

## Increased serum immunoglobulin levels following portacaval shunt in the normal rat

M. KERAAN, O. L. MEYERS<sup>1</sup>, G. H. C. ENGELBRECHT, ROSEMARY HICKMAN, S. J. SAUNDERS, AND JOHN TERBLANCHE

*From the Departments of Medicine and Surgery and the Medical Research Council Liver Research Group, University of Cape Town, and Liver Clinic, Groote Schuur Hospital, Cape Town*

**SUMMARY** Normal rats subjected to end-to-side portacaval shunt showed decreased survival and weight gain, a progressive fall in serum albumin and reciprocal rise in serum gamma globulin when compared with sham-operated controls for 12 weeks. Antibacterial lipopolysaccharide antibody was detected in significant titre at the sixth and twelfth weeks.

It is suggested that the elevated levels of gamma globulin and reversal of albumin/globulin ratios noted in these animals may represent an immune response to bacterial lipopolysaccharides released into the systemic circulation as a result of the portacaval shunt. The hyperglobulinaemia of cirrhosis in human subjects may have a similar aetiology.

The elevation in serum gamma globulin which is frequently noted in patients with chronic liver disease is currently believed to have an immunological origin (Paronetto, 1970). A possible contribution by bowel organisms to these immunological changes was suggested by Bjørneboe (1971), Bjørneboe, Prytz, and Ørskov (1972) who showed a rise in serum immunoglobulins in patients after portacaval shunt. The demonstration of increased antibacterial antibodies in subjects with chronic liver disease by Bjørneboe *et al* (1972) and by Triger, Alp, and Wright (1972) created further interest in bowel microorganisms as a stimulus to the hyperglobulinaemia of chronic liver disease.

Changes in the serum proteins following the creation of a portacaval shunt in the normal rat as described by Kennan (1964) have recently been confirmed by us (Meyers and Keraan, 1973). This paper amplifies the data concerning the changes in serum proteins over a three-month period following portacaval shunt in the rat, and documents the effect of neomycin administration. In addition, survival and weight changes over five months were studied in rats of two weight ranges, namely, 150-250 g and 250-350 g.

### Materials and Methods

Sixty-eight male rats were used to determine the

<sup>1</sup>Address for reprints: O. L. Meyers, Department of Medicine, Medical School, Observatory, Cape, South Africa.

Received for publication 18 March 1974.

following: (1) the effect of standard end-to-side portacaval shunt upon serum protein levels, and the influence of neomycin (25 mg/day by gavage); (2) serum protein changes in unoperated and sham-operated controls, when the sham operation was performed under identical circumstances with the same manoeuvres except for final ligation of the portal vein and its anastomosis to the inferior vena cava; (3) changes in weight gain and survival in small (150-250 g) and large (250-350 g) rats following portacaval shunt, and weekly bleeding of 0.5 ml with or without neomycin administration; (4) the influence of weekly bleeding or neomycin administration upon the weight and survival of sham-operated controls.

All the animals were bled from the tail vein at the start of the study, and at weekly intervals thereafter for six weeks and again at 12 weeks. Serum was separated by centrifugation and stored at  $-20^{\circ}\text{C}$  until assayed for total protein, albumin and globulin, and antibody to bacterial lipopolysaccharide. Total protein was measured using the biuret method, modified for serum volumes of 0.017 ml and a final volume of 1 millilitre. A 3% solution of human albumin was used as a reference standard. The sera were electrophoresed in 1% Noble agar (veronal buffer, pH 8.3) on clean microscope slides. The electrophoresis was continued for 45 minutes under petroleum ether at 60 mA (constant voltage) as described by Wieme (1959). The completed electrophoretograms were dried, stained with Amido black, and read in a Joyce chromoscan densitometer.

Antibodies to rat *Escherichia coli* lipopoly-

saccharide were sought using passive haemagglutination of sheep red cells in a microtitre system. The bacteria were cultured from accumulated rat faeces of all the groups to be studied, and lipopolysaccharide was extracted as follows.

