophoretic run, the block was divided into 1 cm segments and each segment was

eluted with 8 ml P.B.S. using a sintered glass funnel. The eluants were centrifuged to remove fine Pevikon particles and optical activity was determined spectrophotometrically at 280 μ. Eluants with increased optical densities were first reduced in volume to 1-2 ml and then subjected to immuno-electrophoresis. The IgA component was identified (Bazin et al., 1974) and reacted with rabbit antiserum to rat IgG coupled onto cyanogen bromide (Cn Br) - activated Sepharose 4B (Pharmacia) according to the following method (Cuatrecasas, 1970) :

Five ml of rabbit anti-rat IgG antiserum was precipitated with ammonium sulphate and the globulin precipitate was resuspended in 5 ml 0.2M citrate buffer pH 6.5 and dialysed for 48 hours against the same buffer. The dialysate was added to 10-15 ml Cn Br-activated Sepharose (appropriately pretreated according to the manufacturer's instructions) and stirred gently for 2 hours at room temperature and overnight at 4°C. Unbound material was washed away with coupling buffer and any remaining active groups were reacted with 1M ethanolamine pH 8.0 for 1 hour. Non-covalently adsorbed protein was removed by 3 washings with 0.1 M acetate buffer containing 1M sodium chloride pH 4.0 and a final wash with 0.1 M borate buffer containing 1M sodium chloride pH 8.0. One ml of rat IgA solution was reacted with anti-rat IgG coupled onto Cn Br-activated Sepharose and stirred for one hour at room temperature and for 2 days at 4°C. The solution was then centrifuged at 4,000 g for 20 minutes (M.S.E. centrifuge). The supernatant was separated and its volume reduced to 1-2 ml by negative pressure dialysis. After the purity was checked on immuno-electrophoresis and its protein concentration determined, the preparation was used to raise antiserum to rat IgA in rabbits, as described.

Preparation and Partial Purification of Bacterial Lipopolysaccharide (L.P.S.)

A. Faecal pellets were collected and homogenised in a blender with a minimum of phosphate-buffered saline (P.B.S.) pH 7.4 and an aliquot of the resulting suspension was cultured as follows :

An aliquot of the faecal suspension was seeded onto blood agar and bile salt-lactose-agar plates and incubated at 37°C for 18 hours. Lactose and non-lactose fermenting Gram-negative bacilli, in particular, E. coli, were identified according to their biochemical reactions. Staphylococci, Diphtheroids and Bacillus species were identified by standard bacteriological techniques; these Gram-positive organisms were not studied further.

From these mixed cultures, single strains of Gram-negative bacilli were subcultured. A 10-ml suspension of pure culture organisms in its logarithmic growth phase was added to 1 litre of Difco nutrient broth (D.N.B.). After shaking, the bottles were incubated at 37°C for 18 hours. At the end of the incubation period, a small aliquot was withdrawn under sterile conditions from one of the bottles and the samples were recultured to verify the purity of the culture. Bacteria were harvested by centrifugation in a centrifuge (Sorval RC-3) at 5,000 g for 45 minutes at 10°C. The bacteria were resuspended, washed 3 times with sterile isotonic saline and finally recovered by centrifugation at 27,000 g for 10 minutes in a centrifuge (Sorval RC2-B); and

dried under negative pressure in a dessicator containing calcium chloride.

B. Extraction and partial purification of L.P.S.

A suspension of 10 g (dry weight) of bacteria in 160 ml water was cooled to 2°C in a wide-neck conical flask. Two hundred ml of a 75% aqueous phenol solution was added, stirred rapidly and left at 4°C for 30 minutes. The emulsion was centrifuged at 1600 g for 10 minutes at 4°C in a centrifuge (Mistral 4L) and the aqueous supernatant was separated. A further 50 ml of distilled water was added to the phenolic phase, mixed and recentrifuged. The aqueous phase was again separated and added to the first. These combined fractions were dialysed for 3 days against running tap water at 4°C and finally for one day against several changes of distilled water, at 4°C. The dialysate was concentrated at room temperature by negative pressure filtration to a volume of 50 ml and the resultant viscous opalescent solution centrifuged for 5 minutes at 1600 g to remove traces of insoluble material. The solution was poured into 6 volumes of alcohol and 10 ml of alcohol saturated with sodium acetate was added to complete the precipitation. The precipitation was centrifuged for 5 minutes, washed 3 times with alcohol and twice with acetone and then dried in a dessicator under negative pressure.

C. Extraction and purification of lipopolysaccharide

An attempt was made to purify the lipopolysaccharide according to the method of Nimmich (1968).

The dried bacteria were thoroughly ground and then added to 100 ml of 5% aqueous phenol and stirred for 1 hour at room temperature. The emulsion was poured into 600 ml of absolute alcohol to precipitate the crude lipopolysaccharide which was recovered by centrifugation at 1,200 g for 10 minutes at 20°C. The precipitate was dissolved in 20 ml of distilled water and then centrifuged at 27,000 g for 90 minutes at 10°C in a refrigerated centrifuge (Sorval RC2-B). The clear aqueous supernatant was removed and lyophilised.

The lyophilised material was dissolved in a minimum of distilled water, precipitated with 10 ml of a 10% aqueous cetyltrimethyl ammonium bromide ('Cetavlon') solution and left overnight at room temperature. The following morning it was spun at 1,200 g for 10 minutes at room temperature, the aqueous layer discarded and the precipitate dissolved in a minimum of molar sodium chloride. The lipopolysaccharide was precipitated in a volume of absolute alcohol equal to 10 times that of the molar sodium chloride used in the preceding step and recovered again by centrifugation at 1,200 g for 10 minutes at room temperature. The addition of molar sodium chloride to dissolve the precipitate and its consequent precipitation with absolute ethanol followed by centrifugation, was repeated twice. The lipopolysaccharide was dissolved in a minimum of distilled water, dialysed at 4°C against running tap water for 3 days and for 1 day against several changes of distilled water. The dialysate was finally lyophilised.

Passive Haemagglutination Method for Detecting Antibodies to Lipopolysaccharide in Rat Serum

Antibacterial L.P.S. antibodies in rat serum were detected by passive haemagglutination of red cells. Sheep red blood cells (S.R.B.C.) were coated with lipopolysaccharide using glutaraldehyde as a coupling agent (Eskenazy and Petranov, 1971).

Ten mg of lipopolysaccharide were dissolved in 3.2 ml P.B.S. by frequent agitation in a 37°C water-bath. To the dissolved lipopolysaccharide solution was added 0.1 ml of a 50% S.R.B.C. suspension (fresh S.R.B.C. washed 3 times with P.B.S.) and 0.1 ml of a 1% glutaraldehyde solution in P.B.S. The mixture was allowed to incubate for 60 minutes at room temperature and gently agitated at 10-minute intervals. The coated cells were recovered by centrifugation, washed 3 times with 5 ml of a 1% absorbed rabbit serum solution in P.B.S. and resuspended in 2.5 ml of the same solution. A final working solution was prepared by adding 1.7 ml of a 1% absorbed rabbit serum solution to 0.3 ml of coated S.R.B.C. suspension. Uncoated cells were prepared by the addition of 0.1 ml of a 50% S.R.B.C.s suspension and 0.1 ml of a 1% glutaraldehyde solution to 3.2 ml P.B.S. and processed in the same way.

Rat sera, which were to be tested for their anti-lipopolysaccharide antibody titres, were heat-inactivated at 56°C for 20 minutes to destroy complement and then absorbed with an equal volume of packed S.R.B.C. for one hour in a 37°C water-bath and for a minimum of one hour at 4°C. The absorbed sera were separated after centrifugation at 1,600 g for 10 minutes.

The absorbed sera were diluted serially with P.B.S. in a microtitre plate with U-shaped wells (Cooke, Switzerland) using 0.025 ml microdiluters, starting with a 1 : 2 dilution of serum. An equal volume of S.R.B.C. coated with lipopolysaccharide was added. The plates were tapped gently to facilitate adequate mixing, sealed with cellotape and allowed to stand overnight at room temperature. The following controls were included :

i) the initial serum dilution and uncoated cells

ii) P.B.S. and coated cells

The plates were read on the following morning, and the serological reactions were graded as follows :

4+ a smooth mat of cells covered the bottom of the well

3+ a smaller smooth mat of cells with a crenated edge

2+ a small mat of cells with thicker crenated edges

1+ a point of cells lying in a mat with a thick edge

0 (negative) a button of red cells.

The haemagglutination titre was expressed as the logarithm (base 10) of the maximum serum dilution giving a distinct (1+) haemagglutination pattern. This was done to eliminate exaggerated, and often distorted, fluctuations in antibody titres when arithmatic means were used.

The Treatment of Rat Sera with 2-Mercapto-ethanol (2-Me)

2-mercapto-ethanol resistance of antibacterial L.P.S. antibodies was determined according to the method of Nowotny (1968) as follows :

To 0.05 ml of absorbed rat serum with known antibacterial L.P.S. antibody titre, 0.05 ml of 2% 2-Me (v/v in P.B.S.) was added, adequately mixed and allowed to stand at room temperature for 60 minutes. The 2-Me treated and untreated control rat sera were serially diluted in microtitration plates and assayed for antibacterial L.P.S. antibody activity as previously described.

Immune Inhibition with E. Coli L.P.S.

Ten mg aliquots of E. coli (sucrose +ve) L.P.S. and a commercial E. coli L.P.S. preparation (E. coli 0127 : B8 L.P.S., Difco) were each dissolved in 3.2 ml volumes of P.B.S., with stirring. A volume of 0.5 ml of packed sheep erythrocytes and 0.5 ml of 1% glutaraldehyde were added to each of the L.P.S. preparations and the suspensions were incubated at 37°C for 1 hour. The sensitised cells were recovered by centrifugation and washed 3 times with P.B.S. The volume of the sensitised S.R.B.C. suspensions were made up to 2 ml with P.B.S. Aliquots (0.1 ml) of the above suspensions were added to 0.05 ml volumes of rat sera with known antibody titres to E. coli (sucrose +ve) L.P.S., incubated at room temperature for 1 hour and agitated gently. The sera were recovered by centrifugation and were diluted serially together with untreated control sera. Antibodies to sucrose fermenting E. coli L.P.S. were sought using passive haemagglutination of sheep erythrocytes in a microtitre system as previously described.

Haemagglutination plates in all the serological experiments were coded and the haemagglutination reactions were read by a "blinded" observer in an attempt to exclude an element of bias. The same observer was used to read all haemagglutination reactions. This served to introduce an element of 'standardisation' into the reading of the results.

Sera which were collected at weekly or three-weekly intervals, and which were to be tested for L.P.S. antibody activity, were stored at -40°C and assayed at the completion of the experiment. Sufficient bacterial L.P.S. was prepared to ensure that all the sera were tested against the same L.P.S. preparation.

Immunisation of P.C.S., S.O. and N.C. Rats with Human Gamma-Globulin (H.G.G.)

Adult male rats were randomised into three groups and then subjected to end-to-side P.C.S. or sham surgery with the third group serving as normal unoperated controls. Accumulated faeces were collected from all the animals concerned and a suspension of faeces in P.B.S. was submitted to bacteriological analysis. An E. coli L.P.S. was subsequently extracted and partially purified from the faecal material. All the animals were bled pre-operatively and subsequently at three-weekly intervals for the duration of the period of observation.

1. A group of P.C.S., S.O. and N.C. rats were immunised 6 weeks post-operatively with alum precipitated human gamma-globulin (Cohn Fraction II Pentex, Miles Laboratories. Lot 40). The animals were immunised subcutaneously in the nape of the neck and intramuscularly in both thighs, with 0.5 mg alum precipitated human gamma-globulin.

2. At 18 weeks post-operatively the immunisation procedure was repeated with a different batch of P.C.S., S.O. and N.C. rats and blood-letting was continued for at least another 12 weeks subsequent to H.G.G. sensitisation.

The stored sera were assayed for antibody activity against the same H.G.G. preparations used in the above immunisation schedule and antibodies to rat Escherichia coli lipopolysaccharide were sought using passive haemagglutination of sheep cells in a microtitre system.

Cross reactivity between antibodies to human and rat IgG were sought in rat sera with known antibody titres to H.G.G. using passive haemagglutination of S.R.B.C.s coated with rat IgG (Pentex, Miles Laboratories) in a microtitration system. Rabbit anti-rat IgG antiserum was used as a positive control.

The Detection of Antibodies to Human Gamma-Globulin (H.G.G.) by Passive Haemagglutination

Fresh S.R.B.C.s ($\pm$ 3 days old) were coated with H.G.G. (Cohn fraction11, Pentex, Miles Laboratories, Lot 40) at a concentration of 10 mg/3.2 ml P.B.S. using glutaraldehyde as coupling agent. The rat sera were inactivated and absorbed as described, and antibodies to H.G.G. were sought in rat serum using passive haemagglutination of S.R.B.C.

Immune Inhibition with Glutaraldehyde-treated H.G.G.

Two hundred and fifty mg H.G.G. ('Pentex' Miles Laboratories) were dissolved in 5 ml P.B.S. and 1 ml of 2.5% aquous glutaraldehyde was added drop-wise with continuous stirring and allowed to stand for 3 hours at room temperature (Fuchs & Sela, 1973). The insoluble material

was dispersed in 5 ml distilled water and separated by centrifugation. The insolubilised IgG was dispersed in 10 ml 0.2 M phosphate buffer pH 7.4 and homogenised 3 times in a Potter's homogeniser with an additional homogenisation procedure in P.B.S. The immuno-adsorbant was suspended in and washed 3 times with P.B.S. and finally suspended in 5 ml P.B.S. To 0.1 ml of rat serum with known titre to H.G.G. 0.1 ml of immuno-adsorbant was added and stirred gently for 30 minutes at room temperature. Absorbed rat serum was recovered by centrifugation. The untreated and absorbed rat sera were serially diluted and antibodies to H.G.G. were sought using passive haemagglutination of sheep red blood cells in a microtitre system.

Antibodies to Rat 7S Globulin

Antibody activity to rat IgG and denatured rat 7S globulin was determined according to the above method. Rat IgG (Miles Laboratories) at a concentration of 10 mg/3.2 ml P.B.S. was allowed to denature by spontaneous cleavage (Stanworth & Turner, 1973), at $4^{o}C$ for 3 weeks. Fresh and denatured rat IgG were coated onto S.R.B.C. using glutaraldehyde as coupling agent and antibodies against these antigens were sought in P.C.S. rat sera with known titres of anti-H.G.G. antibodies following immunisation with H.G.G.

The Jerne Plaque Technique

A modified Jerne plaque technique (Jerne & Nordin, 1963; Jerne et al., 1963) was used to detect individual antibody-producing cells against E. coli L.P.S.

P.C.S., S.O. and N.C. rats were weighed and exsanguinated by cardiac puncture prior to the removal of their spleens under ether

anaesthesia. The separated sera were assayed for bacterial L.P.S. antibodies, as previously described. The spleen was weighed and the cell contents of each spleen were teased into 2-3 ml cold minimal essential medium (Eagle, Grand Island Biological Co., New York). The volume of the spleen cell suspension was made up to 10 ml in a graduated, conical centrifuge tube with tissue culture medium. After allowing cell clumps and other particulate matter to settle for 5 minutes, the supernatant solution of monodispersed cells was removed and the white cells were counted. The cell count was adjusted by appropriate dilution to contain 5 X 10^6 lymphoid cells per ml. The viability of the spleen cell preparation was determined using trypan blue. The spleen cell suspension was warmed to 37°C in a water-bath and used immediately afterwards. The time lapse between spleen removal and actual plating never exceeded 20 minutes.

A volume of L.P.S. at a concentration of 50 µg/ml (Halliday and Webb, 1965) was dissolved in sterile isotonic saline. The L.P.S. solution was boiled for 2 hours in a 100°C water-bath to facilitate coating onto S.R.B.C. (Neter et al., 1956). S.R.B.C. were collected in sterile Alsevers solution and allowed to age for 7 days in sterile containers (Hübner and Gengozian, 1969) prior to use. The S.R.B.C. were washed 3 times in Eissen's solution (Kern and Eissen, 1959). Twenty ml of the above L.P.S. solution was mixed with 1.4 ml of packed S.R.B.C. and incubated in a 37°C water-bath for 2 hours (Halliday and Webb, 1965). Uncoated control cells were prepared by the addition and incubation of 20 ml sterile isotonic saline with 1.4 ml packed S.R.B.C. The S.R.B.C. suspensions were agitated gently at 15-minute intervals. At the end of the incubation period, the L.P.S.-coated and uncoated S.R.B.C. suspensions were transferred to 15-ml centrifuge tubes and the packed cells recovered by centrifugation at 1,200 g for 10 minutes. Both cell suspensions were washed twice in Eissen's solution and once in Eagle's tissue

culture medium after which the packed cells were suspended in tissue culture medium to a final volume of 20 ml. The coated and uncoated control cell suspensions were pre-warmed in a 37°C water-bath prior to use.

Three ml volumes of melted 1.2% agar solution (Oxoid No. 1, Oxoid Ltd, London) in Eissen's solution containing 0.05% diethylaminoethyl (DEAE)-Dextran (Pharmacia, Uppsala, Sweden) were pipetted into disposable plastic petri dishes, 9 cm in diameter, as underlayers. The latter were usually prepared one day prior to the execution of the experiment. Two-ml aliquots of a 1.8% agar solution in Eissens with DEAE-DEXTRAN (D.D.) were pipetted into test tubes and kept at 45°C in a water-bath. One ml of spleen cell suspension and 0.5 ml 7% S.R.B.C. suspension was added to 2 ml of 1.2% agar solution, poured into petri dishes, and mixed thoroughly. The petri dishes were allowed to stand on a levelling table at room temperature until the agar had solidified. The agar plates were then incubated at 37°C for 2 hours and then stored overnight at 4°C. On the following morning 3 ml aliquots of cold, fresh guinea pig complement diluted 1 : 10 in Eissen's solution were added to the pre-warmed plates and the latter were incubated for 1 hour at 37°C. At the end of the incubation period the plates were removed from the incubator and allowed to stand for 2 - 3 hours at room temperature, after which the guinea pig complement was decanted and the number of plaques per petri dish was counted. Three plates (and a similar number of control plates) were poured from each lymphoid cell suspension and the arithmetic mean number of plaque-forming cells determined. Plaque-counting was facilitated by staining the entire plate with a freshly-prepared solution containing 10 ml of a 2% benzidine solution in a glacial acetic acid, 10 ml 5% H_2O_2 and 80 ml distilled water (Jerne, Nordin and Henry, 1963).

In order to allow comparison of antibacterial L.P.S. antibody titre with L.P.S. plaque-forming cells, antibodies to L.P.S. were sought in P.C.S., S.O. and N.C. rat sera using passive haemagglutination of S.R.B.C. in a microtitre system.

The Effects of Repeated Stimulation of Normal Rats with a Commercial L.P.S. Preparation

Each of 10 normal rats were pre-bled and immunised with a commercial L.P.S. preparation as follows :

i) At zero time rats were immunised with a suspension containing 50 µgm. E. coli 0127:B8 lipopolysaccharide W (Difco, control number 535715) in Freund's complete adjuvant subcutaneously in the nape of the neck and intramuscularly in multiple sites.

ii) After 4 weeks, the animals were boosted intraperitoneally with 50 µgm/1 ml of the same L.P.S. preparation, but without adjuvant.

iii) After 7 weeks, the rats were boosted daily, with 0.25 µgm L.P.S. in 1 ml sterile distilled water administered subcutaneously, for 14 days.

iv) After 11 weeks, the animals were given a final boost of 50 µgm L.P.S.

Rats were bled at weekly intervals throughout the 12-week period following initial immunisation.

The object of this study was to establish whether a normal rat immunised and then repeatedly stimulated with commercial L.P.S. preparation could produce antibody titres comparable to those recorded in P.C.S. rats with an L.P.S. preparation derived from rat faecal material.

The Effect of Neomycin on the Bowel Flora of Rats

Ten adult male rats were housed in two separate cages and fresh accumulated faecal pellets were collected from these animals and a 20% weight/volume (w/v) suspension of faeces in P.B.S. was made. Five ml of the faecal suspension was pipetted into a sterile bijou bottle and submitted to bacteriological analysis, and for the detection of Gram-negative organisms in particular. The following day neomycin sulphate was administered by gavage at a dose of 7.5 mg/100 gm body weight/day and this was continued for 4 weeks. Faecal pellets which were passed while the animals were being handled, were collected at weekly intervals. The pooled faeces were processed immediately, as described above, and submitted to bacteriological analysis. A standard bacteriological technique was used to determine the number of organisms (Coliforms, Enterococci and Staphylococci)/ml.

Splenoportography

Portacaval-shunted rats, untreated and on neomycin therapy, were selected at random, 3 - 36 weeks post-operatively, for splenoportography to demonstrate shunt patency and the extent of collaterals. Five sham-operated controls were also evaluated in order to gauge the effect of laparotomy and I.V.C./portal vein occlusion on the possible

development of a collateral circulation. A small number of unoperated controls were included in order to provide radiological evidence of the relevant vascular anatomy in the normal rat.

Anaesthesia was induced by the intramuscular administration of 0.15 ml pentobarbitone sodium (Sagatal, Maybaker). The anaesthetised rat was strapped supine on a radiolucent tray and the spleen exposed through an upper midline incision. A 23-gauge needle (Plexitron small vein set, Baxter) was carefully inserted into the splenic pulp so as not to transfix the organ. Needle position and splenic vein drainage were checked under screening control after injecting a small volume of sodium iothalamate 70% (Conray 420, Maybaker). Splenoportography was subsequently performed by manually injecting a further 1.0 - 1.5 ml of contrast, near the end of which a single radiograph was taken. Focus-film distance was 42 cm, the rat and tray lying directly on the screen film. Exposure factors of 2.5 mA, 42 Kv and 0.01 seconds, using a focal spot of 0.6 mm, were found to give the best results (Latham, 1975).

The animals were sacrificed following the removal of their livers. The livers were weighed and the weights were expressed as a percentage of the total body weight.

Detection of Fluorescent Auto-antibodies in Portacaval, Sham-operated and Normal Unoperated Control Rats

Fresh rat liver, kidney and stomach were used to prepare a composite block, sections of which were used for the detection of antinuclear, mitochondrial and smooth-muscle antibodies respectively. Rat liver and kidney (cortex and medulla) were cut into 4-mm cubes with a sharp scalpel blade using minimal pressure. A strip of rat

stomach was wrapped around the liver and kidney cubes and the composite block of tissue was quick-frozen onto a metal cryostat chuck. The cryostat temperature was set at -20°C and the microtome adjusted to cut 4 µ sections which were then lifted onto alcohol-cleaned standard microscope slides. The sections were dried for 30 minutes with cold air prior to use.

A standard fluorescent antibody technique was followed based on the method currently in use in Dr D. Doniach's laboratory in the Middlesex Hospital Medical School, London (personal observations). Sera from P.C.S., S.O. and N.C. rats were centrifuged for 10 minutes and then diluted 1 : 10 with P.B.S. The sections were flooded with the diluted rat sera and left to incubate for 20 minutes at room temperature under humid conditions. At the end of the incubation period, the excess sera were washed off with Coons Buffer (APPENDIX V) and the slides were washed in fresh buffer for 15 minutes with contrast agitation. Traces of buffer were carefully wiped off the slides and the sections were covered with a drop of fluorescein isothiocyanate - conjugated rabbit anti-rat immunoglobulin (Wellcome Research Laboratories, England. Lot K 6870), diluted 1 : 40 with Coons Buffer and centrifuged at 1,600 g for 10 minutes prior to use. (In a preliminary experiment, the fluorescent conjugate was diluted 1/10; 1/20; 1/30; 1/40; 1/50 and 1/60, tested against sections of fresh rat liver which had been pre-treated with normal rat sera and then viewed under fluorescent light. A dilution of 1/40 produced an acceptably low level of background fluorescence). AFter allowing the fluorescent conjugate to react with the tissue substrate for 20 minutes, the washing procedure, as described above, was repeated and the sections were mounted in glycine-buffered glycerol (APPENDIX V).

The slides were viewed on the same day under fluorescent light using a WILD M20 microscope (APPENDIX V).

HISTOLOGY

Rat Liver Histology in Portacaval Shunted Rats

The livers of P.C.S., S.O. and N.C. animals were removed under ether anaesthesia and placed in screw-capped jars containing neutral buffered formalin. A standard laboratory procedure including trimming of the liver soon after removal was followed for the processing of rat liver tissue. The sections were stained with Haematoxylin-eosin (APPENDIX VI) and reticulin stain (APPENDIX VI) and examined by ordinary light microscopy.

The Measurement of Aminotransferase Levels in P.C.S., S.O. and N.C. Rat Serum

Aspartate aminotransferase levels in rat sera were determined according to the method of Karmen et al. (1955), modified for a serum volume of 0.05 ml and a total sample of 1.0 ml. The optical density at wavelength 340 u was followed in the spectrophotometer (Beckman model DB) at room temperature for 5 minutes. The A.A.T. activity was expressed as units per ml of serum per minute (Karmen units).

Measurement of Packed Cell Volume (Haematocrit)

The packed cell volume (P.C.V.) of rat blood was measured in the acute stage subsequent to P.C.S. or sham surgery. The animals concerned were bled from the tail vein and a sample of blood was

collected in a heparinised microhaematocrit tube (Hawsley & Sons Ltd, Sussex, England). One end of the capillary tube was sealed and the tube was spun down for 10 minutes at 3 000 g in a desk model centrifuge (ROTO UNI II), fitted with a special head to accommodate the microhaematocrit tubes; after which the height of the P.C.V. was measured with a microhaematocrit reader.

The Effect of Portacaval Shunts on Pig Serum Proteins

Blood samples were collected pre-operatively and 4 weeks after the construction of an end-to-side portacaval anastomosis in the pig. The sera were subjected to serum protein electrophoresis on cellulose acetate membranes.

Statistical Analysis

Significance of differences between experimental and control groups was analysed by the Students' t-test and a probability value of $p < 0.05$ was deemed statistically significant.

Chapter 3

RESULTS

RESULTS

The pre-operative results on P.C.S., S.O. and N.C. animals were pooled throughout this study. The was deemed expedient because :

a) evaluation of changes in a specific parameter between different groups of animals was more meaningful.

b) Since animals were always randomised into groups before each experiment, major variations in pre-operative values in the 3 groups could be expected to be minimised. Justification for this view was obtained when initial statistical evaluation of pre-operative values in the 3 groups showed that no statistical differences could be detected.

Consecutive values were always compared with the pre-operative level of a particular parameter and subjected to statistical analysis. These statistical data are to be found in the relevant Tables. Consecutive data were also examined statistically and differences will be referred to in the text. All the numbered Tables referred to in the text have been included in APPENDIX VII.

Portacaval shunt is well tolerated by the normal rat, and a mortality rate of 10-15% was achieved for the experiments reported in this thesis. Signs of encephalopathy such as slowness of movement and rather torpid behaviour coupled with a tendency to walk on tiptoe (Herz et al., 1971; Herz et al., 1972) were seldom encountered. On occasion, cannibalistic behaviour was also encountered and, although no attempts were made to relate this to ammonia metabolism, it was considered to be a possible manifestation of encephalopathy. This phenomenon, fortunately, was also uncommon. Another consequence of portal shunting in these animals was seen in their undue susceptibility to cold, and early pilot studies were frequently complicated by an excessive post-operative mortality rate until the cold sensitivity was recognised. The most critical period for these animals appeared to be the first 24 - 48 hours after the operation, thereafter their physical condition improved progressively. The metabolic effects of portacaval shunting were not the subject of this investigation and whatever changes were induced by this procedure appeared not to have led to critical metabolic aberrations because the animals in these studies were shown to be capable of surviving in apparent good health for up to 9 months after portacaval shunting.

PART 1

Weight

A. Body Weight

Body weight is expressed throughout this study as a mean percentage of the original weight of animals in each group. This conversion facilitated the comparison of changes in weight of groups of animals in separate or unrelated experiments. Loss of weight was found to be a consistent feature of the rat with a portacaval anastomosis and some workers (Herz et al., 1972) regard the loss of weight, in conjunction with certain other parameters, as a reliable indication of shunt patency.

1. Changes in body weight in Experiment 1

In Experiment 1, the P.C.S. rat lost a significant percentage of body weight ($p < 0.01$) in the 48 hours after surgery (Fig. 8) and this loss of weight was continued for the duration of the experiment (Table 1). An overall loss of 16.4% was observed. In contrast, the sham-control rat also lost weight but the overall loss of 7.8% was not statistically significant (Table 2). It is of interest that the tempo of weight reduction was much more rapid in the P.C.S. animal. Differences in body weight between P.C.S. and S.O. rats reached statistical significance ($p < 0.02$) 48 hours after surgery and this significant difference was maintained for the duration of the experiment.

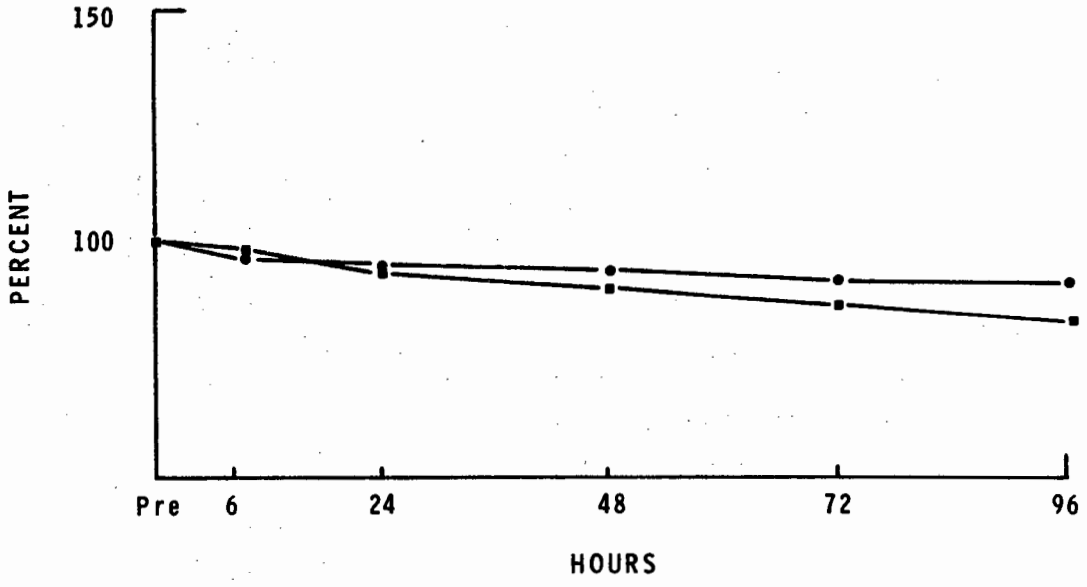

Fig. 8. Mean body weights in P.C.S. (■——■) and S.O. (●——●) rats, expressed as a % of the pre-operative weight. - Experiment I.

2. Changes in body weight in Experiments 2 and 3

Body weight data of Experiments 2 and 3 were not significantly different and were pooled to avoid duplication of results.

The acute loss of weight shown by the P.C.S. rat one week post-operatively, and which amounted to a 12% reduction in pre-operative body weight, was highly significant ($p < 0.001$). A continued loss of weight was observed in the portal-shunted group for a period of 6 weeks at which point the P.C.S. rat had lost up to 31.18% of its initial body weight (Fig. 9). A gradual return of body weight was noted to occur after this period but the rats in the experimental group never regained their initial body weight. All the differences in body weight which were observed were statistically significant when compared with the mean pre-operative weight of the experimental group (Table 3).

The administration of neomycin to the P.C.S. rat did not significantly affect the post-operative body weight changes (Table 4). The body weight changes of neomycin-treated P.C.S. rats closely resembled those of untreated P.C.S. rats (Fig. 9) and the differences in body weight between these 2 groups of animals were not statistically significant. Maximal loss in body weight in neomycin-treated animals was 28.12% which approximates the 31.08% in untreated P.C.S. rats ($p > 0.30$).

S.O. (Table 5) and unoperated N.C. (Table 6) animals continued to gain weight at essentially the same rate and this data stands in marked contrast to the weight changes

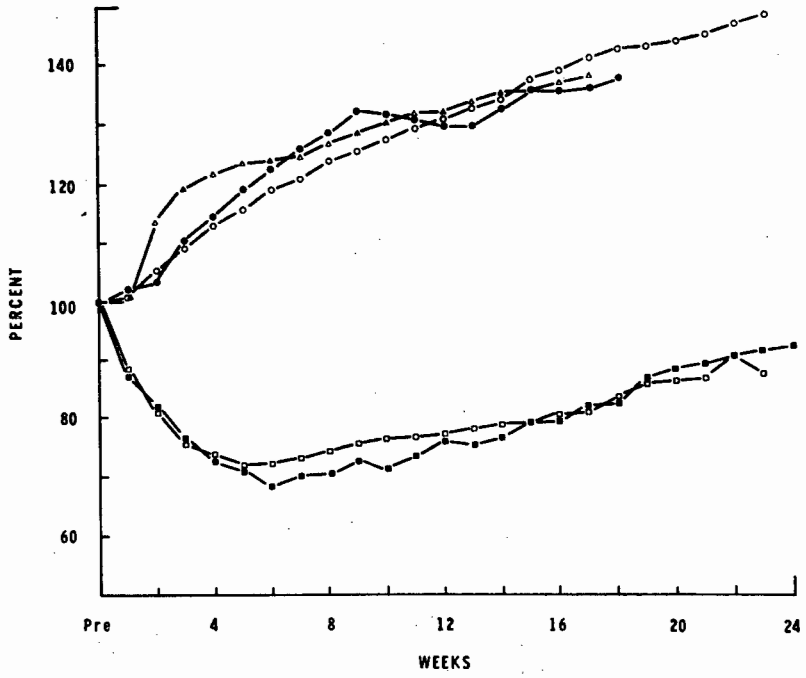

Fig. 9. Mean body weights in P.C.S. (■——■), S.O. (●——●) and N.C. rats (△——△), expressed as a % of the pre-operative weight. Open symbols represent neomycin-treated P.C.S. and S.O. animals. - Experiments 2 and 3.

in P.C.S. animals (Fig. 9). The significant difference ($p < 0.05$) in weight observed between S.O. and N.C. animals up to 3 weeks post-operatively presumably reflect the metabolic effect of surgery in S.O. rats. The administration of neomycin to S.O. animals did not influence the weight curve.

It was of interest to compare weight changes in animals in which the patency of the portal anastomosis was assessed radiographically. The characteristic change in weight was not observed in those animals where the shunt was totally occluded (Fig. 10) (Table 7). In contrast, the weight changes in those animals with totally patent shunts paralleled the data of the experimental portal-shunted group as a whole (Table 8).

Where the shunt was judged to be partially occluded similar changes in weight were observed as in animals with patent shunts (Table 9). It was of interest to note that animals with totally occluded P.C.S. regained their pre-operative body weight 5 weeks after surgery and that the mean weekly weights in such animals were significantly higher throughout the experiment than in animals with totally patent or partially occluded shunts. No significant differences in weight were detected between rate with totally occluded P.C.S. and S.O. controls.

3. Changes in Body weight in Experiment 4

Weight loss was found to be age dependent. Smaller animals lost proportionately more body weight (35.12%) than older animals. An increase of weight was noted earlier in the

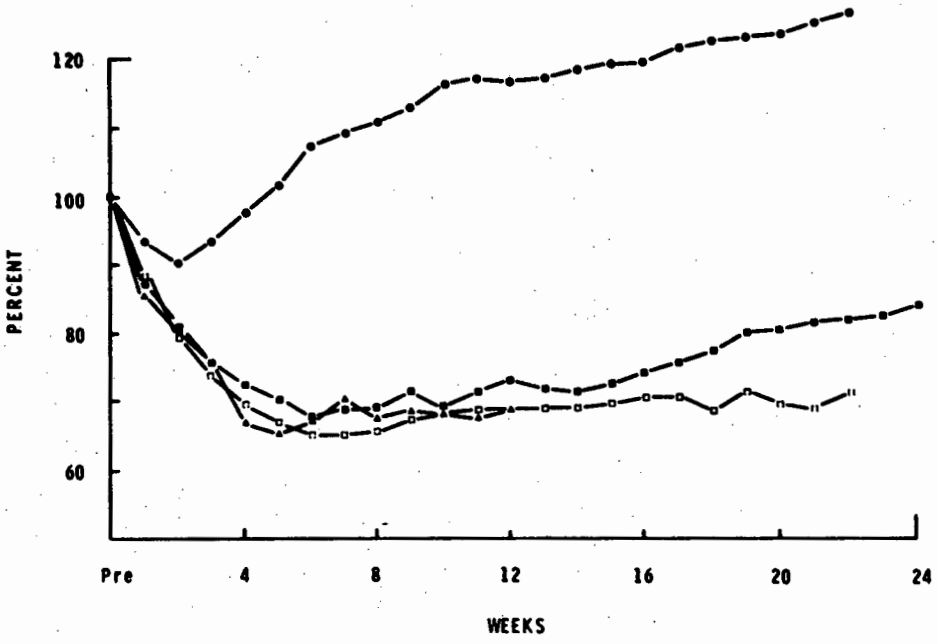

Fig. 10. Mean body weights in rats with patent (■——■), partially occluded (▲——▲), totally occluded (●——●) P.C.S. and patent P.C.S. treated with neomycin (□——□); expressed as a % of the pre-operative weight. - Experiment 3.

case of the small P.C.S. rat (Fig. 11) (Table 10). As with older rats, the administration of neomycin did not influence the change in body weight post-operatively (Table 11).

4. Changes in body weight in Experiment 5

P.C.S. animals subjected to splenectomy showed a similar loss of weight as conventional P.C.S. animals (Fig. 12) (Table 12). An overall loss in body weight of 38% was recorded in the splenectomised P.C.S. rats compared with 31.08% in conventional P.C.S. animals. Differences between these groups of animals could be considered to be due to the loss of splenic weight.

Splenectomy in S.O. animals caused a drop in body weight within the 1st week post-operatively which was comparable to that seen in animals with P.C.S. and splenectomy (Table 13). Unlike the latter group, the S.O. animals regained their initial weight 5 weeks post-operatively. Differences in body weight between S.O. animals and splenectomised S.O. rats were also observed and were significant.

B. Liver Weight

Liver weight was expressed as a percentage of the total body weight and this value was used as an index of liver atrophy. The data on liver weight was obtained at the time when splenectomy was undertaken in Experiment 3 and represents the pooled data on liver weight taken at various intervals after P.C.S. (Fig. 13). In this Experiment, the differences in mean liver weight between P.C.S. (2.345 ± 0.56) and S.O. controls (3.558 ± 0.99) were statistically significant ($p < 0.01$). The liver weight in P.C.S. rats decreased

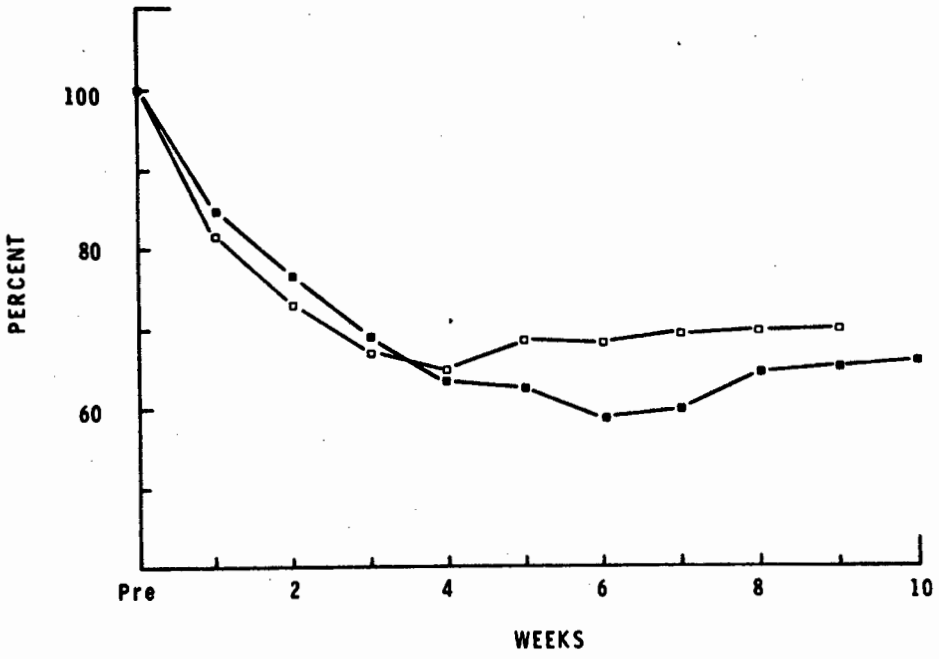

Fig. 11. Mean body weights in small P.C.S. (■——■) and neomycin-treated P.C.S. rats (□——□); expressed as a % of the pre-operative weight. - Experiment 4.

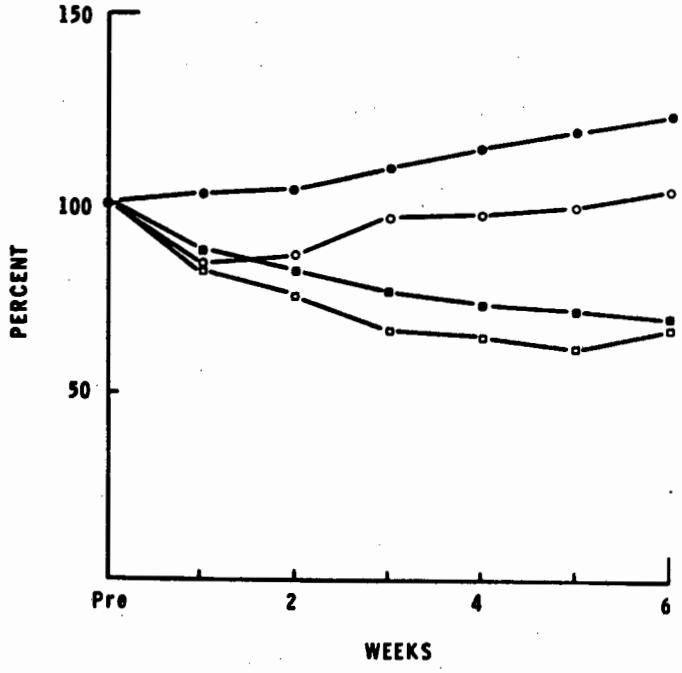

Fig. 12. Mean body weights in P.C.S. (□——□) and S.O. (○——○) rats subjected to splenectomy, and expressed as a % of the pre-operative weight. Closed symbols represent conventional P.C.S. and S.O. animals. - Experiment 5.

by 1/3 relative to that of S.O. controls. (Variations about mean values given in the text describe $\pm$ one standard deviation.)

In Experiment I, the differences in mean liver weight between P.C.S. (2.843 ± 0.54 g) and S.O. rats (3.761 ± 0.64 g) were not significant. (Fig. 13).

C. Spleen Weight

In Experiment 3, spleen weight was similarly expressed as a percentage of total body weight. No statistical differences in spleen weight between P.C.S. (0.821 ± 0.27), S.O. (0.846 ± 0.25) and N.C. rats (0.845 ± 0.25) were detected.

