

Subtitle: Comparative Studies in the Pathology and Host Physiology of Malarial: Renal Function in Mice Infected with P. berghei

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Although acute renal failure is one of the important complications of falciparum malaria, the underlying mechanism (s) has not been fully elucidated. Early workers attributed acute renal failure of malaria to haemoglobin casts in the renal tubules. This explanation was refuted by Foy et al. (1943) and Maegraith and Findlay (1944) who failed to find obstructing casts in the kidneys of malaria patients dying from renal failure. Maegraith (1944) suggested that in common with other conditions such as crush injuries, incompatible transfusions, concealed accidental hemorrhages and cholera, tubular necrosis in malaria results from a shunting of blood from the cortex to the medulla. The recent work of Chongsuphajaisiddhi (1966) in P. knowlesi infected rhesus monkeys supports this concept. By renal artery angiography he demonstrated a marked reduction in perfusion of the kidney, more evident in the cortex than the medulla, and prolonged transit time.

The present study was undertaken to determine if mice infected with P. berghei would offer a suitable and convenient model for the study of renal pathophysiology. It will be shown that renal dysfunction does occur and appears to be the result of a hemodynamic abnormality.

Methods: Twenty to 30 gm female albino mice were infected with 0.1 ml. of pooled heparinized blood from P. berghei infected mice with approximately a 30% parasitaemia. Haematocrit, parasitaemia, blood urea nitrogen (BUN) and per cent excretion of phenolsulfonaphthalein (PSP) were obtained on the 2nd, 4th, 7th, 8th, and 9th days. In addition the kidney was removed for histopathology. Seven animals were studied on day 2, 6 on day 4, 22 on day 7, 25 on day 8 and 9 on day 9. Uninfected control mice were studied by the same methods on each experimental day. On the 11th day, 10 infected and 10 control animals, previously discarded from the above experiments because of PSP dye extravasation at the injection site, were bled to determine BUN and haematocrit. The principles of animal care as promulgated by the National Society for Medical Research were observed.

The procedure was as follows. A 0.25 ml syringe containing PSP was weighed on an analytical balance before and after injection into the tail veins and the exact amount of PSP given was calculated. The dose was usually in the range of 0.02 ml or 0.12 mg of PSP. Pressure was maintained over the injection site for three minutes to insure against loss of dye. Any animal with extravasation of dye was discarded. The mouse was immobilized and a small cup of known weight was appropriately placed to collect faeces-free urine. Sixteen minutes after the injection of dye the animal was anesthetized with chloroform. Residual urine was washed through the urethra by injection of isotonic saline into the bladder and added to the 16 minute collection. The total volume of urine plus saline was obtained by weighing. The PSP concentration was determined on the total collection. Per cent excretion of PSP was calculated by the following formula:

$$\frac{\text{amount excreted (mg)}}{\text{amount injected (mg)}} \times 100.$$

The urinary PSP concentration was determined by a modification of the method of Rowntree and Geraghty (1912) in the 4th U.S. Army Laboratory Manual (1959). The urine was divided into two aliquots; one was alkalinized with 0.5 N NaOH, the other acidified with 0.5 N HCl. The acidified urine was the blank against which the alkalinized specimen was read at 555 m $\mu$  on a Beckman spectrophotometer. Pigments, such as hemoglobin, did not interfere with the determination.

Heart blood was drawn for estimation of parasitaemia, haematocrit, and BUN. The number of parasites counted in 1500 red blood cells was expressed as per cent infected red blood cells. The BUN was measured with a Hyland UN Test Kit Method (Hyland Laboratory, Los Angeles, California) utilizing 20  $\mu$ l of serum.

One kidney from each mouse was fixed in 10% buffered neutral formalin and embedded in paraffin. Sections were cut at seven microns and stained with hematoxylin and eosin. Selected sections were stained by the P.A.S., azure-eosin, and Prussian blue techniques. In addition the liver was removed from mice on the 4th, 7th, 8th and 9th days of study and handled in the same manner as the kidney.