A suspension of bacteria (10 g/160 ml water) was added to 265 ml of a 75% phenol:water solution, stirred rapidly, and left standing for 30 minutes at 3-5°C. The mixture was centrifuged in the cold at 3000 rpm and the aqueous phase separated. The phenolic phase was shaken in 50 ml water and re-centrifuged. The water phase was added to the first collection, and the whole was dialysed against running tap water for two days and for one day against distilled water. The dialysed material was concentrated to 40-50 ml and then centrifuged to remove insoluble material. The viscous solution was poured into 6 volumes of alcohol and 10 ml alcohol saturated with sodium acetate was added to complete the precipitation. The precipitate was centrifuged, washed with alcohol and acetone, and then dried in a dessicator. This produced a partially purified lipopolysaccharide.

Sera from all groups of rats were heat inactivated at 56°C for 30 minutes and absorbed with fresh washed sheep cells. Serial dilutions of 0.025 ml volumes were prepared. Ten milligrams of lipopolysaccharide was dissolved in 3.2 ml buffered saline (pH 7.3) and 0.1 ml of a 50% suspension of washed sheep cells and 0.1 ml of 1% glutaraldehyde were added. The mixture was left at room temperature for one hour being gently agitated at 10-minute

intervals. The passively coated sheep cells were recovered by centrifugation, washed several times with buffered saline containing 1% normal rabbit serum, and resuspended in 2.5 ml of the washing medium. The final working suspension was prepared by mixing 0.3 ml of the sheep cell suspension and 1.7 ml of buffered saline plus 1% rabbit serum solution. This suspension was added to the microtitre wells in volumes of 0.025 ml. The microtitre trays were left at room temperature overnight and read the following morning.

The results from the weekly bleeds from each group were submitted to statistical analysis using Student's t test.

**Results**

**SURVIVAL**

All sham-operated and control animals survived longer than 20 weeks. Mean survival in the large portacaval-shunted animals was much shorter (10 to 14 weeks) and was not influenced by the administration of neomycin. Small animals (150-250 g) lived only  $29.2 \pm 3.3$  days without neomycin but this time was doubled with the administration of the antibiotic.

**WEIGHT LOSS**

Postoperative weekly weights were expressed as a percentage of the preoperative values and mean results are shown in figure 1. While sham-operated animals gained 30% of preoperative weight, all

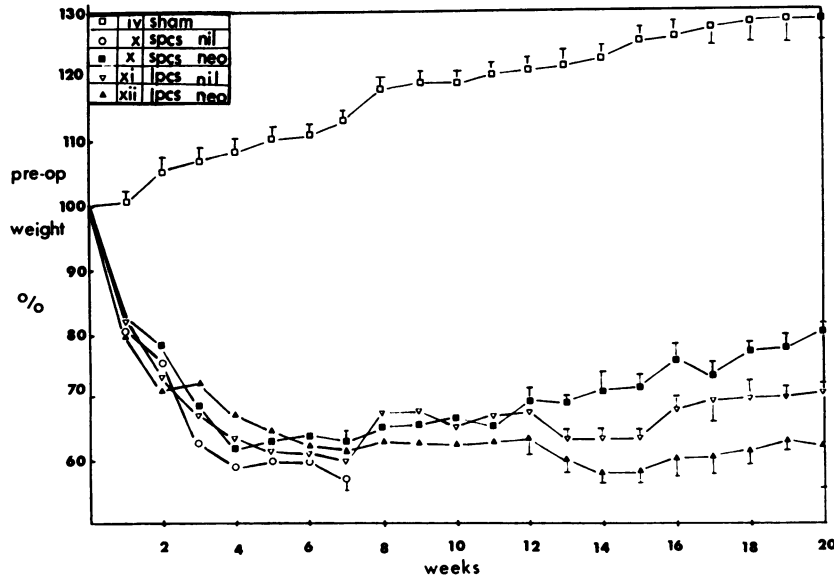


Fig 1 The effects of portacaval shunts upon the weight gain in small and large animals, with and without neomycin, compared with sham-operated controls.

portacaval-shunted animals lost at least 20% within the first six weeks and then gradually gained but preoperative levels were never regained. Neither bleeding nor neomycin affected survival or weight in sham-operated animals, but both factors reduced survival of portacaval-shunted animals.

**SERUM PROTEINS (FIGURES 2 AND 3)**

The total protein levels in all groups fluctuated during the period of study but showed no significant variation.