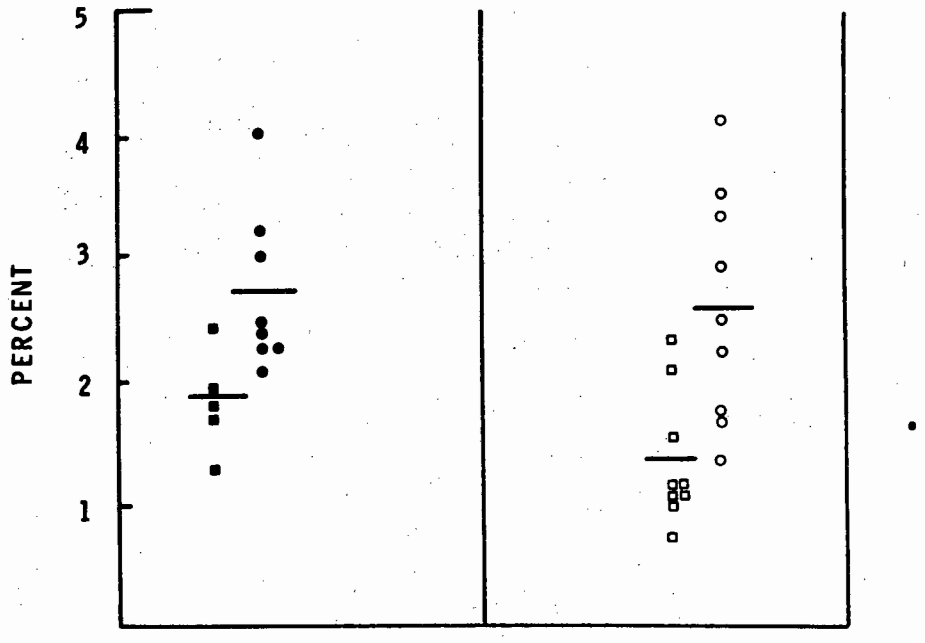

Fig. 13. Liver weight, expressed as a % of total body weight, in P.C.S. (■——■) and S.O. (●——●) rats in Experiment I. Open symbols represent animals in Experiment 3.

DISCUSSION

1. Body Weight

(The term 'weight' was used throughout this thesis in preference to mass because of its continued use in medical parlance).

In all these studies reported here, the animals were weighed regularly and loss of weight was found to be an invariable result of portal shunting. The smaller animals withstood the stress of a portacaval shunt less well and lost more weight. This information is at variance with that reported by Kyu and Cavanagh who showed that smaller animals lost only 10-20% of their body weight as opposed to 20-30% in bigger animals (Kyu and Cavanagh, 1970). It is of interest in this study that the subsequent weight gain was initiated earlier in the smaller animals and in this respect the data confirm the finding of Kyu and Cavanagh. The majority of these small animals, despite their claimed superior resistance to post-operative infections and trauma (Lambert, 1965), could not tolerate the drastic weight loss and indeed Experiment 5 had to be abandoned prematurely because of an excessively high mortality rate.

No advantage was gained by using very large animals (400-450 g) because there was no concomitant increase in the size of the blood vessels (Fisher and Lee, 1965) and because they were said to be less resistant to infection (Farris and Griffith, 1949). Old rats often failed to regain consciousness following the operation but those that survived were able to withstand an acute loss in body weight. It was for these reasons that rats weighing 250-350 g pre-operatively were selected for experimental purposes.

Weight loss recorded in P.C.S. rats in all the Experiments was probably due to anorexia. The extent of post-operative weight loss in P.C.S. rats recorded in these studies supports the findings of several investigators (Henzel et al., 1963; Rubin et al., 1965; Doyle, 1969; Kyu and Cavanagh, 197). The minimal weight loss in P.C.S. rats reported by Assal and co-workers was attributed to precisely controlled environmental conditions (Assal et al., 1971). Reichle and co-workers using a non-suture P.C.S. technique reported no significant weight loss in experimental animals (Reichle et al., 1972A).

It was not the aim of this study to investigate all the conditions that could mitigate against weight loss following P.C.S. Many reasons may be proposed for the loss of weight after portal shunting. The P.C.S. rats appear to eat less for some time following surgery. In this study, food intake was not quantitated but other investigators have reported a significant decrease in food consumption which took 3 months to return to normal (Assal et al., 1971). Similarly, Kyu and Cavanagh reported that food consumption following P.C.S. was one-third less than controls up to 5 weeks post-operatively and not much more than two-thirds of controls 2 months after surgery (Kyu and Cavanagh, 1970). The reasons for this reduced food intake over this length of time are not clear, but suppression of appetite due to a disruption of the hypothalamus-liver satiety axis following portal blood diversion may be suggested. Satiety centres within the lateral hypothalamus are claimed to have neurological links with portal receptors either in the liver or in the portal vein itself and respond to portal injections of glucose. This response was abolished when the neurological connexions from the liver to the hypothalamus were severed in normal rats (Schmitt, 1973). One can only speculate on what disturbances are produced by the surgery of a P.C.S. on such neurological mechanisms, and this is an aspect that needs to be studied further.

The accumulation of oestrogenic compounds in P.C.S. rats (Reichle et al., 1969) may also have contributed to reduction of food intake in experimental animals. Bull and co-workers have reported a significant reduction in food intake in normal rats following the administration of oestrogen (Bull et al., 1974), which tends to suggest that oestrogenic compounds were causally related to appetite suppression. It is of interest that castration in normal male rats has also been claimed to be associated with reduced food intake (Bull et al., 1974). The testicular atrophy which is claimed to follow portacaval shunting (Dordahl et al., 1967; Kyu and Cavanagh, 1970; Chen, 1972; Herz et al., 1972) may be related to this phenomenon.

There are, of course, other reasons for weight loss that do not involve deficient intake of food on the basis of anorexia. Thus Herz and co-workers have reported that substances present in the faeces of P.C.S. rats were detrimental to animals ingesting faecal pellets. Animals which had free access to their faeces lost significantly more weight than animals in which coprophagy was restricted. In contrast, these same workers have shown that coprophagy would appear to be a prerequisite for normal weight gain in non-P.C.S. animals (Herz et al., 1972).

The utilisation of dietary proteins for energy requirements following P.C.S. in rats have been reported by Reichle et al., (1970). It may be suggested that gluconeogenesis from protein contributes to the weight loss in P.C.S. animals. The process of gluconeogenesis involves the transamination of certain amino acids to ketoacid intermediates and the excretion of amino nitrogen in the form of urea (Schimke, 1962). It is of interest in this regard that disturbances in urea synthesis in the P.C.S. liver have been reported (McDermott and Adams, 1954) and it is clear that there are multiple biochemical factors that may contribute to this problem.

The diversion of portal blood from the liver which would allow access of bowel bacterial products, in particular, endotoxin, to the systemic circulation is a central theme in this thesis. It seems pertinent, therefore, to examine the effects of endotoxin on food intake and growth. The administration of endotoxin to mice is accompanied by stunted growth, which is temporary, and a slower gain in body weight (Kind et al., 1967). It is possible that endotoxin had a similar effect in P.C.S. rats. The vigorous and often spectacular antibody levels directed against endotoxin (L.P.S.) lend credence to the view that endotoxin may be present in the systemic circulation. The access of endotoxin to the systemic circulation also raises the possibility of viable micro-organisms gaining similar access and it is not unreasonable to suggest that low-grade bacteraemia and/or chronic immunological stimulation could contribute to the weight loss.

These putative effects cannot be other than temporary because it is the experience of this study, as well as the experience of many others, that weight gain is resumed after a certain period of time. In this investigation some animals lost weight precipitously while others suffered a gradual weight loss, but initial body weight was never regained in animals with fully patent or even partially occluded P.C.S. Experimental animals regaining their pre-operative body weight 4 - 5 weeks after surgery, were analysed separately and their shunt patency queried (serum gamma-globulin levels and anti-bacterial L.P.S. antibody titres were only moderately elevated in these animals). The suspect shunt status of these animals was subsequently confirmed radiologically. Thus, in the absence of radiological evidence of shunt patency, acute post-operative weight loss in P.C.S. rats served as a reliable indication of shunt patency. The limited post-operative weight data presented in this study on P.C.S. rats with partially occluded, or patent, shunts with intermediate

collaterals, do not support the view of Kyu and Cavanagh who emphasised that the extent of weight increase in the P.C.S. rat was dependent on the formation of 'strong adhesions' between the intestine and liver (Kyu and Cavanagh, 1970).

In this study, post-operative weight gain in animals with fully patent P.C.S. began 6 weeks after surgery and supports the findings of other investigators (Henzel et al., 1963; Baldessarini and Fischer, 1967; Doyle, 1969; Kyu and Cavanagh, 1970). Pre-operative weight, however, as mentioned earlier, was never regained and this stands in contrast to reports that P.C.S. animals regained their pre-operative body weight 4 - 5 weeks (Assal et al., 1971), 2 months (Flynn and Kennan, 1968) or 16 - 17 weeks (Chen, 1972) post-operatively.

2. Liver Weight

The 25% loss in liver weight recorded in this study 4 days post-operatively in P.C.S. animals supports the findings of Oudea and Bismuth (1965) who reported a similar drop in liver weight (25-33%) 4 to 14 days after P.C.S. in the normal rat. A slightly bigger loss in liver weight (43%) was reported by Herz et al., (1972) who also demonstrated a 25% reduction in liver weight in normal rats on restricted food intake.

The significant reduction in liver weight following long-term survival after P.C.S. in the normal rat is in agreement with the results documented by Assal et al. (1971) who reported a 22-31% reduction in liver weight in their experimental animals. Loss in liver weight in excess of 50% was reported by other investigators (Fisher et al., 1962; Kyu and Cavanagh, 1970; Fischer et al., 1971).

3. Spleen Weight

The results on post-operative spleen weight in P.C.S. rats confirm those of Kyu and Cavanagh (1970) who reported no change in spleen weight in experimental animals.

PART 2

Serum Proteins

Total Serum Protein Levels in Rats

As mentioned previously the volume of the Biuret method for measuring total serum protein, was modified to a total sample volume of 1 ml. In a pilot study the results obtained with the modified technique were compared statistically with those using standard volumes and colorimeter (Klett) optical density measurements. The following mean values were obtained in 20 randomly selected rat serum samples :

Micro technique	:	6.583 ± 0.381
Standard technique	:	6.319 ± 0.441

The difference between these methods was not statistically significant.

i) Experiment 2

Total serum protein levels decreased significantly ($p < 0.05$) one week after P.C.S. in the normal rat (Fig. 14). The fluctuations in total serum protein level thereafter were minimal and did not deviate significantly from the pre-operative mean value. In S.O. and N.C. rats the serum protein levels fluctuated throughout the period of observation and whatever deviations occurred were neither

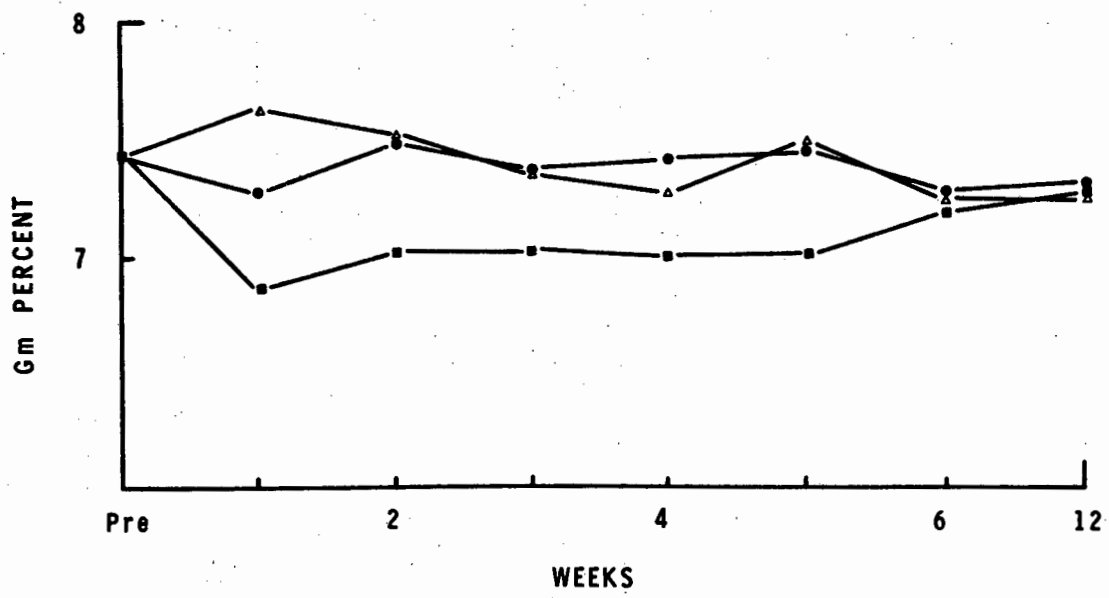

Fig. 14. Serum total protein concentrations in P.C.S. (■——■), S.O. (●——●) and N.C. (△——△) rats. - Experiment 2.

marked nor significant. The difference in total serum protein levels between P.C.S. and N.C. rats was statistically significant ($p < 0.025$) one week post-operatively. Thereafter no significant differences were detected in serum protein levels between the 3 groups of animals.

ii) Experiments 8 and 9

No statistically significant differences were detected in total serum protein levels between the 3 groups of animals after 2 and 7 weeks of pair-feeding. Serum protein levels were, however, lower in pair-fed S.O. and N.C. rats than levels detected in control animals not subjected to pair-feeding. This finding may be accepted as evidence in support of the view that total serum protein depletion in P.C.S. rats is probably due to restricted food intake.

In view of the fact that so many different investigations had to be performed on the limited volume of rat serum, it was decided not to assay for total serum proteins in any of the other experiments.

Changes in Serum Protein Fractions

Rat serum samples in Experiment 2 were electrophoresed initially on agar slides and subsequently on cellulose acetate. Significantly higher levels of serum albumin were detected with the agar slide technique ($p < 0.05$) but gamma-globulin levels were not significantly different. All the rat serum samples in these studies were electrophoresed on cellulose acetate because of the ease and reproducibility of the technique.

The pooled pre-operative levels of rat serum protein fractions in Experiments 1, 2, 3 and 4 did not differ significantly and on this basis it was deemed expedient to compare, at a comparable point in time, the changes in a particular group of animals under varying experimental conditions.

A. Experiment 1 (zero time to 4 days)

i) Serum albumin : In the P.C.S. rat the mean serum albumin level, after an initial rise, decreased so that by the 3rd post-operative day it had reached a level which was significantly lower than the pre-operative value (Fig. 14A) (Table 14). A similar change, but of a greater magnitude, was seen in S.O. rats (Table 15). The difference in serum albumin concentration between P.C.S. and S.O. rats was statistically significant ($p < 0.05$) 4 days post-operatively.

ii) Serum alpha-1 globulins : The serum alpha-1 globulin level, after an initial drop, increased in the P.C.S. rat and achieved statistical significance 48 hours post-operatively (Fig. 15) (Table 16). A sustained rise in serum alpha-1 globulin level was observed in the S.O. rats (Table 17). No significant differences were detected in alpha-1 globulin levels between P.C.S. and S.O. rats.

iii) Serum alpha-2 globulins : In P.C.S. rats the serum alpha-2 globulin level, although showing fluctuations,

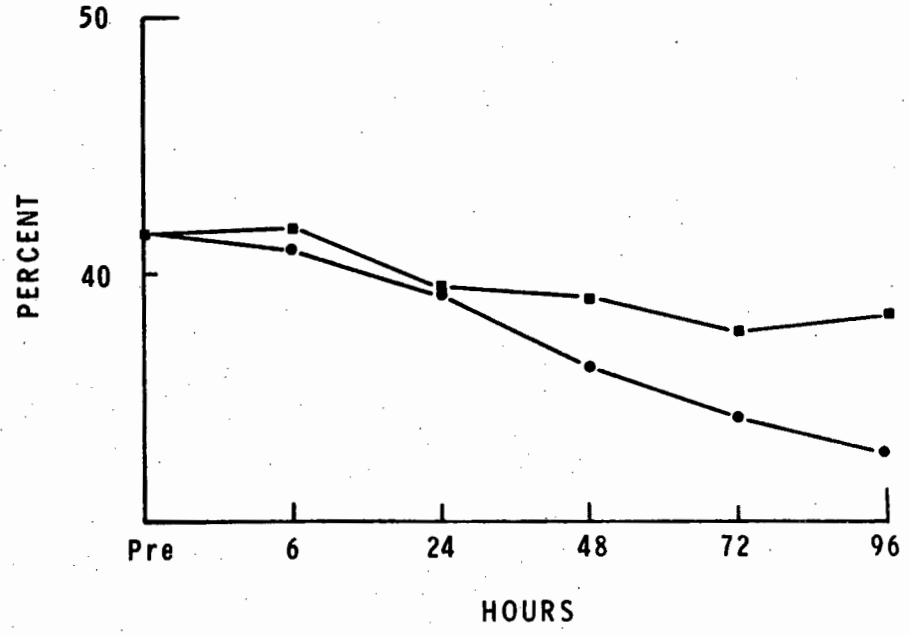

Fig. 14A. Mean serum albumin levels in P.C.S. (■——■) and S.O. (●——●) rats, expressed as a % of the total protein. - Experiment I.

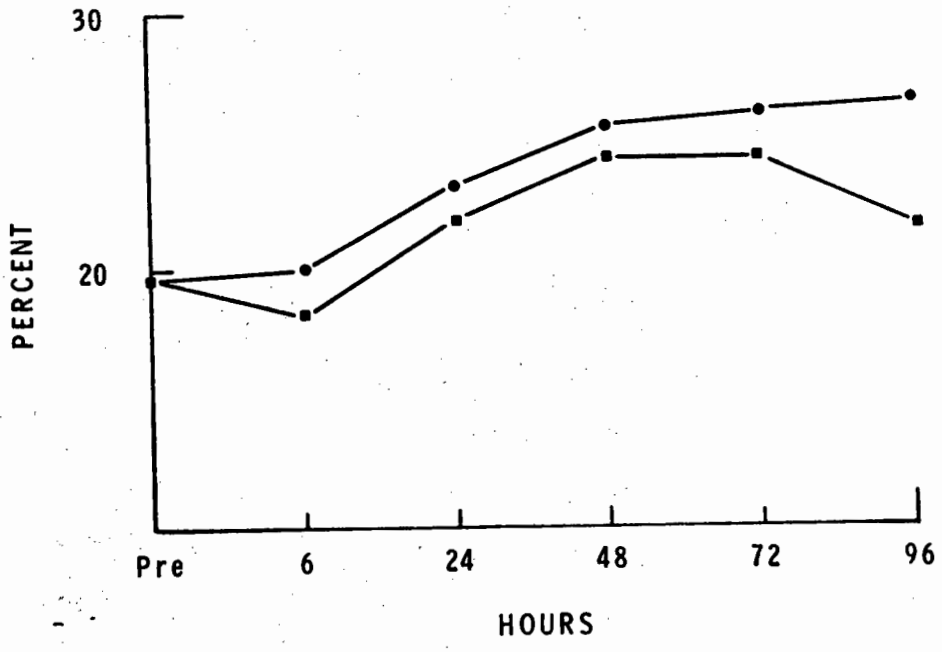

Fig. 15. Mean serum alpha-I globulin levels in P.C.S. (■——■) and S.O. (●——●) rats, expressed as a % of the total protein. - Experiment I.

did not deviate significantly from the initial value (Fig. 16) (Table 18). The alpha-2 globulin level in the S.O. rat fluctuated and became significantly elevated above the initial value 24 hours post-operatively (Table 18A). No significant differences were detected in serum alpha-2 globulin levels between P.C.S. and S.O. animals.

iv) Serum beta-globulins : Serum beta-globulin levels in the P.C.S. rat fluctuated but did not deviate significantly from the pre-operative level and showed an overall decrease for the duration of the Experiment (Fig. 16) (Table 19). The beta-globulin level in the S.O. rat started to increase 48 hours post-operatively but the daily fluctuations thereafter did not deviate significantly from the pre-operative level (Table 20).

v) Serum gamma-globulins : Fluctuations in the serum gamma-globulin levels were seen in P.C.S. and S.O. animals but the variations were neither marked nor significant when compared with the pre-operative value (Fig. 17). There was a tendency for the gamma-globulin level to be maintained in the P.C.S. at the pre-operative value (Table 21) while in the S.O. rat the level declined below this value (Table 22).

B. Experiment 2 (zero time to 12 weeks)

i) Serum albumin : In Experiment 2 there was a striking and statistically significant ($p < 0.01$) decrease in the mean serum albumin level one week after P.C.S. in

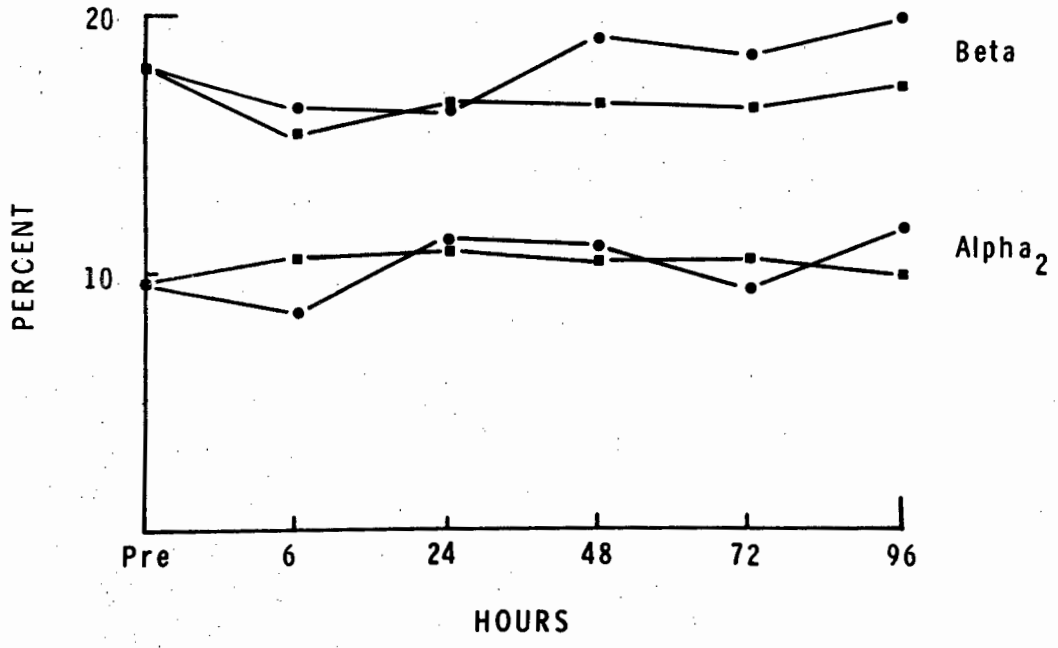

Fig. 16. Mean serum alpha-2 and beta-globulin levels in P.C.S. (■——■) and S.O. (●——●) rats, expressed as a % of the total protein. - Experiment I.

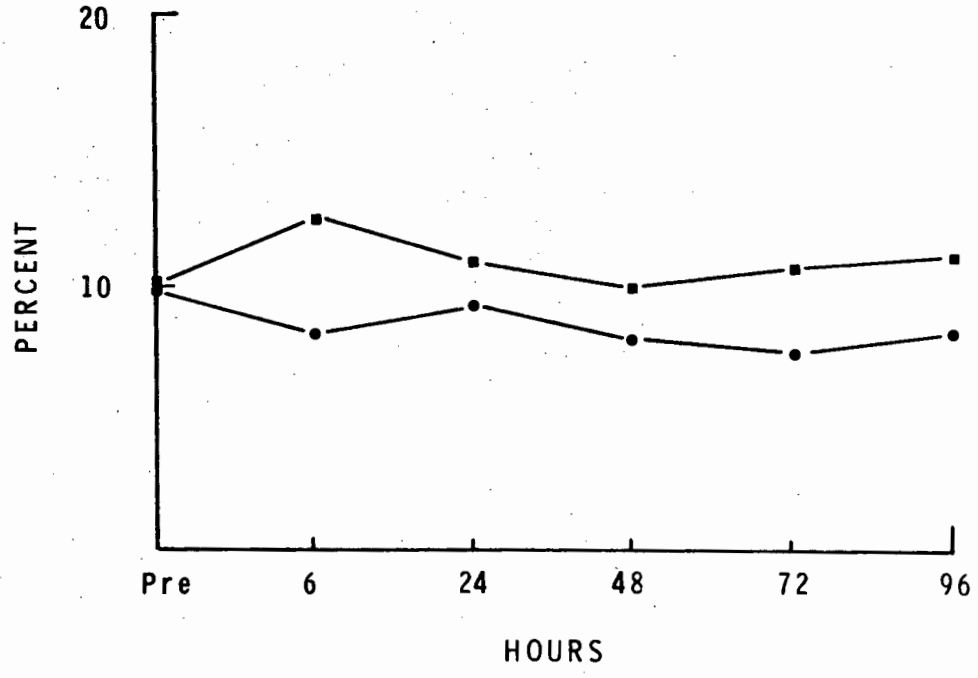

Fig. 17. Mean serum gamma-globulin levels in P.C.S. (■——■) and S.O. (●——●) rats, expressed as a % of the total protein. - Experiment I.

the normal rat (Fig. 18). This drop in serum albumin was observed for a further week although the magnitude of the decrease was less than in the first post-operative week. A gradual increase in serum albumin was noted from the 3rd to the 12th week of the study but the serum albumin level in the terminal bleed was still significantly lower than the pre-operative value (Table 23).

In the S.O. rat a greater decrease in albumin level was detected one week post-operatively but from this point to the completion of the study the serum albumin level increased and by the 8th week post-operatively the initial serum albumin level was reached (Table 24). Serum albumin levels in S.O. rats were significantly higher than those recorded in P.C.S. rats from the 8th week post-operatively until the completion of the study. The serum albumin level in N.C. rats fluctuated during the period of study but what deviations occurred were neither large nor significant (Table 25).

ii) Serum alpha-1 globulins : A striking elevation of serum alpha-1 globulin was noted in P.C.S. rats but the change was not significant (Fig. 19) (Table 26). During the ensuing weeks the serum alpha-1 globulin decreased and remained significantly depressed ($p < 0.001$) for the remaining period of study. In the S.O. rat, a similar rise in serum alpha-1 globulin occurred one week post-operatively and this was followed by a decrease in alpha-1 globulin level 2 weeks post-operatively (Table 27). A comparison of alpha-1 globulin levels

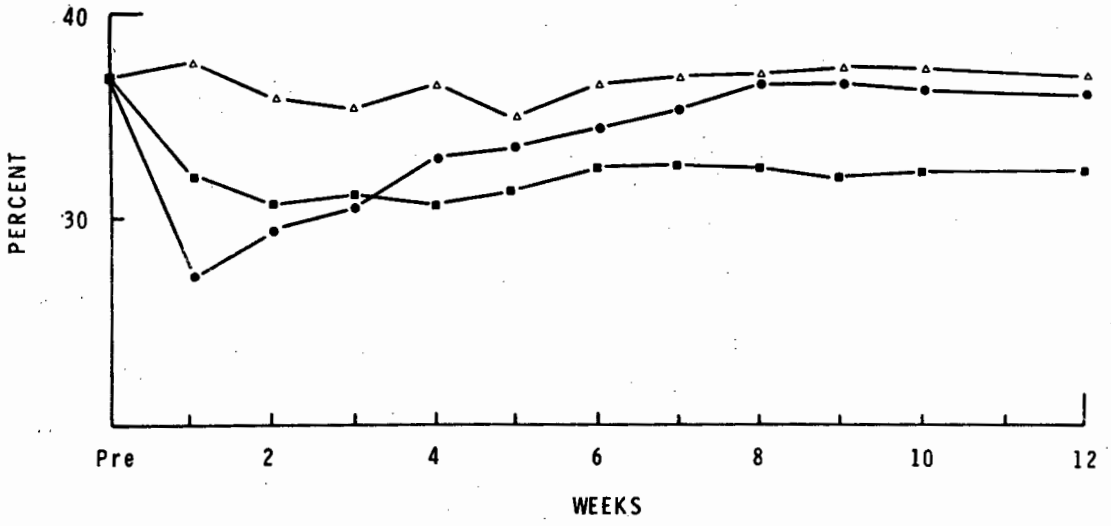

Fig. 18. Mean serum albumin levels in P.C.S. (■——■), S.O. (●——●) and N.C. (△——△) rats, expressed as a % of the total protein. - Experiment 2.

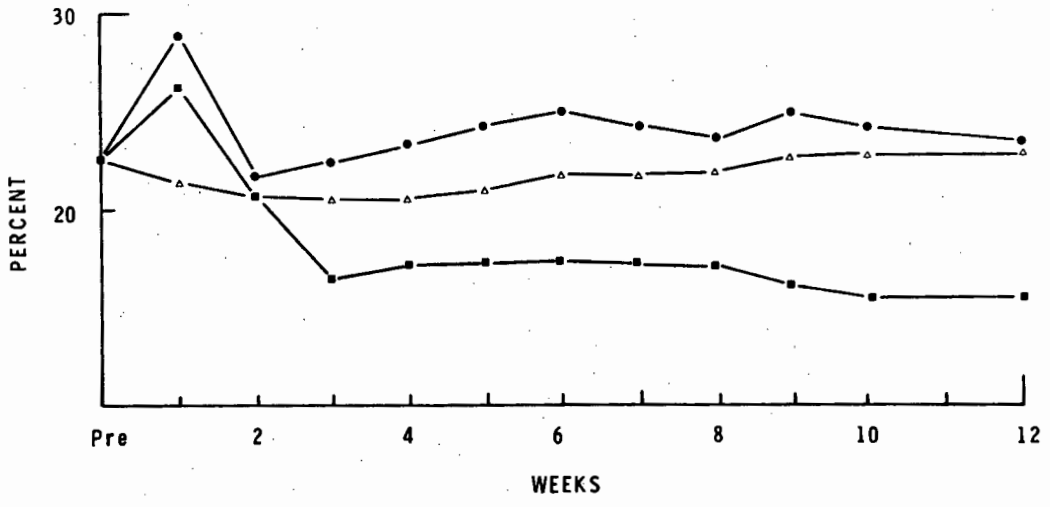

Fig. 19. Mean serum alpha-I globulin levels in P.C.S. (■——■), S.O. (●——●) and N.C. (△——△) rats, expressed as a % of the total protein. - Experiment 2.

in S.O. and P.C.S. rats showed that there was a constantly lower alpha-1 globulin level in the P.C.S. rat and that these differences were, with one exception at 2 weeks post-operatively, statistically significant. In the normal rat little change occurred in the serum alpha-1 globulin level throughout the period of study (Table 28).

iii) Serum alpha-2 globulins : In the P.C.S. rat the serum alpha-2 globulin levels showed minor changes (Fig. 20) (Table 29). The changes in alpha-2 globulin levels in S.O. (Table 30) and N.C. rats (Table 31) were minimal throughout the study. No significant differences in alpha-2 globulin levels were found between P.C.S. and S.O. rats.

iv) Serum beta-globulins : The serum beta-globulin level in the P.C.S. rat decreased for 2 weeks post-operatively. A rise in beta-globulin level was then seen so that by the 11th and 12th weeks of the study serum beta-globulin was significantly higher than the levels seen in S.O. and N.C. rats (Fig. 21) (Table 32). Following sham operation an increase in serum beta-globulins was detected and thereafter the fluctuations of serum beta-globulin levels were minor and showed little variation from the levels seen in normal rats (Table 33). The weekly fluctuations in the serum beta-globulin level in the N.C. rat showed no significant deviation from the initial level throughout the period of study (Table 34).

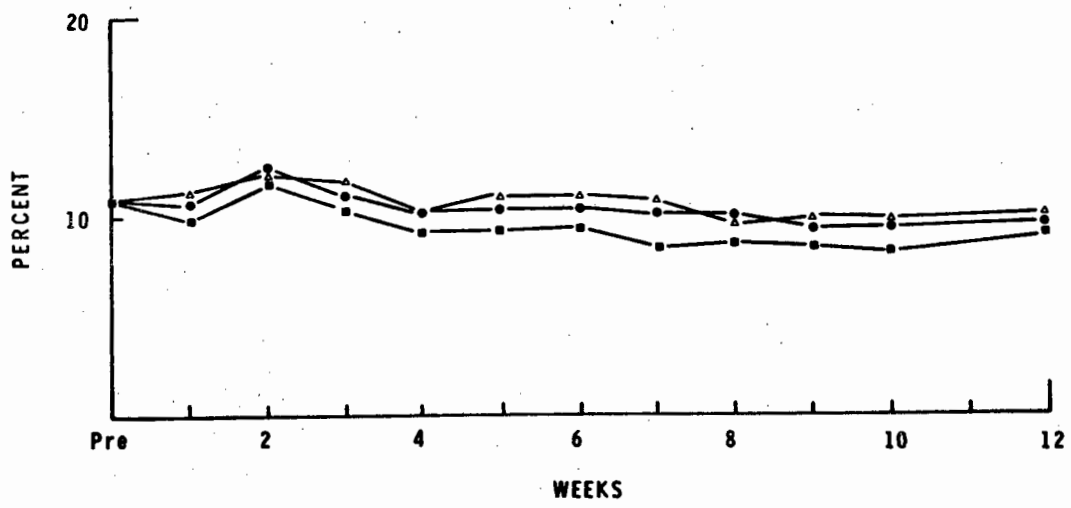

Fig. 20. Mean serum alpha-2 globulin levels in P.C.S. (■——■), S.O. (●——●) and N.C. (△——△) rats, expressed as a % of the total protein. - Experiment 2.

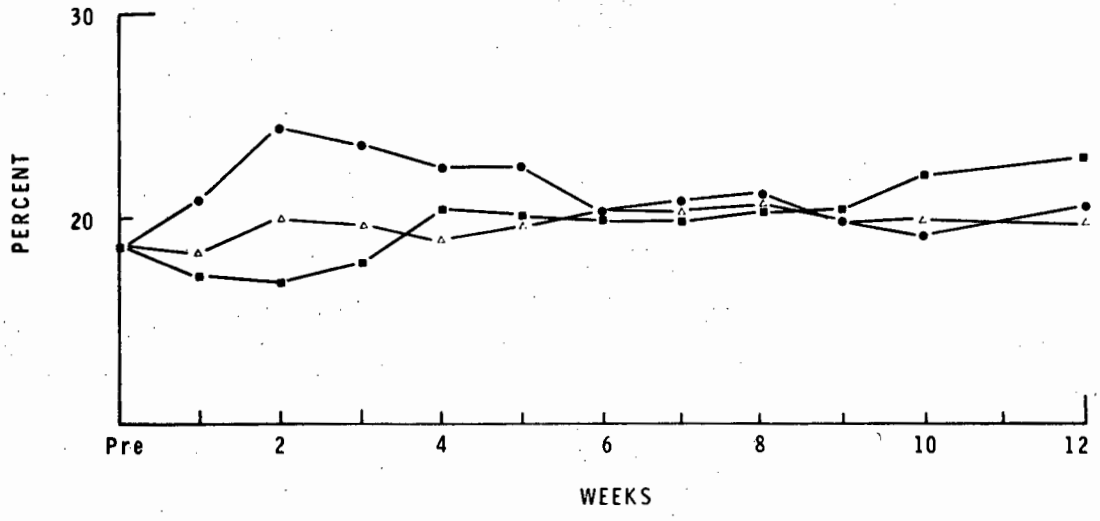

Fig. 21. Mean serum beta-globulin levels in P.C.S. (■——■), S.O. (●——●) and N.C. (△——△) rats, expressed as a % of the total protein. - Experiment 2.

v) Serum gamma-globulins : One week after P.C.S. there was a significant rise in gamma-globulin level ($p < 0.001$). This elevation of gamma-globulin in the P.C.S. rat was sustained, with minor fluctuations, for the duration of the experiment (Fig. 22) (Table 35). Maximum gamma-globulin levels recorded in the 3rd post-operative week, represented at least a two-fold rise in gamma-globulin level. In contrast the gamma-globulin levels seen in S.O. and N.C. rats, although showing minor variations, remained constant throughout the experiment (Tables 36 and 37). The elevated gamma-globulin levels in P.C.S. rats remained significantly above the control animals ($p < 0.001$) throughout the experiment.

C. Experiment 3 (zero time to 36 weeks)

i) Serum albumin : In Experiment 3 the changes in albumin level in P.C.S. rats were similar to that of Experiment 2 over a corresponding period of time. The mean albumin level remained significantly depressed for the first 12 weeks post-operatively (Fig. 23) (Table 38). During the ensuing weeks serum albumin levels showed a continuing rise so that by the 18th week the pre-operative level was reached and maintained for the duration of the experiment.

By contrast a minimal and transient decrease in serum albumin levels was observed in P.C.S. rats where the shunt status was classified as totally occluded or partially occluded on radiographical evidence (Fig. 24). The fluctuations in serum albumin levels in rats with totally occluded and partially occluded anastomosis did not deviate significantly from the mean pre-operative value and this stands in direct contrast to the significantly depressed levels seen in rats with totally patent shunts (Tables

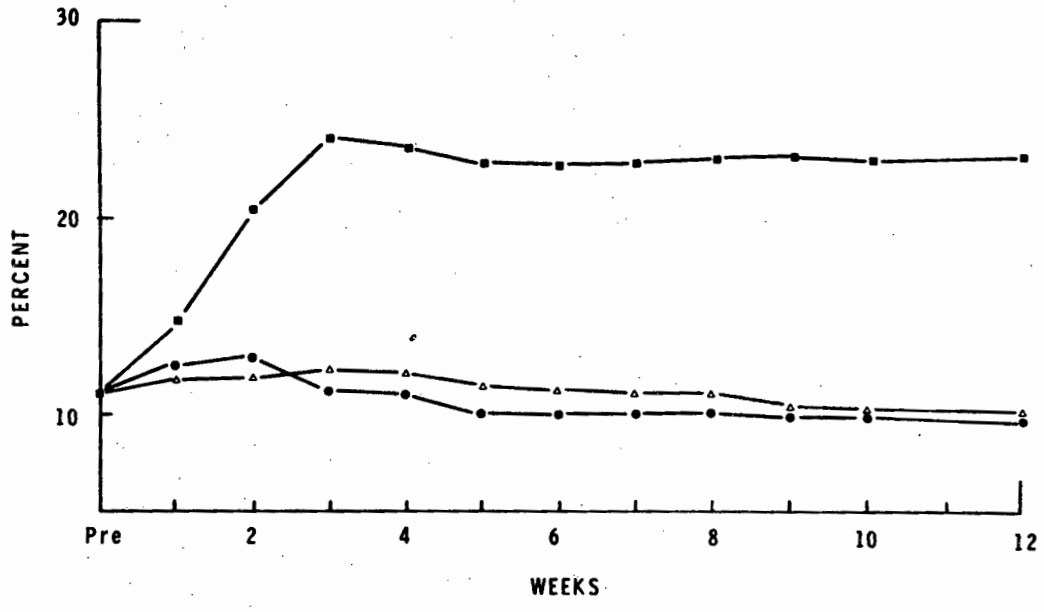

Fig. 22. Mean serum gamma-gobulin levels in P.C.S. (■——■), S.O. (●——●) and N.C. (△——△) rats, expressed as a % of the total protein. - Experiment 2.

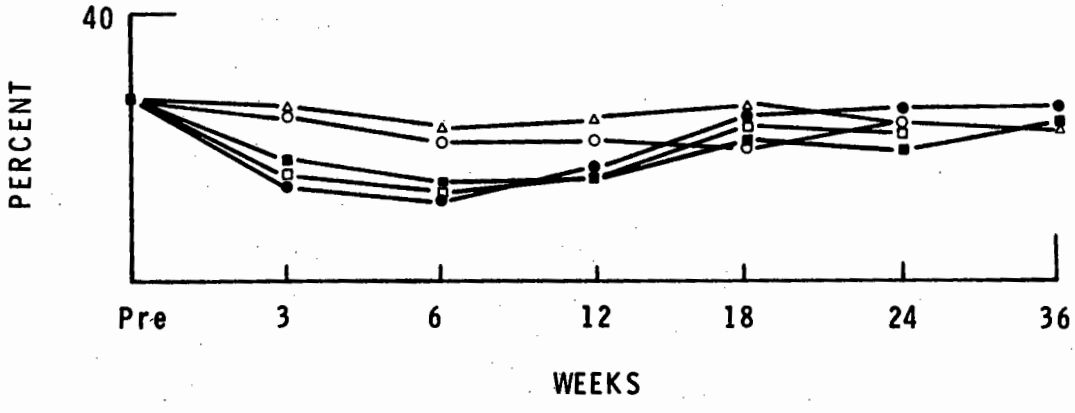

Fig. 23. Mean serum albumin levels in P.C.S. (■——■), S.O. (●——●) and N.C. (△——△) rats, expressed as a % of the total protein. Open symbols represent neomycin-treated P.C.S. and S.O. rats. - Experiment 3.

39 and 40). Albumin levels, however, in rats with totally occluded and partially occluded P.C.S. were not significantly different from those seen in animals with patent shunts.

The administration of neomycin to the P.C.S. rat did not alter the serum albumin changes and indeed a similar significant decrease in serum albumin was noted while the animals were on neomycin (Table 41). In the S.O. rat treated with neomycin no initial drop in serum albumin was detected and, like the N.C. rat, the changes in serum albumin concentration were minimal and did not deviate significantly from the initial level (Tables 42 and 43). Changes observed in serum albumin level in S.O. rats were essentially similar to those seen in Experiment 2 (Table 44).

ii) Serum alpha-1 globulins : In the P.C.S. rat the serum alpha-1 globulin level followed a similar pattern to that of Experiment 2 and the administration of neomycin did not influence the levels of alpha-1 globulin (Fig. 25) (Tables 45 and 45A). The alpha-1 globulin levels in rats with partially occluded P.C.S. were not significantly different from those recorded in animals with totally patent anastomosis (Fig. 26) (Table 46). By contrast the alpha-1 globulin levels in rats with totally occluded P.C.S. were significantly higher than levels detected in animals with a fully patent anastomosis (Table 47). The alpha-1 globulin level did not vary significantly in S.O. and N.C. animals (Tables 48 and 49).

It is of interest to note that the administration of neomycin to S.O. rats was associated with a significant

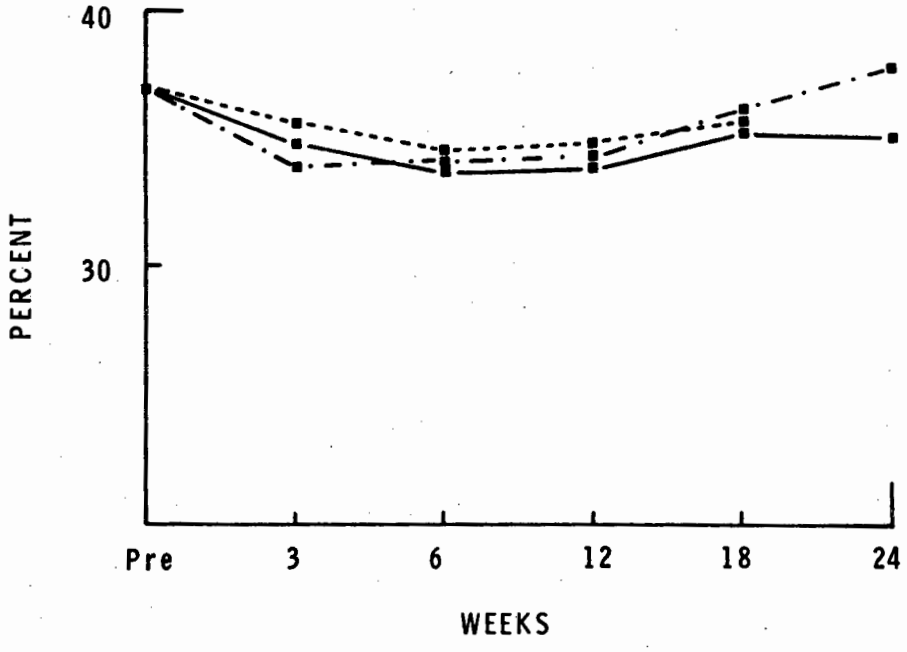

Fig. 24. Mean serum albumin levels in P.C.S. (———) rats and in animals with partially occluded (— — —) and totally occluded (——·——) shunts, expressed as a % of the total protein. - Experiment 3.

