

to portal congestion. Support for this view derives from the finding that protein concentration in liver lymph was abnormally low in each one of 14 consecutive patients with portal hypertension secondary to hepatic cirrhosis, a close relationship which was overlooked in earlier reports based on findings in a smaller number of patients (3, 4). Although more information is needed, particularly with regard to protein content of liver lymph in patients with less advanced stages of cirrhosis, it is tempting to speculate that altered sinusoidal permeability to protein, however, obscure in origin, may be an important component of the mechanism underlying portal hypertension.

### References

- 1 *Starling, E.H.*: Principles of Human Physiology, 2nd ed. Lea and Febiger, Philadelphia (1915) 1014
- 2 *Zimmon, D.S., R.E. Kessler*: The portal pressure-blood volume relationship in cirrhosis. *Gut* 15 (1974) 99
- 3 *Witte, C.L., M.H. Witte, A.E. Dumont, J. Frist, W.R. Cole*: Lymph protein in hepatic cirrhosis and experimental hepatic and portal venous hypertension. *Ann. Surg.* 168 (1968) 567
- 4 *Witte, M.H., A.E. Dumont, W.R. Cole, C.L. Witte, K. Kintner*: Lymph circulation in hepatic cirrhosis: Effect of portacaval shunt. *Ann.Int. Med.* 70 (1969) 303
- 5 *Gorrall, A.G., C.J. Bardawill, M.M. David*: Determination of serum protein by means of the Biuret reaction. *J.Biol.Chem.* 177 (1949) 751
- 6 *Witte, C.L., M.H. Witte, A.E. Dumont, W.R. Cole, J.R. Smith*: Protein content in lymph and edema fluids in congestive heart failure. *Circulation* 40 (1969) 623
- 7 *Popper, H., F. Schaffner*: Capillarization of hepatic sinusoids in man. *Gastroenterology* 44 (1963) 239
- 8 *Starling, E.H.*: The fluids of the body. W.T. Keener, Chicago 1909

*Allan E. Dumont, M.D., Department of Surgery, New York University School of Medicine, 550 First Avenue, New York, New York 10016*

*Lymphology* 8 (1975) 113-122  
© Georg Thieme Verlag Stuttgart

## High Concentration of Bilirubin in Post-Nodal Lymph Associated with Red Blood Cell Catabolism in Lymph Nodes of the Sheep

**J.W. Quin, A.D. Shannon**

Department of Experimental Pathology, John Curtin School of Medical Research, The Australian National University, Canberra, Australia

### Summary

Qualitative and quantitative analysis of post-nodal lymph of the sheep has shown that the distinct yellow colour of this fluid pool is due to the presence of relatively large amounts of bilirubin. In efferent lymph from the popliteal, prefemoral, prescapular, renal and intestinal lymph nodes total bilirubin concentrations were 3-8 times higher than the corresponding concentrations in blood plasma. In contrast the total bilirubin concentrations in afferent lymph from the lower leg and kidney were less than the corresponding concentrations in blood plasma. Histological examination of several popliteal and mesenteric lymph nodes revealed the presence of free iron and bilirubin in the cytoplasm of cells located near the lymphatic sinuses of the node. In addition, the concentration of bilirubin in efferent lymph from the popliteal node was observed to increase following an induced rise in the number of red blood cells reaching the node by way of the afferent lymphatic duct. These latter observations suggest that the bilirubin in post-nodal lymph is associated with the catabolism of extravascular red cells by reticulo-endothelial cells within the lymph nodes.

### *Introduction*

Bilirubin in tissues and fluid pools in the body is largely derived from the catabolism of erythrocyte haemoglobin (1, 2). Cells capable of processing erythrocyte haemoglobin to bilirubin occur in almost all tissues but are present in high numbers in the spleen, bone marrow, liver and lymph nodes (3). However, the actual sites of catabolism of senescent red blood cells in the intact animal are at present uncertain. Under experimental conditions, red blood cells altered by immunological, chemical or physical means are largely removed by the liver and spleen in both animals and man (4, 5, 6, 7, 8).

Notwithstanding, it would appear that significant catabolism of red blood cells and erythrocyte haemoglobin may take place in peripheral sites including lymph nodes (9). Indeed, it is known that peripheral lymph nodes in the dog and the rabbit remove autologous red cells when these cells are infused into the node by way of the afferent lymph (10, 11, 12), and the mediastinal lymph nodes in a number of animals have been shown to be capable of removing some of the red cells introduced into the peritoneal cavity (13).