In order to determine the relationship of anaemia to haemoglobinuria ten additional mice were infected at the same time as the previously described animals. Haematocrits from tail blood and urine haemoglobin determinations by the hemacombistix (Ames Company, Inc., Elkhart, Indiana) were performed daily on these animals until death.

## Results

Uninfected control animals. The average haematocrit of 35 mice was 51.6 (S.D.  $\pm$  3.5) per cent. The average BUN of 44 mice was 21 (S.D.  $\pm$  4) mg per cent. For 35 mice the median per cent excretion of PSP was 20 (range, 12-37 per cent).

Course of infection. Anaemia was first observed on the 4th day of infection and was accompanied by the haemoglobinuria in 2 of 10 animals. During subsequent days as the haemolytic anaemia increased in severity, the haemoglobinuria was marked and was observed in 8 out of 10 animals studied. The haematocrit reached a mean of approximately 20 per cent on the 7th, 8th and 9th days. The parasitaemia rose very rapidly. By the 4th day the mean per cent of red blood cells infected was 55. Though the mean parasitaemia did not change in subsequent days, there were some mice with virtually every red blood cell infected.

Blood urea nitrogen (BUN) (Table I and fig. 1). The mice studied on the 2nd and 4th days of infection had a normal BUN. By the seventh day, however, 15 per cent (3 out of 20) had a mild elevation of BUN. The per cent of animals with an abnormal BUN increased to 67 per cent (6 out of 9) by the 9th day and 80 per cent (8 out of 10) by the 11th. This trend of increasing BUN after day 7 is clearly shown in fig. 1.

Per cent excretion of PSP (Table I and fig. 1). On the 2nd and 4th days of infection the mice had a normal per cent excretion of PSP. Abnormal PSP excretion was observed in 36 per cent (8 of 22 mice) on the 7th day. On the 8th day 56 per cent (14 out of 25), and by the 9th day 78 per cent (7 out of 9) were abnormal. The increased percentage of animals with very low PSP excretion after day 7 was concurrent with the increasing level of BUN (fig. 1). The correlation between BUN and per cent excretion of PSP of individual mice is shown in fig. 2. As the per cent excretion of PSP approached zero the BUN became increasingly elevated. In those mice with zero excretion of PSP the BUN was markedly elevated, with a range of 27-88 mg per cent.

### Pathology. Kidney morphology:

Kidneys secured on day 2 resembled normal controls except for mild swelling of the glomerular epithelium and the presence of an occasional parasitized erythrocyte in a glomerular capillary. Kidneys secured on day 4 appeared slightly swollen. The perivascular lymphatics were increased in prominence, containing scattered pigment laden mononuclear cells.

Periarterial and periarteriolar groups of active appearing reticuloendothelial cells were present in all animals although there was considerable variation in their distribution and number. Kidneys from animals studied on days 7, 8 and 9 appeared markedly swollen, with cortical pallor and variable medullary engorgement. Many of the glomeruli appeared collapsed and bloodless (fig. 3A). The juxtamedullary glomeruli in general appeared to contain more erythrocytes than those in the more superficial portions of the cortex (fig. 3B). The basement membrane was not altered. There was no evident necrosis of tubular epithelium, although occasional mitotic figures suggested the possibility of an increase in epithelial turnover rate. The

cortical perivascular lymphatics were dilated and contained large numbers of cells. The intramedullary veins and peritubular capillary plexus showed patchy engorgement (fig. 3C). In many animals the intramedullary portions of Henle's loop also appeared distended. These anatomic changes are believed to suggest significant alteration of cortical blood flow and considerable interstitial edema. An attempt at quantitation of anatomic alteration and correlation with observed physiologic changes was unsuccessful.

#### Liver morphology:

At 4 days an occasional animal had moderately severe hydropic changes in the cytoplasm of the liver cells surrounding the central vein. In material obtained on days 7, 8, and 9 this change was fairly constant and irregular foci of centrilobular necrosis were also present (fig. 4). No extensive fatty metamorphosis was seen. These lesions probably are related to varying degrees of centrilobular ischemia.