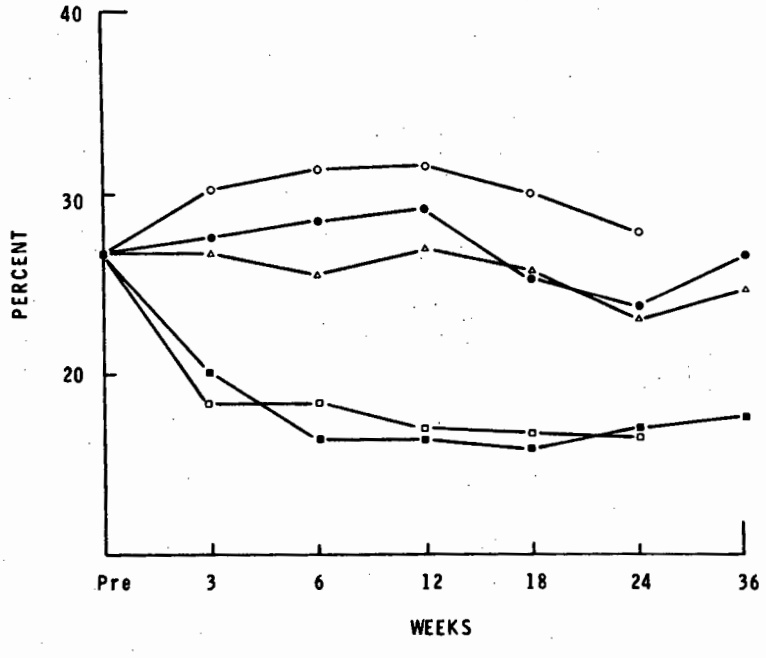

Fig. 25. Mean serum alpha-I globulin levels in P.C.S. (■——■), S.O. (●——●) and N.C. (△——△) rats, expressed as a % of the total protein. Open symbols represent neomycin-treated P.C.S. and S.O. animals. - Experiment 3.

rise in alpha-1 globulins which persisted for 12 weeks (Table 50). Serum alpha-1 globulins in the 2 groups of P.C.S. animals were significantly lower than levels in the control animals.

iii) Serum alpha-2 globulins : As in Experiment 2, the serum alpha-2 globulins in P.C.S., S.O., S.N. and N.C. rats did not deviate significantly from the pooled pre-operative level (Tables 51, 52, 53, and 54). There were also no significant differences in alpha-2 globulin levels between rats with patent anastomosis, partially occluded P.C.S., totally occluded shunts and S.O. animals (Tables 55 and 56). The administration of neomycin to P.C.S. rats was associated with an initial significant increase ($p < 0.05$) in alpha-2 globulin level which then fluctuated for the duration of the experiment (Table 57).

iv) Serum beta-globulins : In the P.C.S. rat the serum beta-globulins increased and this rise in beta-globulin level achieved statistical significance 36 weeks post-operatively (Fig. 27) (Table 58). The administration of neomycin to the P.C.S. rat had no effect on serum beta-globulin levels (Table 59). Fluctuations in beta-globulin levels were minimal in rats with totally occluded and partially occluded P.C.S. (Fig. 28) (Tables 60 and 61). No significant differences were detected in beta-globulin levels between rats with fully patent anastomosis, partially occluded P.C.S., totally occluded P.C.S. and S.O. animals. The changes in beta-globulin levels in N.C., S.O. and S.N. rats were neither marked nor statistically significant (Tables 62, 63 and 64). No statistically significant differences were detected in beta-

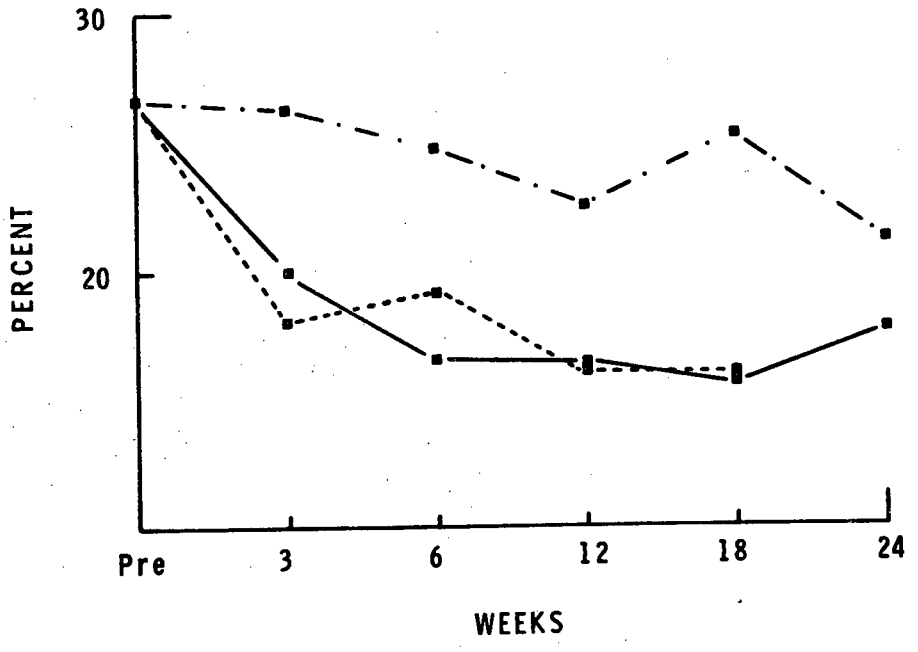

Fig. 26. Mean serum alpha-I globulin levels in rats with patent (———), partially occluded (— — —) and totally occluded (——•——) P.C.S., expressed as a % of the total protein. Experiment 3.

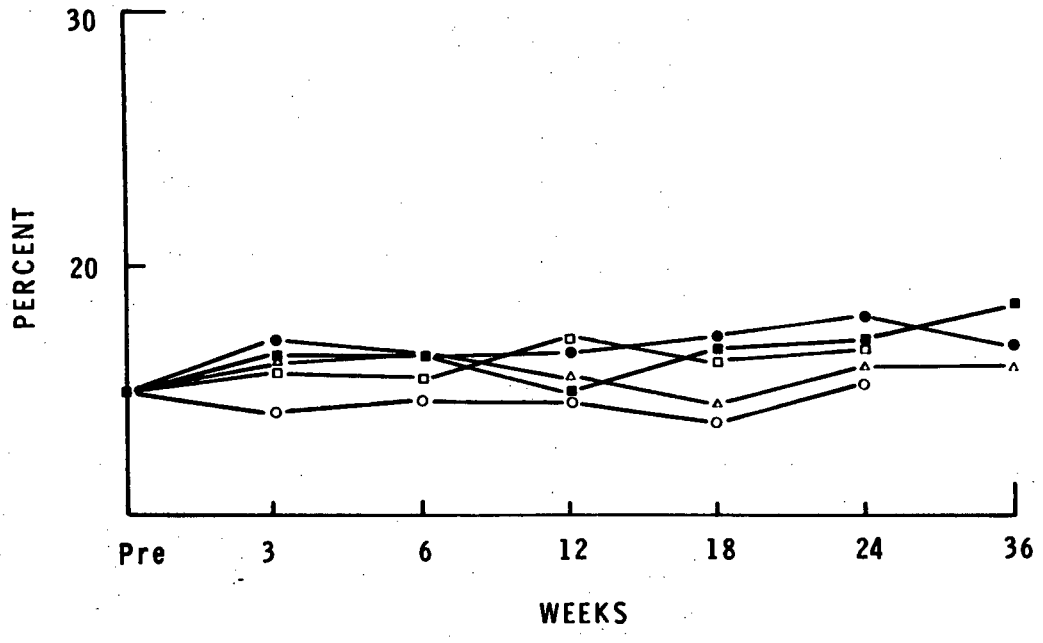

Fig. 27. Mean serum beta-globulin levels in P.C.S. (■——■), S.O. (●———●) and N.C. (△———△) rats, expressed as a % of the total protein. Open symbols represent neomycin-treated P.C.S. and S.O. rats. Experiment 3.

globulin levels between P.C.S. rats and controls.

v) Serum gamma-globulins : In P.C.S. rats a similar rise in the mean gamma-globulin level, which was sustained throughout the study, was observed (Fig. 29). This sustained rise remained significant for the duration of the study (Table 65). The administration of antibiotic appeared not to affect the serum gamma-globulin level in the first 12 post-operative weeks but from 12 weeks onwards an overall decline in gamma-globulin level was seen in P.C.S. animals treated with neomycin (Table 66). This decline was of sufficient magnitude to cause the levels to drop significantly below that seen in untreated P.C.S. animals 18 weeks post-operatively ($p < 0.05$). The mean serum gamma-globulin level in P.C.S. rats treated with neomycin remained significantly higher than the pre-operative value throughout the experiment ($p < 0.001$). No significant differences were detected in the serum gamma-globulin levels between rats with fully patent anastomosis and animals with partially occluded P.C.S. (Fig. 30) (Table 67). By contrast, the rise in serum gamma-globulin levels in rats with totally occluded P.C.S. was of a much lower magnitude than those recorded in rats with fully patent or partially occluded anastomosis (Table 68). Consequently the differences in serum gamma-globulin levels between rats with totally occluded P.C.S., on the one hand, and animals with fully patent or partially occluded shunts on the other, were statistically significant throughout the study. In N.C. and S.O. rats, minimal variation in the serum gamma-globulin was seen and in sham rats treated with neomycin the gamma-globulin level was also depressed (Tables 69, 70 and 71).

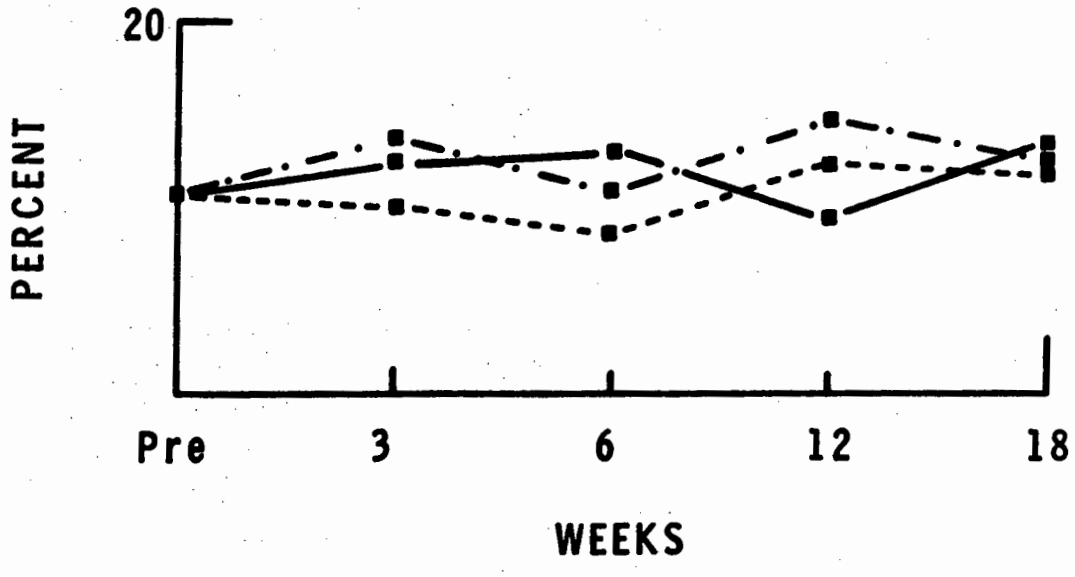

Fig. 28. Mean serum beta-globulin levels in rats with patent (————), partially occluded (— — —) and totally occluded (——·——) P.C.S., expressed as a % of the total protein. - Experiment 3.

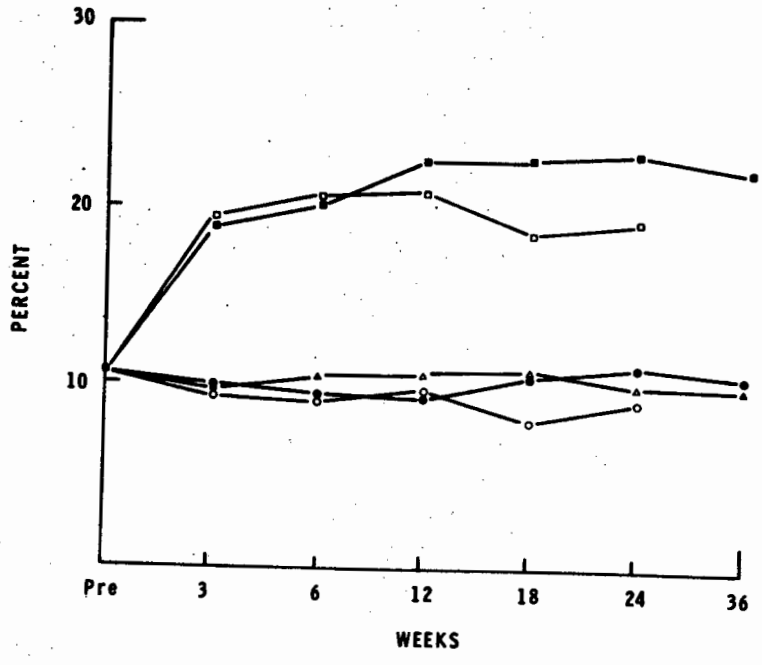

Fig. 29. Mean serum gamma-globulin levels in P.C.S. (■——■), S.O. (●——●) and N.C. (△——△) rats, expressed as a % of the total protein. Open symbols represent neomycin-treated P.C.S. and S.O. rats. - Experiment 3.

D. Experiment 4 (zero time to 6 weeks - splenectomy in P.C.S. and S.O. rats)

i) Albumin : In the P.C.S. rat subjected to splenectomy an acute decrease in the serum albumin was seen (Fig.31). This decrease in albumin, although of greater magnitude than those recorded in P.C.S. rats, was essentially similar to that seen in Experiments 2 and 3. Serum albumin in the splenectomised animals showed a tendency to return to pre-operative levels earlier than P.C.S. animals so that by the end of the experiment, i.e. 6 weeks post-operatively serum albumin levels had returned to the pre-operative level (Table 72). This was in direct contrast to the expected and persistent low serum albumin levels which were seen to follow P.C.S. alone. The difference between splenectomised P.C.S. and P.C.S. animals was not significant.

In S.O. rats (Table 73) subjected to splenectomy a decrease in mean serum albumin occurred similar to that seen in Experiments 2 and 3 but it is of interest that the magnitude of the decrease was smaller in the splenectomised animals (6.14% in splenectomised animals compared with 9.65% in S.O. rats in the Experiment 2). The differences in the mean serum albumin level between S.O. rats and sham controls subjected to splenectomy were, with the exception of the first post-operative week, statistically significant throughout. No significant differences were detected in albumin levels between P.C.S. and S.O. animals subjected to splenectomy.

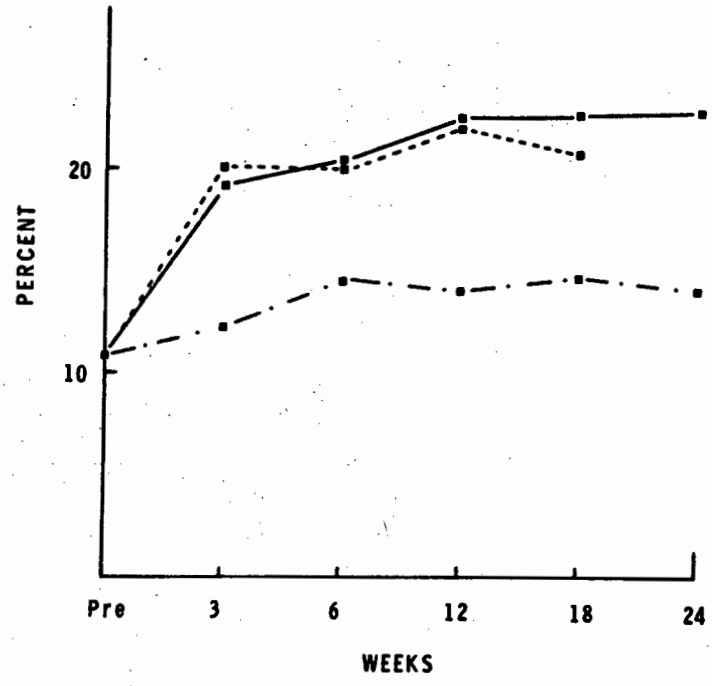

Fig. 30. Mean serum gamma-globulin levels in rats with patent (———), partially occluded (— — —) and totally occluded (—— • ——) P.C.S., expressed as a % of the total protein.- Experiment 3.

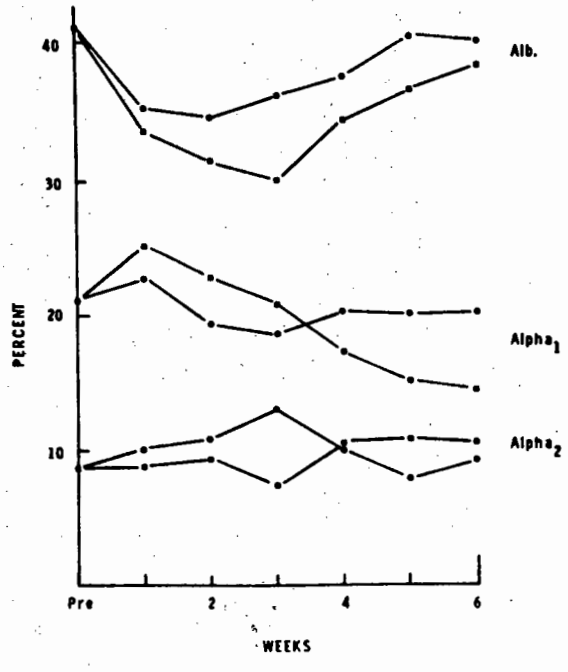

Fig. 31. Mean serum albumin, alpha-I and alpha-2 globulin levels in P.C.S. (■——■) and S.O. (●——●) rats subjected to splenectomy, expressed as a % of the total protein. - Experiment 4.

ii) Serum alpha-1 globulins : Serum alpha-1 globulin levels in P.C.S. animals subjected to splenectomy paralleled those seen in Experiments 2 and 3 and the levels in S.O. rats subjected to splenectomy did not change significantly during the course of the experiment (Fig. 31) (Tables 74 and 75). Six weeks post-operatively serum alpha-1 globulin levels in the splenectomised P.C.S. rats were significantly lower ($p < 0.005$) than those seen in S.O. animals subjected to splenectomy.

iii) Serum alpha-2 globulins : Serum alpha-2 globulin levels in P.C.S. rats subjected to splenectomy did not differ significantly from the pre-operative level (Fig. 31) (Table 76). In this respect, the behaviour of alpha-2 globulins was not different from those observed in Experiments 2 and 3. No significant differences were detected in serum alpha-2 globulin levels between P.C.S. and S.O. rats (Table 77) subjected to splenectomy.

iv) Serum beta-globulins : Serum beta-globulin levels in P.C.S. rats subjected to splenectomy did not show any significant variations from the pre-operative level and resembled those seen in P.C.S. rats (Fig. 32) (Table 78). In contrast, the level of serum beta-globulins in S.O. rats subjected to splenectomy did rise during the first 2 weeks post-operatively ($p > 0.05$) and then returned to normal (Table 79). The differences in serum beta-globulins between P.C.S. and S.O. rats subjected to splenectomy were not statistically significant throughout the study.

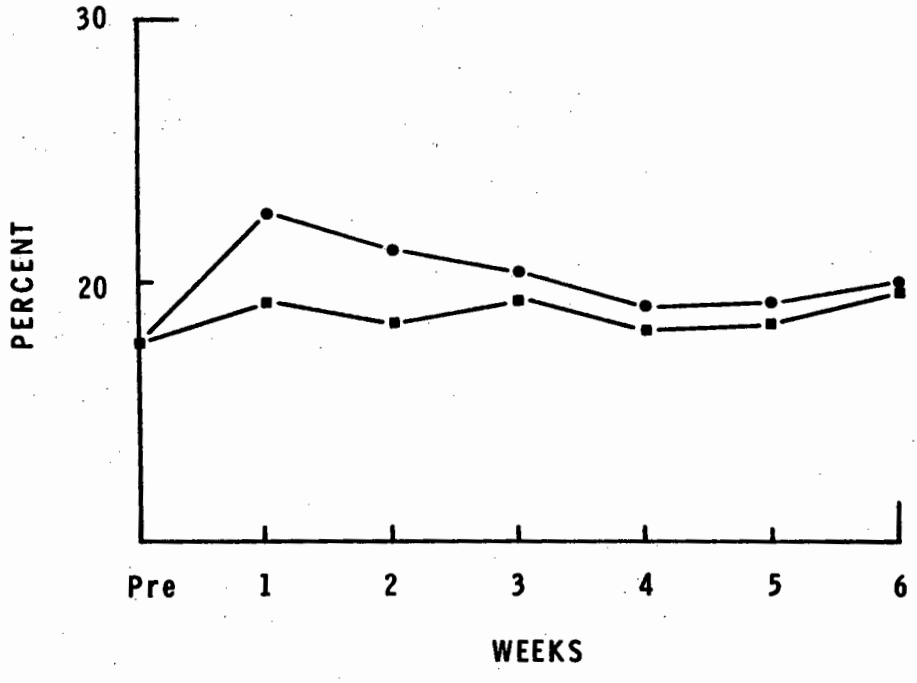

Fig. 32. Mean serum beta-globulin levels in P.C.S. (■——■) and S.O. (●——●) rats subjected to splenectomy, expressed as a % of the total protein. - Experiment 4.

v) Serum gamma-globulins : A significant rise in serum gamma-globulin levels was seen following splenectomy in P.C.S. rats (Fig. 33) (Table 80). This rise was sustained for the duration of the experiment and of comparable magnitude to the increase in serum gamma-globulin levels recorded in P.C.S. rats in Experiments 2 and 3. In contrast to these changes were the minor variations in gamma-globulin level which were seen in S.O. rats subjected to splenectomy. Following splenectomy gamma-globulin levels in P.C.S. rats were higher than those recorded in S.O. rats subjected to splenectomy and this difference achieved statistical significance 2 weeks post-operatively.

E. Experiment 8 (Pair-fed P.C.S. rats bled 2 weeks post-operatively)

There were no significant differences in the total serum protein concentrations between pair-fed P.C.S., S.O. and N.C. rats 2 weeks post-operatively (Tables 81, 82 and 83).

The mean serum albumin levels in pair-fed N.C. rats were higher than those recorded in P.C.S. and S.O. animals but the differences were not statistically significant (Fig. 34). The differences in alpha-1 globulin, alpha-2 globulin and beta-globulin levels between the 3 groups of animals were not statistically significant despite the fact that the alpha-2 globulin level in S.O. rats was higher than in the other 2 groups. Serum gamma-globulin levels in pair-fed P.C.S. rats were significantly higher ($p < 0.005$) than those recorded in the 2 control groups.

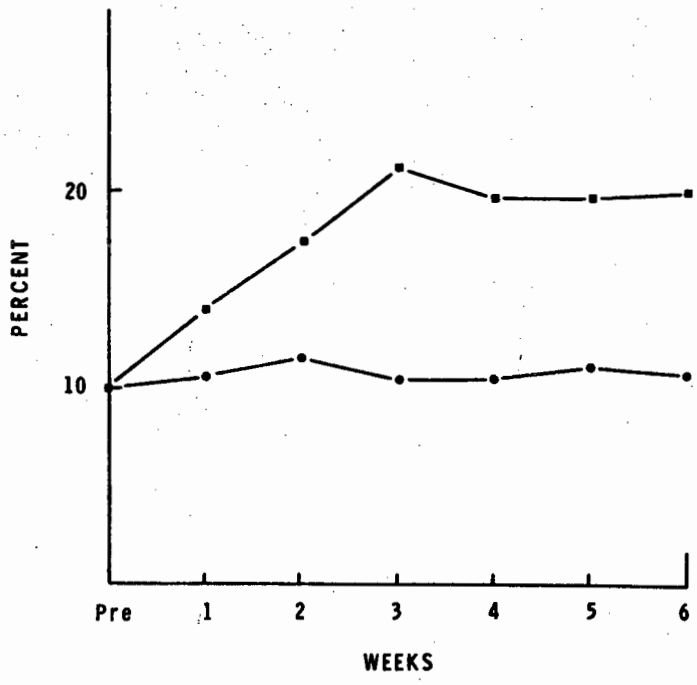

Fig. 33. Mean serum gamma-globulin levels in P.C.S. (■——■) and S.O. (●——●) rats subjected to splenectomy, expressed as a % of the total protein. - Experiment 4.

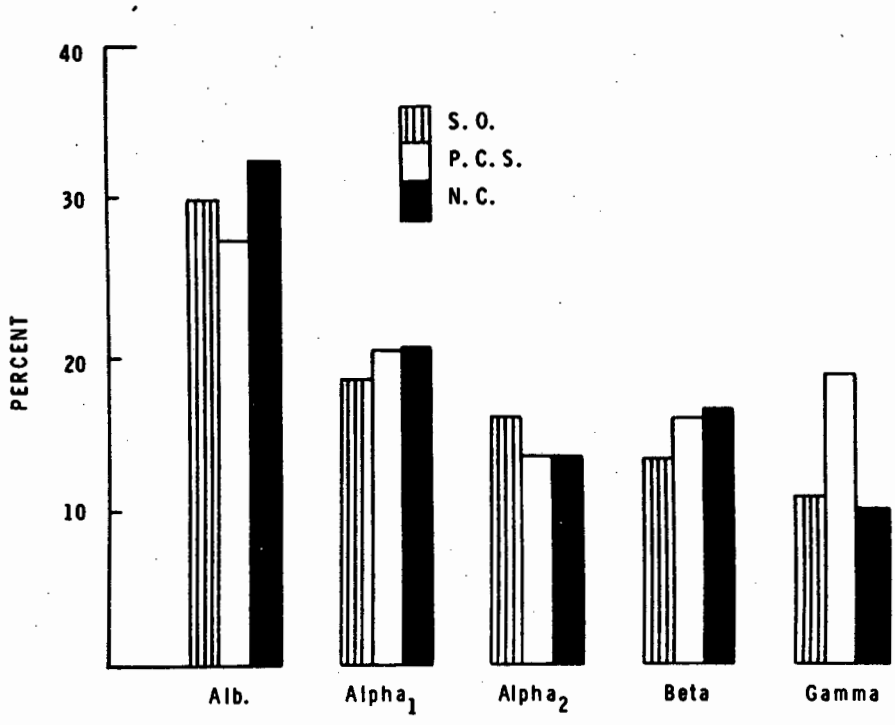

Fig. 34. Mean serum albumin, alpha-I, alpha-2, beta- and gamma-globulin levels in pair-fed P.C.S., S.O. and N.C. rats, expressed as a % of the total protein. - Experiment 8.

F. Experiment 9 (Pair-fed P.C.S. rats bled 7 weeks post-operatively)

Total serum protein concentrations in pair-fed P.C.S., S.O. and N.C. rats were not significantly different (Tables 84, 85 and 86).

i) Serum albumin : Serum albumin levels in P.C.S., S.O. and N.C. rats did not differ significantly (Fig. 35). The serum albumin level increased in all 3 groups of animals, and statistically significantly in the case of the P.C.S. groups ($p < 0.005$), when the period of pair-feeding was extended from 2 to 7 weeks.

ii) Serum alpha-1 globulin : Serum alpha-1 globulin levels in the pair-fed P.C.S. rat was significantly lower than those recorded in S.O. ($p < 0.05$) and N.C. ($p < 0.02$) animals (Fig. 35). In P.C.S. and N.C. rats, pair-feeding for an additional 5-week period produced lower levels of alpha-1 globulins.

iii) Serum alpha-2 globulins : Alpha-2 globulin levels in P.C.S., S.O. and N.C. rats were not significantly different (Fig. 35). In the S.O. rat, lower levels of alpha-2 globulins were found in the 7-week pair-feeding experiment aa compared with the 2-week pair-feeding experiment.

iv) Serum beta-globulins : There were no statistically significant differences in the beta-globulin levels in P.C.S., S.O. and N.C. rats and the changes from

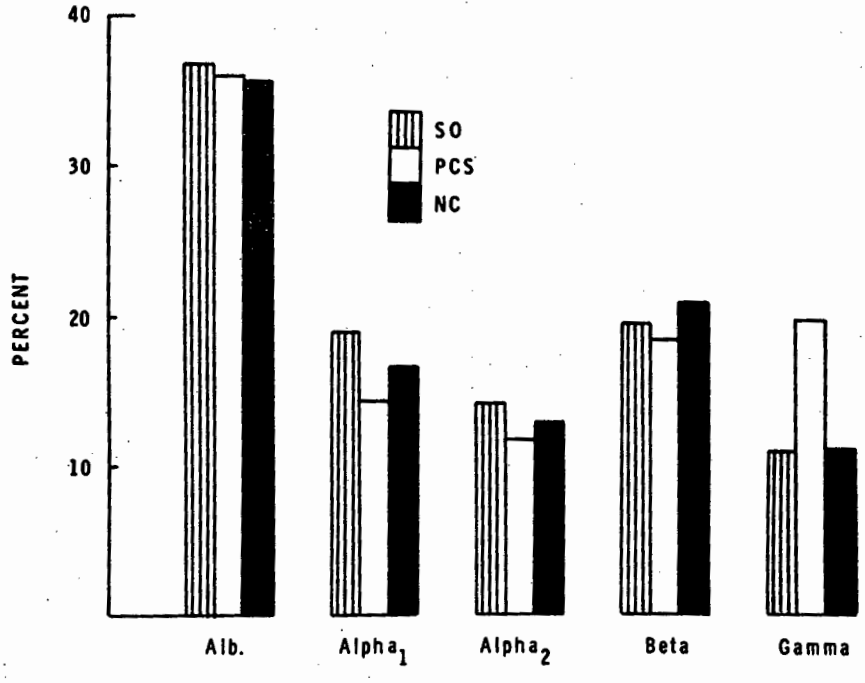

Fig. 35. Mean serum albumin, alpha-I, alpha-2, beta- and gamma-globulin levels in pair-fed P.C.S., S.O. and N.C. rats, expressed as a % of the total protein. - Experiment 9.

their respective values recorded after 2 weeks of pair-feeding, were minimal.

v) Serum gamma-globulins : Mean serum gamma-globulin levels in pair-fed P.C.S. rats were significantly higher ($p < 0.005$) than those recorded in S.O. and N.C. rats (Fig. 35). The gamma-globulin levels in P.C.S. rats increased slightly and those in S.O. and N.C. rats decreased minimally over an additional 5-week period of pair-feeding.

G. Experiment 10 (serum protein fractions in P.C.S. pigs)

The serum albumin level in the pig decreased significantly ($p < 0.001$) following P.C.S. (Fig. 36) (Table 87). Changes in alpha-1, alpha-2 and beta-globulins were minimal and did not deviate significantly from the pre-operative levels.

The serum gamma-globulin level rose significantly after P.C.S. in the pig ($p < 0.01$). The rise in gamma-globulin level in the P.C.S. pig corresponds to a 48% increase above the pre-operative level and was not as impressive as the increase in gamma-globulins subsequent to the diversion of portal venous blood in the rat.

Serum IgG Concentrations in Experiment 2

Serum IgG concentrations increased significantly ($p < 0.001$) 48 hours after P.C.S. in the normal rat (Table 88).

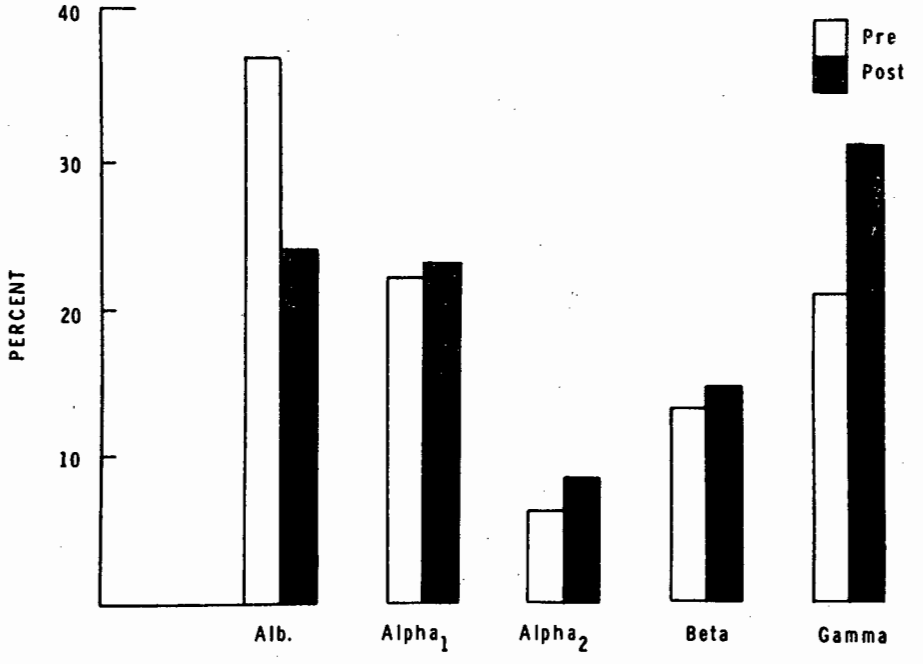

Fig. 36. Mean serum albumin, alpha-I, alpha-2, beta- and gamma-globulin levels in pre- and post-P.C.S. pigs, expressed as a % of the total protein. - Experiment 10.

This elevated IgG level was sustained for the duration of the experiment (Fig. 37). Maximum serum IgG activity was detected 4 weeks post-operatively. This increase represents a 59% rise over the initial value.

In S.O. and N.C. rats the IgG concentration did not deviate significantly from the initial value (Tables 89 and 90). From the 3rd post-operative week, the serum IgG concentration in P.C.S. rats was significantly higher than levels recorded in S.O. and N.C. rats ($p < 0.02$) and a similar level of significance was maintained throughout the period of study.

Serum IgM Concentrations in Experiment 2

There was a striking and statistically significant ($p < 0.001$) rise in serum IgM levels after P.C.S. in the normal rat and this level of significance was sustained throughout the 6-week period of study (Fig. 38) (Table 91). Subsequent evidence showed that this trend was maintained for 12 weeks post-operatively and that a maximum IgM activity, which was equivalent to a 112% increase above the initial value, was attained post-operatively.

In S.O. and N.C. rats the serum IgM concentration did not deviate significantly from the mean pre-operative level (Tables 92 and 93). Serum IgM concentrations in P.C.S. rats were significantly higher throughout than levels recorded in S.O. and N.C. animals ($p < 0.001$).

Serum IgA Concentrations in Experiment 2

The IgA level increased rapidly after P.C.S. in the rat (Fig. 39). Following the rise the IgA levels fluctuated and the initiation of a more gradual increase in serum IgA concentration was observed thereafter. No serum IgG was detected in some P.C.S.

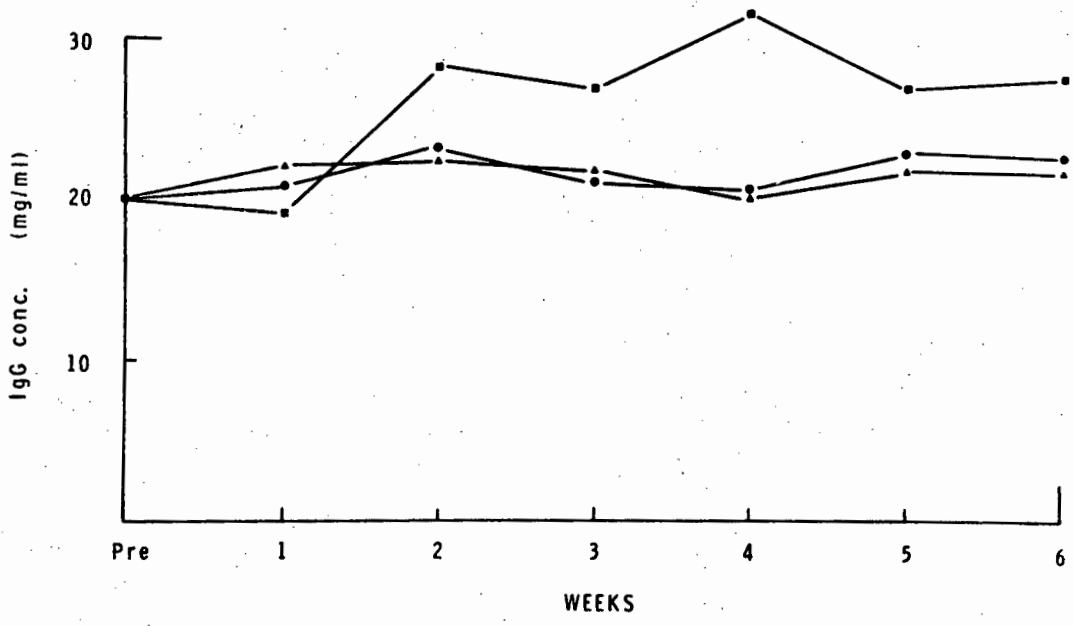

Fig. 37. Mean serum IgG levels in P.C.S. (■——■), S.O. (●——●) and N.C. (▲——▲) rats. - Experiment 2.

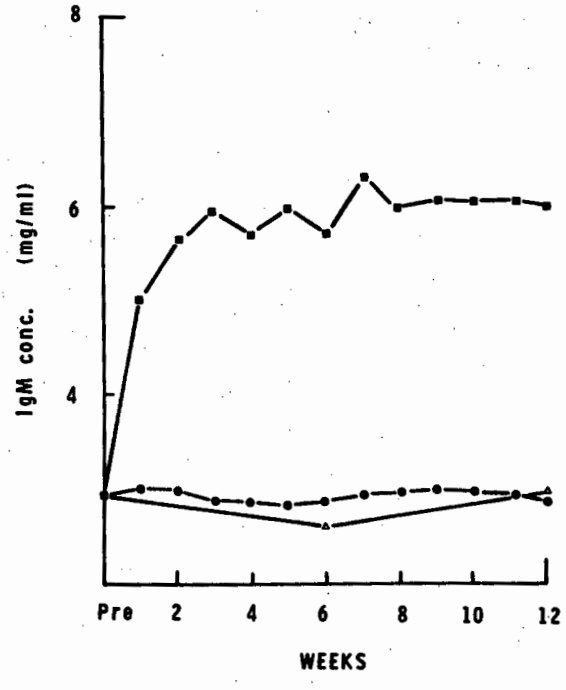

Fig. 38. Mean serum IgM levels in P.C.S. (■——■), S.O. (●——●) and N.C. (△——△) rats. Experiment 2.

rats. Similarly, attempts to detect serum IgA in pre-operative P.C.S. rat serum samples were also without success. No serum IgA was detected in S.O. and N.C. rats throughout the period of study.

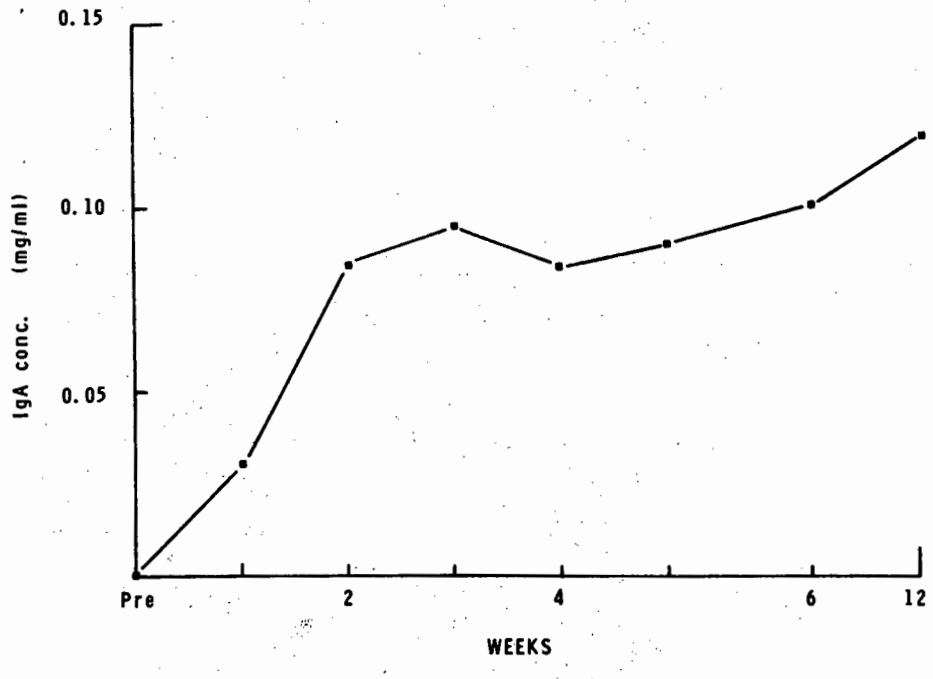

Fig. 39. Mean serum IgA levels in P.C.S. rats. Experiment 2.

DISCUSSION

A significant drop in total serum protein concentration was observed one week after P.C.S. in the rat. Following this rapid depletion, the serum protein levels increased gradually and 12 weeks post-operatively were within the normal range. A consistent decrease in serum albumin was detected following P.C.S. in rats (Experiments 1, 2, 3 and 4) and the pre-operative level was only regained 18 weeks post-operatively. Serum alpha-1 globulin levels were significantly depressed in P.C.S. rats whereas minor fluctuations were detected in serum alpha-2 globulins. A slight increase in beta-globulins was detected following portal shunting in rats. Serum gamma-globulins increased significantly and consistently in the P.C.S. rat and these elevated levels were sustained for up to 36 weeks.

A decrease in total serum protein concentration is a frequent accompaniment of surgical trauma in man (Wollheim et al., 1969; Aronsen et al., 1972) and it is also a consequence of experimental injury in rats (Neuhaus et al., 1961; Owen, 1967). The decreased levels of total serum proteins which persisted for 5 weeks in P.C.S. rats in this study may be due to restricted food intake since experimental protein restriction in rats has been shown to cause a significant drop in total protein concentration (Metcoff et al., 1948; Weimer, 1959; Weimer, 1961). It was of interest to note that 6 weeks post-operatively, when the P.C.S. rat started to gain weight and food intake apparently improved, total serum protein levels increased to within the normal range and that this trend was still evident 12 weeks post-operatively. The utilisation of dietary protein for energy purposes as well as the diversion of splanchnic blood containing amino acids essential for protein synthesis may also be implicated in the reduced

total serum protein levels in P.C.S. rats.