The serum albumin levels in the normal and sham-operated rats showed weekly fluctuations but no significant deviation from the initial level. In the portacaval-shunted rats, there was a striking change in the albumin level. From an initial value of 4.7 g/100 ml there was a progressive fall to 3.9 g/100 ml by the twelfth week. The fall was significant from the third week after the shunt ( $P = 0.02$ ). In contrast, the serum albumin levels in the portacaval-shunted rats which were treated with neomycin did not change significantly.

There was minimal variation in the serum gamma globulin levels in normal and sham-operated rats over the period of study, but a progressive increase

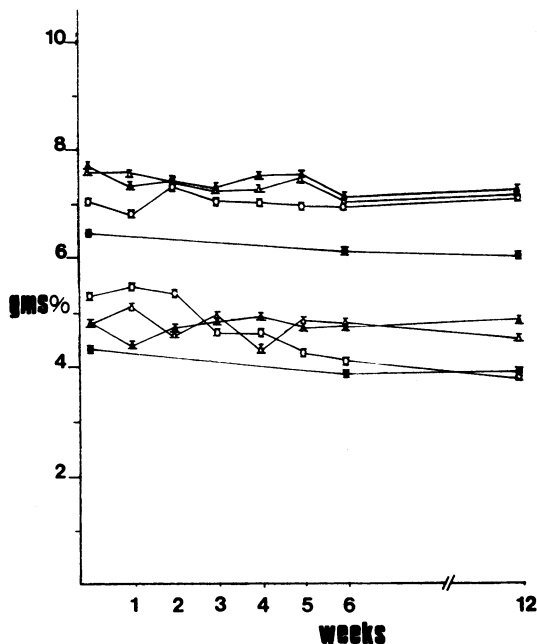


Fig 2 The total protein and serum albumin levels in normal, sham-operated, and portacaval-shunted rats with and without neomycin.

- ● portacaval shunt with neomycin
- ○ portacaval shunt without neomycin
- ▲ ▲ normal
- △ △ sham operated

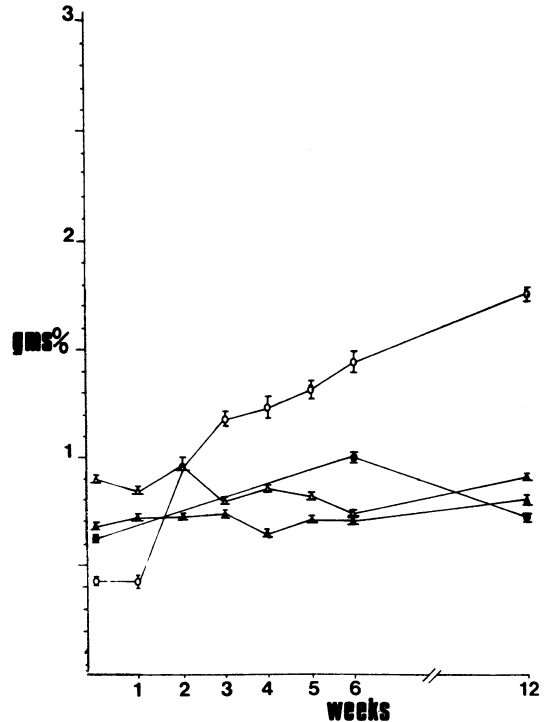


Fig 3 Serum gamma globulin levels in normal, sham-operated, and portacaval-shunted rats with or without neomycin.

- ● portacaval shunt with neomycin
- ○ portacaval shunt without neomycin
- ▲ ▲ normal
- △ △ sham operated

in portacaval-shunted animals to a mean final value which was four times higher than the initial levels ( $P < 0.001$ ). In rats, given neomycin following portacaval shunt, there was an initial rise in serum gamma globulin which was significant by the sixth week ( $P < 0.02$ ), but which returned to preoperative levels by the twelfth week ( $P < 0.04$ ).

**ANTIBACTERIAL LIPOPOLYSACCHARIDE ANTIBODY (FIGURE 4)**

The mean titre of antibody in the normal and sham-operated rats remained less than 20 throughout the study. In the portacaval-shunted rats the mean titre rose from less than 20 at the start of the study to reach a mean value of 640 by the sixth week and dropped by the twelfth week to a mean of 120 ( $P < 0.001$ ).