#### Discussion:

In contrast to the more moderate infection in rats in which renal function was normal (Keeler et al., 1960), the results of this study clearly demonstrated alterations in renal function in P. berghei infected mice. Sadun et al. (1965) found that P. berghei infected mice exhibited an elevation in serum non-protein nitrogen in the absence of an elevated serum creatinine. This would suggest an increased protein catabolism and urea production but not necessarily indicate an abnormality in renal physiology. However, in the present study a low or absent excretion of PSP in those mice with an elevated BUN makes this explanation untenable. PSP is excreted mainly by transport across the proximal tubule. Any reduction of blood flow to this area of the kidney or dysfunction of the proximal tubule would lead to a reduction in excretion of this substance. The absence of any tubular pathology would implicate blood flow as the primary abnormality. One other possible explanation for a reduced PSP excretion is a low urine flow due to lower solute excretion or antidiuresis in mice with malaria. This might explain a mild reduction in PSP excretion but would be an unlikely explanation for an excretion of zero. Secondly and more important, the combination of an extremely low PSP excretion and an elevated BUN would reflect a true abnormality in renal function.

The sequence of events leading to azotaemia and renal failure in malaria is unknown. Some mechanism which causes decreased blood flow to the kidney or shunting of blood from the cortex to the medulla, as originally proposed by Maegraith and Findlay (1944), may be involved. The histopathologic findings of a relatively bloodless cortex and congested medullary vessels are compatible with intra-renal shunting of blood, although the dynamic events can not be reconstructed from pathologic examination. A similar pathologic findings has been demonstrated in fatal cases of falciparum malaria (Maegraith and Findlay, 1944) and in other examples of ischemic renal disease (Merrill, 1962). Sitprija et al. (1967) found decreased inulin-PAH clearances in three azotaemic patients with severe falciparum malaria. These patients had no functional evidence of tubular necrosis (low urinary sodium excretion and a high urine osmolality). They felt that in the absence of morphologic changes in the glomerulus or tubular epithelium the best explanation for the azotaemia was an altered renal blood flow. Similarly, Desowitz et al. (1967) found that an elevated BUN and serum creatinine may be found without renal histopathology in the rhesus monkey with P. coatneyi malaria. Undoubtedly the physiologic alterations precede a demonstrable histopathologic lesion. It is believed that on the basis of available evidence the most reasonable hypothesis for renal pathology is a haemodynamic abnormality, although other factors such as toxins or lesions of antigen-antibody complexes must also be considered.

While only renal pathology was considered in this discussion, functional and anatomical changes in other organs, e.g. liver dysfunction with centrilobular necrosis, may well be the result of a similar underlying mechanism(s).

Summary:

(1). Albino mice infected with P. berghei were studied sequentially for changes in BUN, per cent excretion of PSP, renal histopathology, parasitaemia, and haematocrit.

(2). By the 7th day the BUN increased and the per cent excretion of PSP had decreased. These values became progressively more abnormal on subsequent days. In individual mice, as the PSP excretion decreased, the BUN increased. The pathologic abnormality consisted of a bloodless cortex and congested medullary vessels.

(3). On the basis of functional tests and renal histopathology, it is postulated that hemodynamic changes are responsible for the renal disease.

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Legends for figures:

Fig. 1. The per cent distribution of PSP excretion and BUN for mice studied on various days after infection with P. berghei.

Fig. 2. The relationship between BUN and per cent excretion of PSP on days 7,8, and 9.

Fig. 3. Kidney, mouse 135.

- A. Collapsed superficial glomerulus.
- B. Juxtamedullary glomerulus, erythrocytes seen in capillaries. Malaria pigment present in endothelium.
- C. Engorged medullary vessel. Hematoxylin and eosin x 430.

Fig. 4. Liver, mouse 135. Focal necrosis, hydropic degeneration. Hematoxylin and eosin x 250.

TABLE I — The Parasitaemia, Haematocrit, BUN, and Per cent Excretion of PSP on various days of Infection with P. Berghel.