In the present experiments, high concentrations of bilirubin in post-nodal lymph in the sheep are reported and the suggested role of lymph nodes in the catabolism of red cells has been investigated in further detail.

### *Materials and Methods*

#### *Animals*

Adult merino and merino cross-bred sheep were used in the experiments. These sheep were a mixture of wethers and non-pregnant ewes. The animals were housed indoors in metabolism cages and fed lucerne chaff and oats *ad libitum*.

Various lymphatic ducts in 25 sheep were cannulated in the following manner: An efferent lymphatic duct of the popliteal node in each of 15 animals was cannulated by the method of *Hall and Morris* (14). In 9 of these sheep, an afferent lymphatic duct of the popliteal node was cannulated in the opposite leg enabling simultaneous collections of efferent and afferent lymph to be made in each of these sheep. Intestinal lymph was collected from 7 sheep following the procedure of *Lascelles and Morris* (15). The intestinal lymph was recirculated in each sheep through an indwelling jugular cannula. In 3 sheep, an efferent lymphatic duct of each of the prefemoral and prescapular nodes were cannulated as described by *Hall and Morris* (6) and *Pedersen and Morris* (17), respectively. Following the surgical procedure described by *McIntosh* (18), an afferent and efferent lymphatic duct of the renal lymph node were cannulated in each of 2 sheep. Difficulty was experienced in maintaining lymph flow from the renal afferent duct due to the presence of large numbers of red blood cells in this lymph following cannulation (cf. Table 3). Thus additional renal afferent lymph from a transplanted kidney autograft was obtained as described by *Pedersen and Morris* (17).

An indwelling jugular cannula was inserted in each sheep at the time of the lymphatic cannulations to facilitate blood collection without disturbance to the animal.

In all except 1 sheep, a recovery period of at least 3 days and up to 10 days was allowed post-operatively before samples of blood plasma and lymph were collected for analysis. In 1 sheep with a renal afferent cannulation, samples were collected at 6-8 hr after the operation.

For comparative purposes, the thoracic ducts of 4 Wistar strain (random bred, closed colony) rats were cannulated by the method of *Bollmann, Cain and Grindlay* (19) and samples of thoracic duct lymph were collected over the following 1-5 days for analysis. Blood samples were obtained from an indwelling femoral vein cannula in each rat.

Lymph samples were collected into graduated glass measuring cylinders containing 0.01 ml of a 1000 i. u./ml heparin solution (Pularin, Evans Medical Australia Ltd, Boronia, Victoria). Lymph was collected in this manner at intervals for periods of 10 min to 1 hr. Samples of blood plasma were taken in the middle of each period of lymph collection.

### *Cell Counting*

Total lymphocytes and red blood cells (RBC) in lymph were determined using a model ZBI Coulter Counter (Coulter Electronics, England). Immediately after collection, 0.05 ml of lymph was diluted to 25 ml with 0.9% sterile saline. A total cell count was carried out on the diluted lymph sample before 3 drops of Zaponin (Coulter Electronics, England) were added to lyse the RBC in the lymph. A total lymphocyte count was then carried out and the number of RBC in the lymph was defined as the difference between the total cell count and the total lymphocyte count.

### *Biochemical Analysis*

#### (a) *Total Bilirubin*

Total bilirubin in lymph and plasma was determined using a modification of the *Jendrassik and Grofts* alkaline diazo-coupling method as described by *Brodersen and Jacobsen* (20). A bilirubin standard was prepared from pure bilirubin (Sigma Chemical Co., USA) according to the methods previously described (21, 22).

#### (b) *Thin Layer Chromatography*

The yellow pigment from post-nodal lymph from the intestine and the popliteal node was extracted with 20 volumes of 95% ethanol. The extracted pigments were separated by thin layer chromatography (TLC) on 0.5 mm layer of silica gel with liquefied phenol:water, 41:9 (v/v) as solvent (23).

#### (c) *Albumin Determination*

The single radial immunodiffusion technique (24) was used to determine the concentration of albumin in lymph and blood samples. Monospecific anti-ovine albumin was prepared by intramuscular injections of 5 mg of pure ovine albumin together with 1 ml Freund's complete adjuvant D (Difco, USA) into the hind leg of an outbred rabbit.

Pure ovine albumin was prepared by pooling fractions from the major optical density peak of a DEAE Sephadex gradient elution (0.1 M to 0.4 M phosphate buffer, pH 8.0) of sheep albumin fraction V (Schwarz/Mann, USA).