The effect of weekly bleeding of 0.5-1 ml of blood also needs to be considered as contributing to a fall in total protein. Such weekly bleedings can be estimated to have depleted the experimental animals of 35-70 mg of protein per week. It is probably that it contributed little to the S.O. and N.C. animals where serum protein levels remained constant but its effects in the P.C.S. animal need to be considered. Similar reduction in serum proteins have been reported in P.C.S. dogs (Doberneck et al., 1963).

It has been suggested that the drop in serum protein level following experimental protein depletion in rats may be due to a decrease in serum albumin concentration (Metcoff et al., 1948). It has also been postulated that serum albumin serves as the primary source of amino acids for the synthesis of other body proteins in rats fed a low protein diet (Jeffay and Winzler, 1958). Studies on experimental protein calorie malnutrition in rats supports the view that protein malnutrition leads to a decrease in serum albumin (Kirsch et al., 1968).

A consistent finding in all the experiments on P.C.S. has been a significant reduction in albumin in the P.C.S. rat (Experiments 1, 2, 3, 8 and 9). Decreased levels of serum albumin were repeatedly found in P.C.S. rats and this supports the data of Kennan (1964) who reported a mean serum albumin level of 31.2% in P.C.S. rats compared with 42.9% in normal control animals. Similar significant reductions in serum albumin were found in P.C.S. pigs (Table 87) and are in agreement with other reported studies (Hickman et al., 1974; Cuschieri et al., 1974). P.C.S. in the normal dog (Bollman, 1961; Doberneck et al., 1963; Kirsch et al., 1965) has been shown to produce a decrease in serum albumin. The decrease in serum albumin levels following P.C.S.

is so consistent in these studies that it may be thought of as a characteristic of portal diversion in experimental animals.

In broad principle low levels of serum albumin may be due to deficient food intake, liver disease or excessive loss. Several workers have drawn attention to decreased food intake following P.C.S. in rats (Rubin et al., 1965; Kyu and Cavanagh, 1970; Assal et al., 1971). Although measurement of food intake was not undertaken in these studies the data on pair-feeding of P.C.S. rats (Experiments 8 and 9) support the view that food intake is reduced in P.C.S. rats and, as a consequence of this, serum albumin levels are decreased since albumin levels are a sensitive index of nutritional status. The reasons for reduced food intake in the P.C.S. rat over several weeks are not clear but attention should be drawn to the work of Bull et al., (1974) on the suppressive effect of oestrogenic hormones on appetite in rats. In this regard it is of interest that Reichle et al. (1969) have reported an accumulation of oestrogenic compounds in the P.C.S. rat. An effect of P.C.S. on the putative hypothalamus-hepatic satiety axis (Schmitt, 1973) is also possible.

The central and vital role which the liver plays in albumin synthesis has been well documented (Müting and Reikowsky, 1965; Rothchild et al., 1972). In these studies portal diversion has been shown to produce liver necrosis which is, however, short-lived (Experiment 1) and no significant morphological alterations were seen in the long-term study (Experiment 3). The preservation of normal liver histology argues against overt liver disease as an important factor in the genesis of hyperglobulinaemia. Hepatic resection in man (Wollheim et al., 1969) leads to a decrease in serum albumin presumably as a result of decreased liver cell mass. The possibility that portal diversion and the consequent hepatic arterial compensation

may lead to a relatively deficient perfusion in areas of liver, remains speculative and will be difficult to prove. Such a theoretical mal-perfusion of liver might be expected to reduce serum albumin but this is perhaps more theoretical than real in view of the relatively well-preserved liver architecture in the long-term study.

Studies on liver weight showed that a degree of liver atrophy occurs after P.C.S.. What contribution this makes to the decreased serum albumin levels can only be speculative. Excessive loss of serum albumin may occur as a result of increased catabolism or loss into tissue fluids. A decrease in serum albumin is well documented following experimental injury in rats and probably represents one of the many complex facets of the metabolic response to injury. In P.C.S. rats the loss in serum albumin found in Experiment 1 could be attributed to the metabolic consequence of trauma but the long continued changes (Experiments 2 and 3) argue against this possibility.

Hypo-albuminaemia is well recognised following bacterial infection in man (Peterman, 1961). In the P.C.S. rat, no direct evidence of bacterial infection was seen or looked for but in view of the dramatic increase in anti-L.P.S. antibodies, continuing low-grade bacteraemia remains a possible factor in the genesis of the low serum albumin. If such a putative bacterial infection is accepted it could be expected to operate for the first 6 weeks following P.C.S., and to be contained thereafter since the albumin levels begin to return to normality after this period.

The loss of albumin to tissue fluids has been documented with gastric mucosal disease (Citrin et al., 1957; Tarver et al., 1961). Erosive gastric mucosal changes have been reported in the P.C.S. rat (Day et al., 1963). Gastric hypersecretion associated with elevated

histamine levels are also well known in the P.C.S. rat (Day et al., 1963; Fischer and Snyder, 1965). These aspects have not been studied as part of this thesis and their contribution to the serum albumin changes is unknown. The loss of albumin to the wound oedema which has been suggested as operative in the hypo-albuminaemia following surgery in humans (Mouridsen, 1967; Werner, 1969) is also conjectural in the P.C.S. rat. A small daily loss of albumin into the faeces can be inferred from studies with I^{131}-labelled albumin (Tarver et al., 1961). What contribution this made in the P.C.S. rat is also unknown because no information exists on the physiological alteration which may occur in the bowel following P.C.S. in the rat. No literature exists on pathological changes in the small or large bowel following portal shunting in the rat and this was not looked for in these studies.

The behaviour of alpha-1, alpha-2 and beta-globulins in the immediate post-operative period following experimental procedures in rats (Owen, 1967; Neuhaus et al., 1961; Jamieson and Ashton, 1973) and surgery in humans (Peterman, 1961; Werner, 1969; Wollheim et al., 1969) is well documented and an extensive literature exists on the subject. In the acute study (Experiment 1) alpha-1 globulin levels in both P.C.S. and S.O. rats were significantly elevated while minimal changes were detected in alpha-2 and beta-globulin levels.

Limited data exist on the long-term effects of P.C.S. on serum alpha-1, alpha-2 and beta-globulin levels in the rat. Kennan (1964) reported no change in alpha-1 globulin levels, a slight increase in alpha-2 globulins (from 6.0% in normal animals to 9.4% in P.C.S. rats) and a reduction in beta-globulin levels (28.6% in normal rats compared with 18.8% in portal-shunted animals) 6 months after P.C.S. in the rat. In this thesis reduced alpha-1 globulin levels were recorded which persisted for 9 months following P.C.S. in the normal rat and this data is at variance with the observations of Kennan.

Whereas the latter reported a drop in serum beta-globulin levels in P.C.S. rats, a steady rise in the serum protein fraction was recorded in these studies. Differences in electrophoretic techniques may account for some of these discrepancies. Kennan, for example, electrophoresed rat serum samples on filter paper for 16 hours at 2.5 V/cm. In these studies the electrophoretic separation of rat serum protein fractions was achieved within 30 minutes. As shown earlier the utilisation of different electrophoretic techniques can result in significantly different levels of serum protein fractions from the same serum sample.

In these studies no significant change in serum gamma-globulin was detected in the 4 days after P.C.S. in the normal rat (Experiment 1). A significant rise in serum gamma-globulin level was seen in P.C.S. rats 7 days post-operatively and this trend was maintained to the 3rd post-operative week when maximum gamma-globulin levels were recorded in the experimental animals. The elevated levels of gamma-globulin were sustained for the duration of the study (Experiment 2). The rise represented a two-fold increase over the initial gamma-globulin level. The early rise in serum gamma-globulins confirms the data of Bismuth et al. (1964) who reported a significant rise in serum gamma-globulin levels 2 weeks after P.C.S. in the normal rat. Similar levels of serum gamma-globulins were seen in P.C.S. rats subjected to splenectomy (Experiment 4) and in pair-fed P.C.S. rats (Experiments 8 and 9).

The serum gamma-globulins followed a similar trend in the long-term study (Experiment 3) and significantly elevated levels were detected 36 weeks post-operatively. This data supports the findings of Kennan (1964) who detected elevated levels of gamma-globulin 6 months post-operatively in P.C.S. rats. The administration of neomycin to the portal-shunted animals had no immediate effect on the serum gamma-globulin level. The continued administration of neomycin to P.C.S.

rats 18 weeks post-operatively associated with a drop in serum gamma-globulin which permits of no ready explanation.

Hyperglobulinaemia was a consistent finding after P.C.S. in the normal rat and in this respect resembles changes in weight which were regarded as a reliable index of shunt patency. The difference between these parameters, however, is in their disparity in the long-term i.e. weight is regained but gamma-globulins remain elevated. Hyper-globulinaemia was also associated with P.C.S. in the pig (Experiment 10) and this data supports the findings of Hickman et al. (1974).

The elevation of serum gamma-globulin levels in human liver disease seems to be the result of increased synthesis rather than a decrease of catabolism (Eisenmenger and Slater, 1953; Havens et al., 1954; Cohen, 1963). Attention has already been drawn to the fact that experimental evidence tends to suggest that the hyperglobulinaemia of chronic liver disease is not due to a reciprocal rise in serum gamma-globulins consequent upon hypo-albuminaemia. Hoffenberg et al. (1966) reported significantly decreased levels of serum albumin following repeated plasmapheresis in rabbits but no changes in serum gamma-globulins were detected. Similarly, Kirsch et al. (1968) reported hypo-albuminaemia without gamma-globulin elevation in protein calorie malnutrition in rats. It may therefore be suggested that the hyperglobulinaemia following P.C.S. in the rat is also not directly due to a decrease in albumin. Evidence in support of this was derived from data on pair-fed rats where gamma-globulin levels were elevated in pair-fed P.C.S. rats only and not in S.O. and N.C. animals despite the similarity in albumin levels in all 3 groups. Similarly, when the serum albumin levels started to increase in P.C.S. rats 18 weeks post-operatively (Experiment 3) no corresponding drop in serum gamma-globulins was detected.

The possibility of immunological factors operating in the genesis of the hyperglobulinaemia deserves attention because :

i) the rise is in gamma-globulins and, to a lesser extent, in beta-globulins;

ii) the rise in gamma-globulins has the time sequence of an immunological response.

Chronic liver disease in man, irrespective of its aetiology, is generally associated with a polyclonal increase in gamma-globulins (Law et al., 1966; Eliakim et al., 1972; Finlayson, 1972). A similar polyclonal hyperglobulinaemia in the P.C.S. rat has been repeatedly shown in these studies and in Experiment 2 elevated IgM, IgG and to a lesser extent, IgA levels have been detected. The immune response to bacterial L.P.S. is usually characterised by a prolonged synthesis of IgM (Protell et al., 1971; Möller et al., 1973) and L.P.S. antibodies are generally confined to the IgM antibody class (Britton and Möller, 1968; Friedman, 1973). Studies on repeated L.P.S. stimulation (Experiment 12) tend to support this, and in the serological studies with mercaptoethanol (Experiment 14) and Sephadex fractionation (Experiment 13) this has again been confirmed.

The purity of the IgM preparation was confirmed on ultracentrifugation and a single peak, with a sedimentation co-efficient of 18.25, was isolated (Fig.65 APPENDIX VIII). The detection of elevated levels of serum IgM in P.C.S. rats lends further credence to the view that antibacterial L.P.S. antibody production played an important role in the genesis of hyperglobulinaemia in the P.C.S. rat. Increased levels of IgM may be due to increased production or decreased catabolism. The rate of IgM catabolism was not investigated in these studies but sufficient evidence has been presented in support of increased production.

A rise in serum IgG levels has also been shown in these studies after portacaval shunt and supports the data of Benjamin et al. (1974). The mechanism for this rise is not clear but it may represent the response to other antigenic constituents of bacteria, antigenic material from food or an anamnestic/adjuvant response to the absorption of L.P.S. No attempt was made to define the antigenic stimulus which led to the increase of IgG, but it would seem to be unrelated to liver cell disease in view of the normal morphological status of the P.C.S. rat liver in the long-term.

Of the three major immunoglobulin classes in the mammalian system, IgA in the normal rat is present in the lowest concentration (Basin et al., 1974). The studies on IgA (Experiment 2) confirmed the consistently lower levels of this immunoglobulin when compared with IgM and IgG. A rise in IgA was neither large nor consistent when compared with the changes in IgM and IgG. Despite the apparent purity and monospecificity of the IgA preparation on immuno-electrophoresis, five components with the following sedimentation co-efficients were demonstrated on ultracentrifugation (Fig.66APPENDIX VIII) :

14.46S, 12.0S, 7.6S, 5.6S and 3.0S.

The 14.46S and 12.0S components probably represent polymerisation of the IgA molecule while the 5.6S and 3.0S components may be due to the dissociation of IgA.

No data exist on the effect of neomycin on serum proteins and, in particular, on immunoglobulin levels in rats. The oral administration of neomycin leads to alterations of the jejunal mucous membrane (Thompson et al., 1971) and results in a malabsorption. The effect of jejunal abnormality is to reduce its permeability to molecules which

ordinarily traverse it with ease (Jacobson et al., 1960). The administration of neomycin to P.C.S. rats had no significant effect on the gamma-globulin level in these animals. Studies, however, have shown that the dose of neomycin used in all the experiments had no effect on the bowel flora of rats.

PART 3

Antibodies

ANTIBACTERIAL L.P.S. ANTIBODIES

I. Antibody titres to purified L.P.S.

In these series of serological experiments the influence of the purity of the antigen on the final antibody titre was investigated. Because of the small volumes of sera available, a comparison of anti-bacterial L.P.S. antibody titres to partially purified antigen, and to purified antigen was only obtained in selected sera 3 and 6 weeks after P.C.S.

Three weeks after P.C.S. on the normal rat the mean log antibody titre to purified E. coli (sucrose positive) L.P.S. was 0.262 ± 0.142 compared with a log titre of 0.836 ± 0.217 in the same sera using partially purified E. coli (sucrose positive) L.P.S. This difference was statistically significant ($p < 0.005$). Similar findings were obtained in the 6-week sera where the mean log antibody titre to purified E. coli (sucrose positive) L.P.S. was 0.285 ± 0.171 compared with a log titre of 0.933 ± 0.360 in the same sera using partially purified L.P.S. The difference was also statistically significant ($p < 0.001$). In the light of these findings an experiment was designed to determine the effect of concentration of purified L.P.S. on anti-L.P.S. antibody titres.

Experiment 11

Ten P.C.S. rat sera with known anti-L.P.S. antibody titres were selected at random and anti-E. coli (sucrose positive) L.P.S. antibody

activity were sought to :

A. purified E. coli (sucrose positive) L.P.S. at a concentration of 10 mg/3.2 ml

B. purified E. coli (sucrose positive) L.P.S. at a concentration of 20 mg/3.2 ml

C. purified E. coli (sucrose positive) L.P.S. at a concentration of 30 mg/3.2 ml

D. partially purified E. coli (sucrose positive) L.P.S. at a concentration of 10 mg/3.2 ml.

The following results were obtained :

Mean log antibody titres to purified and partially purified L.P.S.

	A	B	C	D
Mean titre	0.262	0.650	0.876	1.352
n	10	10	10	10
S.E.	0.442	0.375	0.509	0.546

A vs D	$p < 0.005$
B vs D	$p > 0.30$
C vs D	$p > 0.50$

When the concentration of purified L.P.S. was doubled or trebled the resulting antibacterial antibody titres were lower than those obtained with partially purified L.P.S. Better antibacterial L.P.S. antibody titres were obtained with partially purified antigenic material and it was decided to test antibacterial L.P.S. antibody activity in Experiments 1, 2 and 3 using partially purified L.P.S. material.

II. Antibody titres to partially purified L.P.S.

A. Anti-L.P.S. antibody titres in Experiment 1

The post-operative rise in anti-E. coli (sucrose positive) L.P.S. antibody titres in P.C.S. and S.O. rats was not statistically significant and differences in antibody titres between the 2 groups of animals were also not significant (Fig. 40).

B. Anti-L.P.S. antibody titres in Experiment 2

A striking rise in anti-L.P.S. (E. coli) antibody titres was observed 6 weeks after P.C.S. in the normal rat (Fig. 41). By the 12th post-operative week the antibody titre decreased but the level was still significantly elevated above the pre-operative value (Table 94). In S.O. and N.C. rats the fluctuations in mean antibody titres were minimal and did not deviate significantly from the pooled pre-operative value (Tables 95 and 96). Antibacterial L.P.S. antibody titres in P.C.S. rats were significantly higher than those recorded in S.O. and N.C. animals ($p < 0.001$).

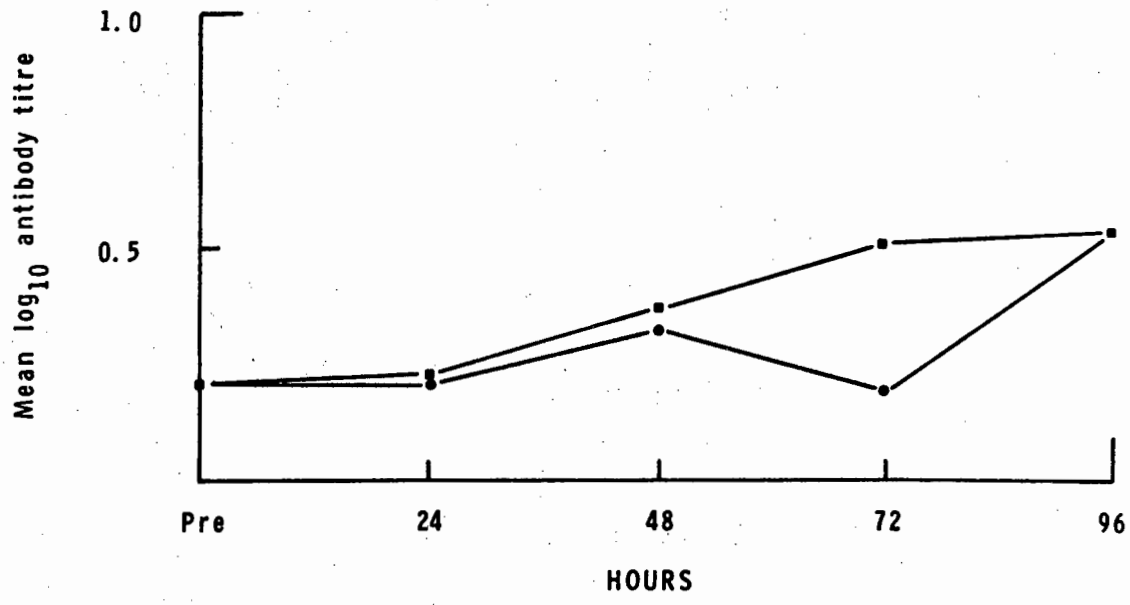

Fig. 40. Mean log anti-L.P.S. (E. coli) antibody titres in P.C.S. (■——■) and S.O. (●——●) rats. - Experiment I.

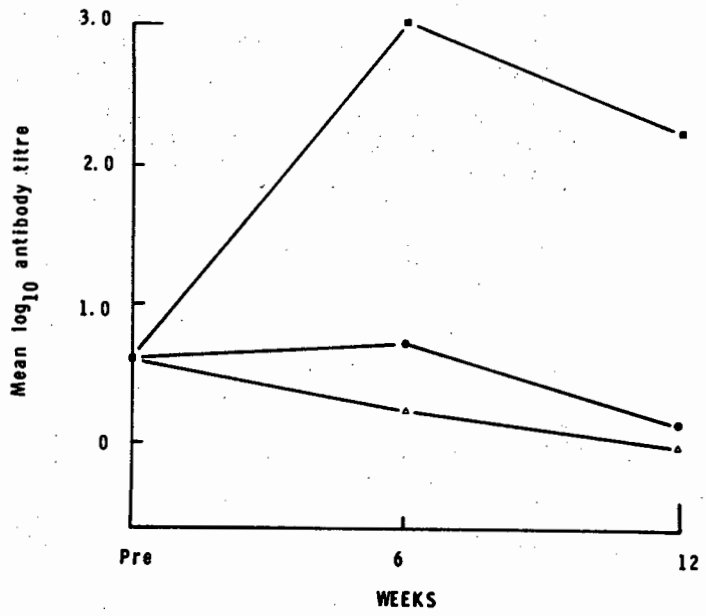

Fig. 41. Mean log anti-L.P.S. (E. coli) antibody titres in P.C.S. (■——■), S.O. (●——●) and N.C. rats(△——△). Experiment 2.

C. Anti-L.P.S. antibody titres in Experiment 3

Two strains of E. coli which were differentiated according to their sucrose-fermenting properties, and a Proteus species were selected from the microbial flora of rats in this Experiment and antibody activity to partially purified L.P.S. preparations was determined. Antibacterial L.P.S. antibody titres could not be determined in some rats with partially occluded P.C.S. and this group of animals had to be excluded from the subsequent series of results.

i) Anti-L.P.S. (E. coli sucrose negative) antibody titres

The rise in anti-L.P.S. (E. coli sucrose negative) antibody titres in the P.C.S. rat was highly significant ($p < 0.001$) and this level of statistical significance was maintained throughout the period of observation. (Fig. 42) (Table 97). Three months post-operatively a gradual decline in antibody activity was observed in P.C.S. rats but antibody titres in the terminal bleed still represented a 3- to 4-fold increase above the mean pre-operative value.

The rise in antibacterial L.P.S. antibody titres in neomycin-treated P.C.S. rats was significant throughout ($p < 0.001$) but the initial increase in antibody activity was not as impressive as in the untreated experimental animal (Fig. 42) (Table 98). The rise in antibody activity which was noted from the 12th post-operative week in neomycin-treated P.C.S. animals was in direct contrast to the trend observed in conventional P.C.S. rats. The difference in anti-L.P.S. antibody titres between neomycin-treated and conventional P.C.S. rats was statistically significant 12 weeks post-operatively ($p < 0.005$).

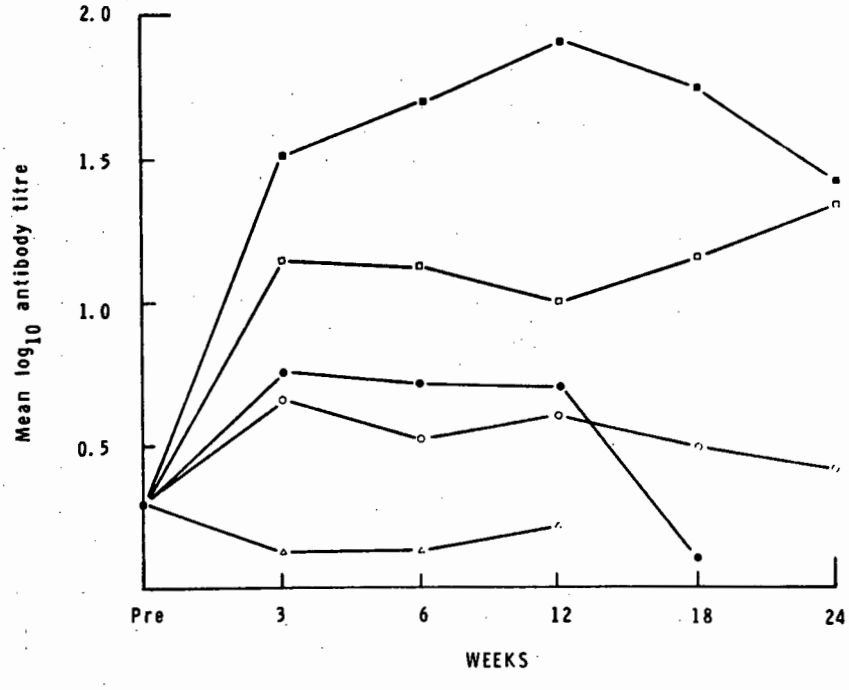

Fig. 42. Mean log anti-L.P.S. (E. coli sucrose Neg.) antibody titres in P.C.S. (■——■), S.O. (●——●) and N.C. (△——△) rats. Open symbols represent neomycin-treated P.C.S. and S.O. rats. - Experiment 3.

The differences in antibody titres in the ensuing weeks were, however, not significant.

The rise in antibacterial L.P.S. antibody titres in rats with totally occluded P.C.S. 3 weeks post-operatively was statistically significant ($p < 0.02$). A gradual decrease in antibody level was observed in the ensuing weeks and 12 weeks post-operatively the anti-L.P.S. antibody level was not significantly different from the initial value (Fig. 43) (Table 99). By contrast, the antibacterial L.P.S. antibody levels in rats with fully patent shunts were significantly elevated ($p < 0.001$) throughout the study (Table 100). Twelve weeks post-operatively the difference in antibody levels between rats with totally occluded P.C.S. and those with patent anastomosis was statistically significant ($p < 0.005$). No significant differences were detected in antibody levels between rats with totally occluded P.C.S. and S.O. controls.

In S.O., S.N. and N.C. rats the fluctuations in anti-L.P.S. antibody titres were minimal and did not deviate significantly from the pre-operative level (Tables 101, 102 and 103). Differences in antibody titres between the 3 control groups of animals were not statistically significant.

ii) Anti-L.P.S. (E. coli sucrose positive) antibodies

In P.C.S. rats the rise in anti-L.P.S. (E. coli sucrose positive) antibody titres was significant (Table 104). The antibody level was significantly elevated above the pre-operative value throughout the study despite an appreciable drop in antibody acitivity 12 weeks post-operatively (Fig. 44). The rise in anti-L.P.S.

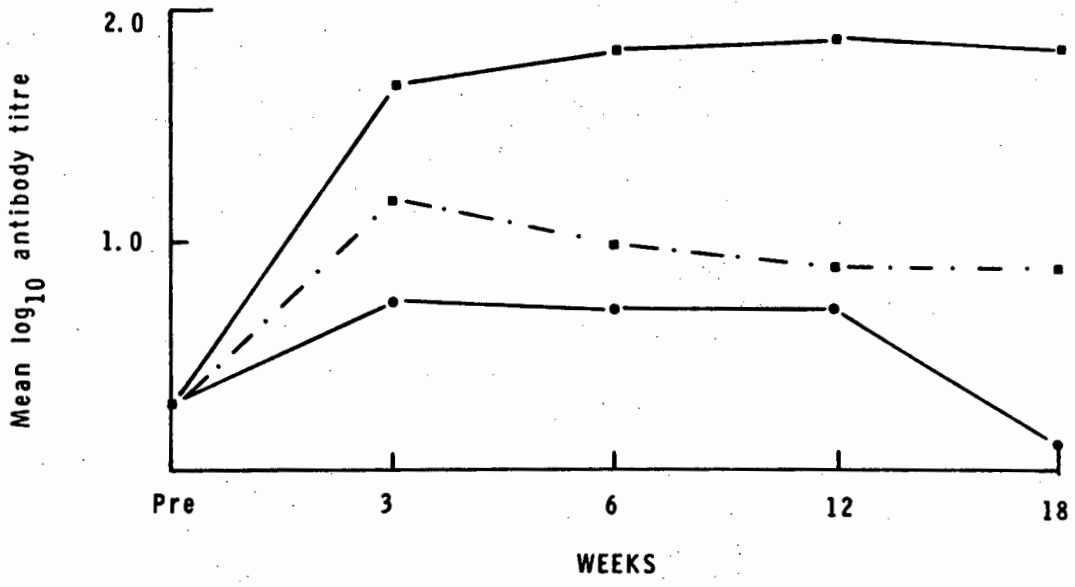

Fig. 43. Mean log anti-L.P.S. (E. coli sucrose Neg.) antibody titres in rats with patent (■———■), totally occluded (■—·—■) P.C.S. and in S.O. (●——— ●) animals. Experiment 3.

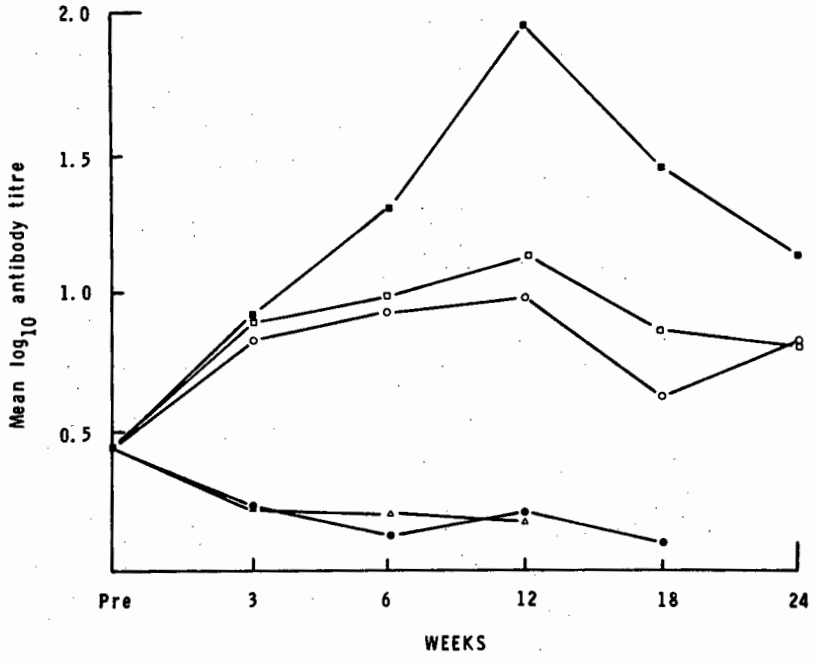

Fig. 44. Mean log anti-L.P.S. (E. coli sucrose Pos.) antibody titres in P.C.S. (■———■), S.O. (●——— ●) and N.C. (△——△) rats. Open symbols represent neomycin-treated P.C.S. and S.O. animals. - Experiment 3.

antibody titres in neomycin-treated P.C.S. rats was statistically significant 6 to 12 weeks post-operatively (Table 105). Three months post-operatively, when maximum antibody titres were recorded in both groups of P.C.S. animals, the antibody level in the neomycin-treated P.C.S. animals was only 56% of the titre recorded in the conventional experimental animals. The response of the S.O. rat treated with neomycin was at variance with other data because these animals showed antibody levels equivalent to those seen in P.C.S. rats treated with neomycin (Table 106). No significant differences were detected in antibacterial L.P.S. antibdoy titres between these 2 groups. Anti-L.P.S. antibody titres in P.C.S. rats were significantly higher than those recorded in P.C.S. rats treated with neomycin and S.N. animals 12 weeks post-operatively.

In rats with totally occluded P.C.S. the initial rise in antibacterial L.P.S. antibody titre was significant 2 weeks post-operatively (Fig. 45) (Table 107). Although the rise in antibody titres was significant 12 and 18 weeks post-operatively in rats with totally occluded P.C.S., antibody titres were significantly lower than those recorded in animals with fully patent P.C.S. Maximum antibody titres in rats with totally occluded P.C.S. represented a 145% increase above the initial value in comparison with an increase of 431% recorded in rats with patent anastomosis (Table 108).

iii) Anti-L.P.S. (Proteus Sp.) antibody titres

In the P.C.S. rat a post-operative rise in antibacterial L.P.S. antibody activity was seen and this rise was statistically significant ($p < 0.001$). The elevated antibody levels were

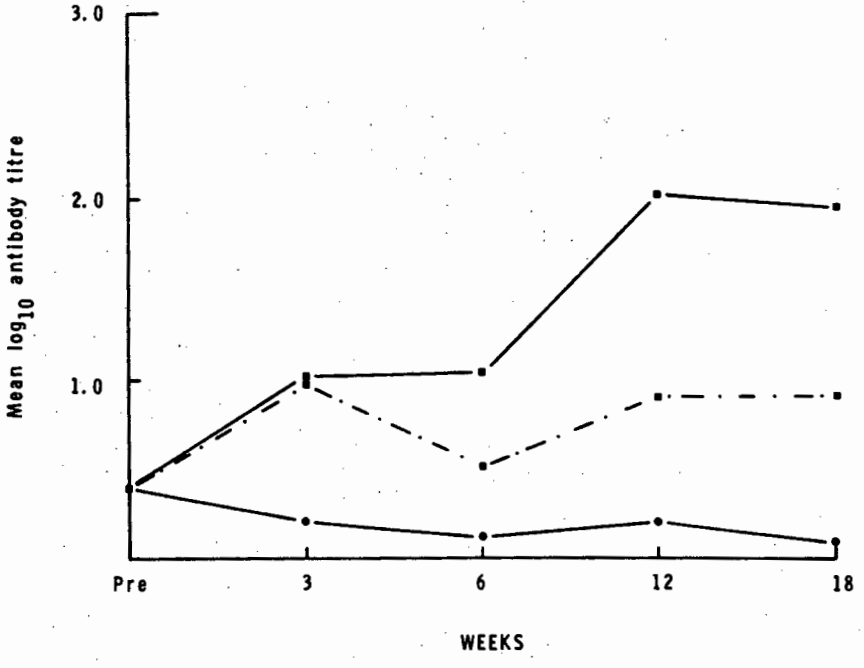

Fig. 45. Mean log anti-L.P.S. (E. coli sucrose Pos.) antibody titres in rats with patent (■——■), totally occluded (■—·—■) P.C.S. and in S.O. (●——●) animals. - Experiment 3.

sustained throughout the period of observation (Fig. 46) (Table 109). Antibody titres in P.C.S. rats treated with neomycin rose sharply, reached a peak 12 weeks post-operatively, and then declined progressively (Table 110). This was in direct contrast with increasing antibody titres that were noted in animals not treated with neomycin. In S.N. rats the antibody titres rose significantly and then fluctuated for the duration of the experiment (Table 111). In S.O. and N.C. rats the fluctuations in antibody titres did not deviate significantly from the pre-operative level (Tables 112 and 113).

The rise in anti-L.P.S. (Proteus Sp.) antibody titres in rats with totally occluded P.C.S. was not significant (Fig. 47) (Table 114). Twelve and 18 weeks post-operatively antibody titres in rats with totally occluded P.C.S. were significantly lower than titres recorded in animals with fully patent anastomosis but significantly higher than levels in S.O. rats. Maximum antibacterial antibody titres in rats with totally occluded P.C.S. represented an increase of 154% above the initial value compared with an increase of 355% in rats with patent shunts (Table 115).

D. Experiment 7A (anti-L.P.S. antibody titres in rats immunised 6 weeks post-operatively with HGG)

There was an immediate and highly significant rise ($p < 0.001$) in antibody titre to bacterial L.P.S. in P.C.S. rats. The significantly elevated antibody titres were maintained throughout (Fig. 48) (Table 116). Antibacterial L.P.S. antibody titres in P.C.S. rats were significantly higher than those recorded in S.O. and N.C. animals (Table 117 and 118). The immunisation of P.C.S. rats with H.G.G. 6 weeks post-operatively did not stimulate antibacterial L.P.S. antibody production.

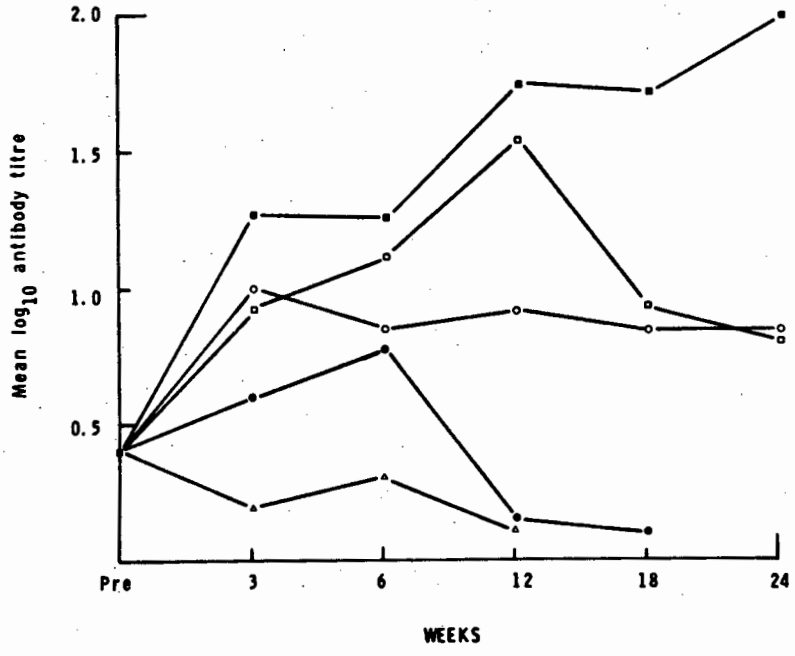

Fig. 46. Mean log anti-L.P.S. (Proteus sp.) antibody titres in P.C.S. (■——■), S.O. (●——●) and N.C. (△——△) rats. Open symbols represent neomycin-treated P.C.S. and S.O. animals. - Experiment 3.

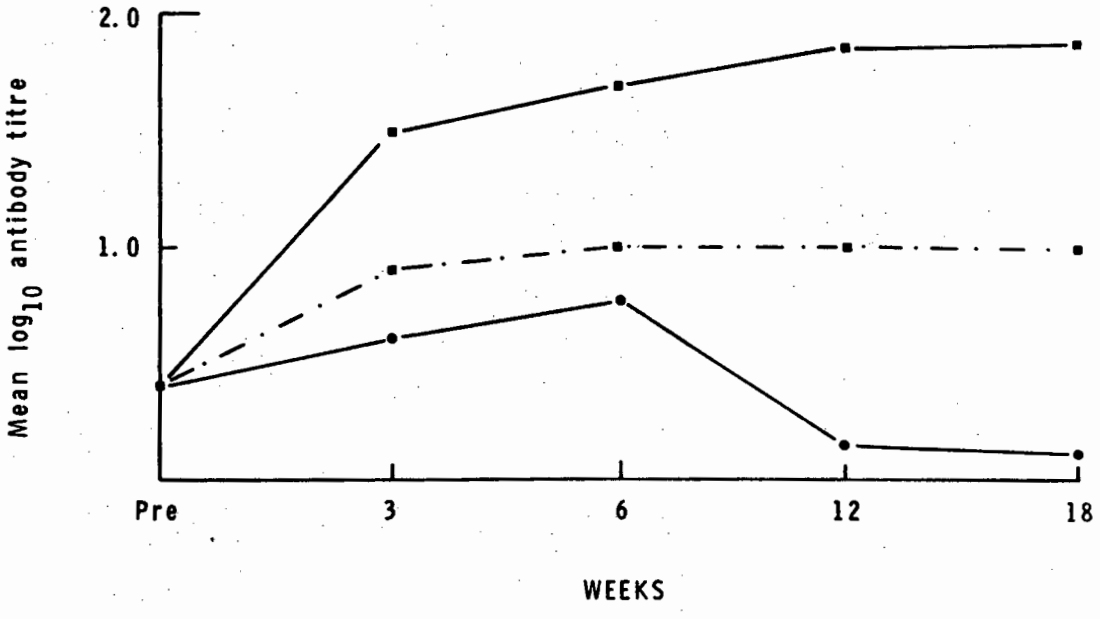

Fig. 47. Mean log anti-L.P.S. (Proteus sp.) antibody titres in rats with patent (■——■), totally occluded (■—·—■) P.C.S. and in S.O. (●——●) animals. - Experiment 3.

Fluctuations in mean log antibody titre to E. coli (sucrose positive) L.P.S. in S.O. and N.C. animals did not deviate significantly from the pooled pre-operative value.

Experiment 7B (anti-L.P.S. antibody titres in rats immunised 18 weeks post-operatively with H.G.G.)

A significant rise ($p < 0.001$) in antibacterial antibody titres was observed in the P.C.S. rats. The significantly elevated antibody levels were sustained throughout the period of observation (Fig. 49) (Table 119). Antibody titres in P.C.S. rats were significantly higher than that of S.O. and N.C. animals (Tables 120 and 121). No increase in antibacterial L.P.S. antibody titres was observed in P.C.S. rats subsequent to immunisation with H.G.G. 18 weeks post-operatively.

In S.O. and N.C. rats fluctuation in antibacterial L.P.S. antibody titres did not deviate significantly from the initial level and the differences in antibody titres between these 2 groups of animals were not statistically significant.

E. Experiment 12 : Mean log antibody titres in rats following repeated stimulation with E. coli 0127 L.P.S.

In normal rats immunised with a commercial L.P.S. (E. coli 0127) preparation the antibacterial antibody titre increased significantly and remained significantly elevated above the pre-operative value (Table 122). Boosting of these animals 4 weeks after primary immunisation produced a significant rise ($p < 0.001$) in antibacterial antibody titres which peaked at 7 days (Fig. 50). These same animals showed

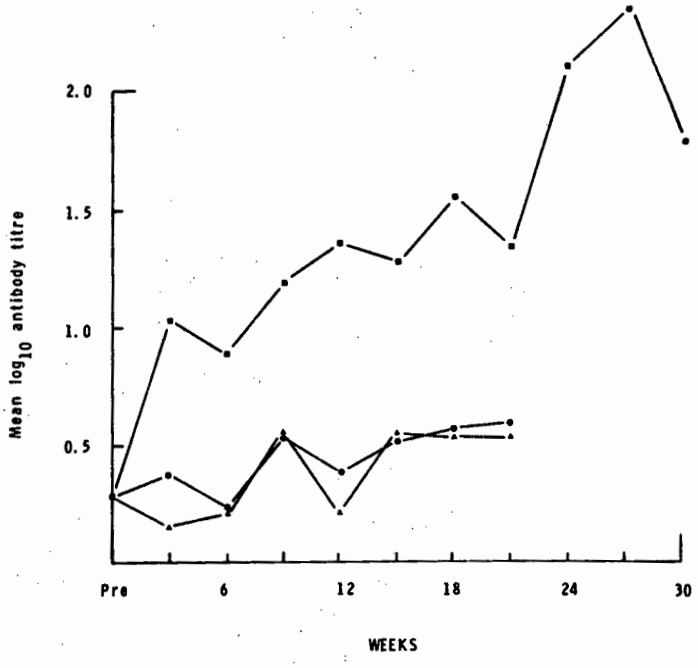

Fig. 48. Mean log anti-L.P.S. (E. coli sucrose Pos.) antibody titres in P.C.S. (■——■), S.O. (●——●) and N.C. (▲——▲) rats immunised 6 weeks post-operatively with H.G.G. - Experiment 7A.