Days After Inoculation with <u>P. berghel</u>	2	4	7	8	9	11
Number of Mice	7	6	22	25	9	10
Parasitaemia* (% infected rbc)	8±2	55±9	50±5	52±5	64±4	—
Haematocrit* (%)	52±1	45±2	20±2	18±1	18±1	16±1
BUN** (% mice abnormal)	0	0	15 <sup>†</sup>	41 <sup>†</sup>	67	80
PSP excretion <sup>+</sup> (% mice abnormal)	0	0	36	56	78	—

\* The results are expressed as means± standard error of the mean.

\*\* The mean± 2SD for BUN in 44 uninfected mice was 21±8 mg per cent. Any determination above 29 mg cent was considered abnormal.

† The number of mice with BUN determination on days 7 and 8 were 20 and 22, respectively.

+ The range for per cent excretion of PSP in 35 uninfected mice was 12–37 per cent. Any determination below 12 per cent was considered abnormal.

Fig. 1. BUN and PSP excretion in *P. berghei* infected mice.

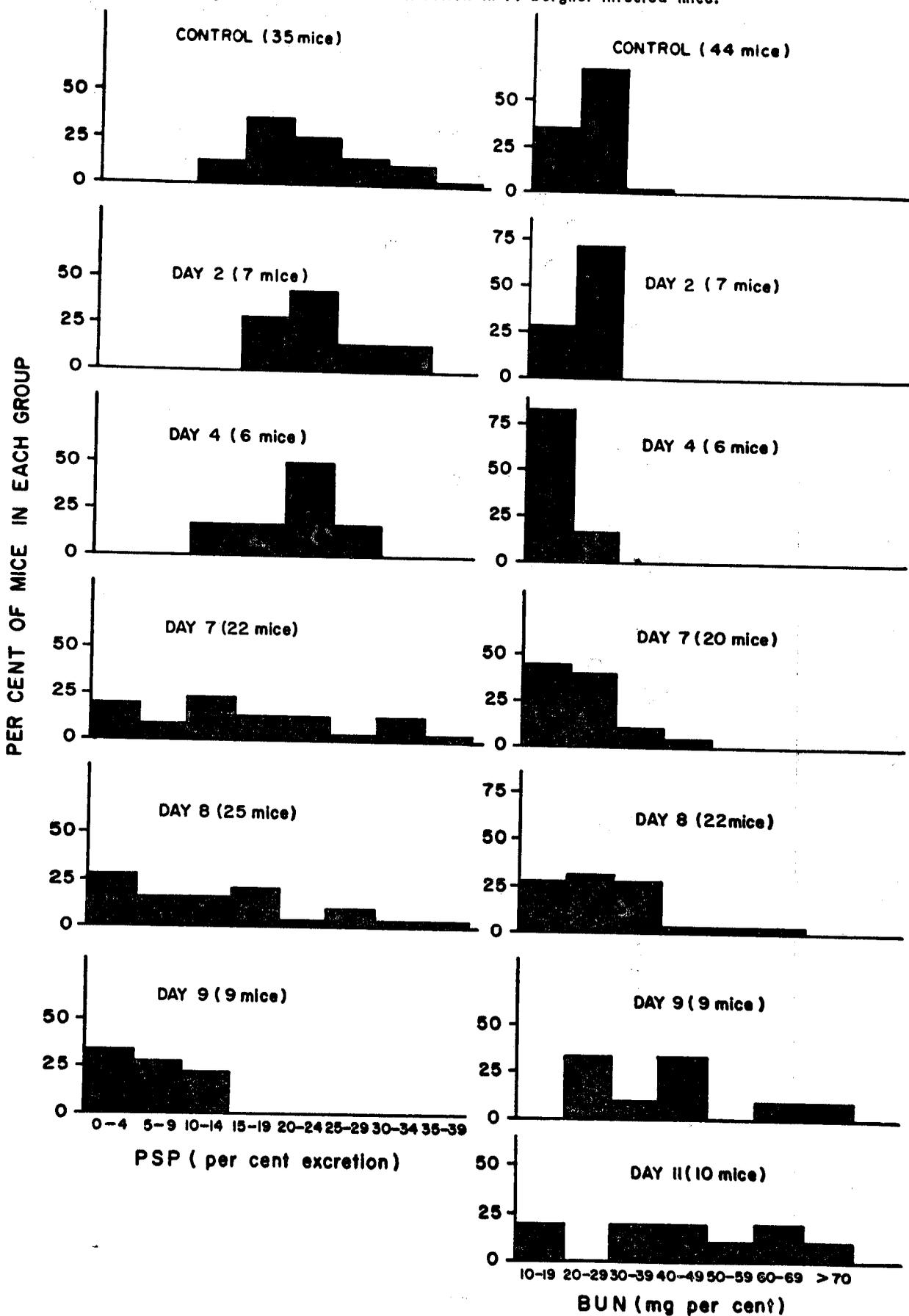
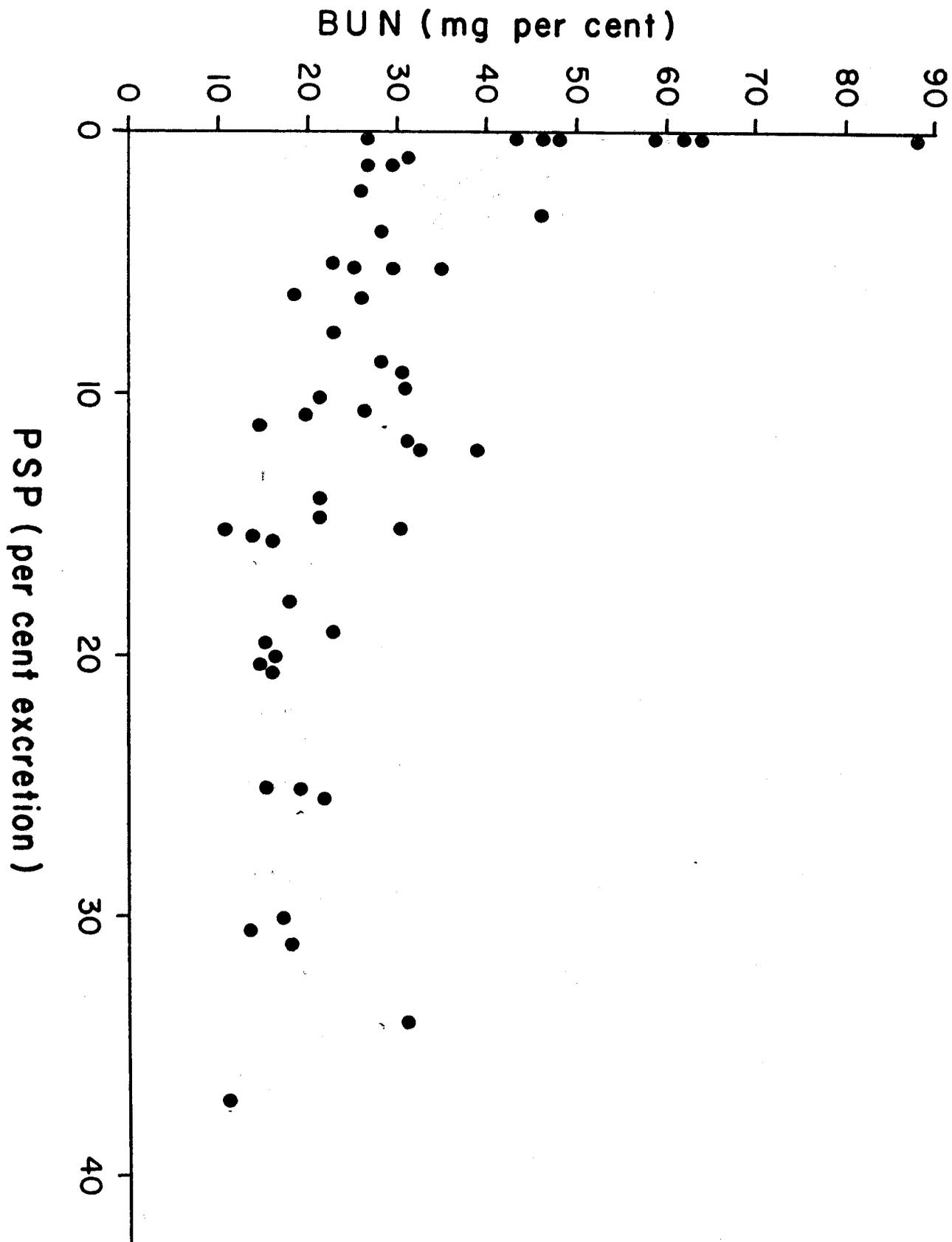