**Discussion**

The data confirm the observations by Kennan (1964) that the creation of a portacaval shunt in the

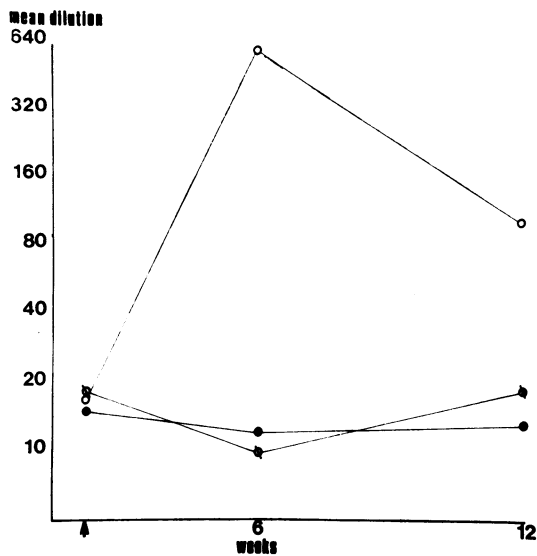


Fig 4 Serological responses to rat *E. coli* lipopolysaccharide in normal ●-●, sham and portacaval shunt ○-○, rats.

normal rat resulted in a fall in the level of serum albumin and a rise in serum gamma globulin. In addition, it was confirmed that considerable weight loss occurred and that survival was shorter than in sham-operated controls.

The apparent reciprocal changes in serum albumin and gamma globulin require explanation. Whilst the weekly bleeding of 0.5 millilitres from each animal could be estimated to have depleted 35 mg protein per week, no change in serum protein levels was noted in normal or sham-operated animals, but this procedure may have contributed to the decreasing levels of serum albumin in the portacaval-shunted animals. The progressive increase in gamma globulin in these rats cannot be reconciled with putative protein depletion.

Weight loss following portacaval shunt has been noted to varying degree; despite there being no long-term major morphological effects, liver weight may be reduced by one-third over a period of 12 weeks (Assal, Levrat, Cahn, and Renold, 1971). Kyu and Cavanagh (1970) have reported in detail the feeding habits of rats after portacaval shunt and showed that the food intake of such animals was a third of normal for several weeks after surgery, and others have confirmed this (Bismuth, Benhamou, and Lataste, 1963; Herz, Robert, and Bircher, 1971). The decreasing albumin levels may therefore have arisen from protein calorie malnutrition. Studies on experimental protein calorie malnutrition in rats by Kirsch, Brock, and Saunders (1968) confirmed that

protein malnutrition led to a decrease in serum albumin but there was no reciprocal increase in gamma globulin. The similar but smaller changes seen in rats which were treated with neomycin suggest that infection rather than protein malnutrition may have been operative.

In addition, the decreased levels of serum albumin in animals on neomycin may have been due to relatively large doses of antibiotic used and the complication of inducing malabsorption if given in large doses (Jacobsen, Prior, and Faloon, 1960).

One of the major effects of the operation is to exclude the reticuloendothelial system of the liver from contact with the portal circulation. This would result in unlimited access of bowel organisms or their antigenic constituents to the immunocompetent cells in the spleen and elsewhere. It is suggested that such stimulation of the immune system could cause a rise in gamma globulin. Thomas, MacSween, and White (1973) showed that the induction of cirrhosis in rats led to a reduced capacity of the liver to trap antigen and an increase in antigen in the spleen. The data obtained in the present study of antibacterial lipopolysaccharide antibodies support this view and lend further credence to the suggestion that the prime reason for the rise in gamma globulin in chronic liver disease is reduction in the filtering or trapping capacity of the liver consequent upon the development of portosystemic shunts at an intra- or extrahepatic level.

It is suggested that the portocaval-shunted rat may be a useful model in which to study the inter-relationship between bowel microorganisms and/or endotoxin and the immunocompetent cells.

Financial assistance was received from the Medical Research Council of South Africa, the Cape Provincial Administration, and the Herman Caporn bequest to the University of Cape Town. We are indebted to Dr Sun Lee of La Jolla, California, who taught two of us (M.K. and G.H.C.E.) the technique of portacaval shunt during a microvascular workshop in Cape Town in 1972.

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