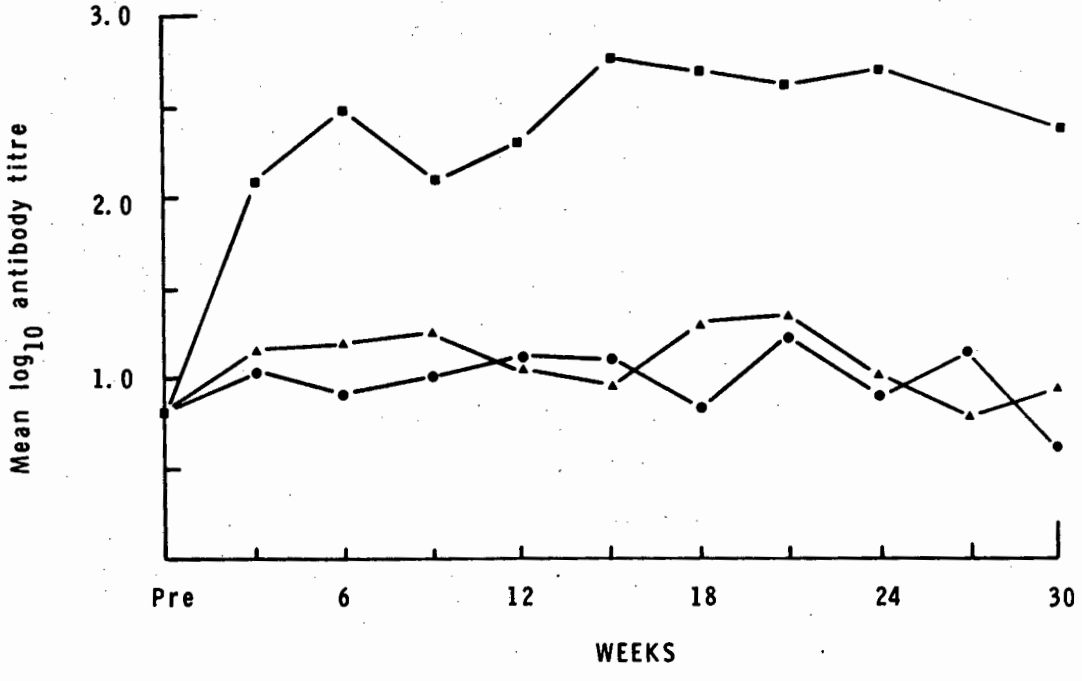

Fig. 49. Mean log anti-L.P.S. (E. coli sucrose Pos.) antibody titres in P.C.S. (■——■), S.O. (●——●) and N.C. (▲——▲) rats immunised 18 weeks post-operatively with H.G.G. - Experiment 7B.

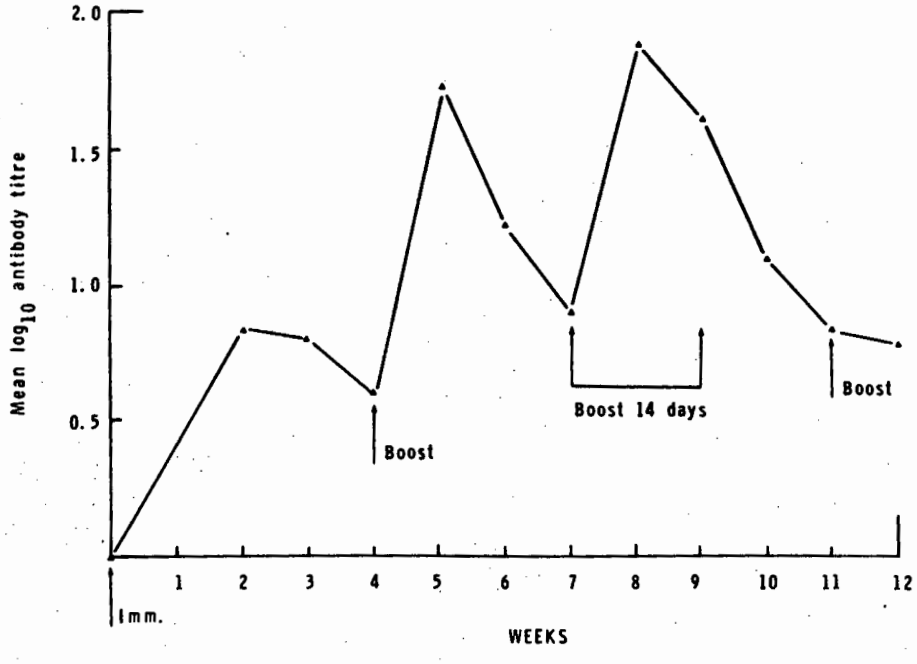

Fig. 50. Mean log antibacterial antibody titres in normal rats repeatedly stimulated with a commercial L.P.S. (E. coli 0127) preparation.

a similar antibody response following a daily antigenic stimulation for a period of 14 days. The antibody response to repeated antigenic stimulation resulted in antibody titres being maintained at a higher level for a longer time and it is of interest that 2 weeks after the final challenge a repeat single antigenic stimulus failed to evoke an antibody response. The antibody response to this L.P.S. preparation was lower than the response in the experimental animals subjected to P.C.S. and tested with an L.P.S. preparation derived from their own bowel microflora.

F. Experiment 13 : Antibacterial L.P.S. antibody activity in fractionated rat serum

Antibody activity to bacterial L.P.S. was found in the IgM fraction of rat serum as indicated in the Table :

Anti-L.P.S. (E. coli sucrose positive) antibody titres in fractionated rat serum

	Whole serum	IgM fraction	IgG fraction
Mean titre	2.006	1.705	0.000
n	3	3	3
S.E.	0.100	0.100	-

Whole serum vs IgM $p > 0.05$

G. Experiment 14 : Antibacterial L.P.S. antibody titres in rat sera treated with 2-Me

As indicated in the Table below and in Fig. 51, the antibacterial L.P.S. antibody titre decreased significantly ($p < 0.001$) in rat sera pretreated with 2-Mercaptoethanol (2-Me) when compared with that of untreated sera. A 7-fold depression of antibody titres to Proteus and E. coli (sucrose positive) L.P.S. preparations was obtained and a 20-fold decrease in antibody activity to E. coli (sucrose negative) L.P.S. was recorded.

Antibacterial L.P.S. antibody titres

	Anti-L.P.S. (Proteus) antibodies		Anti-L.P.S. (E.coli sucr.pos.) antibodies		Anti-L.P.S. (E.coli sucr. neg.)antibodies	
	un-treated	2-Me treated	un-treated	2-Me treated	un-treated	2-Me treated
Mean titre	1.634	0.258	2.167	0.331	2.270	0.109
n	7	7	10	10	11	11
S.E.	0.261	0.121	0.117	0.170	0.123	0.109
p	< 0.001		< 0.001		< 0.001	

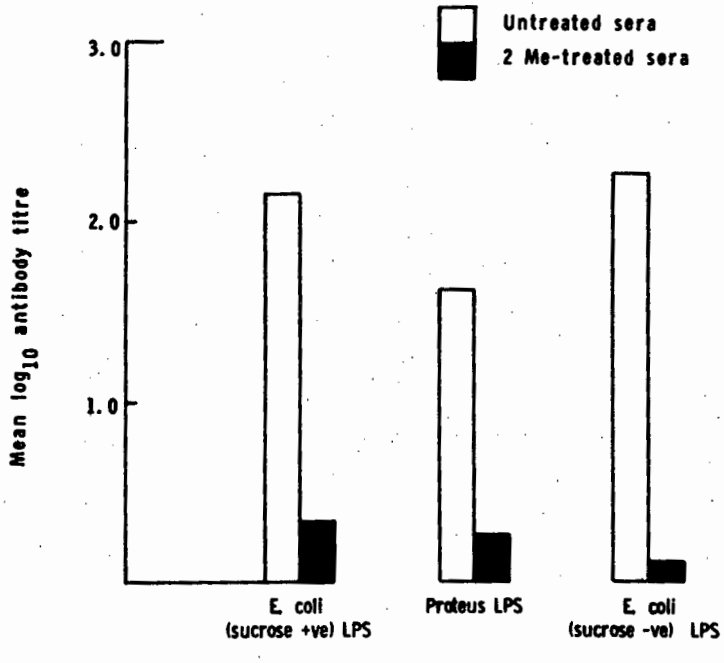

Fig. 51. The effect of 2-Mercaptoethanol on antibacterial L.P.S. antibody titres. Experiment 14.

H. Experiment 15 : Immune inhibition with bacterial L.P.S.

The prior treatment of rat serum containing anti-L.P.S. (E. coli sucrose positive) antibodies with its specific antigen produced immune inhibition whereas pre-treatment of serum with another L.P.S. preparation (E. coli 0127) produced a lesser degree of immune inhibition as indicated in the following Table :

Mean log anti-L.P.S. (sucrose positive) antibody titres :

	A sera treated with E. coli L.P.S. (specific)	B sera treated with E. coli 0127 L.P.S. (non-specific)	C untreated sera
Mean titre	0.109	1.680	1.792
n	22	22	22
S.E.	0.075	0.176	0.095

A vs B $p < 0.001$

A vs C $p < 0.001$

B vs C $p > 0.20$

Experiment 6 : Jerne Plaque Studies

In a preliminary experiment normal rats were immunised, subcutaneously and intramuscularly, with 50 µgm E. coli 0127 - B8 L.P.S.

and plaque-forming cells in rat spleen, to this L.P.S., were sought. The effects of coating different concentrations of L.P.S. onto target cells, as well as boiling the L.P.S. preparation for 2 hours prior to coupling, on the number of plaque-forming cells were investigated. A boiled L.P.S. preparation at a concentration of 50 µgm/ml evoked maximal haemolytic plaque-forming antibody response. An investigation in the number of plaque-forming cells in P.C.S. and S.O. was undertaken. P.C.S. animals had survived the operation for 14-42 (mean 27.87 $\pm$ 8.26) weeks. S.O. rats of identical survivorship served as controls. A number of normal control rats were included in these experiments.

No differences in plaque-forming cells to E. coli (sucrose positive) L.P.S. were detected as shown in the Table below :

L.P.S. plaque-forming cells :

	P.C.S.	S.O.	N.C.
Mean	5.233	5.140	4.861
n	12	7	7
S.E.	0.601	1.080	0.619

P.C.S.	vs	S.O.	$p > 0.90$
P.C.S.	vs	N.C.	$p > 0.50$
N.C.	vs	S.O.	$p > 0.60$

The number of L.P.S. plaque-forming cells was very similar to and not statistically different from the number of background plaques. While plaque-forming cells were being enumerated the serum of these animals was tested for anti-L.P.S. (E. coli sucrose positive) antibodies and

the following antibody levels were obtained :

Mean log anti-L.P.S. (E. coli sucrose positive) antibody titres

	P.C.S.	S.O.	N.C.
Mean titre	1.870	0.903	0.926
n	12	7	7
S.E.	0.170	0.199	0.157

P.C.S.	vs	S.O.	$p < 0.001$
P.C.S.	vs	N.C.	$p < 0.001$
N.C.	vs	S.O.	$p > 0.90$

Experiment 16 : Effect of neomycin on the bowel flora of rats

This experiment was undertaken to investigate the effectiveness of the dosage of neomycin used in previous experiments.

The attempt to sterilise the bowel of rats by daily oral administration of 7.0 mg/100 g body weight of neomycin, proved ineffective. The antibiotic did not reduce the total number of microbial organisms nor the number of colonies present in rat faecal material. The results of approximate counts, expressed as organisms per ml of rat faeces are shown in the following Table :

Number of organisms per ml

	Pre	1 week	2 weeks	3 weeks
Coliforms	500,000	100,000	50,000	1,000,000
Enterococci	130,000	100,000	1,000,000	1,000,000
Staphylococci	100,000	100,000	150,000	300,000

It is clear from these studies that the dosage of neomycin used in these experiments was insufficient to sterilise the bowel.

ANTI-HUMAN GAMMA-GLOBULIN ANTIBODIES

Prior to these experiments with H.G.G. in P.C.S. rats, a pilot study showed that normal rats immunised with 500 ugm of alum-precipitated H.G.G. produced good titres of anti-H.G.G. antibodies.

Experiment 7A : (Rats immunised 6 weeks post-operatively with H.G.G.)

Following immunisation with H.G.G the anti-H.G.G antibody titres rose significantly in S.O. and N.C. rats and remained elevated for 6 weeks (Tables 123, 124 and 126). Thereafter a progressive decline in antibody titres was found so that 9 weeks after the primary immunisation no anti-H.G.G. antibodies were detected in the sera (Fig. 52).

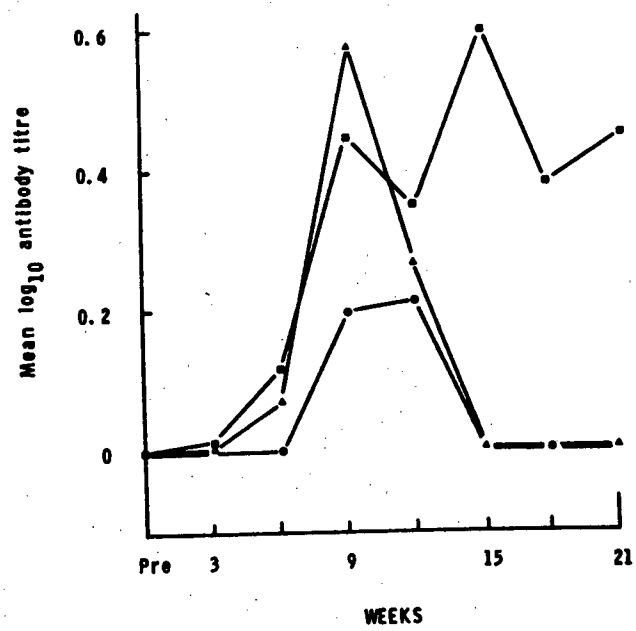

Fig. 52. Mean log anti-H.G.G. antibody levels in P.C.S. (■——■), S.O. (●——●) and N.C. (▲——▲) rats immunised 6 weeks post-operatively with H.G.G.. - Experiment 7A.

A similar rise in anti-H.G.G antibody titres was detected in P.C.S. rats following immunisation with H.G.G. (Table 125). The anti-H.G.G. antibody activity was sustained throughout at a higher level and for a longer period of time than in S.O. and N.C. animals. Nine weeks following immunisation with H.G.G. the anti-H.G.G. antibody levels were still significantly higher than the pre-immunisation level and significantly higher than levels recorded in S.O. and N.C. animals.

Experiment 7B (Rats immunised 18 weeks post-operatively with H.G.G.)

When animals were immunised with H.G.G. 18 weeks after P.C.S., similar sustained levels of anti-H.G.G. antibodies were detected in P.C.S. rats (Fig. 53). Anti-H.G.G antibody titres in P.C.S. rats were significantly higher than titres recorded in S.O. and N.C. rats 3 and 6 weeks after immunisation with H.G.G.

Experiment 17 : (Immune inhibition with H.G.G.)

The specificity of the anti-H.G.G. antibodies was investigated in sera with known anti-H.G.G antibody titres and tested for immune inhibition as indicated in the Table below :

Mean log anti-H.G.G antibody titres

	A Untreated controls	B Imm. Inh. H.G.G.
Mean titre	2.686	1.504
n	19	19
S.E.	0.134	0.216

A vs B $p < 0.001$

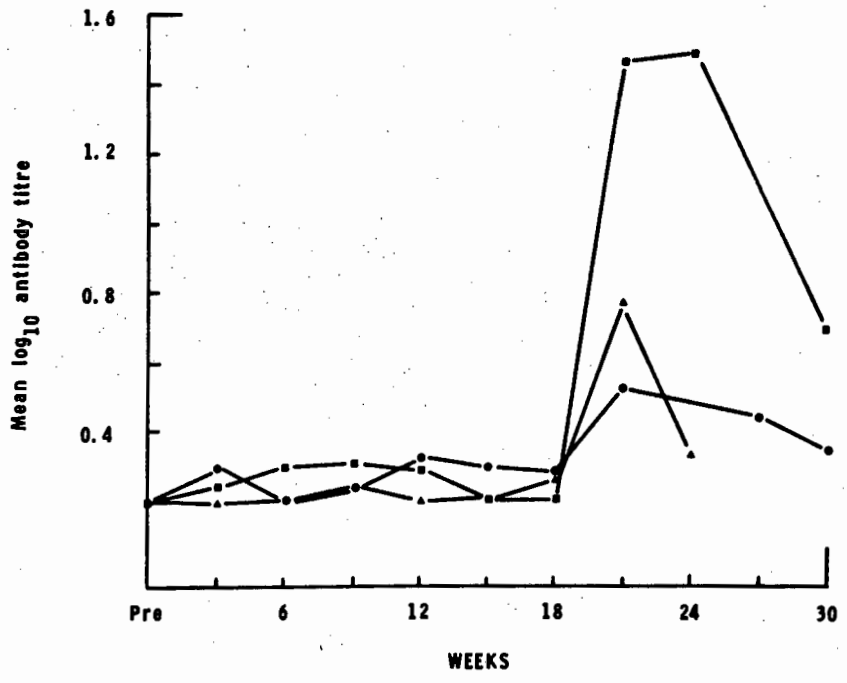

Fig. 53. Mean log anti-H.G.G. antibody levels in P.C.S. (■——■), S.O. (●——●) and N.C. (▲——▲) rats immunised 18 weeks post-operatively with H.G.G. - Experiment 7B.

Anti-H.G.G. antibody titres were significantly reduced ($p < 0.001$) following immune inhibition with H.G.G.

Experiment 18 : (Antibodies to rat IgG)

Antibody activity to rat IgG was sought in rat sera showing good antibody titres to H.G.G. No anti-rat IgG antibodies were detected in the 15 samples analysed.

Experiment 19 : (Antibodies to denatured rat IgG)

No antibody activity to denatured rat IgG was detected in rat serum with known antibody titres to bacterial L.P.S.

Experiment 20 : (Fluorescent auto-antibodies in rat serum)

No fluorescent auto-antibodies were detected in P.C.S. and S.O. rat sera 1 to 36 weeks post-operatively.

DISCUSSION

Bacterial L.P.S. is a powerful B-cell mitogen and can initiate a primary immune response without T-cell co-operation (Möller and Michael, 1971). The serological response to B-cell stimulation can in most instances be measured in the IgM fraction (Britton and Möller, 1968; Friedman, 1973; Möller et al., 1973). Antibacterial anti-L.P.S. antibody activity also occurs in the IgG fraction but this phenomenon is poorly understood (Möller et al., 1973), and this preferential stimulation of IgM antibody class is largely unknown.

In the studies reported here a characteristic feature was the demonstration of increased levels of antibacterial L.P.S. antibody following P.C.S. in the rat. The rise in antibacterial antibody was not seen in the immediate post-shunt period but became evident from the 3rd week onward and persisted for the duration of observation, 12-30 weeks (Experiments 2, 3, 7A and 7B). Changes in antibacterial L.P.S. antibodies were consistently demonstrated in 4 separate experiments. These changes were detected in response to several organisms, i.e. 2 E. coli strains and one Proteus species. In one experiment (Experiment 3) the serological response to all 3 organisms was studied simultaneously and although the tempo and the magnitude of the serological response varied with the 3 L.P.S. preparations they followed a consistent pattern of continued elevation of L.P.S. antibodies. The response to the Proteus L.P.S. was slower and peaked at a much later time period.

In selected sera the mercaptoethanol sensitivity of the anti-L.P.S. antibodies was tested and a significant reduction in antibody activity was detected (Experiment 14). In a smaller number

of experiments Sephadex gel filtration also served to localise the antibody class in the IgM fraction (Experiment 13), once again demonstrating the propensity for L.P.S. to produce IgM antibodies. All bacterial L.P.S. preparations which were used in these studies were tested for endotoxin activity with the Limulus lysate assay which confirmed the presence of functional endotoxin activity (Levin et al., 1970). The specificity of these antibody responses was assessed by immune inhibition (Experiment 15) and the data shows that apart from minor cross-reactivity with a commercial L.P.S. preparation (E. coli 0127) the serological responses were specific. Repeated stimulation of rats with a commercial preparation of L.P.S. did not produce comparable levels in antibacterial L.P.S. antibodies, but in view of the previously documented requirement of higher concentration of pure antigen in the haemagglutination studies, these findings are to be viewed with reserve. One of the effects of L.P.S. is to cause the cyclical appearance of IgM antibodies which is believed to be due to an antibody-dependent feedback system (Britton and Möller, 1968). This cyclical phenomenon could possibly explain the variation in antibacterial antibody titres which was observed in a Proteus L.P.S. in Experiment 3 and in E. coli L.P.S. in Experiment 7A.

The major source for endotoxin can be expected to be the microflora of the bowel. In these studies on L.P.S. antibodies, the antigen was derived from organisms from the experimental rats and it may be assumed that these organisms were shared by all experimental animals (prior randomisation and the pooling of the faeces from all the experimental groups were utilised to ensure this). No definitive serological typing of the organisms was undertaken. The specificity and the duration of the serological responses to a given L.P.S. suggest that the antigenic source was derived from specific bowel flora. The other experimental data which can be offered in support of this assumption is the finding of significantly lower titres to a given L.P.S. in those animals when occlusion of the shunt could be demonstrated,

and the generally lower levels of antibodies in animals treated with neomycin (Experiment 3). The magnitude and the duration of the serological response to a given bacterial L.P.S. preparation can be related to the patency of the portacaval shunt and in this respect this phenomenon is similar to changes in weight and total gamma-globulin.

In all the experiments control animals i.e. normal and S.O. showed low levels of antibacterial L.P.S. antibody which presumably reflects prior contact with bacterial L.P.S. from endogenous microflora. Michael et al. (1962) suggested the endogenous microflora as a major cause for endotoxin antibodies, and also raised the possibility of ingested endotoxins as an alternative source.

The only possible route for absorbed bacterial products is via the portal venous system to the liver. Evidence in support of this suggestion comes from experiments on Thiry Vella loops in P.C.S. rats which have demonstrated absorption of L.P.S. from the isolated loops and its presence in the portal vein (Gans and Matsumoto, 1975). Bacterial L.P.S. has been demonstrated in the liver of normal rabbits and Ravin et al. (1961) suggested on the basis of this finding that there was continuous absorption of bacterial L.P.S. from the gastro-intestinal tract of normal animals.

Antibacterial L.P.S. antibody levels in normal animals and in man tend to be maintained at low levels except when infection with Gram-negative organisms supervenes. This apparent paradox between an assumed continuous antigenic stimulus from the bowel and a rather low profile antibody response may be resolved by assuming that L.P.S. is trapped in the liver, where it is either degraded or sequestered. The putative hepatic barrier to the free circulation of absorbed L.P.S. may be expected to be breached under conditions of liver injury. In this respect, it may be suggested that the temporarily

elevated antibacterial L.P.S. antibodies which were encountered in S.O. animals in all these experiments were on the basis of transient hepatic injury. Histological evidence of liver cell damage was obtained in S.O. animals following temporary occlusion (10 minutes) of the portal vein (Experiment 1). Confirmation for this hypothesis comes from the finding of free-circulating L.P.S. in the systemic circulation following portal vein occlusion in normal rats (Olkay et al., 1974). Portal venous diversion is a predictable way of removing the hepatic barrier to bacterial L.P.S.

The administration of neomycin to P.C.S. rats might be expected to reduce the antigenic load by-passing the liver and indeed lower levels of antibacterial L.P.S. antibodies were recorded in the neomycin-treated P.C.S. rat, but total suppression of the characteristic L.P.S. antibody response was not achieved. The assumption of a partial reduction in intraluminal bacterial load may at first sight seem reasonable but such an assumption needs to be tempered by data obtained on the effects of neomycin on bacterial colony counts from the bowel and antibacterial L.P.S. antibody levels in S.O. rats.

The experiments to assess the effect of neomycin on bowel flora in the rat were developed in order to gauge the efficacy of the dose of neomycin which was employed in reducing total members of bacteria (Experiment 16). In these studies the sensitivity of most of these organisms to neomycin was established. Studies on colony counts failed, however, to show any significant reduction in bowel microflora while animals were being treated with neomycin. The design of these experiments itself may have contributed to the apparent lack of effect i.e. bacterial counts were not made with reference to a standard faecal weight. The dose of neomycin which was employed was an arbitrary one scaled down from that which was regarded as

sufficient to control bowel micro-organisms in humans. This is possibly a naïve assumption.

In the S.N. animals, contrary to expectation, increased levels of antibacterial L.P.S. antibodies were detected which was of an order of magnitude comparable to that seen in neomycin-treated P.C.S. animals. This apparent contradiction permits of no ready explanation. Several possibilities may be advanced. The possible ineffectiveness of the antibiotic has already been mentioned. A second possibility is that the antibody responses reflect the acquisition of neomycin resistance by a particular population of organisms i.e. E. coli sucrose positive, E. coli sucrose negative. All L.P.S. antibody studies were done with L.P.S. preparations derived from endogenous organisms within the first 3 weeks of the experiment. No attempts were made to examine bowel microflora after the administration of neomycin. It is therefore possible that neomycin-resistant organisms with close serological identity were allowed to proliferate. If either of these two latter possibilities are operative, the data on the effect of neomycin on L.P.S. antibody levels needs to be viewed in a different light i.e. the lower levels in P.C.S., assumed to result from neomycin administration, and the increased levels seen in the S.N. rat may represent an entirely different serological response. Until this matter can be clarified by further careful studies and cross-absorption experiments with different L.P.S. preparations it is perhaps wise not to draw many conclusions from these experiments with neomycin.

The free access of L.P.S. to the systemic circulation can be expected to have several effects. Direct stimulation leading to the formation of antibacterial L.P.S. antibodies was already described and discussed. The immune responses in the P.C.S. rat are reflected by an increase in IgG, IgM and, to a lesser extent, IgA levels (Experiment 2). The antibacterial L.P.S. antibodies may be a

part or whole explanation for the changes that have been seen in IgM but the mechanisms of total elevation of IgG cannot be explained on the basis of antibody to bacterial L.P.S.

Bacterial L.P.S. is known to influence immune reactions. Thus it is believed to enhance immune responses early in the formation of antibody (Kind and Johnston, 1959), and that it leads to a general potentiation of all phases of antibody production (Munoz, 1964). The mechanisms whereby these effects are realised are largely unknown but the potent effect of L.P.S. on cell membranes (Thomas, 1959; Munoz, 1964) may be a key to its effects. This would explain its well-known effects on the R.E.S. (Biozzi et al., 1955; Thomas, 1959). In view of the far-reaching effects of L.P.S. on antibody formation it could be expected that P.C.S. would influence immune responses.

In experiments on the serological responses to H.G.G. such an effect was sought i.e. a new immune response to H.G.G. was heightened and prolonged by a P.C.S. whether the P.C.S. was 6 or 18 weeks old (Experiments 7A and 7B). The specificity of these anti-H.G.G. antibodies was checked by immune inhibition and no cross-reactivity with a commercial rat IgG preparation was detected (Experiment 18).

In view of the association of hyperglobulinaemia and rheumatoid factor in human disease, an attempt was made to look for rat rheumatoid factor with negative results (Experiment 19). Similarly, because of the known associations of certain antinuclear factors, antimitochondrial and anti-smooth muscle antibodies with prolonged bacterial infections in man, attempts were made to find these auto-antibodies in rat serum, with negative results (Experiment 20).

The site of formation of antibody to L.P.S. might be expected in the spleen since it is a major antibody-forming organ. Preliminary studies in which rats were immunised with a commercial

preparation of L.P.S. showed that individual antibody-forming cells to the L.P.S. were detectable in the spleen. However, when antibody-forming cells were looked for in the spleen of P.C.S. rats no differences were detected when compared with control animals. In contrast to these low numbers of plaque-forming cells to L.P.S. were the high levels of antibacterial L.P.S. antibodies in the sera of these rats. The Jerne-plaque technique has many technical pitfalls which would vitiate against obtaining reliable data. Technical problems should certainly be considered to explain this apparent paradox. An alternative explanation would imply that antibody formation to L.P.S. does not occur in the spleen to any significant extent. The absence of changes in splenic weight in these studies may be proposed in support of this argument but it should, however, be noted that there is little correlation between spleen weight and gamma-globulin levels (Glasgov and Popper, 1959). Of interest is the data on splenectomy and P.C.S. which showed that the removal of the spleen at the same time that P.C.S. is performed, affects neither the magnitude nor the duration of the gamma-globulin changes, and this is perhaps the strongest argument in favour of a minor contribution of the spleen to L.P.S. antibody formation. Johnson et al. (1956) investigating the immune response to L.P.S. were also unable to demonstrate antibody formation in the spleen using a direct technique. More definitive studies on the effect of splenectomy in the P.C.S. rat are needed to define the immunoglobulin changes since it might be expected that non-L.P.S. antibody production could be curtailed by splenectomy (Wolfe et al., 1950; Rowley, 1950; De Carvalho et al., 1967).

PART 4

Packed Cell Volume,

Aminoaspartate Transferase and

Liver Histology

A. PACKED CELL VOLUME

Experiment 1

An immediate and statistical significant drop in packed cell volume was recorded in P.C.S. and S.O. rats (Tables 126 and 127). Whereas the decrease in the S.O. rat was evenly distributed throughout the period of observation, the experimental animal showed a marked depression in P.C.V. 24 hours post-operatively (Fig. 54). The differences in packed cell volume between P.C.S. and S.O. rats were statistically significant on the first and second post-operative days.

B. ASPARTATE AMINOTRANSFERASE LEVELS

i) Experiment 1

The increase in A.A.T. level, which occurred 6 hours after P.C.S. in rats was highly significant ($p < 0.001$). Following this increase, enzyme levels decreased rapidly to within the normal range 72 hours post-operatively (Fig. 55) (Table 128).

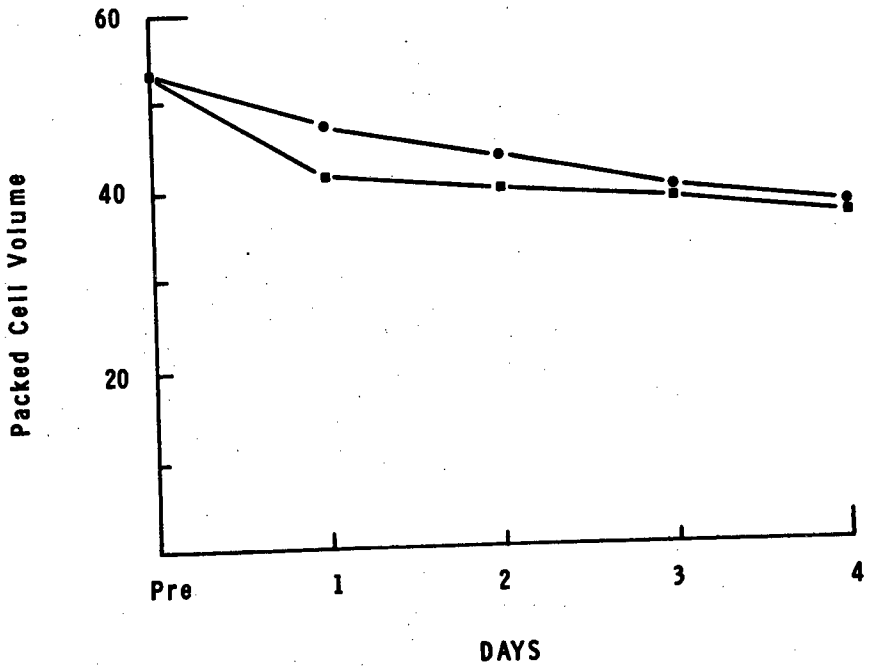

Fig. 54. Mean packed cell volumes in P.C.S. (■——■) and S.O. (●——●) rats. - Experiment 1.

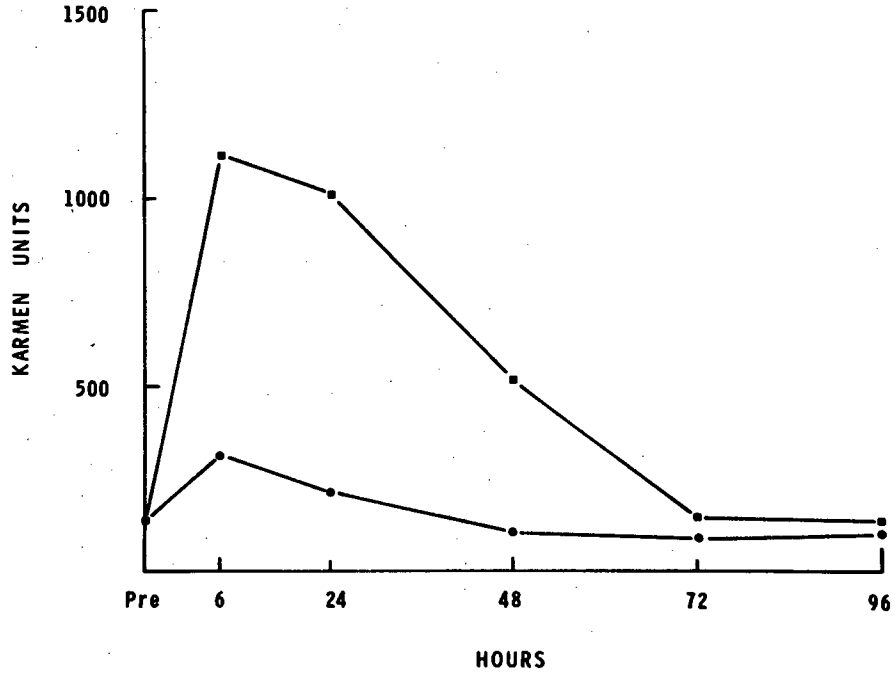

Fig. 55. Mean A.A.T. levels in P.C.S. (■——■) and S.O. (●——●) rats. - Experiment 1.

A much lower rise in A.A.T. occurred in S.O. rats 6 hours post-operatively, and the A.A.T. levels remained significantly elevated above the initial value 24 hours post-operatively (Fig. 55). After being significantly depressed below the pre-operative value the A.A.T. levels returned to normal 96 hours after sham surgery (Table 129). Differences in A.A.T. levels between P.C.S. and S.O. rats were statistically significant 24 and 48 hours post-operatively.

ii) Experiment 3

In P.C.S. rats the mean A.A.T. level fluctuated throughout but did not deviate significantly from the pre-operative value (Fig. 56) (Table 130). Similar changes in A.A.T. activity were observed in neomycin-treated P.C.S. animals but the rise in enzyme level 3 weeks post-operatively was more pronounced than in conventional experimental animals (Table 131). The differences in A.A.T. levels between these 2 groups of animals were not statistically significant.

In S.O. rats a significant decrease in A.A.T. activity was observed and significantly depressed enzyme levels were recorded for 3 months (Table 132). The administration of antibiotic to the S.O. rat had no immediate effect on A.A.T. levels relative to the enzyme levels in untreated S.O. controls (Table 133). But whereas A.A.T. levels in S.O. rats increased to beyond the initial level 18 weeks post-operatively, enzyme levels in S.N. rats were significantly depressed throughout (Fig. 56). Consequently, the differences in A.A.T. levels between the 2 groups of S.O. controls were statistically significant 18 weeks post-operatively ($p < 0.05$). Fluctuations in A.A.T. levels in N.C. rats did not deviate significantly from the pooled pre-operative value.

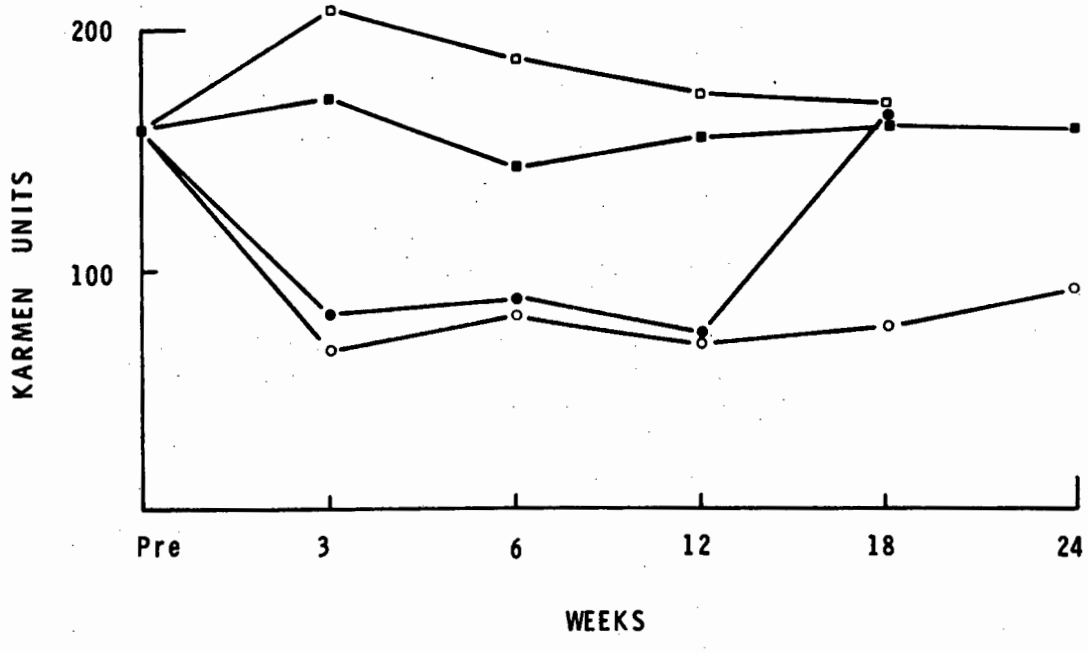

Fig. 56. Mean A.A.T. levels in P.C.S. (■——■) and S.O. (●——●) rats. Open symbols represent neomycin-treated P.C.S. and S.O. animals. - Experiment 3.

Minimal fluctuations in enzyme levels were detected in P.C.S. rats where the shunts were classified as partially occluded (Fig. 57) (Table 134). This was followed by a sharp decline in enzyme activity 6 weeks post-operatively. No significant differences were detected in A.A.T. levels between rats with fully patent P.C.S. and those with partially occluded anastomosis.

A.A.T. levels in rats with totally occluded P.C.S. dropped precipitously after surgery and 6 weeks post-operatively the enzyme levels were significantly lower than the initial value (Fig. 57) (Table 135). Enzyme levels in rats with fully patent, partially occluded and totally occluded P.C.S. were not significantly different. No significant differences were detected in A.A.T. levels between rats with totally occluded P.C.S. and S.O. animals.

C. RAT LIVER HISTOLOGY

i) Experiment 1

The liver specimens taken from rats 6 and 24 hours after P.C.S. showed marked areas of hepatic necrosis (Fig. 58) which were arbitrarily graded 1+ to 4+ according to the degree of involvement of 3 randomly selected specimens. These areas were seen to be undergoing phagocytosis with regeneration and infiltration of polymorphonuclear leucocytes (Fig. 59), 48 and 96 hours post-operatively.

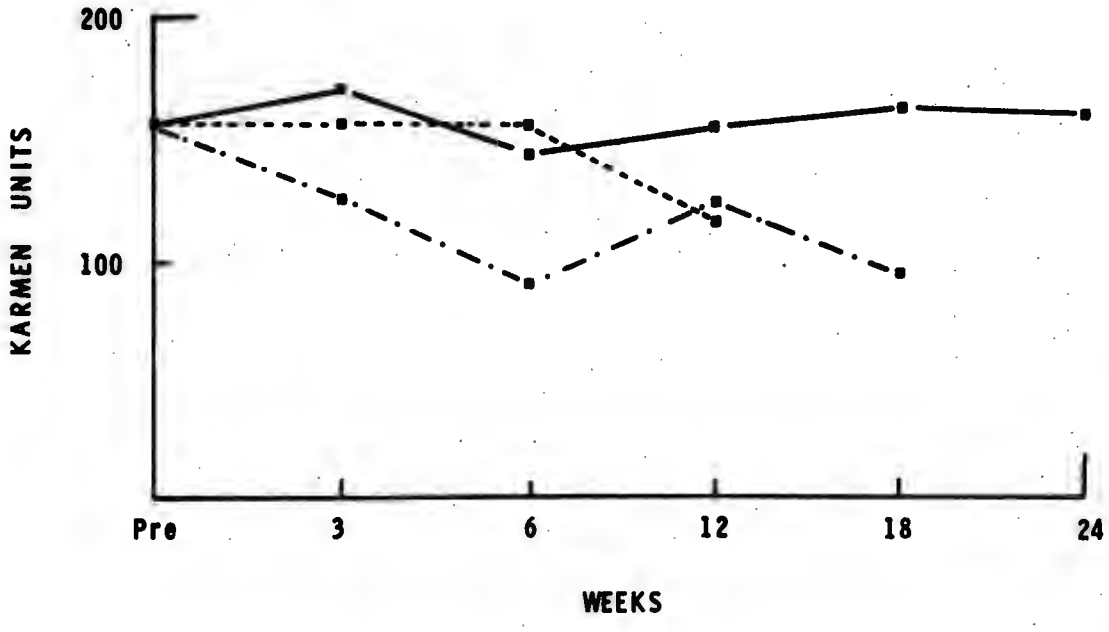

Fig. 57. Mean A.A.T. levels in rats with patent (———), partially occluded (— — —) and totally occluded (——•——) P.C.S. Experiment 3.

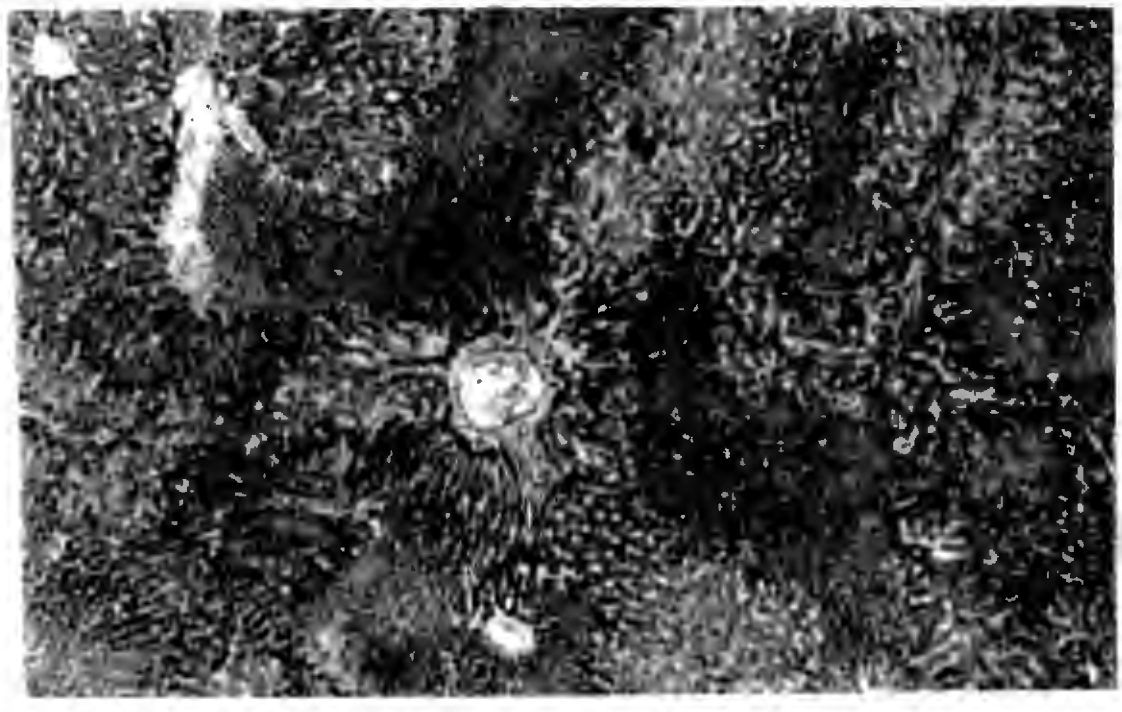

Fig. 58. Liver from a P.C.S. rat, showing marked areas of hepatic necrosis. - Experiment 1. (H & E stain - Magnification X 160.)