Fig. 2. Correlation between BUN and PSP excretion in *P. berghel* infected mice



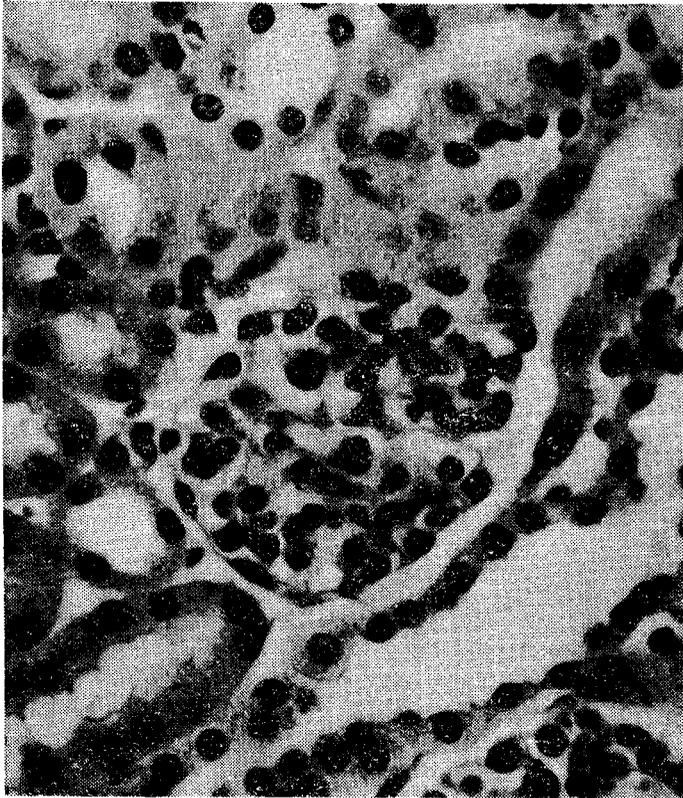


Fig. 3a. Histopathology of the kidney of P. berghei mice.

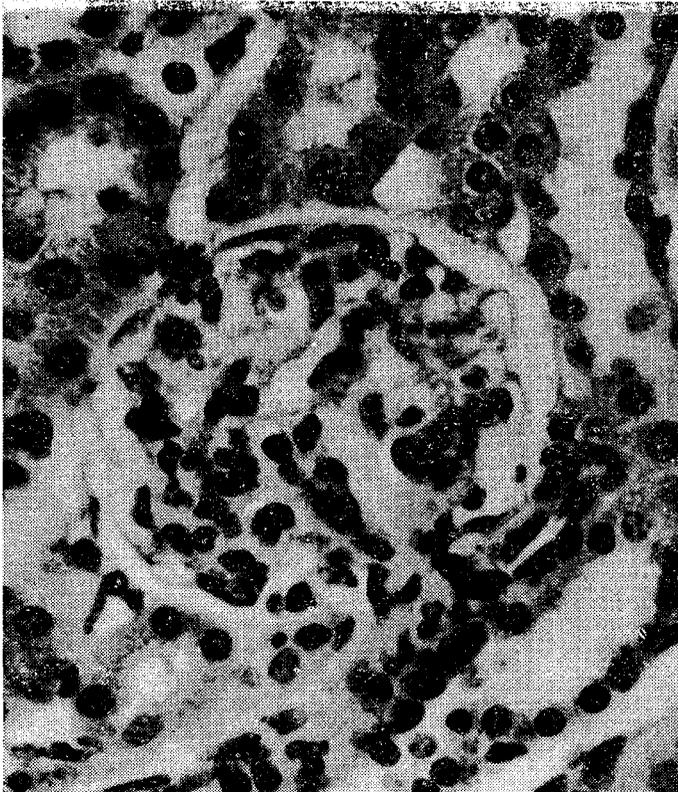


Fig. 3b. as Fig. 3a.