The areas of necrosis were almost always in relation to central veins. Fatty change was found in only 3 specimens, and minimal changes were seen in S.O. rats. Small circumscribed areas of polymorphonuclear leucocyte infiltration were seen in all specimens, whether from P.C.S. or S.O. animals. Reticulin staining demonstrated minimal collapse of architecture 5 days post-operatively, despite the appearance of haematoxylin-eosin-stained specimens.

ii) Experiment 3

Livers were examined histologically at several intervals after P.C.S. surgery and all showed similar features. In the P.C.S. animals, the principal features were mild fatty change, and infiltration of chronic inflammatory cells into the portal tracts (Fig. 60). These biopsies in sequentially sacrificed animals showed that there was no evidence of linking of portal tracts in any biopsy. The cellular infiltrate into the portal tracts might perhaps have significance in this regard and may indicate persisting liver damage. No particular features of note were seen in biopsies from S.O. and N.C. animals.

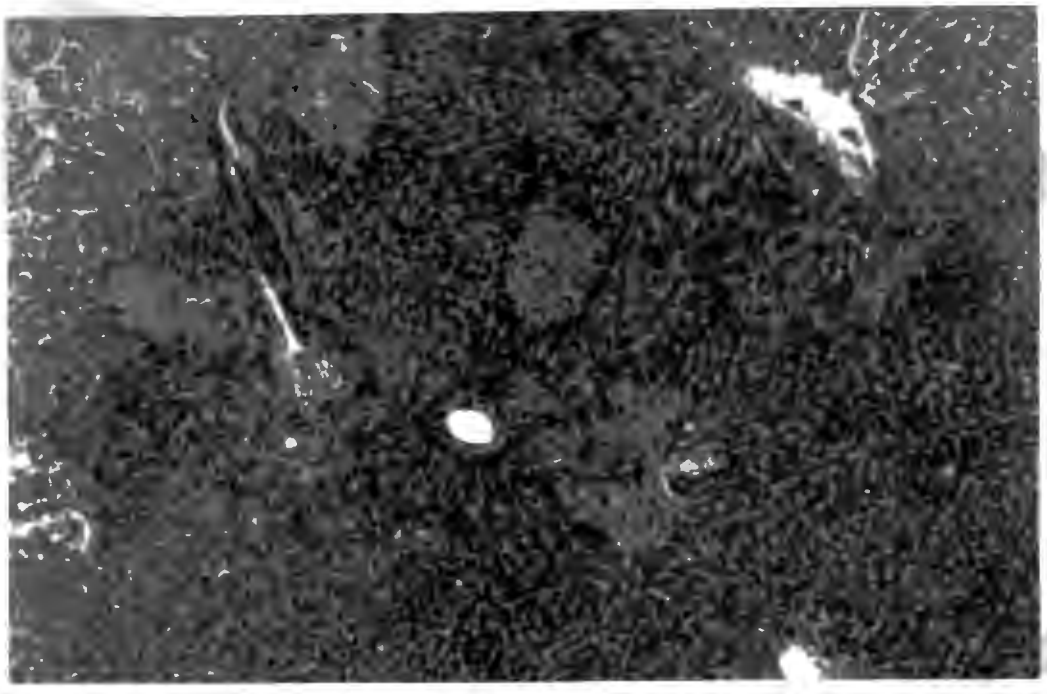

Fig. 59. Liver from a P.C.S. rat, showing areas undergoing phagocytosis with regeneration and infiltration of polymorphonuclear leucocytes. - Experiment 1. (H & E stain - magnification X 160).

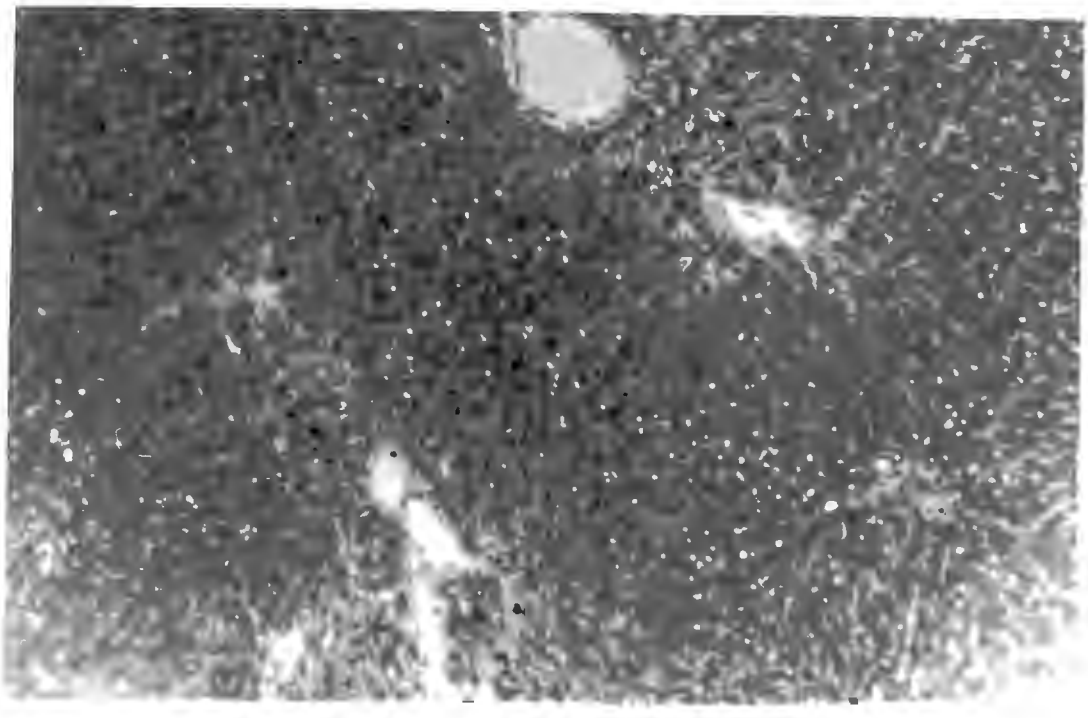

Fig. 60. Liver from a P.C.S. rat, showing mild fatty change. Experiment 3. (H & E stain magnification X 160).

DISCUSSION

The P.C.V. dropped significantly following P.C.S. and sham surgery in rats (Experiment 1). This decrease may be accounted for by haemodilution in the P.C.S. rat because of the intravenous administration of Ringer's lactate solution at the completion of the anastomosis. The effect of irrigating the exposed viscera, in both P.C.S. and S.O. rats, with Ringer's solution and its subsequent absorption needs also to be considered. Changes in P.C.V. were not documented in the long-term but Chen (1972) has reported a significant reduction in P.C.V. 16 weeks post-operatively. This decrease may be accounted for by the fact that haemopoiesis was shown to be reduced following P.C.S. in dogs (Doberneck et al., 1965) and that a significant shortening in erythrocyte life span, indicative of haemolysis, was observed in P.C.S. humans (Da Silva et al., 1963). Increased erythrocyte destruction and the consequent storage of iron in the Kupffer cells was also noted by Castro et al. (1963) following P.C.S. in dogs. Several investigators have also reported an increase in haemosiderosis following P.C.S. (Doberneck et al., 1963; Rubin et al., 1964; Grace and Balint, 1966; Grace et al., 1966; Doberneck, 197). The restricted availability of iron for haemoglobin synthesis could also have contributed to the decrease in P.C.V., but does not explain the acute changes adequately.

No documented data exists on the acute phase and long-term changes in A.A.T. levels following P.C.S. in the normal rat. The sharp rise in A.A.T. activity within 6 hours of P.C.S. (Experiment 1) is probably due to liver cell damage. Some degree of post-shunt necrosis was detected in the majority of P.C.S. livers. Ozawa et al. (1973) reported that A.A.T. levels increased 90-fold within 12 hours of portal vein ligation in normal rats and then decreased rapidly to within normal limits at 24 hours. It is possible that the initial

PART 5

Splenoportography

SPLENOPORTOGRAPHY IN RATS

The unequivocal demonstration of the I.V.C. was used as the criterion for shunt patency in P.C.S. rats. Of 21 P.C.S. animals subjected to splenoportography, 17 had patent shunts (Fig. 61), and of these 10 rats showed radiological evidence of collateral formation (Fig. 62) as illustrated in the following Table :

Rat	Weeks after P.C.S.	Weight Change(gm)	Shunt Status	Collaterals
M1	6	-100	P	+
M2	6	-118	P	+
M6	12	-136	P	-
M9	3	- 46	P	++
M10	6	-110	P	-
M11	18	-114	P	-
M12	36	+ 65	O	+++
M14	3	- 69	PO	+++
M15	24	- 27	P	-
M20	6	-100	P	+
M22	3	- 39	P	+
M23	24	- 54	PO	+
M24	3	-114	P	+
M25	12	- 97	P	++
M26	3	- 59	P	-
M27	36	+147	O	+++
M28	18	- 77	P	-
M32	3	- 90	P	+
M38	12	- 15	P	+
M42	24	- 85	P	++
M44	18	-143	P	-

P = patent PO = partially occluded O = totally occluded

Fig. 61. Splenoportogram showing a patent shunt. I.V.C. - inferior vena cava; S - splenic vein; C - collaterals.

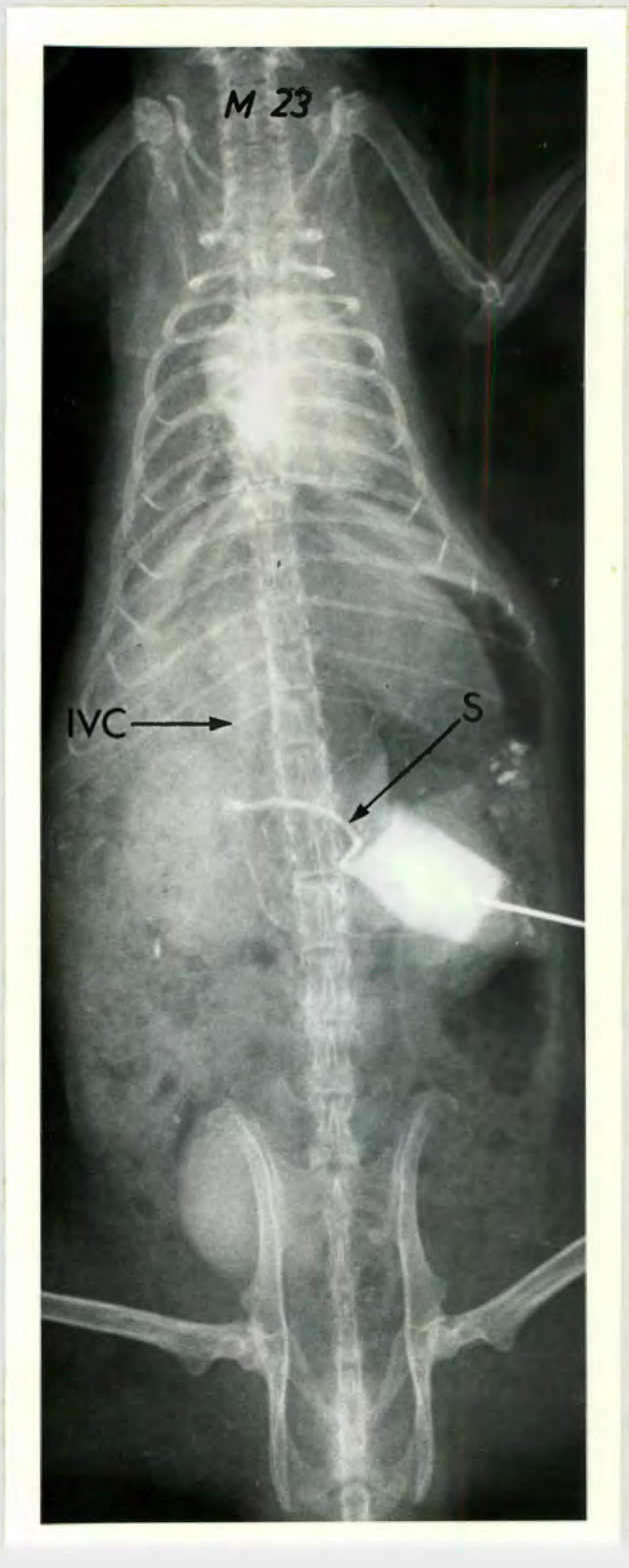

Fig. 62. Splenoportogram showing partial shunt occlusion with collaterals.

Collaterals, which were demonstrated in all but 7 animals and which were related to the site of the anastomosis, were graded as follows :

+	=	Few and fine
++	=	Intermediate
+++	=	Multiple and providing significant splenic drainage

Totally occluded P.C.S. were detected in 2 rats where radiological contrast was seen in the hepatic portal system but not in the I.V.C. (Fig. 63). Multiple collaterals which by-passed the shunt to restore portal venous blood flow to the liver, were detected in these animals. In two P.C.S. rats the anastomoses were classified as partially occluded on the basis of poor contrast of the I.V.C. and absence of contrast in the hepatic portal system. Six N.C. rats were subjected to splenoportography to demonstrate the relevant vascular anatomy (Fig. 64) and no radiological differences were detected between these and the splenoportograms of five S.O. rats.

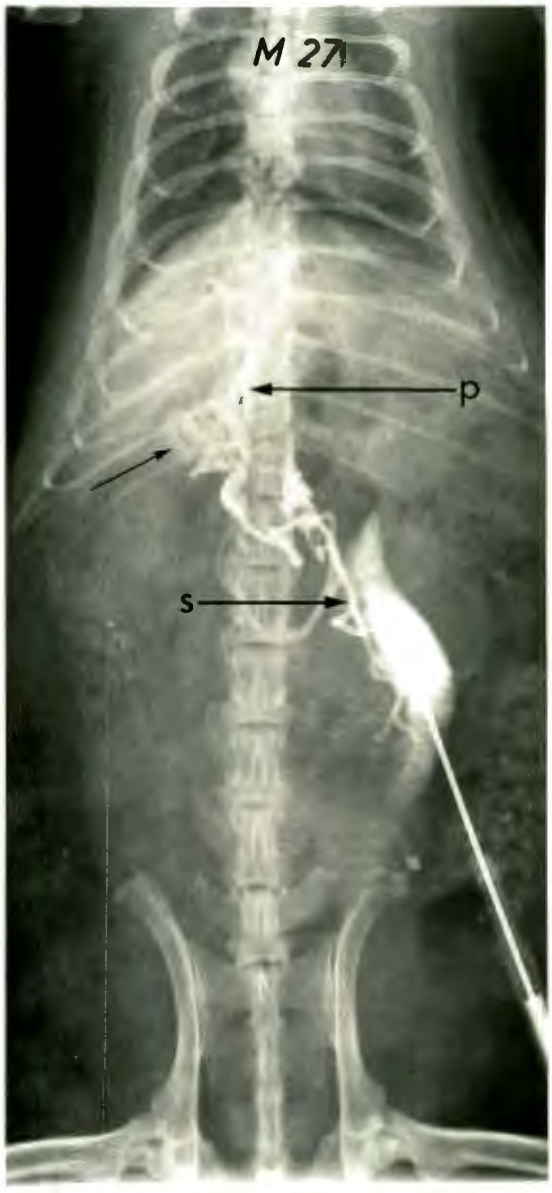

Fig. 63. Splenoportogram showing total shunt occlusion. Arrow indicates extensive collateral formation. P - portal vein.

Fig. 64. Splenoportogram of a normal rat.

DISCUSSION

The functioning of a P.C.S. is of paramount importance in any study aimed at determining the effects of P.C.S. The most direct and informative approach to determine the functional status of a P.C.S. is the use of splenoportography to outline the splanchnic venous system. This method has been recommended and utilised by Lee and Fisher (1961), Henzel et al. (1963) and Latham (1974) and the data from Experiment 3 have shown a surprising degree of variation from total shunt occlusion to shunt patency and an equally variable degree of collateral circulation. The formation of collaterals seems to bear little relationship to the degree of operative interference and the careful attention to keeping the serosal surfaces of the bowel moist while the shunt was in progress, seems not to have been a particularly important precaution. It is of interest that the formation of collaterals was not associated with differences in weight, gamma-globulin or antibacterial L.P.S. antibody. The collaterals were generally thought to be portal-systemic rather than an attempt at re-establishing splanchnic blood flow through the liver and this once again suggests the importance of the exclusion of the liver in the genesis of some of these phenomena.

Various indirect methods for determining the patency of the P.C.S. have been described. Pearlman et al. (1966) measured blood ammonia levels in the I.V.C. while Khanna (1967) used a differential glucose tolerance test. Both methods are cumbersome and involve cannulation of the venous system. The experimental data from this thesis has demonstrated a clear relationship between shunt patency and weight loss in the first 6 weeks; shunt patency and elevated gamma-globulin levels over 36 weeks and high titres of antibacterial L.P.S. antibody. These 3 parameters have been demonstrated with such regularity that it is proposed that they could be useful measures of indirect shunt patency.

CONCLUSIONS

The common laboratory rat is a suitable experimental subject and its almost legendary resistance allows for experimental procedures that are not possible in larger animals. The construction of a P.C.S. can be accomplished with minimal equipment and apparent discomfort to the experimental animal. Following the construction of the shunt a series of characteristic phenomena ensue. Marked weight loss is a dramatic and arresting development which has origins in anorexia of undetermined aetiology and is attended by a degree of hypo-albuminaemia. Equally important, and no less dramatic, is a rise in serum gamma-globulins and, to a lesser extent, of serum beta-globulins. At least three immunoglobulin classes, IgM, IgG and IgA contribute to this elevated gamma-globulin and give the elevated gamma-globulin its polyclonal character. A further characteristic of the portal-shunted rat is the development of considerably increased antibacterial L.P.S. antibody levels which probably contribute to a significant degree to the elevation in IgM. The elevation in IgG is also probably contributed to by the immune response to bacterial products but the direct contribution of bacterial L.P.S. to elevated IgG is of small consequence. The formation of antibacterial L.P.S. antibodies appears to be the result of the free access of L.P.S. to the systemic circulation and the stimulation of immuno-competent cells. The site of formation of these antibodies is not clear but a significant contribution from the spleen is unlikely. Another effect of portal shunting is on new antibody responses where the evidence seems to suggest potentiation of the antibody response to antigens.

These changes in weight following P.C.S. are well known and these studies have served only to highlight them and emphasise the deficiency in knowledge concerning its causation. The alterations

in gamma-globulin are also not new but these studies have served to characterise these changes more fully and to document the polyclonal nature of the gamma-globulin elevation. The demonstration of bacterial L.P.S. antibodies following P.C.S. in the rat, although predictable has never been demonstrated before and a putative adjuvant effect of P.C.S. on other immune responses has also not been studied before. The data reported here is also the first to correlate the patency of the shunt with changes in weight, gamma-globulins and antibacterial L.P.S. antibodies. The information documented in this thesis is also the first to examine the antibody response to constituents of bowel bacteria serially following P.C.S. A study of P.C.S. in the pig, the dog and in man serves to draw attention to the uniformity of these changes that have been documented most fully in the rat.

The postulates which were advance and formulated into an hypothesis were :

i) the changed hepatic cell metabolism and possibly hepatic cell death may account for the observed hyperglobulinaemia.

Marked liver cell necrosis was observed following P.C.S. in the rat in the acute phase study (Experiment 1) but these morphological changes were short-lived and reversible. The absence of marked histological changes in the P.C.S. rat liver but the sustained elevated gamma-globulin levels in these animals (Experiment 3) argues against altered cell metabolism and hepatic cell death as major factors in the genesis of hyperglobulinaemia in the P.C.S. rat.

ii) the exclusion of a major portion of the R.E.S. might be expected to lead to the diversion of antigenic material from the bowel. Such putative antigenic material could either

be derived from ingested food or resident microflora of the bowel.

No attempts were made to detect antibodies to food constituents and whatever contribution these could have made to the hyperglobulinaemia in the P.C.S. rat, remains speculative. Significantly elevated titres of antibacterial antibodies to L.P.S. isolated from the endogenous bowel flora were, however, consistent findings following portal shunting in rats. Attention has already been drawn to the effects of shunt occlusion and neomycin administration on antibacterial L.P.S. antibody titres and on gamma-globulin levels in P.C.S. rats.

iii) The ready access of bacteria or their products into the systemic circulation would be expected, under some circumstances, to act as adjuvants in the immune response.

Experimental evidence was documented in this thesis on the possible adjuvant effect of L.P.S. on the potentiation of new immune responses in P.C.S. rats (Experiments 7A and 7B). The effect of P.C.S. on existing immune responses was not investigated and this is an aspect which requires investigation.

The hypothesis that hyperglobulinaemia in the P.C.S. rat is a direct consequence of the diversion of splanchnic blood from the liver has been proved. Experimental evidence supports the view that antibacterial L.P.S. antibodies are responsible, at least in part, for the hyperglobulinaemia which has consistently been shown to follow

portal shunting in the rat. Antibacterial L.P.S. antibodies cannot, however, be regarded as the only contributor to the elevated gamma-globulin levels, and the role of antibodies to other antigens, of whatever origin, needs to be investigated. It would be of interest to determine the antibacterial L.P.S. antibody levels in P.C.S. pigs in view of the moderate increase (48%) in gamma-globulins following portal diversion in these animals. The determination of the optimal dose of neomycin which will effectively sterilise the bowel of the rat without inducing acute malabsorption, is another aspect which requires further definitive study. Whether hyperglobulinaemia will ensue after P.C.S. in such animals, is a question which would be of interest to resolve. In this respect, the construction of a P.C.S. in the germ-free rat is another exciting possibility. Since the role of the spleen in anti-L.P.S. antibody production and in the genesis of hyperglobulinaemia in the P.C.S. rat is probably of minor importance, an investigation into other possible sites for antibacterial L.P.S. antibody production viz. the bone marrow and lymphoid tissue of the gut, would be of great interest. Further definitive studies on IgA production in P.C.S. rats are also required and, in view of the low levels detected, a more senstive technique such as radio-immunoassay may be necessary for the quantitation of serum IgA in rats. New immune responses are potentiated in the P.C.S. rat and it would be of interest to determine the effect of portal shunting on existing immune reactions in the rat.

Finally it was hoped that an animal model could be developed which would explain the hyperglobulinaemia of liver disease. Hyperglobulinaemia of liver disease is well known for its polyclonal nature and the regularity with which it occurs. Considerable data exists on the contribution of various immunoglobulin classes to this polyclonal increase and variations in the contribution of various fractions have been proposed as useful diagnostic indices in searching for the causation of primary liver disease. Little was known about

antigens which evoked these polyclonal responses until the demonstration by Bjørneboe of increased levels of IgG and antibacterial antibody levels following P.C.S. in human cirrhosis. This very important contribution set the stage for other investigators. Thus Triger et al. showed not only increased antibacterial antibody levels but also antiviral antibodies in humans with cirrhosis, and the multiplicity of antibodies correlates with the polyclonal gamma-globulin elevation. What is remarkable about this series of antibodies against infectious agents is not their multiplicity but their magnitude. The immunological capacity of the human cirrhotic has been the subject of investigation intermittently since the demonstration by Havens of superior antibody production in cirrhotics. The importance of the liver as a barrier to uncontrolled access of antigenic material from the bowel could be inferred from the rich population of reticulo-endothelial cells and the result of impairment of the function of these cells could also be predicted. It is surprising that impairment of hepatic reticulo-endothelial function in human liver disease received so little attention until Bjørneboe reawakened interest in the filter activity of the liver R.E.S. Since then, experimental work has amply confirmed this important function.

The production of a P.C.S. in the rat is a caricature of what is believed to occur in chronic liver disease in humans. Whereas in humans the shunts are at multiple levels, in the experimental rat it is a single, large shunt. The total effect in humans of multiple small shunts is probably little different from the experimental animal. The effect in both circumstances is to influence profoundly the distribution of portal blood and, by inference, any antigenic material. The relative, as in man or absolute exclusion of the R.E.S. in the experimental rat is the same. In man it is believed that the failure of the reticulo-endothelial filtering capacity permits antigenic simulation with consequent increase in antibacterial antibodies and immunoglobulin levels. In the experimental animal total diversion

of portal blood has the same effect and the changes are almost identical, except for the relatively greater increase in IgM and the smaller and less consistent changes in IgA.

Whereas anorexia and weight loss are tell-tale symptoms of liver cirrhosis in man (Patek, 1963), reduced food intake and weight loss are, similarly, consistent features following portal shunting in the rat. Whereas in human cirrhosis increased antibacterial antibody levels have been demonstrated so in the P.C.S. rat a similar increase in antibacterial L.P.S. antibodies has been demonstrated. Whereas hyperglobulinaemia is a consistent feature of human cirrhosis so has an elevation in gamma-globulins been repeatedly demonstrated in the P.C.S. rat. Whereas in human cirrhosis better antibodies are produced, so in the experimental animal P.C.S. appears to potentiate antibody levels (no avidity studies were, however, done on the antibacterial L.P.S. antibodies).

Thus it can be concluded that the P.C.S. rat may be used as an animal model for studying the phenomenon of hyperglobulinaemia of chronic liver disease in humans.

APPENDIX

A P P E N D I X I

Total Serum Protein Determination

Reagents

Biuret reagent

A. Dissolve 17.3 gm Cu SO_4. $5H_2O$ in 100 ml hot distilled water.

B. Dissolve 173 gm sodium citrate and 100 gm anhydrous Na_2 CO_3 in 800 ml water with heating.

Allow to cool then pour B into A and stir. Dilute to one litre with distilled water.

A P P E N D I X II

Electrophoresis of Rat Serum Samples on Agar Slides

Buffer Solution

Seventeen gm Barbitone sodium was dissolved in 900 ml distilled water and the pH adjusted to 8.4 by the addition of approximately 15 ml of 1N HCl. The volume was made up to one litre with distilled water and the stock buffer solution was stored at $4^{o}C$.

An equal volume of distilled water was added to an appropriate volume of stock buffer solution and the pH of the working buffer solution was checked and adjusted, if necessary.

Ethanol-glacial acetic acid solution

Ethanol	70 ml
Glacial acetic acid	5 ml
Distilled water to	100 ml

Amido Black stain

Amido Black 10B	0.5 gm
Mercurochloride	5.0 gm
Glacial acetic acid	5.0 ml
Distilled water to	100.0 ml and filtered before use

A P P E N D I X III

Electrophoresis of rat serum proteins on cellulose acetate membranes

a) Beckman Buffer

2.76 gm Diethyl Barbituric acid)
15.40 gm Sodium Diethylbarbiturate) dissolved in 1 litre of distilled water and the pH adjusted to 8.6

b) Fixative dye

0.5 gm Ponceau-S stain)
7.5 gm Trichloroacetic acid) dissolved in 250 ml distilled water
7.5 gm Sulfosalicyclic acid)

c) Clearing Solution

25 ml Glacial acetic acid made up to 100 ml with absolute ethanol

APPENDIX IV

The Bleeding of Rabbits

The rabbit was immobilised in a standard wooden restraining box. The hair on the dorsal surface of the posterior border of the ear was shaved and then swabbed with absolute alcohol. A diagonal cut was made across the marginal vein, care being taken to avoid severing the vein, and blood was collected into 15 ml centrifuge tubes. Haemostasis was effected by the application of slight pressure on a piece of cotton-wool positioned over the incised vein.

The blood was allowed to clot at room temperature for 1 hour after which the clot was wrung with a wooden applicator. The clotted blood was kept at $4^{o}C$ overnight and the serum separated by centrifugation and stored at $-40^{o}C$.

A P P E N D I X V

Coons Buffer (pH 7.2)

Sodium barbitone	20.6 g
Sodium chloride	85.0 g
1N hydrochloride acid	80.6 ml
Distilled water to	5 litres

The above stock buffer solution was diluted with an equal volume of distilled water before use.

Mounting Medium (Buffered glycerol)

Glycine	14.0 g
Sodium Hydroxide	0.7 g
Sodium Chloride	17.0 g
Sodium Azide	1.0 g

Made up to 1 litre with distilled water.

One part of buffer to 2 parts of glycerol was used for mounting.

A P P E N D I X V

Fluorescent Microscope

A high pressure mercury vapour lamp OSRAM HB200 was used for UV observations using the following filters :

A violet-blue filter U.G.I. was used to cut out unwanted wave-lengths.
A heat absorption filter K G 1 was positioned in front of the lamp.
A barrier filter G G 13c was used for the eye-pieces.

Condensor:

A dark-field immersion condenser was used. Immersion oil (nd 1.515) was applied between the condenser and the slide.

Lenses :

Eye-piece

Wide-angle compensating eye-pieces 10 x with a field number of 18 mm and exit pupil distance of 15.5 mm were used.

Objectives

The microscope was equipped with Fluotar objectives 10 x with a numerical aperture of 0.40 and a working distance of 4.9 mm.

APPENDIX VI

Gordon & Sweets' Reticulin Stain (Gordon and Sweets, 1936)

Reagents :

A. Silver solution

Strong ammonia (.880) was added drop-wise to 5 ml of a 10.2% aqueous silver nitrate solution until the precipitate, which was first formed, just dissolved. Five ml of 3.1% sodium hydroxide was added and strong ammonia (.880) was added drop-wise until the resulting precipitate was just dissolved (the solution should not be completely clear). The volume was made up to 50 ml with fresh distilled water.

B. Acidified potassium permanganate

0.5% potassium permanganate	95 ml
3.0% sulphuric acid	5 ml

Method : The sections were :

1. fixed onto gelatine-coated slides and then hydrated
2. oxidised in acidified potassium permanganate for 7 minutes
3. washed in water and bleached in 1% oxalic acid for 3-5 minutes
4. rinsed in a) distilled water
   b) washed in tap water, and
   c) 2-3 changes of distilled water
5. Mordanted in 2.5% iron alum for 10 minutes to 2 hours
6. Washed in 2-3 changes of distilled water
7. Treated with silver solution ($\pm$ 60 seconds) until the sections became transparent

A P P E N D I X VI

Haematoxylin-Eosin Staining Procedure

1. The tissue was fixed in 10% neutral buffered formalin for 12-24 hours.

2. The liver tissue was processed in a Shandon-Elliot tissue processor and dehydrated as follows :

    a) in 70% alcohol for 2 hours
    b) in 8% phenol (in 96% alcohol) for 2 hours
    c) 96% alcohol for 2 hours
    d) and finally for 8 hours in 4 changes of absolute alcohol.

3. The tissue was cleared for 4 hours in 2 changes of xylol and wax impregnated for 2 hours each in 3 changes of wax (Paraplast M.pt 57^{o}C : Baird & Tatlock).

4. The tissue was embedded in Paraplast (kept at 60^{o}C prior to use) using a Leukard embedding box.

5. The block of tissue was sectioned at 2-3u on a JUNG sliding microtome and fixed onto gelatin-coated slides (2% gelatine in 1% phenol).

6. The sections were processed as follows :

    a) the sections were hydrated, stained with Meyer's haematoxylin for 10 minutes and then briefly rinsed in water.

    The slides were
    b) briefly differentiated in 1% acid alcohol
    c) rinsed in water
    d) blued in Scotch tap water substrate
    e) rinsed in water

7. The sections were counter-stained for 3 minutes in 1% Eosin-1% Phloxem solution mixed in the proportions 2:1 respectively, dehydrated and mounted in Canada-Balsam.

10% neutral buffered formalin (pH 7)

sodium dehydrogen phosphate (anhydrous)	3.5 g
disodium hydrogen phosphate (anhydrous)	6.5 g
formaldehyde (40%)	100 ml
distilled water	900 ml

Scotch tap-water substrate

sodium bicarbonate	3.5 g
magnesium sulphate	20.0 g
distilled water to 1 litre	

8. Washed well in several changes distilled water
9. Reduced in 10% aqueous formalin for 1 minute
10. Washed in tap water and then in distilled water
11. Toned in 0.2% gold chloride for 10-15 minutes
12. Rinsed in distilled water
13. Fixed in 5% sodium thiosulphate for 5 minutes
14. Washed in water for 1-2 minutes
15. Dipped into Van Giesson's solution for 2 minutes
16. Dehydrated, cleared and mounted in the usual way.

A P P E N D I X VII

Table 1

Changes in Body Weight in P.C.S. rats - Expt. 1

	6 hr	Day 1	2	3	4
Mean	98.903	93.942	90.123	87.395	83.606
n	6	19	13	12	6
S.E.	0.309	0.534	0.767	1.026	1.936
p	>0.80	>0.50	<0.01	<0.001	<0.001

Table 2

Changes in Body Weight in S.O. rats - Expt. 1

	6 hr	Day 1	2	3	4
Mean	98.680	94.352	94.105	92.765	92.218
n	6	19	12	12	6
S.E.	0.473	0.811	1.401	1.607	2.401
p	>0.80	>0.50	>0.50	>0.10	>0.05

Table 3

Changes in Body Weight in P.C.S. rats - Experiment 2/3

Weeks	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Mean	87.63	81.97	76.49	73.11	71.33	68.92	70.05	70.31	72.94	71.14	73.83	76.18	75.91	76.75	79.13	79.85	81.86	82.90	86.41	88.08	89.48	90.31	91.09	91.99
n	35	35	35	32	32	32	28	27	25	21	16	12	9	7	6	6	6	6	5	4	4	4	4	4
S.E.	0.81	1.13	1.37	1.71	1.79	1.83	2.31	2.43	2.39	2.97	3.28	4.03	4.83	6.31	7.58	7.10	7.15	7.11	6.79	8.31	8.77	9.28	9.46	8.96
p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.005	<0.005	<0.005

Table 4

Changes in Body Weight in P.C.S. rats treated with neomycin

Expt. 2/3

Weeks	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Mean	88.07	81.05	75.93	73.30	31.88	72.43	73.12	74.39	75.64	76.63	76.83	77.04	78.31	79.11	79.47	81.06	81.44	83.00	86.40	86.21	86.21	90.58	87.27
n	19	19	19	16	16	16	14	13	13	13	13	13	12	12	12	11	11	9	8	8	8	7	4
S.E.	1.35	1.47	2.17	3.17	3.82	4.62	5.18	5.74	5.67	5.92	5.91	5.93	6.39	6.38	6.31	6.88	7.19	8.90	9.60	9.83	10.02	11.02	16.33
p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.005	<0.005	<0.01	<0.05	<0.05	<0.05	>0.10	>0.05

Table 5

Changes in Body Weight in S.O. rats - Expt. 2/3

Weeks	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Mean	102.35	103.83	110.18	114.69	119.44	123.00	125.83	128.36	132.36	131.64	131.21	129.95	129.83	132.37	135.96	135.46	136.21	137.42
n	5	5	5	5	5	5	5	5	5	5	5	5	3	3	3	3	3	3
S.E.	2.53	3.38	2.87	3.24	3.80	4.20	4.40	4.62	3.40	2.67	2.94	3.57	6.27	6.46	6.04	6.34	6.18	6.33
p	> 0.30	> 0.20	< 0.01	< 0.005	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

Table 6

Changes in Body Weight in N.C. rats - Expt. 2/3

Weeks	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Mean	100.87	113.42	119.10	121.72	123.55	123.61	124.63	126.67	128.64	130.25	131.42	132.44	133.54	134.70	135.72	136.67	137.62
n	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
S.E.	0.15	1.78	1.83	1.16	1.51	2.53	2.43	2.56	2.90	3.16	3.27	3.32	3.48	3.23	2.97	2.73	2.76
p	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

Table 7

Changes in Body Weight in rats with partially-occluded P.C.S. - Expt. 2/3

Weeks	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Mean	93.62	90.52	93.446	97.79	101.79	107.89	109.69	111.10	112.99	116.50	117.01	116.92	117.43	118.62	119.41	119.59	121.80	122.86	122.97	123.62	125.01	126.61
n	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
S.E.	1.72	2.57	3.58	5.45	6.50	8.51	7.56	7.90	6.62	5.38	5.53	5.79	6.02	6.28	5.30	5.94	5.83	5.89	6.69	6.89	6.34	5.95
p	<0.025	<0.025	>0.10	>0.70	>0.70	>0.40	>0.20	>0.20	>0.10	<0.05	<0.05	<0.05	<0.05	<0.05	<0.025	<0.05	<0.025	<0.02	<0.05	<0.05	<0.02	<0.02

Table 8

Changes in Body Weight in rats with Patent P.C.S. - Expt. 2/3

Weeks	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Mean	87.37	81.44	75.82	72.61	70.76	68.20	69.12	69.33	71.83	69.37	71.63	73.40	71.96	71.83	73.09	74.23	76.15	77.24	80.62	80.92	81.86	82.15	82.64	84.30
n	32	32	32	31	31	31	27	26	24	20	15	11	8	6	5	5	5	5	4	3	3	3	3	3
S.E.	0.87	1.19	1.42	1.69	1.75	1.74	2.20	2.31	2.17	2.51	2.60	3.19	3.44	4.67	5.62	5.32	5.28	5.26	4.57	6.00	6.14	6.25	6.01	6.48
p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Table 9

Changes in Body Weight in rats with Partially-occluded P.C.S. - Expts. 2/3

	1	2	3	4	5	6	7	8	9	10	11	12
Mean	85.50	80.96	75.82	67.03	65.31	67.90	70.54	67.58	68.10	68.34	67.99	68.94
n	5	5	5	3	3	3	3	3	3	3	3	3
S.E.	2.30	2.32	2.72	1.34	1.64	5.61	2.09	2.18	0.61	0.52	0.95	1.05
p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Table 10

Changes in Body Weight in P.C.S. rats - Expt. 5

Weeks	1	2	3	4	5	6	7	8	9
Mean	81.23	72.99	66.82	64.77	68.23	67.91	69.08	69.41	69.60
n	19	18	17	13	10	10	8	6	3
S.E.	1.27	1.24	1.36	1.99	2.97	2.97	3.91	5.24	5.63
p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Table 11

Changes in Body Weight in P.C.S. rats treated with Neomycin - Expt. 5

Weeks	1	2	3	4	5	6	7	8	9	10
Mean	84.74	76.56	68.78	63.58	62.43	58.95	59.87	64.06	65.08	65.47
n	15	15	15	13	10	7	6	4	3	3
S.E.	1.69	1.92	1.80	2.17	2.33	3.46	3.13	3.20	1.90	2.33
p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	< 0.001

Table 12

Changes in Body Weight in P.C.S. rats subjected to Splenectomy - Expt. 4

Weeks	Pre	1	2	3	4	5	6
Mean	310.722	82.156	75.361	66.340	64.271	61.570	66.755
n	36	16	12	9	7	7	4
S.E.	5.573	1.905	1.906	2.819	3.664	4.731	4.520
p		< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.001

Table 13

Changes in Body Weight in S.O. rats subjected to Splenectomy - Expt. 4

Weeks	Pre	1	2	3	4	5	6
Mean	310.722	82.889	86.670	96.350	96.770	99.078	102.306
n	36	10	8	7	6	5	5
S.E.	5.573	1.807	2.110	3.761	2.211	2.090	1.601
p		< 0.001	< 0.001	< 0.05	< 0.001	> 0.20	< 0.001

Table 14

Changes in mean serum Albumin levels in P.C.S. rats - Expt. 1

	Pre	6hr	Day 1	2	3	4
Mean	41.332	41.736	39.448	38.811	37.762	38.383
n	48	6	18	13	12	6
S.E.	0.673	2.099	0.944	2.228	1.616	2.208
p		>0.80	>0.10	>0.10	<0.05	>0.10

Table 15

Changes in mean serum Albumin levels in S.O. rats - Expt. 1

	Pre	6hr.	Day 1	2	3	4
Mean	41.332	41.041	39.256	36.110	34.156	32.956
n	48	6	18	12	11	6
S.E.	0.673	1.839	1.374	2.188	2.409	2.354
p		>0.80	>0.10	<0.005	<0.001	<0.001

Table 18

Changes in mean serum α_2 - globulin levels in P.C.S. rats - Expt. 1

	Pre	6 hr	Day 1	2	3	4
Mean	9.961	10.573	10.975	10.485	10.631	9.991
n	48	6	18	13	12	6
S.E.	0.293	0.892	0.371	0.531	0.551	0.690
p		>0.40	>0.05	>0.40	>0.50	>0.95

Table 18A

Changes in mean serum α_2 - globulin levels in S.O. rats - Expt. 1

	Pre	6 hr	Day 1	2	3	4
Mean	9.961	8.536	11.310	10.960	9.530	11.791
n	48	6	18	12	11	6
S.E.	0.293	0.483	0.664	0.918	1.059	1.468
p		>0.10	<0.05	>0.10	>0.50	>0.30

Table 19

Changes in mean serum β - globulin levels in P.C.S. rats - Expt. 1

	Pre	6 hr	Day 1	2	3	4
Mean	17.985	15.341	16.709	16.626	16.540	17.303
n	48	6	18	13	12	6
S.E.	0.430	0.961	0.397	0.593	0.629	0.648
p		<0.05	>0.05	>0.10	>0.50	>0.10

Table 20

Changes in mean serum β - globulin levels in S.O. rats - Expt. 1

	Pre	6 hr	Day 1	2	3	4
Mean	17.985	16.481	16.418	19.250	18.684	20.311
n	48	6	18	12	11	6
S.E.	0.430	0.805	0.433	0.703	0.603	1.228
p		>0.20	>0.05	>0.10	>0.40	>0.05

Table 23

Changes in mean serum Albumin levels in P.C.S. rats - Expt. 2

	Pre	1	2	3	4	5	6	7	8	9	10	12
an	36.841	32.061	30.709	31.105	30.760	31.420	32.583	32.701	32.568	32.062	32.394	32.356
	20	12	14	14	10	14	14	13	13	11	11	5
E.	0.836	0.881	1.098	0.735	1.505	0.807	0.777	0.841	0.940	0.926	0.621	1.788
		<0.01	<0.001	<0.001	<0.001	<0.001	<0.02	<0.05	<0.02	<0.005	<0.005	<0.05

Table 24

Changes in mean serum Albumin levels in S.O. rats - Expt. 2

	Pre	1	2	3	4	5	6	7	8	9	10	12
an	36.841	27.194	29.468	30.628	32.977	33.332	34.287	35.354	36.579	36.456	36.212	36.001
	20	9	10	10	10	10	9	10	10	9	8	8
E.	0.836	2.039	0.767	0.899	0.842	0.649	1.061	0.601	0.961	1.435	1.298	1.291
		<0.001	<0.001	<0.001	<0.01	<0.02	>0.05	>0.20	>0.50	>0.80	>0.60	>0.50

Table 25

Changes in mean serum Albumin levels in N.C. rats - Expt. 2

Pre	1	2	3	4	5	6	7	8	9	10	12
36.841	37.426	35.831	35.289	36.374	34.853	36.420	36.850	36.897	37.231	37.125	37.075
20	9	10	10	10	9	8	8	9	6	7	7
0.836	0.800	0.993	1.466	1.011	1.342	0.829	0.539	0.993	1.423	0.969	1.654
	>0.40	>0.40	>0.20	>0.70	>0.20	>0.50	>0.995	>0.70	>0.80	>0.80	>0.80

Table 26

Changes in mean serum α_1 - globulin levels in P.C.S. rats - Expt. 2

Pre	1	2	3	4	5	6	7	8	9	10	12
22.890	26.406	20.745	16.590	17.061	17.240	17.419	17.273	17.034	16.175	15.380	15.474
20	12	14	17	10	14	14	13	13	11	11	5
1.462	0.760	0.670	0.465	0.530	0.493	0.986	0.530	0.646	0.823	0.547	0.829
	>0.05	>0.20	<0.001	<0.02	<0.005	<0.01	<0.005	<0.01	<0.01	<0.01	>0.05