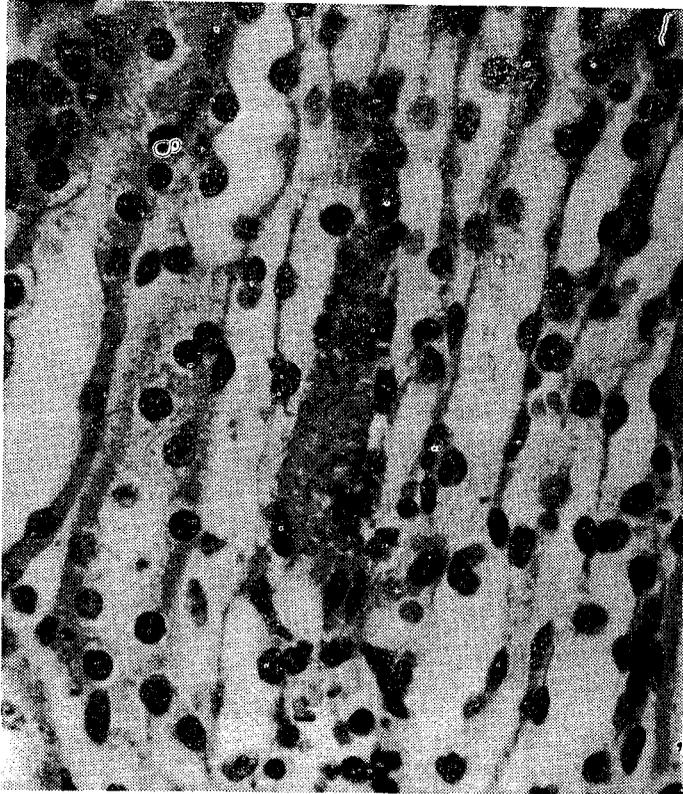


Fig. 3c. same title as Fig. 3a

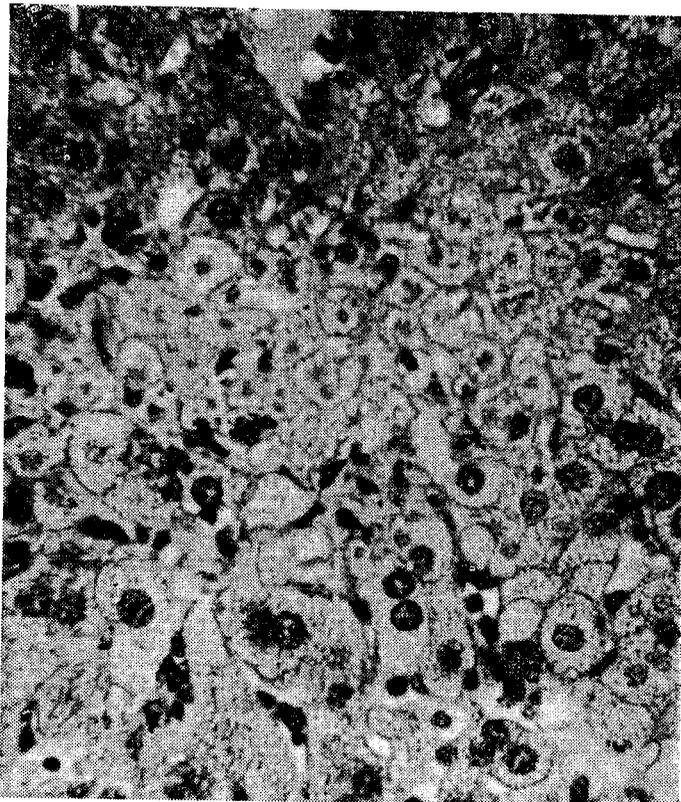


Fig. 4. The liver of a P. berghei infected mouse showing centrilobular necrosis