Table 27

Changes in mean serum α_1 - globulin levels in S.O. rats - Expt. 2

	Pre	1	2	3	4	5	6	7	8	9	10	12
n	22.890	28.906	21.880	22.571	23.523	24.302	24.992	24.249	23.771	24.828	24.251	23.546
	20	9	10	10	10	10	9	10	10	9	8	8
.	1.462	2.411	0.686	0.757	0.670	1.024	1.447	0.773	0.727	1.694	1.078	1.235
		<0.001	>0.60	>0.80	>0.70	>0.50	>0.40	>0.60	>0.70	>0.40	>0.60	>0.80

Table 28

Changes in mean serum α_1 - globulin levels in N.C. rats - Expt. 2

	Pre	1	2	3	4	5	6	7	8	9	10	12
n	22.890	21.431	20.763	20.634	20.671	20.954	21.858	21.650	21.951	22.601	22.648	22.869
	20	9	10	10	10	9	8	9	9	6	7	7
.	0.836	1.253	0.954	1.098	0.664	1.103	0.749	0.501	1.006	0.944	1.330	0.643
		>0.50	>0.30	>0.30	>0.30	>0.30	>0.60	>0.50	>0.60	>0.70	>0.80	>0.90

Table 29

Changes in mean serum α_2 - globulin levels in P.C.S. rats - Expt. 2

	Pre	1	2	3	4	5	6	7	8	9	10	12
Mean	10.966	9.890	11.850	10.375	9.223	9.282	9.344	8.383	8.987	8.528	8.322	9.342
n	20	12	14	14	10	14	14	13	13	11	11	5
S.E.	0.552	0.459	0.407	0.400	0.882	0.262	0.477	0.545	0.366	0.957	0.376	1.678
p		>0.10	>0.20	>0.40	>0.05	<0.05	<0.05	>0.05	<0.02	<0.05	<0.05	>0.30

Table 30

Changes in mean serum α_2 - globulin levels in S.O. rats - Expt. 2

	Pre	1	2	3	4	5	6	7	8	9	10	12
Mean	10.966	10.817	11.274	10.951	10.058	10.261	10.568	10.192	9.786	9.272	9.571	9.483
n	20	9	10	10	10	10	9	10	10	9	8	8
S.E.	0.552	0.727	0.646	0.680	0.484	0.486	0.447	0.470	1.135	0.894	0.605	0.864
p		>0.80	>0.70	>0.98	>0.20	>0.40	>0.60	>0.30	>0.20	>0.10	>0.10	>0.10

Table 31

Changes in mean serum α_2 - globulin levels in N.C. rats - Expt. 2

	Pre	1	2	3	4	5	6	7	8	9	10	12
n	10.966	11.088	12.057	11.851	10.161	11.128	11.073	10.716	9.720	9.623	9.697	9.877
	20	9	10	10	10	9	8	9	9	6	7	7
.	0.552	1.213	0.548	0.447	0.513	0.655	0.530	0.566	0.938	0.982	1.186	1.132
		>0.90	>0.20	>0.30	>0.30	>0.80	>0.60	>0.30	>0.20	>0.20	>0.20	>0.10

Table 32

Changes in mean serum β - globulin levels in P.C.S. rats - Expt. 2

	Pre	1	2	3	4	5	6	7	8	9	10	12
n	18.256	17.509	17.089	17.863	20.437	20.080	20.097	19.924	20.490	20.440	22.205	22.976
	20	12	14	14	10	14	14	13	13	11	11	5
.	0.842	0.817	0.549	0.674	1.068	0.532	0.555	0.875	0.487	0.658	0.931	0.941
		>0.40	>0.30	>0.40	<0.05	>0.10	>0.05	>0.05	<0.05	>0.10	<0.005	<0.001

Table 33

Changes in mean serum β - globulin levels in S.O. rats - Expt. 2

	Pre	1	2	3	4	5	6	7	8	9	10	12
ean	18.256	21.071	24.456	23.857	22.413	22.753	20.026	20.956	20.945	20.063	19.490	20.620
	20	9	10	10	10	10	9	10	10	9	8	8
.E.	0.842	1.364	0.610	1.124	0.534	0.564	1.203	0.648	0.439	0.823	0.898	0.701
		>0.10	<0.001	<0.001	<0.005	<0.001	>0.30	>0.05	>0.05	>0.20	>0.40	>0.10

Table 34

Changes in mean serum β - globulin levels in N.C. rats - Expt. 2

	Pre	1	2	3	4	5	6	7	8	9	10	12
ean	18.256	18.426	20.083	19.831	19.237	19.941	20.585	20.298	20.568	19.971	19.841	20.098
	20	8	10	10	10	9	8	9	9	6	7	7
.E.	0.842	0.443	0.876	1.304	1.027	0.845	0.522	0.680	0.355	0.882	0.688	0.538
		>0.98	>0.20	>0.50	>0.50	>0.20	>0.20	>0.10	>0.10	>0.70	>0.50	>0.20

Table 35

Changes in mean serum γ - globulin levels in P.C.S. rats - Expt. 2

	Pre	1	2	3	4	5	6	7	8	9	10	12
Mean	11.098	14.865	20.229	23.895	23.455	22.607	22.310	22.506	22.656	22.959	22.715	22.960
n	20	12	14	14	10	11	11	12	13	11	10	5
S.E.	0.588	0.567	0.762	0.869	0.975	0.670	0.549	0.791	0.644	1.030	0.693	0.513
P		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Table 36

Changes in mean serum γ - globulin levels in S.O. rats - Expt. 2

	Pre	1	2	3	4	5	6	7	8	9	10	12
Mean	11.098	12.415	12.820	11.253	10.869	9.909	10.000	9.956	10.008	9.975	9.908	9.756
n	20	9	10	10	10	10	9	10	10	9	7	8
S.E.	0.588	0.882	0.400	0.649	0.347	0.862	0.652	0.602	0.356	0.778	0.678	0.472
P		>0.30	>0.10	>0.975	>0.10	>0.10	>0.10	>0.05	>0.10	>0.10	>0.10	>0.05

Table 37

Changes in mean serum γ - globulin levels in N.C. rats - Expt. 2

	Pre	1	2	3	4	5	6	7	8	9	10	12
Mean	11.095	11.705	11.742	12.197	11.791	11.272	11.037	10.902	10.851	10.148	10.100	9.924
n	20	9	10	10	10	9	8	9	9	6	7	7
S.E.	0.558	0.669	0.607	0.362	0.440	0.392	0.533	0.545	0.522	0.645	0.433	0.578
p		>0.80	>0.70	>0.40	>0.40	>0.70	>0.70	>0.70	>0.50	>0.20	>0.10	>0.10

Table 38

Changes in mean serum Albumin levels in P.C.S. rats - Expt. 3

	Pre	3	6	12	18	24	36
Mean	36.970	34.635	33.888	34.002	35.289	35.093	36.125
n	45	18	14	10	10	8	4
S.E.	0.477	0.950	0.744	1.060	1.264	0.597	0.855
p		<0.02	<0.005	<0.01	>0.10	>0.10	>0.60

Table 39

Changes in mean serum Albumin levels in rats with T.O. P.C.S. - Expt. 3

	Pre	3	6	12	18	24
Mean	36.970	33.833	33.950	34.310	36.173	37.793
n	45	3	3	3	3	3
S.E.	0.477	3.308	2.002	3.928	2.514	3.211
p		>0.10	>0.10	>0.20	>0.60	>0.60

Table 40

Changes in mean serum Albumin levels in rats with P.O. P.C.S. - Expt. 3

	Pre	3	6	12	18
Mean	36.970	35.352	34.313	34.823	35.379
n	45	5	3	3	3
S.E.	0.477	2.622	1.868	2.102	1.973
p		>0.30	>0.10	>0.20	>0.30

Table 41

Changes in mean serum Albumin levels in P.C.S. rats treated with neomycin -Expt.3

	Pre	3	6	12	18	24
Mean	36.970	34.228	33.451	33.940	35.854	35.730
n	45	15	12	10	7	3
S.E.	0.477	1.178	1.435	1.260	1.256	1.539
p		<0.02	<0.005	<0.02	>0.30	>0.95

Table 42

Changes in mean serum Albumin levels in S.N. rats - Expt. 3

	Pre	3	6	12	18	24
Mean	36.970	36.708	35.411	35.350	35.188	36.155
n	45	10	10	10	8	6
S.E.	0.477	0.998	0.883	0.637	0.869	2.080
p		>0.80	>0.10	>0.10	>0.10	>0.56

Table 43

Changes in mean serum Albumin levels in N.C. rats - Expt. 3

	Pre	3	6	12	18	24	36
Mean	36.970	36.727	35.772	36.168	36.686	36.024	36.120
n	45	10	10	10	6	7	6
S.E.	0.477	1.615	1.042	1.033	1.844	1.654	1.149
p		>0.80	>0.60	>0.70	>0.80	>0.95	>0.50

Table 44

Changes in mean serum Albumin levels in S.O. rats - Expt. 3

	Pre	3	6	12	18	24	36
Mean	36.970	33.833	33.370	34.257	36.158	36.566	36.625
n	45	10	10	10	12	10	8
S.E.	0.477	1.439	1.073	1.451	0.949	1.051	0.851
p		<0.02	<0.02	>0.10	>0.40	>0.70	>0.70

Table 45

Changes in mean serum α_1 - globulin levels in P.C.S. rats - Expt. 3

	Pre	3	6	12	18	24	36
Mean	26.770	20.125	16.467	16.385	15.846	17.007	17.600
n	45	18	14	10	10	8	4
S.E.	0.579	0.837	1.006	1.188	1.292	0.540	2.994
p		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Table 45A

Changes in mean serum α_1 - globulin levels in P.C.S. rats treated with neomycin - Expt. 3

	Pre	3	6	12	18	24
Mean	26.770	18.873	18.401	16.951	16.862	16.530
n	45	14	12	10	7	3
S.E.	0.579	1.644	1.625	1.881	2.499	0.719
p		<0.001	<0.001	<0.001	<0.001	<0.001

Table 46

Changes in mean serum α_2 - globulin levels in rats with P.O. P.C.S. - Expt. 3

	Pre	3	6	12	18
Mean	26.770	18.014	19.155	16.300	15.935
n	45	5	3	3	3
S.E.	0.579	1.271	0.064	0.954	0.174
p		<0.001	<0.01	<0.001	<0.001

Table 47

Changes in mean serum α_1 - globulin levels in rats with T.O. P.C.S. - Expt. 3

	Pre	3	6	12	18	24
Mean	26.770	26.340	24.686	22.606	25.253	21.160
n	45	3	3	3	3	3
S.E.	0.579	2.162	4.410	2.494	3.621	1.882
p		>0.80	>0.40	>0.20	>0.50	<0.02

Table 48

Changes in mean serum α_1 - globulin levels in S.O. rats - Expt. 3

	Pre	3	6	12	18	24	36
Mean	26.770	27.706	28.490	29.162	25.396	23.732	26.525
n	45	10	10	10	12	10	8
S.E.	0.579	1.921	0.559	1.655	1.000	0.966	0.875
p		>0.60	>0.30	>0.10	>0.20	<0.02	>0.80

Table 49

Changes in mean serum α_1 - globulin levels in N.C. rats - Expt. 3

	Pre	3	6	12	18	24	36
Mean	26.770	26.867	25.694	27.064	25.725	23.066	24.760
n	45	10	10	10	6	7	6
S.E.	0.579	0.273	1.090	1.023	0.356	1.561	0.978
p		>0.90	>0.50	>0.40	>0.50	<0.02	>0.20

Table 50

Changes in mean serum α_1 - globulin levels in S.N. rats - Expt. 3

	Pre	3	6	12	18	24
Mean	26.770	30.260	31.362	31.574	29.913	27.970
n	45	10	10	10	8	6
S.E.	0.579	1.794	0.773	1.140	2.186	2.194
p		<0.025	<0.001	<0.001	>0.05	>0.50

Table 51

Changes in mean serum α_2 - globulin levels in P.C.S. rats - Expt. 3

	Pre	3	6	12	18	24	36
Mean	9.582	9.762	10.720	10.292	10.227	10.487	8.650
n	45	18	14	10	10	8	4
S.E.	0.273	0.486	0.841	1.063	0.609	0.569	0.856
p		>0.70	>0.20	>0.60	>0.50	>0.30	>0.20

Table 52

Changes in mean serum α_2 - globulin levels in S.O. rats - Expt. 3

	Pre	3	6	12	18	24	36
Mean	9.582	10.900	11.920	10.530	9.613	9.822	8.825
n	45	10	10	10	12	10	8
S.E.	0.273	0.608	0.514	0.943	0.661	0.503	0.642
p		>0.30	<0.02	>0.40	>0.60	>0.80	>0.10

Table 53

Changes in mean serum α_2 - globulin levels in S.N. rats - Expt. 3

	Pre	3	6	12	18	24
Mean	9.582	8.877	9.084	9.297	10.623	10.971
n	45	10	10	10	8	6
S.E.	0.273	0.160	0.605	0.575	0.974	0.692
p		>0.10	>0.20	>0.30	>0.30	>0.10

Table 54

Changes in mean serum α_2 - globulin levels in N.C. rats - Expt. 3

	Pre	3	6	12	18	24	36
Mean	9.582	11.302	10.394	11.152	10.250	9.125	9.540
n	45	10	10	10	6	7	6
S.E.	0.273	1.174	0.506	0.912	1.304	0.510	0.267
p		>0.10	>0.50	>0.10	>0.60	>0.20	>0.70

Table 55

Changes in mean serum α_2 - globulin levels in rats with P.O. P.C.S. - Expt. 3

	Pre	3	6	12	18
Mean	9.582	10.734	11.535	10.350	11.275
n	45	5	3	3	3
S.E.	0.273	0.482	2.145	0.648	0.094
p		>0.20	>0.20	>0.60	>0.20

Table 56

Changes in mean serum α_2 - globulin levels in rats with T.O. P.C.S. - Expt. 3

	Pre	3	6	12	18	24
Mean	9.582	10.676	11.620	11.193	10.040	10.360
n	45	3	3	3	3	3
S.E.	0.273	1.657	2.002	0.806	0.591	0.451
p		>0.30	>0.10	>0.05	>0.80	>0.60

Table 57

Changes in mean α_2 - globulin levels in P.C.S. rats treated with neomycin - Expt. 3

	Pre	3	6	12	18	24
Mean	9.582	10.857	11.146	10.968	11.371	10.865
n	45	15	12	10	7	3
S.E.	0.273	0.497	0.625	0.711	0.433	0.244
p		<0.05	<0.05	>0.05	<0.05	>0.40

Table 58

Changes in mean serum β - globulin levels in P.C.S. rats - Expt. 3

	Pre	3	6	12	18	24	36
Mean	15.425	16.266	16.608	14.953	16.719	16.941	18.505
n	45	18	14	10	10	8	4
S.E.	0.410	0.482	0.553	0.485	0.671	1.321	0.579
p		>0.20	>0.10	>0.70	>0.10	>0.20	<0.05

Table 59

Changes in mean serum β - globulin levels in P.C.S. rats treated with neomycin - Expt. 3

	Pre	3	6	12	18	24
Mean	15.425	15.866	15.540	17.126	16.137	16.805
n	45	15	12	10	7	3
S.E.	0.410	0.854	0.555	0.876	0.669	0.424
p		>0.60	>0.80	>0.05	>0.50	>0.40

Table 60

Changes in mean serum β - globulin levels in rats with T.O. P.C.S. - Expt. 3

	Pre	3	6	12	18	24
Mean	15.425	16.813	15.536	17.413	15.850	16.370
n	45	3	3	3	3	3
S.E.	0.410	1.801	1.821	1.696	0.989	0.759
p		>0.40	>0.90	>0.20	>0.70	>0.50

Table 61

Changes in mean serum β - globulin levels in rats with P.O. P.C.S. - Expt. 3

	Pre	3	6	12	18
Mean	15.425	15.132	14.435	16.346	16.085
n	45	5	3	3	3
S.E.	0.410	1.011	1.014	0.937	2.005
p		>0.80	>0.60	>0.50	>0.70

Table 62

Changes in mean serum β - globulin levels in S.O. rats - Expt. 3

	Pre	3	6	12	18	24	36
Mean	15.425	17.023	16.380	16.682	17.102	17.930	16.740
n	45	10	10	10	12	10	8
S.E.	0.410	0,717	0.726	1.378	0.605	0.600	0.690
p		>0.30	>0.30	>0.30	<0.05	<0.01	>0.20

Table 63

Changes in mean serum β - globulin levels in S.N. rats - Expt. 3

	Pre	3	6	12	18	24
Mean	15.425	14.003	14.714	14.572	13.844	15.435
n	45	10	10	10	9	6
S.E.	0.410	0.973	0.441	0.846	1.125	0.239
p		>0.10	>0.40	>0.40	>0.10	>0.99

Table 64

Changes in mean serum β - globulin levels in N.C. rats - Expt. 3

	Pre	3	6	12	18	24	36
Mean	15.425	16.175	16.540	15.670	14.375	15.914	16.020
n	45	10	10	10	6	7	6
S.E.	0.410	1.103	0.604	1.231	1.349	0.646	1.524
p		>0.60	>0.30	>0.80	>0.40	>0.60	>0.50

Table 65

Changes in mean serum γ-globulin levels in P.C.S. rats - Expt. 3

	Pre	3	6	12	18	24	36
Mean	10.819	19.148	20.392	22.610	22.919	23.097	22.225
n	45	18	14	10	10	8	4
S.E.	0.322	0.876	0.796	0.966	0.971	0.879	0.728
p		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Table 66

Changes in mean serum γ - globulin levels in P.C.S. rats treated with neomycin - Expt. 3

	Pre	3	6	12	18	24
Mean	10.819	19.405	20.684	20.897	18.752	19.433
n	45	15	12	10	7	3
S.E.	0.322	1.517	1.732	1.155	1.575	0.862
p		<0.001	<0.001	<0.001	<0.001	<0.001

Table 67

Changes in mean serum γ - globulin levels in rats with P.O. P.C.S. - Expt. 3

	Pre	3	6	12	18
Mean	10.819	20.122	20.210	22.246	20.975
n	45	5	3	3	3
S.E.	0.322	1.032	1.241	1.021	1.075
p		<0.001	<0.001	<0.001	<0.001

Table 68

Changes in mean serum γ - globulin levels in rats with T.O. P.C.S. - Expt. 3

	Pre	3	6	12	18	24
Mean	10.819	12.206	14.513	14.086	14.886	14.150
n	45	3	3	3	3	3
S.E.	0.322	1.344	1.588	1.054	0.536	1.770
p		>0.20	<0.01	<0.02	<0.005	<0.02

Table 69

Changes in mean serum γ - globulin levels in N.C. rats - Expt. 3

	Pre	3	6	12	18	24	36
Mean	10.819	9.667	10.610	10.638	10.925	10.122	10.156
n	45	10	10	10	6	7	6
S.E.	0.322	0.517	0.434	0.599	2.165	0.569	0.520
p		>0.30	>0.40	>0.80	>0.90	>0.30	>0.70

Table 70

Changes in mean serum γ - globulin levels in S.O. rats - Expt. 3

	Pre	3	6	12	18	24	36
Mean	10.819	10.040	9.780	9.705	10.727	11.170	10.726
n	45	10	10	10	12	10	8
S.E.	0.322	1.312	0.620	0.720	0.501	0.604	0.658
p		>0.50	>0.10	>0.30	>0.80	>0.60	>0.80

Table 71

Changes in mean serum γ - globulin levels in S.N. rats - Expt. 3

	Pre	3	6	12	18	24
Mean	10.819	9.702	9.432	9.884	8.226	9.301
n	45	10	10	10	8	6
S.E.	0.322	0.584	0.518	0.524	0.725	0.631
p		>0.10	>0.05	>0.20	<0.005	>0.05

Table 72

Changes in mean serum Albumin levels in P.C.S. rats subjected to Splenectomy - Expt. 4

Weeks	Pre	1	2	3	4	5	6
Mean	41.522	33.915	31.710	31.040	34.186	36.287	38.400
n	29	12	10	8	5	7	4
S.E.	0.675	1.334	2.145	2.522	3.086	2.523	2.777
p		<0.001	<0.001	<0.001	<0.001	<0.001	>0.10

Table 73

Changes in mean serum Albumin levels in S.O. rats subjected to Splenectomy - Expt. 4

Week	Pre	1	2	3	4	5	6
Mean	41.522	35.635	35.381	36.255	37.915	40.877	40.800
n	29	7	8	6	6	4	4
S.E.	0.675	3.885	2.339	1.684	1.522	3.128	2.299
p		<0.02	<0.005	<0.005	>0.05	>0.70	>0.70

Table 74

Changes in mean serum α_1 - globulin levels in P.C.S. rats subjected to Splenectomy - Expt. 4

Weeks	Pre	1	2	3	4	5	6
Mean	21.746	25.325	22.722	20.588	17.692	15.387	14.602
n	29	12	10	8	5	7	4
S.E.	0.796	2.182	2.011	1.966	1.662	1.310	0.752
p		>0.05	>0.50	>0.50	>0.05	<0.001	<0.005

Table 75

Changes in mean serum α_1 - globulin levels in S.O. rats subjected to Splenectomy - Expt. 4

Weeks	Pre	1	2	3	4	5	6
Mean	21.746	22.765	19.298	18.843	20.615	20.022	20.780
n	29	7	8	6	6	4	5
S.E.	0.796	2.219	1.380	0.975	0.417	1.612	0.549
p		>0.60	>0.10	>0.10	>0.50	>0.40	>0.60

Table 76

Changes in mean serum α_2 - globulin levels in P.C.S. rats subjected to Splenectomy - Expt. 4

Weeks	Pre	1	2	3	4	5	6
Mean	8.678	8.726	9.496	7.775	10.470	10.974	10.940
n	29	12	10	8	5	7	4
S.E.	0.456	0.506	0.969	0.292	0.687	1.348	0.881
p		>0.95	>0.30	>0.30	>0.10	>0.05	>0.105

Table 77

Changes in mean serum α_2 - globulin levels in S.O. rats subjected to Splenectomy - Expt. 4

Weeks	Pre	1	2	3	4	5	6
Mean	8.678	10.308	11.397	13.271	10.111	8.122	9.782
n	29	7	8	6	6	4	4
S.E.	0.456	0.913	1.668	2.098	0.843	0.511	0.770
p		>0.10	<0.05	<0.005	>0.10	>0.60	>0.30

Table 78

Changes in mean serum β - globulin levels in P.C.S. rats subjected to Splenectomy - Expt. 4

Weeks	Pre	1	2	3	4	5	6
Mean	17.583	19.360	18.427	19.268	18.024	18.411	19.802
n	29	12	10	8	5	7	4
S.E.	0.605	1.044	0.928	1.181	1.840	1.056	1.433
p		>0.10	>0.40	>0.20	>0.70	>0.50	>0.20

Table 79

Changes in mean serum β - globulin levels in S.O. rats subjected to Splenectomy - Expt. 4

Weeks	Pre	1	2	3	4	5	6
Mean	17.583	22.765	21.348	20.438	19.067	19.380	19.757
n	29	7	8	6	7	4	4
S.E.	0.605	1.923	1.399	1.580	1.327	1.132	1.075
p		<0.005	<0.01	>0.05	>0.20	>0.20	>0.20

Table 80

Changes in mean serum γ - globulin levels in P.C.S. rats subjected to Splenectomy - Expt. 4

Weeks	Pre	1	2	3	4	5	6
Mean	10.211	14.002	17.673	21.255	19.828	19.752	19.933
n	29	12	10	8	5	7	4
S.E.	0.388	1.144	1.896	2.100	2.302	0.814	1.157
p		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Table 80A

Changes in mean serum γ - globulin levels in S.O. rats subjected to Splenectomy - Expt. 4

Weeks	Pre	1	2	3	4	5	6
Mean	10.211	10.750	11.735	10.633	10.565	11.150	10.855
n	29	7	8	6	6	4	4
S.E.	0.456	0.981	0.577	1.057	1.039	1.257	0.865
p		>0.50	>0.05	>0.60	>0.70	>0.40	>0.50

Table 81

Serum Total Proteins and serum protein fractions in Pair-fed P.C.S. rats - Expt. 8

	T.Proteins	Albumin	α_1	α_2	β	γ
Mean	6.820	27.587	20.465	13.862	21.030	18.922
n	4	4	4	4	4	4
S.E.	0.121	2.031	3.482	1.359	3.835	0.727

Table 82

Serum Total Proteins and serum protein fractions in Pair-fed S.O. rats - Expt. 8

	T.Proteins	Albumin	α_1	α_2	β	γ
Mean	6.522	30.355	18.642	21.405	18.427	10.975
n	4	4	4	4	4	4
S.E.	0.188	1.803	2.378	4.046	0.484	0.918

Table 83

Serum Total Proteins and serum protein fractions in Pair-fed N.C. rats - Expt. 8

	T.Proteins	Albumin	α_1	α_2	β	γ
Mean	6.893	32.823	20.623	13.526	21.553	10.070
n	3	3	3	3	3	3
S.E.	0.219	0.586	4.317	3.017	2.292	0.697

Table 84

Serum Total Proteins and serum protein fractions in Pair-fed P.C.S. rats - Expt. 9

	T.Proteins	Albumin	α_1	α_2	β	γ
Mean	6.865	35.953	14.316	11.686	18.348	19.693
n	6	6	6	6	6	6
S.E.	0.156	1.056	0.729	0.754	1.437	1.100

Table 85

Serum Total Proteins and serum protein fractions in Pair-fed S.O. rats - Expt. 9

	T.Proteins	Albumin	α_1	α_2	β	γ
Mean	6.895	36.733	18.945	14.196	19.550	10.873
n	6	6	6	6	6	6
S.E.	0.401	1.345	1.840	1.123	1.114	0.847

Table 86

Serum Total Proteins and serum protein fractions in Pair-fed N.C. rats - Expt. 9

	T.Proteins	Albumin	α_1	α_2	β	γ
Mean	6.998	35.676	16.601	12.988	20.836	10.825
n	6	6	6	6	6	6
S.E.	0.094	1.268	1.663	0.515	1.960	0.725

Table 87

Pre and Post-operative serum protein fractions in P.C.S. Pigs - Expt. 10

		Albumin	α_1	α_2	β	γ
Mean	pre	37.276	22.412	6.402	13.440	21.222
	post	24.027	23.225	8.538	14.826	31.516
n	pre	8	8	8	8	8
	post	8	8	8	8	8
S.E.	pre	2.071	1.645	0.497	1.145	2.404
	post	1.341	1.634	1.178	2.544	2.023

Table 88

Changes in mean serum IgG levels in P.C.S. rats - Expt. 2

Weeks	Pre	1	2	3	4	5	6
Mean	20.017	19.221	28.350	27.107	31.833	27.000	27.500
n	34	14	14	14	9	14	14
S.E.	0.820	1.561	2.447	1.509	3.077	1.510	1.182
p		>0.60	<0.001	<0.001	<0.001	<0.001	<0.001

Table 89

Changes in mean serum IgG levels in S.O. rats - Expt. 2

Weeks	Pre	1	2	3	4	5	6
Mean	20.017	20.990	23.200	21.050	20.500	22.800	22.450
n	34	10	10	10	10	10	10
S.E.	0.820	1.393	1.360	1.640	1.683	0.746	1.055
p		>0.50	>0.05	>0.50	>0.70	>0.05	>0.10

Table 91

Changes in mean serum IgM levels in P.C.S. rats - Expt. 2

	Pre	1	2	3	4	5	6	7	8	9	10	12
Mean	2.981	5.082	5.665	5.986	5.740	5.985	5.688	6.344	6.080	6.213	6.276	6.224
n	20	12	14	14	10	14	14	13	13	11	11	5
S.E.	0.150	0.280	0.195	0.165	0.232	0.196	0.163	0.237	0.132	0.282	0.263	0.149
p		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Table 92

Changes in mean serum IgM levels in S.O. rats - Expt. 2.

	Pre	1	2	3	4	5	6	7	8	9	10	12
Mean	2.981	3.170	3.027	2.866	2.832	2.825	2.875	2.941	2.960	3.056	2.990	2.877
n	20	9	10	10	10	10	9	10	10	10	8	8
S.E.	0.150	0.261	0.264	0.268	0.205	0.209	0.146	0.170	0.190	0.168	0.243	0.150

Table 93

Changes in mean serum IgM levels in N.C. rats - Expt. 2

	Pre	6	12
Mean	2.981	2.600	2.971
n	20	9	7
S.E.	0.150	0.230	0.278

Table 94

Mean log anti-L.P.S. (E.coli) antibody titres in P.C.S. rats - Expt. 2

Weeks	Pre	6w	12w
Mean	0.604	3.055	2.254
n	33	14	10
S.E.	0.167	0.158	0.339
p		<0.001	<0.001

Table 95

Mean log anti-L.P.S. (E. coli) antibody titres in S.O. rats - Expt. 2

Weeks	Pre	6w	12w
Mean	0.604	0.740	0.162
n	33	10	8
S.E.	0.167	0.253	0.162
p		>0.60	>0.20

Table 96

Mean log anti-L.P.S. (E. coli) antibody titres in N.C. rats - Expt. 2

Weeks	Pre	6w	12w
Mean	0.604	0.260	0.000
n	33	10	7
S.E.	0.167	0.173	0.000
p		>0.20	>0.10

Table 97

Mean log anti-L.P.S. (E. coli sucrose -ve) antibody titres in P.C.S. rats

Expt. 3

Weeks	Pre	3	6	12	18	24
Mean	0.324	1.505	1.705	1.906	1.756	1.429
n	38	13	9	6	6	4
S.E.	0.071	0.212	0.229	0.100	0.143	0.144
p		<0.001	<0.001	<0.001	<0.001	<0.001

Table 98

Mean log anti-L.P.S. (E. coli sucrose -ve) antibody titres in P.C.S. rats (neom)

Expt. 3

Weeks	Pre	3	6	12	18	24
Mean	0.324	1.163	1.128	1.103	1.204	1.354
n	38	15	12	9	9	3
S.E.	0.071	0.194	0.284	0.173	0.265	0.150
p		<0.001	<0.001	<0.001	<0.001	<0.001

Table 99

Mean log anti-L.P.S. (E. coli sucrose +ve) antibody titres in rats with totally-occluded P.C.S. - Expt. 3

Weeks	Pre	3	6	12	18
Mean	0.324	1.204	1.053	0.903	0.903
n	38	3	3	3	3
S.E.	0.071	0.300	0.451	0.300	0.300
p		<0.02	<0.05	>0.05	>0.05

Table 100

Mean log anti-L.P.S. (E. coli sucrose -ve) antibody titres in rats with patent P.C.S. - Expt. 3

Weeks	Pre	3	6	12	18
Mean	0.324	1.696	1.849	1.906	1.866
n	13	7	7	6	5
S.E.	0.113	0.191	0.274	0.100	0.112
p		<0.001	<0.001	<0.001	<0.001

Table 101

Mean log anti-L.P.S. (E. coli sucrose -ve) antibody titres in S.O. rats

Expt. 3

Weeks	Pre	3	6	12	18
Mean	0.324	0.752	0.722	0.702	0.10
n	38	4	5	5	5
S.E.	0.071	0.313	0.204	0.200	0.100
p		>0.50	>0.05	>0.10	>0.30

Table 102

Mean log anti-L.P.S. (E.coli sucrose -ve) antibody titres in S.N. rats -

Expt. 3

Weeks	Pre	3	6	12	18	24
Mean	0.324	0.668	0.524	0.602	0.489	0.401
n	38	9	9	10	8	6
S.E.	0.071	0.223	0.169	0.167	0.188	0.265
p		>0.05	>0.20	>0.05	>0.30	>0.70

Table 103

Mean log anti-L.P.S. (E. coli sucrose -ve) antibody titres in N.C. - Expt. 3

Weeks	Pre	3	6	12
Mean	0.324	0.125	0.125	0.202
n	38	5	5	5
S.E.	0.071	0.083	0.098	0.033
p		>0.20	>0.20	>0.10

Table 104

Mean log anti-L.P.S. (E. coli sucrose +ve) antibody titres in P.C.S. rats - Expt. 3

Weeks	Pre	3	6	12	18	24
Mean	0.408	0.920	1.313	1.956	1.454	1.128
n	42	17	11	6	6	4
S.E.	0.087	0.162	0.270	0.277	0.359	0.643
p		<0.005	<0.001	<0.001	<0.001	<0.05

Table 105

Mean log anti-L.P.S. (E. coli sucrose +ve) antibody titres in P.C.S rats (neom) - Expt. 3

Weeks	Pre	3	6	12	18	24
Mean	0.408	0.903	0.995	1.113	0.865	0.802
n	42	15	13	10	8	3
S.E.	0.087	0.160	0.251	0.261	0.165	0.200
p		>0.01	<0.01	<0.05	>0.10	>0.70

Table 106

Mean log anti- L.P.S. (E. coli sucrose +ve) antibody titres in S.N. rats - Expt. 3

Weeks	Pre	3	6	12	18	24
Mean	0.408	0.836	0.933	0.993	0.639	0.817
n	42	9	10	10	8	7
S.E.	0.087	0.217	0.113	0.119	0.192	0.215
p		0.05	0.01	0.05	0.20	0.50

Table 107

Mean log anti-L.P.S. (E. coli sucrose +ve) antibody titres in rats with totally-occluded P.C.S. - Expt. 3

Weeks	Pre	3	6	12	18
Mean	0.408	1.003	0.501	0.903	0.903
n	42	3	3	3	3
S.E.	0.087	0.530	0.501	0.173	0.173
p		<0.01	>0.60	<0.02	<0.02

Table 108

Mean log anti-L.P.S. (E. coli sucrose +ve) antibody titres in rats with patents P.C.S. - Expt. 3

Weeks	Pre	3	6	12	18
Mean	0.408	1.043	1.605	2.167	1.956
n	16	13	9	8	5
S.E.	0.127	0.157	0.229	0.221	0.260
p		<0.001	<0.001	<0.001	<0.001

Table 109

Mean log anti-L.P.S. (Proteus sp.) antibody titres in P.C.S. rats - Expt. 3

Weeks	Pre	3	6	12	18	24
Mean	0.413	1.273	1.264	1.755	1.745	2.006
n	40	13	10	6	5	3
S.E.	0.076	0.145	0.214	0.180	0.199	0.361
p		<0.001	<0.001	<0.001	<0.001	<0.001

Table 110

Mean log anti-L.P.S. (Proteus sp.) antibody titres in P.C.S. rats (neom)-Expt. 3

Weeks	Pre	3	6	12	18	24
Mean	0.413	0.924	1.122	1.572	0.936	0.802
n	40	14	11	9	9	3
S.E.	0.076	0.217	0.292	0.164	0.221	0.437
p		<0.01	<0.005	<0.001	<0.01	>0.30

Table 111

Mean log anti-L.P.S. (Proteus sp.) antibody titres in S.N. rats - Expt. 3

Weeks	Pre	3	6	12	18	24
Mean	0.413	1.003	0.872	0.933	0.865	0.852
n	40	9	10	10	8	6
S.E.	0.076	0.141	0.158	0.113	0.132	0.180
p		<0.005	<0.02	<0.005	<0.02	<0.05

Table 112

Mean log anti-L.P.S. (Proteus sp.) antibody titres in S.O. rats - Expt. 3

Weeks	Pre	3	6	12	18
Mean	0.413	0.602	0.782	0.150	0.100
n	40	4	5	5	5
S.E.	0.076	0.245	0.073	0.150	0.100
p		>0.70	>0.05	>0.10	<0.05

Table 113

Mean log anti-L.P.S. (Proteus sp.) antibody titres in N.C. rats - Expt. 3

Weeks	Pre	3	6	12
Mean	0.413	0.200	0.302	0.120
n	40	5	5	5
S.E.	0.076	0.091	0.261	0.120
p		>0.10	>0.30	>0.10

Table 114

Mean log anti-L.P.S. (Proteus sp.) antibody titres in rats with totally-occluded P.C.S. - Expt. 3

Weeks	Pre	3	6	12	18
Mean	0.413	0.903	1.053	1.003	1.053
n	40	3	3	3	3
S.E.	0.076	0.459	0.150	0.100	0.150
p		>0.10	>0.05	>0.05	>0.05

Table 115

Mean log anti-L.P.S. (Proteus sp.) antibody titres in rats with patent P.C.S.

Expt. 3

Weeks	Pre	3	6	12	18
Mean	0.413	1.505	1.705	1.866	1.881
n	15	10	7	5	4
S.E.	0.145	0.100	0.148	0.175	0.189
p		<0.001	<0.001	<0.001	<0.001

Table 118

Mean log anti-L.P.S. (E. coli sucrose +ve) antibody titres in N.C. rats - Expt. 7A

Weeks	Pre	3	6	9	12	15	18	21
Mean	0.273	0.150	0.218	0.571	0.200	0.602	0.526	0.541
n	43	12	11	10	9	9	8	5
S.E.	0.057	0.078	0.152	0.151	0.132	0.122	0.168	0.147
p		>0.20	>0.60	>0.05	>0.50	>0.05	>0.10	>0.10

Table 119

Mean log anti-L.P.S. (E. coli sucrose +ve) antibody titres in P.C.S. rats - Expt. 7B

Weeks	Pre	3	6	9	12	15	18	21	24	27	30
Mean	0.827	2.105	2.498	2.130	2.329	2.789	2.704	2.642	2.703		2.403
n	44	16	13	12	8	7	6	5	5		3
S.E.	0.082	0.203	0.128	0.200	0.237	0.258	0.327	0.358	0.327		0.596
p		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001		<0.001

Table 120

Mean log anti-L.P.S. (E. coli sucrose +ve) antibody titres in S.O. rats - Expt 7B

Weeks	Pre	3	6	9	12	15	18	21	24	27	30
Mean	0.827	1.070	0.940	1.003	1.128	1.143	0.852	1.254	0.903	1.161	0.652
n	44	9	8	9	8	5	6	6	4	7	6
S.E.	0.082	0.052	0.088	0.070	0.168	0.060	0.080	0.050	0.388	0.078	0.211
p		>0.10	>0.50	>0.30	>0.10	>0.20	>0.90	>0.05	>0.70	>0.10	>0.40

Table 121

Mean log anti-L.P.S. (E. coli sucrose +ve) antibody titres in N.C. rats - Expt. 7B

Weeks	Pre	3	6	9	12	15	18	21	24	27	30
Mean	0.827	1.161	1.204	1.279	1.094	0.963	1.304	1.354	1.003	0.790	0.963
n	44	14	11	12	11	10	6	4	6	8	5
S.E.	0.082	0.113	0.157	0.105	0.234	0.194	0.126	0.194	0.228	0.188	0.112
p		>0.05	>0.05	>0.05	>0.10	>0.40	>0.10	>0.05	>0.40	>0.80	>0.50

Table 122

Mean log anti-L.P.S. (E. coli 0127) antibody titres in N.C. rats - Expt. 12

Weeks	Pre	2	3	4	5	6	7	8	9	10	11	12
Mean	0.00	0.842	0.812	0.602	1.745	1.234	0.903	1.896	1.625	1.113	0.842	0.782
n	10	10	10	10	10	10	10	10	10	10	10	10
S.E.	0.00	0.227	0.168	0.179	0.204	0.151	0.167	0.127	0.201	0.110	0.098	0.136
p		<0.005	<0.001	<0.005	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Table 123

Mean log anti-H.G.G. antibody titres in S.O. rats - Expt. 7A

	Pre	3	6	9	12	15	18	21
Mean	0.000	0.000	0.000	0.200	0.218	0.000	0.000	0.000
n	37	12	11	9	11	6	10	4
S.E.	0.00	0.00	0.00	0.132	0.167	0.00	0.00	0.00
p				<0.001	<0.02			

Table 124

Mean log anti-H.G.G. antibody titres in N.C. rats - Expt. 7A

	Pre	3	6	9	12	15	18	21
Mean	0.000	0.000	0.070	0.580	0.267	0.000	0.000	0.000
n	37	12	11	5	9	3	7	4
S.E.	0.00	0.00	0.054	0.204	0.105	0.00	0.00	0.00
p		-	>0.05	<0.001	<0.001	-	-	-

Table 125

Mean log anti-H.G.G. antibody titres in P.C.S. rats - Expt. 7A

	Pre	3	6	9	12	15	18	21
Mean	0.000	0.054	0.164	0.451	0.301	0.602	0.376	0.451
n	37	11	11	8	7	3	4	4
S.E.	0.00	0.073	0.084	0.139	0.146	0.202	0.225	0.251
p		>0.05	<0.001	<0.001	<0.001	<0.001	<0.001	<0.005

Table 126

Changes in mean P.C.V. in P.C.S. rats - Expt. 1

Days	Pre	1	2	3	4
Mean	50.378	42.153	40.428	39.166	37.666
n	37	13	7	6	6
S.E.	0.468	1.049	0.782	0.307	0.614
p		<0.001	<0.001	<0.001	<0.005

Table 127

Changes in mean P.C.V. in S.O. rats - Expt. 1

Days	Pre	1	2	3	4
Mean	50.378	47.888	44.083	40.666	38.000
n	37	18	12	12	6
S.E.	0.468	0.704	0.656	0.466	0.447
p		<0.005	<0.001	<0.001	<0.001

Table 128

Changes in mean serum A.A.T. levels in P.C.S. rats - Expt. 1

Hours	Pre	6'	24'	48'	72'	96'
Mean	139.795	1129.166	1014.684	513.076	142.083	134.166
n	49	6	19	13	12	6
S.E.	8.049	16.543	19.011	15.653	19.271	15.078
p		<0.001	<0.001	<0.001	>0.90	>0.80

Table 129

Changes in mean serum A.A.T. levels in S.O. rats - Expt. 1

Hours	Pre	6'	24'	48'	72'	96'
Mean	139.795	313.500	217.058	102.916	98.833	101.000
n	49	6	17	12	12	5
S.E.	8.049	63.811	24.021	12.514	16.926	12.980
p		<0.001	<0.001	>0.05	>0.05	>0.10

Table 130

Changes in mean serum A.A.T. levels in P.C.S. rats - Expt. 3

Weeks	Pre	3	6	12	18	24
Mean	159.695	172.500	145.812	156.666	163.750	161.666
n	46	16	16	6	4	3
S.E.	8.363	14.252	19.035	13.944	22.488	19.649
p		>0.40	>0.40	>0.80	>0.80	>0.95

Table 131

Changes in mean serum A.A.T. levels in P.C.S. rats (neom.) - Expt. 3

Weeks	Pre	3	6	12	18
Mean	159.695	209.545	188.125	175.000	171.000
n	46	11	8	6	5
S.E.	8.363	43.251	27.757	44.721	22.670
p		>0.05	>0.70	>0.50	>0.60

Table 132

Changes in mean serum A.A.T. levels in S.O. rats - Expt. 3

Weeks	Pre	3	6	12	18
Mean	159.695	81.250	87.000	73.000	165.000
n	46	4	5	5	3
S.E.	8.363	23.037	14.456	21.365	45.000
p		<0.02	<0.01	<0.005	>0.80

Table 133

Changes in mean serum A.A.T. levels in S.N. rats - Expt. 3

Weeks	Pre	3	6	12	18	24
Mean	159.695	67.000	82.500	71.500	78.125	93.000
n	46	10	10	10	8	5
S.E.	8.363	6.798	10.144	7.151	12.815	17.000
p		<0.001	<0.001	<0.001	<0.001	<0.02

Table 134

Changes in mean serum A.A.T. levels in rats with partially-occluded P.C.S. - Expt. 3

Weeks	Pre	3	6	12
Mean	159.695	158.333	158.333	116.666
n	46	6	3	3
S.E.	8.363	13.944	24.551	21.666
p		>0.95	>0.95	>0.20

Table 135

Changes in mean serum A.A.T. levels in rats with totally-occluded P.C.S. - Expt. 3

Weeks	Pre	3	6	12	18
Mean	159.695	125.000	91.666	125.000	95.000
n	46	3	3	3	3
S.E.	8.363	36.170	22.047	12.583	7.637
p		>0.30	<0.05	>0.30	>0.05

Fig. 65. Analytical centrifuge pattern of rat IgM used in Experiment 2 - 56,100 r.p.m.; 20°C; solvent : P.B.S. pH 7.4. Photographs taken at 8-minute intervals.

Fig. 66. Analytical centrifuge pattern of rat IgA used in Experiment 2 - 56,100 r.p.m.; 20°C; solvent : P.B.S. pH 7.4. Photographs taken at 8-minute intervals.

REFERENCES

A

Aldrete, J.S., McIlrath, D.C., and Hallenbeck, G.A. (1967). Effect of portal ligation and of Eck's fistula on hepatic blood flow and concentration of blood ammonia. J. Surg. Res. 7 : 504.

Aronson, K.F., Ekelund, G., Kindmark, C.O., and Laurel, C-B. (1972). Sequential changes of plasma proteins after surgical trauma. Scand. J. clin. Lab. Invest. 29 suppl. 124 : 127.

Asheron, G.L. (1959). Antibodies against nuclear and cytoplasmic cell constituents in systemic lupus erythematosus and other diseases. Brit. J. Expt. Path. 40 : 209.

Ashikari, H., Dreyling, D.A. (1968). The effect of portacaval shunting on the exocrine pancreatic maximum secretory capacity. Amer. J. Gastroenterol. 49 : 312.

Assal, J.P., Levrat, R., Cahn, T., and Renold, A.E. (1971). Metabolic consequences of portacaval shunting in the rat. Z. ges. expt. Med. 154 : 87.

B

Baldessarini, R.J., and Fischer, J.E. (1967). S-adenosylmethionine following portacaval anastomosis. Surgery 62 : 311.

Bazin, H., Beckers, A., and Querinjean, P. (1974). Three classes and four subclasses of rat immunoglobulins. Europ. J. Immunol. 4 : 44.

Becher, M., Freud, M., Kott, I., Kravvic, H., Stern, J., Sandbank, U., and Bornstein, B. (1970). Hepatic cirrhosis with post-shunt myelopathy. J. Neurol. Sci. 11 : 101.

Beeson, P.B., Brannon, E.S., and Warren, J.V. (1945). Observations on sites of removal of bacteria from the blood in patients with bacterial endocarditis. J. Expt. Med. 81 : 9.

Bekoe, S., Mizock, B., Rone, A., and Peskin, G.W. (1972). Comparison of liver mitochondrial function after end-to-side and side-to-side portacaval shunting. Amer. J. Surg. 123 : 43.

Benjamin, I.S., Ryan, C.J., McLay, A.L.C., Horne, C.H.W., and Blumgart, L.H. (1974). The effect of portacaval shunting and portacaval transposition on serum gamma globulin levels in the rat. Gut 15 : 528.

Bensinger, T.A., Keller, A.R., Merrel, L.F., and O'Leary, D.S. (1971). Thorotrast-induced reticulo-endothelial blockade in man. Amer. J. Med. 51 : 663.

Bernstein, J.E., Nutting, R.O., and Orloff, M.J. (1968). Comparison of the effect of end-to-side and side-to-side portacaval shunts on liver function, liver blood flow, and ammonia metabolism in dogs and man. Surg. Forum 19 : 320.

Biozzi, G., Benecerraf, B., and Halpern, B.N. (1953). Quantitative study of granulopectic activity of reticulo-endothelial system : study of kinetics of granulopeotic activity of R.E.S. in relation to dose of carbon injected. Relationship between weight of organs and their activity. Brit. J. Expt. Path. 34 : 441.

Biozzi, G., Benecerraf, B., and Halpern, B.N. (1955). The effect of Salm. typhi and its endotoxin on the phagocytic activity of the reticulo-endothelial system in mice. Brit. J. Expt. Path. 36 : 226.

Bismuth, H., Benhamou, J.P., and Lataste, J. (1963). L'anastomose porto-cave expérimentale chez le rat normal. Presse med. 71 : 1859.

Bismuth, H., Berthelot, P., Desbuquois, B., Benhamou, J.P. and Fauvert, R. (1964). L'anastomose porto-cave expérimentale chez le rat normal. Etude de quelques anomalies biologiques. Rev. Franc. Etud. Clin. Biol. 9 : 608.

Bjørneboe, M., Prytz, H., and Ørskov, F. (1972). Antibodies to intestinal microbes in serum of patients with cirrhosis of the liver. Lancet i : 58.

Blakemore, A.H., Lord, J.W., and Stefko, P.L. (1942). The severed primary artery in the war wounded : a non-suture method of bridging arterial defects. Surgery 12 : 488.

Bollman, J.L. (1961). The animal with an Eck fistula. Physiol. Rev. 41 : 607.

Bono, R.F., Moreno, A.H., Rousselot, L.M. and Panke, W.F. (1960). Studies on portal hypertension. Surgery 48 : 119.

Bouchier, I.A.D., Rhodes, K., and Sherlock, S. (1964). Serological abnormalities in patients with liver disease. Brit. Med. J. 1 : 592.

Britton, S., and Möller, G. (1968). Regulation of antibody synthesis against E. coli endotoxin. Suppressive effect of endogenously produced and passively transferred antibodies. J. Immunol. 100 : 1326.

Brown, H., and Brown, J. (1965). Urea cycle enzymes after portacaval shunting. J. Amer. Med. Assoc. 192 : 382.

Brown, H., Trey, C., and McDermott, W.V., (1970). Encephalopathy after portacaval shunting managed with lactulose. Amer. J. Surg. 119 : 132.

Bull, S.L., Hurley, W.L., Kennett, W.S., Tamplin, C.B., and Williams, W.F. (1974). Effect of sex hormones on food intake in rats. J. Nutr. 104 : 968.

Burnett, W.E., Rosemond, G.P., Weston, J.K., and Tyson, R.R. (1952). Studies in hepatic response to changes in blood supply. Surg. Forum 3 : 147.

C

Callow, A.D., Lloyd, J.B., Ishihara, A., Ponsdomenech, E., O'Hara, E.T., Chalmers, T.C., and Garaan, A.J. (1965). Interim study with a controlled study of prophylactic portacaval shunt. Surgery 57 : 123.

Cannon, P.R., Wisler, R.W., Woolridge, R.L., and Benditt, E.P. (1944). The relation of protein deficiency to surgical infection. Ann. Surg. 120 : 514.

Castro, S., Vontz, F.K., and Foraker, A.G. (1963). Effect of Eck fistula on the dog liver. Arch. Path. 75 : 350.

Cavanagh, J.B., and Kyu, M.H. (1969). Colchicine-like effect on astrocytes after portacaval shunt in rats. Lancet ii : 620.

Cavanagh, J.B., Blakemore, W.F., and Kyu, M.H. (1971). Fibrillary accumulations in oligodendioglial processes of rats subjected to portacaval shunt anastomosis. J. Neurol. Sci. 14 : 143.

Cavanagh, J.B., and Kyu, M.H. (1971). Type II Alzheimer change experimentally produced in astrocytes in the rat. J. Neurol. Sci. 12 : 63.

Cavanagh, J.B., Lewis, P.D., Blakemore, W.F., and Kyu, M.H. (1972). Changes in the cerebellar cortex in rats after portacaval anastomosis. J. Neurol. Sci. 15 : 13.

Chauvand, S., Grange, D., Franco, D., and Bismuth, H. (1973). Does hepatic blood flow decrease after portal systemic shunt? A study in the rat. Europ. Surg. Res. 5 : 458.

Chen, F.C.K. (1972). Blood cholinesterase levels in experimental portacaval anastomosis in the male rat. Comp. Biochem. Physiol. 42B : 227.

Citrin, Y., Sterling, K., and Halstead, J.A. (1957). The mechanism of hypoproteinaemia associated with giant hypertrophy of the gastric mucosa. New Engl. J. Med. 247 : 906.

Clarke, J.S., Ozeran, R.S., Hart, J.C., Cruze, K., and Crevling, V. (1958). Peptic ulcer following portacaval shunt. Ann. Surg. 148 : 551.

Cohen, S. (1963). Gamma globulin metabolism. Brit. Med. Bull. 19 : 202.

Condon, R.E. (1971). Blood galactose clearance in normal and Eck fistula rats. J. Surg. Res. 11 : 135.

Conn, H.O., and Lindemuth, W.W. (1968). Prophylactic portacaval anastomosis in cirrhotic patients with oesophageal varices. New Engl. J. Med. 279 : 725.

Conn, H.O. (1972). Unilateral edema and jaundice after portacaval anastomosis. Ann. Intern. Med. 76 : 459.

Covelli, V.H., Ackroyd, F.W., Brown, M.E., Katayama, I., McDermott, W.V. (1967). Portacaval shunt - colectomy preparations in dogs. Surg. Forum 18 : 326.

Cuatrecasas, P. (1970). Protein purification by affinity chromatography. Derivatizations of agarose and polyacrylamide beads. J. Biol. Chem. 245 : 3059.

Cuschieri, A., Baker, P.R., Holley, M.P., and Hanson, C. (1974). Portacaval shunt in the pig. Effect on survival, behaviour, nutrition and liver function. J. Surg. Res. 17 : 387.

D

Da Silva, L.C., Jamra, M.A., Maspes, V., Pontes, J.F., Pieroni, R.R., and De Ulhoa Centra, A.B. (1963). Pathogenesis of indirect reacting bilirubinaemia after portacaval anastomosis. Gastroenterology 44 : 117.

Dawson, A.M., McLaren, J., and Sherlock, S. (1957). Neomycin in the treatment of hepatic coma. Lancet ii : 1263.

Day, S.B., Szeth, A.M., Skoryna, S.C., Webster, D.R., and Maclean, L.D. (1963). Tissue and blood histamine levels after portacaval shunt. Surg. Forum 14 : 80.

Day, S.B., Skoryna, S.C., Webster, D.R., and Maclean, L.D. (1963)A Gastric hypersecretion and histamine levels in liver, stomach, duodenum and blood following portacaval shunt in rats. Surgery 54 : 764.

De Carvalho, I.F., Borel, Y., and Miescher, P.A. (1967). Influence of splenectomy in rats on the formation of 19 S and 7 S antibodies. Immunology 12 : 505.

Delaney, J.P., Goodale, R.L., Cheng, J., Wangensteen, O.H. (1964). Portacaval shunting and upper gastrointestinal blood flow. J. Amer. Med. Assoc. 192 : 125.

Doberneck, R.C., Kline, D.G., Morse, A.S. and Berman, A. (1963). Relationship of haemosiderosis and portacaval shunting. Surgery 54 : 912.

Doberneck, R.C., Morse, A.S., Conrad, M.E., and Bermann, A.R. (1965). Hepatic iron storage and erythrokinetics after portacaval shunt. Surgery 57 : 800.

Doberneck, R.C. (1971). A comparison of haemosiderosis and haemopoiesis after end-to-side and side-to-side portacaval shunt. Surgery 70 : 428.

Doniach, D., Roitt, I.M., Walker, J.G., and Sherlock, S. (1966). Tissue antibodies in primary biliary cirrhosis, active chronic (lupoid) hepatitis, cryptogenic cirrhosis and other liver disease and their clinical implications. Clin. Expt. Immunol. 1 : 237.

Dordahl, E., Seymour, G., and Debarros, T.S. (1967). Testicular atrophy in rats with portacaval anastomosis. Arch. Path. 83 : 49.

Doyle, D. (1969). Gastric erosion and gastro-intestinal bleeding in rats with portacaval anastomosis. Brit. J. Expt. Path. 50 : 196.

Dragstedt, L.R. (1931). Gradual obliteration of the portal vein as a substitute for Eck-fistula. Science 73 : 315.

E

Eck, N.V. (1877). Vogen Med. J. 130 (11) section 2 : 1 (Translated in Surg. Gynec. Obstet. (1953). 96 : 375.

Eisenmenger, J., and Slater, R.J. (1953). Distribution and decay of I^{131} tagged albumin and gamma globulin in patients with cirrhosis. J. Clin. Invest. 32 : 564.

Eliakim, M., Zlotnick, A., and Slavin, S. (1972). Gammopathy in liver disease. In Progress in Liver Disease 4 : 403. Ed. by H. Popper and F. Schaffner. Grune and Stratton, New York and London.

Eskenazy, M. and Petrunov, B. (1971). Sensitisation of erythrocytes with lipopolysaccharides using glutaraldehyde as a coupling agent. Immunology 20 : 311.

F

Fahey, J.L., and McKelvey, E.M. (1965). Quantitative determination of serum immunoglobulins in antibody-agar plates. J. Immunol. 94 : 84.

Fahey, J.L., and McLaughlin, C. (1963). Preparation of antisera specific for 6.6 S γ-globulins, β 2a-globulins, γ_1-macroglobulins, and for Type I and II common γ -globulin determinants. J. Immunol. 91 : 484.

Fahey, J.L., and Robinson, A.G. (1963). Factors controlling γ-globulin concentration. J. Expt. Med. 118 : 845.

Farris, E.J., and Griffith, J.Q. eds. (1949). The rat in laboratory investigation. J.P. Lippincott, London and Montreal.

Fauvert, R. (1967). Haemodynamic aspects of porta-systemic shunting operation. In The Liver, p. 227. Ed. Read, A.E. Butterworths, London.

Fernandez, A., Sibel, C., and Goldenberg, H. (1966). An improved method for determining serum albumin and globulin. Clin. Chem. 12 : 194.

Finlayson, N.D.C. (1972). Immunological aspects of chronic liver disease. In Symposium : Liver Disease. Ed. Robertson, R.F. Royal College of Physicians, Edinburgh.

Fischer, J.E., and Snyder, S.H. (1965). Histamine synthesis and gastric secretion after portacaval shunt. Science 150 : 1034.

Fischer, J.E., Myers, A., and James, H. (1971). Ornithine decarboxylase: a defect in liver regeneration following portacaval shunt. Surgery 70 : 182.

Fisher, B., Lee, S.H., Fisher, E.R., and Saffer, E. (1962). Liver regeneration following portacaval shunt. Surgery 52 : 88.

Fisher, E.R. and Fisher, B. (1963). Ultrastructural hepatic changes following partial hepatectomy and portacaval shunt on the rat. Lab. Invest. 12 : 929.

Fisher, B., and Lee, S. (1965). Microvascular surgical technique in research, with special reference to renal transplantation in the rat. Surgery 58 : 904.

Fisher, B., Fisher, E.R., and Lee, S. (1967). Experimental evaluation of liver atrophy and portacaval shunt. Surg. Gynec. Obstet. 125 : 1253.

Flynn, P.J., and Kennan, A.L. (1968). The rat with a portacaval anastomosis. Arch. Path. 85 : 138.

Freeman, T., and Gordon, A.H. (1964). Metabolism of albumin and γ-globulin in protein deficient rats. Clin. Sci. 26 : 17.

Friedman, H.(1973). Cellular and molecular aspects of the immune response to a somatic bacterial antigen. J. Infect. Dis. 128 : 561.

Fuchs, S. and Sela, M. (1973). Immunoadsorbents. In Handbook of Experimental Immunology 11. 4. Ed. Weir, D.M. Blackwells Scientific Publications, London.

G

Gans, H., and Matsumoto, K. (1975). On the escape of endotoxin from the intestine. In Gram-negative Bacterial Infections, p. 429. Eds. Urbaschek, B., Urbaschek, R., and Neter, E. Springer-Verlag, New York.

Gjessing, E.C., and Chanutin, A. (1947). An electrophoretic study of plasma and plasma fractions of normal and injured rats. J. Biol. Chem. 169 : 657.

Glasgov, S., Kent, G., and Popper, H. (1959). Relationship of splenic and lymph node changes to hyperglobulinaemia in cirrhosis. Arch. Path. 67 : 9.

Gordon, A., and Sweets, H.H. (1936). A simple method for the silver impregnation of reticulum. Amer. J. Path. 12 : 545.

Grace, N.D., and Balint, J.A. (1966). Hemochromatosis associated with E-S P.C.S. anastomosis. Amer. J. Dig. Dis. 11 : 351.

Grace, N.D., Muench, H., and Chalmers, T.C. (1966). The present status of portal hypertension in cirrhosis. Gastroenterology 50 : 684.

Grange, D., Chauvand, S., Thangapreggassam, M.J., and Bismuth, H. (1974). The effect of colectomy on encephalopathy in rats with portacaval shunt. Surg. Gynec. Obstet. 138 : 537.

Gustafson, B.E., and Laurel, C.B. (1958). Gamma-globulins in germ-free rats. J. Expt. Med. 108 : 251.

H

Hallet, E.B., Holton, G.W., Patersen, S.C.S., and Schilling, J.A. (1952). Liver blood flow, hepatic glucose production, and splanchnic oxygen consumption in normal dogs and following Eck fistula. Liver blood flow after splenectomy. Surg. Gynec. Obstet. 95 : 401.

Halliday, W.J., and Webb, M. (1965). A plaque technique for counting cells which produce antibacterial antibody. Aust. J. Expt. Biol. Med. Sci. 43 : 163.

Havens, W.P., Dicken Sheets, J., Brierly, J.N., and Eberhard, T.P. (1954). The half life of I^{131} labelled normal gamma globulin in patients with cirrhosis. J. Immunol. 73 : 256.

Havens, W.P., Schaffer, J.M., and Hopke, C.J. (1951). The production of antibodies by patients with chronic hepatic disease. J. Immunol. 67 : 347.

Hearn, G.W., and Paton, A. (1963). Development of diabetes mellitus after portacaval anastomosis. Brit. Med. J. 2 : 96.

Henzel, J., Turcotte, J.G., and Child, C.G. (1963). The response of normal rats to portacaval shunt. Arch. Surg. 86 : 342.

Herbert, W.J. (1974). Laboratory animal techniques for immunology. In Handbook of Experimental Immunology, section A 3.15. Ed. Weir, D.M. Blackwells Scientific Publications, London.

Herz, R., Robert, F., and Bircher, J. (1971). Die enzephalopathie der Ratte mit Porto-Kavelem Shunt : ein Tiermodel für therapeutische Versuche. Schweiz. med. wschr. 101 : 739.

Herz, R., Sautter, V., Robert, F., and Bircher, J. (1972). The Eck fistula rat : definition of an experimental model. Europ. J. Clin. Invest. 2 : 390.

Hickman, R., Crosier, J.H., Saunders, S.J., and Terblanche, J. (1974). Transhepatic metabolism after end-to-side portacaval shunt in the young pig. Surgery 76 : 601.

Hoffenberg, R., Black, E., and Brock, J.F. (1966). Albumin and γ-globulin tracer studies in protein depletion states. J. Clin. Invest. 45 : 143.

Hübner, K.F., and Gengozian, N. (1969). Critical variables of the Jerne plaque technique as applied to rodent antibody-forming systems responding to heterologous red cell antigens. J. Immunol. 102 : 155.

J

Jackson, F.C., Perrin, E.B., and Felix, R. (1971). A clinical investigation of portacaval shunt. Survival analysis of the therapeutic operation. Ann. Surg. 174 : 672.

Jacobson, E.D., Prior, J.T., and Faloon, W.W. (1960). Malabsorption syndrome induced by neomycin: morphological alterations in the jejunal mucosa. J. Lab. Clin. Med. 56 : 245.

James, I.M., Macdonell, L., and Xanalatos, C. (1972). The effect of acute portacaval shunting in dogs on cerebral and peripheral blood flow and metabolism. Clin. Sci. 42 : 769.

Jamieson, J.C., and Ashton, F.E. (1973). Studies on acute phase proteins of rat serum: Pathway of secretion of albumin and α_1-acid glycoprotein from liver. Canad. J. Biochem. 51 : 1281.

Jeffay, H., and Wingler, R.J. (1958). The metabolism of serum proteins. J. Biol. Chem. 231 : 111.

Jerne, N.K., Nordin, A.A., and Henry, C. (1963). In Conference on Cell Bound Antibodies, Washington, D.C. Ed. by Amos, B. and Koprowski, H., p. 109. Wistar Institute Press, Philadelphia, U.S.A.

Jerne, N.H., and Nordin, A.A. (1963). Plaque formation in agar by single antibody-producing cells. Science 140 : 405.

Johnson, A.G., Gaines, S. and Landy, M. (1956). Studies on the O antigen of Salmonella typhosa. Enhancement of antibody response to protein antigens by the purified lipopolysaccharide. J. Expt. Med. 103 : 225.

K

Kaplan, E.L., and Peskin, G.W. (1966). Factors influencing the absorption and deposition of iron after portal systemic shunting procedures. Surg. Forum 17 : 379.

Karmen, A., Wroblewski, F. and La Duc, J. (1955). Transaminase activity in human blood. J. Clin. Invest. 34 : 126.

Kennan, A.L. (1964). Changes in plasma proteins after portacaval shunt in the rat. Proc. Soc. Expt. Biol. Med. 116 : 543.

Kennan, A.L. (1964)A Glutamine synthetase activity in rat liver after portacaval shunt. Endocrinology 74 : 805.

Keraan, M.M., Meyers, O.L., Engelbrecht, G.H.C., Hickman, R., Saunders, S.J., and Terblanche, J. (1974). Increased serum immunoglobulin levels following portacaval shunt in the normal rat. Gut 15 : 468.

Kern, M., Eissen, H.N. (1959). The effect of antigenic stimulation on incorporation of phosphate and methionine into proteins of isolated lymph node cell. J. Expt. Med. 110 : 207.

Khanna, S.K., Saini, V.K., Das, T. (1967). Differential glucose tolerance: a method for determining portacaval shunt patency. Canad. J. Surg. 10 : 325.

Kind, P., and Johnson, A.J. (1959). Studies on the adjuvant effect of bacterial endotoxins on antibody formation. J. Immunol. 82 : 415.

Kind, P., Campbell, P., and Rowland, D.T. (1967). Endotoxin-induced wasting disease in mice: a temporary condition explained by endotoxin tolerance. Proc. Soc. Expt. Biol. Med. 125 : 495.

Kirsch, M.M., Knapp, W., Sherrol, A.N., Coon, W., Zumeida, G.D. and Arbor, A. (1965). Portal systemic shunting in experimental ammonia intoxication. Arch. Surg. 91 : 640.

Kirsch, R.E. (1968). The kinetics of albumin metabolism in experimental protein calorie malnutrition. M.D. thesis, University of Cape Town.

Kirsch, R.E., Brock, J.F., and Saunders, S.J. (1968). Experimental protein-calorie malnutrition. Amer. J. Clin. Nutr. 21 : 820.

Klages, F., and Kurten, H.F. (1938). Die anlegung der eckshen fistel beim kleintier. Arch. f. expt. path. u pharm. 188 : 489.

Kline, D.G., Doberneck, R.C., Chun, B.K., Rutherford, R.B. (1965). Encephalopathy in graded portacaval shunts. Surg. Forum 16 : 282.

Kline, D.G., and Nance, F.C. (1969). Eck's fistula encephalopathy: long term studies in primates and germ-free dogs. Surg. Forum 19 : 358.

Kline, D.G., Crook, J.N., and Nance, F.C. (1971). Eck fistula encephalopathy. Ann. Surg. 173 : 97.

Koenemann, L.C. and Ceballos, R. (1966). Massive hepatic necrosis following portacaval shunt. J. Amer. Med. Assoc. 198 : 138.

Kowalewski, K., Russel, J.C., and Kohell, A. (1971). Excretion of ^{3}H-histamine in urine of rats with P.C.S. Proc. Soc. Expt. Biol. Med. 136 : 760.

Kyu, M.H., and Cavanagh, J.B. (1970). Some aspects of portacaval anastomosis in the male rat. Brit. J. Expt. Path. 51 : 217.

L

Lambert, R. (1965). Surgery of the Digestive System of the Rat (Translated by B. Julien). Charles C. Thomas, Springfield, Illinois.

Latham, J.B. (1975). Determination of portacaval shunt patency in rats by splenoportography. S. Afr. Med. J. 49 : 757.

Law, D.H., Perrine, J.D., and Baumgarther, L.F. (1966). Diagnostic significance of myeloma-like peaks on paper electrophoresis. Clin. Res. 14 : 303.

Lee, S.H., and Fisher, B. (1961). Portacaval shunt in the rat. Surgery 50 : 688.

Lee, S., Condon, J.K., Chandler, J.G., Koopmans, H., Ehara, Y., Yen, S.S.C., and Orloff, M.J. (1972). The effect of Eck fistula upon intrasplenic ovarian neoplasm formations. Surg. Forum 23 : 110.

Lee, S., Chandler, J.G., Broelsch, C.E., Flamant, Y.M., and Orloff, M.J. (1974). Current research review: portal-systemic anastomosis in the rat. J. Surg. Res. 17 : 53.

Leevy, C.M. (1957). Practical Diagnosis and Treatment of Liver Disease, p. 83. Hoeber and Harper, New York.

Levin, J., Tomasulo, P.A., and Oser, R.S. (1970). Detection of endotoxin in human blood and demonstration of an inhibitor. J. Lab. Clin. Med. 75 : 903.

Lo Grippo, G.A., Anselm, K., and Hayashi, H. (1971). Serum immunoglobulins and five serum proteins in extrahepatic jaundice and alcoholic cirrhosis. Amer. J. Gastroenterol. 56 : 357.

M

Macbeth, W.A.A.G., Kass, E.H., and McDermott, W.V. (1965). Treatment of hepatic encephalopathy by alteration of intestinal flora with Lactobacillus acidophilus. Lancet i : 399.

Masland, W.S. (1964). Interrelationship between diet and weight gain in rats with portacaval shunts. Amer. J. Physiol. 206 : 304.

Mazzoni, G. (1974). Data presented at 2nd International Microvascular Surgery Congress, Rome.

McDermott, W.V., and Adams, R.D. (1954). Episodic stupor associated with an Eck fistula in the human with particular reference to the

metabolism of ammonia. J. Clin. Invest. 33 : 1.

McDermott, W.V., Victor, M., and Point, W.W. (1962). Exclusion of the colon in the treatment of hepatic encephalopathy. New Engl. J. Med. 267 : 850.

McKay, I.R., Taft, L.I., and Cowling, D.C. (1956). Lupoid hepatitis. Lancet ii : 1323.

Megysi, C., Samols, E., and Marks, V. (1967). Glucose tolerance and diabetes in chronic liver disease. Lancet ii : 1051.

Metcoff, J., Darling, D.S., Scanlon, M.S., and Stare, F.J. (1948). Nutritional status and infection response. J. Lab. Clin. Med. 33 : 47.

Meyers, O.L., and Keraan, M. (1973). Experimental hyperglobulinaemia. In Liver, pp. 78-79. Eds. Saunders, S.J. and Terblanche, J. Pitman Medical Books, London.

Michael, J.G., Whitby, J.L., and Landy, M. (1962). Studies on natural antibodies to Gram-negative bacteria. J. Expt. Med. 115 : 131.

Möller, G., and Michael, G. (1971). Frequency of antigen-sensitive cells to thymus-independent antigens. Cell Immunol. 2 : 309.

Möller, G., Sjöberg, O. and Anderson, J. (1973). Immunogenicity, tolerogenicity and mitogenicity of lipopolysaccharides. J. Infect. Dis. 128 : 552.

Morgan, E.H., and Brackenridge, C.J. (1962). Serum protein changes and plasma total iron binding capacity after laparotomy or partial hepatectomy and in anaemia in the albino rat. Quart. J. Expt. Physiol. 47 : 66.

Mouridsen, H.T. (1967). Turnover of human serum albumin before and after operations. Clin. Sci. 33 : 345.

Muller-Eberhard,H.(1960). A new supporting medium for preparative electrophoresis. Scand. J. Clin. Invest. 12 : 33.

Munoz, J. (1964). Antibody response. In Advances in Immunology 4 : 411. Eds. Dixon, F.J. and Humphrey, J.M. Academic Press, New York and London.

Mutchnick, M.G., Learner, E., and Conn, H.O. (1974). Portosystemic encephalopathy and portacaval anastomosis: a prospective controlled investigation. Gastroenterology 66 : 1005.

Müting, D., and Reikowsky, H. (1965). Protein metabolism in liver disease. In Progress in Liver Diseases 2 : 85. Eds. Popper, H. and Schaffner, F. Grune and Stratton, New York and London.

N

Nance, F.C. and Kline, D.G. (1971). Eck fistula encephalopathy in germ-free dogs. Ann. Surg. 174 : 856.

Nance, F.C., Batson, R.C., and Kline, D.G. (1971). Ammonia production in germ-free Eck fistula dogs. Surgery 70 : 169.

Neter, E., Westphal, O., Lüderitz, O., Gorzynok, E.A., and Eichenberger, E. (1956). Studies on enterobacterial lipopolysaccharides. J. Immunol. 76 : 377.

Neuhaus, O.W., Balegus, H.F., and Milauskas, A.T. (1962). Hepatic production of serum α_2 and β -globulins in rats after injury. Amer. J. Physiol. 202 : 257.

Nicol, T., Vernon-Roberts, B., and Quantock, D.C. (1965). The influence of various hormones on the reticulo-endothelial system: endocrine control of body defence. J. Endocrinol. 33 : 365.

Nimmich, W. (1968). Zur Isolierung und qualitativen Bausteinanalyse der K-antigen von Klebsiellen. Z. med. mikrobiol. u immunol. 154 : 117.

Nowotny, A. (1968). Immunogenicity of toxic and detoxified endotoxin preparation. Proc. Soc. Expt. Biol. Med. 127 : 745.

O

Olkay, I., Kitahama, A., Miller, R.H., Drapanas, T., Trejo, R.A., and Di Luzio, N.R. (1974). Reticuloendothelial dysfunction and endotoxaemia following portal vein occlusion. Surgery 75 : 64.

Orloff, M.J., Wall, M.H., Hickman, E.B., and Neesby, T. (1963). Influence of stomal size of portacaval shunt on peripheral blood ammonia levels. Ann. Surg. 158 : 172.

Orloff, M.J., Chandler, J.G., Keiter, J.E., Rosen, H. (1969). Gastric secretion and peptic ulcer following portacaval shunt in man. Ann. Surg. 170 : 515.

Ossenberg, F.W., Pointard, L., Benhamou, J.P. (1972). Effect of portacaval shunt and portacaval transposition on hepatic cytochrome P-450 in the rat. Rev. Europ. Etudes Clin et Biol. 27 : 791.

Ouchterlony, Ö., and Nilsson, L.A.. (1974). Immunodiffusion and immunoelectrophoresis. In Handbook of Experimental Immunology, section 19.10. Ed. Weir, D.M. Blackwell Scientific Publications, London.

Oudea, P., and Bismuth, H. (1965). L'anastomose porto-cave experimentale chez le rat normal. Etude des lesions hepatiques par la microscopie electronique. Path. Biol. 13 : 288.

Owen, J.A. (1967). Effect of injury on plasma proteins. In Advances in Clinical Chemistry, 9 : 2. Ed. by Sobotka, H., and Stewart, C.P. Academic Press, New York and London.

Ozawa, K., Kitamura, O., Yamaoka, Y., Kamano, T., Mizukami, T., Takeda, H., Takasan, H., and Houjo, I. (1973). Metabolic response of the liver of human beings and other mammals to deprivation of portal blood supply. Surg. Gynec. Obstet. 137 : 623.

P

Paranetto, F., Schaffner, F., and Popper, H. (1964). Immunological and serological observations in primary biliary cirrhosis. New Engl. J. Med. 271 : 1123.

Patek, A.J. (1963). Portal cirrhosis. In Diseases of the Liver, p. 573. Ed. Schiff, L. Pitman, London.

Pearlman, D.M., Coomaraswamy, R.P., and Dargan, E.L. (1966). Blood ammonia levels in the inferior vena cava: a simple test of portacaval shunt patency. Surgery 60 : 541.

Perez, V., Schaffner, F., and Popper, H. (1972). Hepatic drug reactions. In Progress in Liver Disease, 4 : 607. Ed. by Popper, H., and Schaffner, F. Grune and Stratton, New York and London.

Peterman, M.L. (1961). Plasma proteins in bacterial infections. Ann. N.Y. Acad. Sci. 94 : 144.

Protell, R.L., Soloway, R.D., Martin, W.J., Schoenfield, L.J., and Summerskill, W.H.J. (1971). Anti-Salmonella agglutinins in chronic active liver disease. Lancet ii : 330.

Prytz, H., Bjørneboe, M., Johansen, T.S., and Ørskov, F. (1974). The influence of portasystemic shunt operation on the immunoglobulins and E. coli antibodies in patients with cirrhosis of the liver. Acta Med. Scand. 196 : 109.

R

Ramsoe, K., Westergaard, H., Jarnum, S., Jorgensen, M., Schiodt, T., and Tygstrup, N. (1970). Albumin and IgG turnover studies in patients with cirrhosis. Scand. J. Gastroenterol. 5 : 25.

Ravin, H.A., Rowley, D., Jenkins, C., and Fine, J. (1961). On the absorption of bacterial endotoxin from the gastro-intestinal tract of the normal and shocked animal. J. Expt. Med. 112 : 783.

Reichle, F.A., Gruenstein, H., Meranze, D.R., Rosemond, G.P., and Shimkin, M.B. (1969). The effect of portacaval shunt on 7,12-Dimethylbenz (a) anthracene-produced mammary carcinoma in the rat. J. Surg. Res. 9 : 559.

Reichle, F.A., Brigham, M.P., Tyson, R.R., Reichle, R.M., Reilly, M., and Rosemond, G.P. (1970). Serine metabolism after portacaval shunt. Arch. Surg. 100 : 163.

Reichle, F.A., Alloy, S., Reichle, R.M., Fruenstein, M., Essa, N., and Rosemond, G.P. (1972). Portacaval shunt in established experimental mammary carcinoma. Surg. Forum 23 : 107.

Reichle, F.A., Gruenstein, M., Reichle, R.M., Meraze, D.R., Rosemond, G.P., and Shimkin, M.B. (1972)A. Inhibition of experimental mammary carcinoma. Arch. Surg. 104 : 206.

Rothchild, M.H., Oratz, M., and Schreiber, S.S. (1972). Albumin metabolism. In Progress in Liver Diseases, 4 : 19. Eds. Popper, H., and Schaffner, F. Grune and Stratton, New York and London.

Rowley, D.A. (1950). The formation of circulating antibody in the splenectomised human being following intravenous injection of heterologous erythrocytes. J. Immunol. 65 : 515.

Rubin, E., Kohan, P., Tomita, F., and Jacobson, J.H. (1964). Experimental hepatic siderosis following portacaval shunt. Proc. Soc. Expt. Biol. Med. 115 : 350.

Rubin, E., Gevirtz, N.R., Cohan, P., Tomita, F., and Jacobson, J.H. (1965). Liver cell damage produced by portacaval shunts. Proc. Soc. Expt. Biol. Med. 118 : 235.

Rubin, E., Oshiro, T., and Jacobson, J.H. (1967). Effect of experimental portacaval shunt on drug metabolising enzymes. Fed. Proc. 26 : 690.

Rubin, E. (1968). Studies in hepatic injury induced by experimental portacaval shunts. Israel J. Med. Sci. 4 : 155.

S

Saadi, M.H. (1967). Gangrene of the ileum as an unusual complication of portacaval shunt. Brit. J. Clin. Pract. 21 : 622.

Seager, S.E., Blumgart, L.H., and Boyns, A.R. (1972). Ovarian implantation into the spleen of normal and portacavally shunted rats. Brit. J. Surg. 59 : 907.

Schimke, R.T. (1962). Adaptive characteristics of urea cycle enzymes in the rat. J. Biol. Chem. 237 : 459.

Schmitt, Mary (1973). Influences of hepatic portal receptors on hypothalamic feeding and satiety centres. Amer. J. Physiol. 225 : 1089.

Sell, S. (1964). Immunoglobulin of germ-free guinea pigs. J. Immunol. 93 : 122.

Sell, S., and Fahey, J.L. (1964). Relationship between γ-globulin metabolism and low serum γ-globulin in germ-free mice. J. Immunol. 93 : 81.

Selye, H., Tuchweber, B., and Bertok, L. (1966). Effect of lead acetate on the susceptability of rats to bacterial endotoxins. J. Bact. 91 : 884.

Sherlock, S. (1970). The immunology of liver disease. Amer. J. Med. 49 : 693.

Silen, W., Harper, H., Mawdsley, D.L., and Weinrich,W.L. (1955). Effects of antibacterial agents on ammonia production within the intestines. Proc. Soc. Expt. Biol. Med. 88 : 138.

Silen, W., Mawdsley, D.L., Weinrich, W.L. and Harper, H.A. (1957). Studies of hepatic function in dogs with Eck fistula or portacaval transposition. Arch. Surg. 74 : 964.

Smith, G.W., and Mouzas, G.L. (1970). The metabolic response of the liver to portacaval shunts. Surgery 68 : 341.

Song, C.S., and Kappas, A. (1970). The influence of hormones on hepatic function. In Progress in Liver Diseases, vol. 3 : 94. Eds. Popper, H., and Schaffner, F. Grune and Stratton, New York and London.

Souhami, R.L. (1972). The effect of colloidal carbon on the organ distribution of sheep red cells and the immune response. Immunology 22 : 685.

Souhami, R.L., Addison, I.E., and Bradfield, J.W.B. (1975). Increased antibody production following depression of hepatic phagocytes. Clin. Expt. Immunol. 20 : 155.

Stanworth, D.R., and Turner, M.W. (1973). Immunochemical analysis 10.22. In Handbook of Experimental Immunology. Ed.Weir, D.M. Blackwell Scientific Publications, London.

Starzl, T.E., Chase, P. H.P., Putnam, C.W., Porter, K.A. (1973). Portacaval shunt in hyperlipoproteinaemia. Lancet ii : 940.

Starzl, T.E., Putnam, C.W., Porter, K.A., Halgrimson, C.G., Corman, J.C., Brown, B.I., Gotlin, R.W., Rodgerson, D.O., Greens, H.L. (1973)A. Portal diversion for treatment of glycogen storage disease in humans. Ann. Surg. 178 : 525.

T

Tarver, H., Armstrong, F.B., Debro, J.R., and Margen, S. (1961). Catabolism of plasma protein in the gut. Ann. N.Y. Acad. Sci. 94 : 23.

Thomas, L. (1959). In Cellular and Humoral Aspects of Hypersensitivity States. N.Y. Acad. Med. Symp. p. 451. Ed. Lawrence, H.S. Harper (Hoeber), New York.

Thomas, H.C., McSween, R.N.M., and White, R.G. (1973). Role of the liver in controlling immunogenicity of commensal bacteria in the gut. Lancet i : 1288.

Thompson, R.G., Barrowman, J., Gutierriz, L., and Dowling, R.H. (1971). Action of neomycin on the ultraluminal phase of lipid absorption. J. Clin. Invest. 50 : 319.

Trejo, R.H., Di Luzio, N.R., Loose, L.D., and Hoffman, E. (1972). Reticulo-endothelial and hepatic functional alterations following lead acetate administration. Expt. Molec. Path. 17 : 145.

Triger, D.R., Alp, M.H., and Wright, R. (1972). Bacterial and dietary antibodies in liver disease. Lancet i : 601.

Triger, D.R., Kartz, J.B., MacCullam, F.O., and Wright, R. (1972). Raised antibody titres to measles and rubella viruses in chronic active hepatitis. Lancet i : 665.

Triger, D.R., and Wright, R. (1973). Hyperglobulinaemia in liver disease. Lancet i : 1494.

Triger, D.R., Cynamon, M.H., and Wright, R. (1973). Studies on hepatic uptake of antigen - Comparison of inferior vena cava and portal vein routes of immunisation. Immunology 25 : 941.

Triger, D.R., Kurtz, J.B., and Wright, R. (1974). Viral antibodies and autoantibodies in chronic liver disease. Gut 15 : 94.

Turney, S.W., Foster, J.H., Lanier, V.C., and Younger, R.K. (1966). Hepatic parenchyma: the major determinant of blood ammonia levels. Surg. Forum 17 : 360.

V

Van Breda Vriesman, P.J.C., and Feldman, J.D. (1972). Rat γ-M immunoglobulin: Isolation and some biological characteristics. Immunochemistry 9 : 525.

Vidal, M. (1903). Traitement chirurgical des ascites. Presse Med.11 : 747.

Villar-Valdes, H., Thompson, H.G., Chandler, J.G., and Orloff, M.J. (1969). Endogenous origin of the humoral agent responsible for gastric hypersecretion associated with portacaval shunt. Surg. Forum 20 : 360.

W

Weech, A.A., Goettsch, E., and Reeve, E.B. (1934). Nutritional oedema in the dog. Development of hypoproteinaemia on a diet deficient in proteins. J. Expt. Med. 61 : 299.

Weimer, H.E. (1959). Effect of protein depletion and repletion on serum electrophoretic patterns of adult rats. Proc. Soc. Expt. Biol. Med. 102 : 689.

Weimer, H.E. (1961). The effect of protein depletion and repletion on the concentration and distribution of serum proteins and protein-bound carbohydrates of the adult rat. Ann. N.Y. Acad. Sci. 94 : 225.

Weinbren, K. (1955). The portal blood supply and regeneration of the rat liver. Brit. J. Expt. Path. 36 : 583.

Werner, M. (1969). Serum protein changes during the acute phase reaction. Clin. Chim. Acta 25 : 299.

Whitaker, W.L. (1946). Portal vein ligation and the Eck Fistula in the rat. Proc. Soc. Expt. Biol. Med. 61 : 420.

Wieme, R.J. (1959). An improved method of electrophoresis. Clin. Chim. Acta 4 : 317.

Wilkens, B.J., Hunt, D.G., and Hardy, J.D. (1969). Serum gastrin-like activity following P.C.S. in dogs. Brit. J. Surg. 56 : 695.

Wolfe, H.R., Norton, S., Springer, E., Goodman, M., and Herrick, C.A. (1950). Precipitin production in chickens: The effect of splenectomy on antibody formation. J. Immunol. 64 : 179.

Wollheim, F.A., Pihl, B., Ekelund, G., and Aronsen, K.F. (1969). Immunoglobulins and acute phase reactants in man after major hepatic resection. Scand. J. Gastroenterol. 4 : 249.

Wostmann, B.S., and Gordon, A.A. (1960). Electrophoretic studies on the serum protein pattern of the germ free rat and its changes upon exposure to a conventional bacterial flora. J. Immunol. 84 : 27.

Z

Zeldis, L.J., Alling, E.L., McCoord, A.B., and Kulka, J.P. (1945). Plasma protein metabolism - electrophoretic studies. Chronic depletion of circulating proteins during low protein feeding. J. Expt. Med. 82 : 157.

Zieve, L., Mendelson, D.F., and Goepfert, M. (1960). Shunt encephalomyelopathy - occurrence of permanent myelopathy. Ann. Intern. Med. 53 : 53.