#### (d) *Precipitation of Albumin Using Antisera*

Efferent lymph from a popliteal node of one sheep was slowly added to rabbit monospecific anti-ovine albumin serum until a final ratio of 1 to 5 (lymph:antisera) was achieved. Albumin and total bilirubin determinations were carried out on the lymph before and after precipitation of the albumin. Corrections were made for dilution effects and the small increment in total bilirubin associated with the addition of the rabbit antisera. Efferent lymph added to normal rabbit serum was used as a control. No change in total bilirubin or albumin was observed in the control.

### *Injection of Isologous Red Blood Cells*

In each of 2 experiments, 10 ml of sterile whole donor blood was collected into capped glass centrifuge tubes containing 10 ml of sterile Alsevers solution (25). The 'buffy coat' and serum

were removed by centrifugation at 1100 g for 10 mins. The red blood cells were washed 4 times in Alsever's solution and serially diluted to approximately  $10^7$  RBC/ml.

A 1 ml volume containing  $10^7$  RBC was injected subcutaneously below the tarsus of the same sheep from which it had been collected and the recovery of RBC and bilirubin in efferent and afferent popliteal lymph determined.

### Histochemistry

Intestinal and popliteal lymph nodes were removed from 3 sheep. The nodes were fixed in formal-saline, embedded in paraffin and 4  $\mu$ m serial sections stained for ferric ion (26) and bilirubin (27). A serial section was also stained with haematoxylin and eosin to determine structure and cell differentiation.

### Results

Characterisation of the distinct yellow colour in postnodal lymph of the sheep by standard physico-biochemical methods revealed that the yellow pigment was bilirubin. A summary of the main characteristics is as follows:

- (a) The post-nodal lymph collected from the various regions gave a positive, indirect Van den Bergh test (28).
- (b) The light absorption spectrum of efferent lymph from the popliteal node was a slightly skewed (to the right) parabolic curve with an absorption maximum between 460 and 470 nm. This light absorption spectrum was similar to the bilirubin-albumin complex reported by *Blauer, Harnatz and Snir* (29).
- (c) The Rf values of 0.70-0.85 contained by TLC separation of the ethanol extracted pigment from efferent lymph of the popliteal and intestinal nodes were similar to those observed for a bilirubin standard run on the same plate. In addition the pigment exhibited red-orange fluorescence in long wave ultraviolet light.
- (d) A significant correlation ( $r$  value = 0.96, probability < 0.01) was observed between the intensity of the pigment (absorbance per cm at 460 nm) and the total bilirubin concentration in efferent lymph of the popliteal node.
- (e) G 200 sephadex fractionation of efferent lymph from the popliteal node showed that the bilirubin was entirely associated with compounds in the third major optical density peak. The results presented in Table 1 show that a 33% reduction in total bilirubin accompanied the precipitation of 37% of the albumin in efferent lymph from the popliteal node. Further precipitation was not carried out due to the error in determining total bilirubin and albumin concentrations below 1  $\mu$ g/ml and 2 mg/ml, respectively, in the diluted samples. However, the results suggest that the bilirubin in post nodal lymph is largely, if not entirely, associated with albumin.

The results in Table 2 show that the total bilirubin concentrations in post-nodal lymph from 5 different regions of the sheep and the thoracic duct of the rat were 3-8 times higher than the corresponding concentrations found in blood plasma. In contrast, the concentration in afferent lymph from the 2 regions of the sheep examined was generally less than that of blood plasma. Despite the large variation in total bilirubin concentrations of post-nodal lymph between animals, the total bilirubin concentrations observed in all of the sheep and 3 of the 4 rats were significantly higher than that of blood plasma. In addition, a significant correlation ( $r = 0.90$ ,  $P < 0.05$ ) was observed between the total bilirubin concentration in efferent popliteal lymph of the sheep and the number of RBC in this lymph.

Since the bilirubin in efferent lymph is bound to albumin, similar lymph:plasma concentration ratios for both bilirubin and albumin could be expected. However, a comparison between the results for albumin and bilirubin presented in Table 2 point to the fact that the lymph:plasma

Table 1. Changes in the total bilirubin and albumin concentrations in efferent lymph of the popliteal node following precipitation of the albumin with monospecific anti-ovine albumin. Values have been corrected for dilution effects and the increment in total bilirubin associated with the addition of the rabbit antisera

	Total Bilirubin ( $\mu\text{g/ml}$ )	Albumin ( $\text{mg/ml}$ )
<b>Efferent lymph</b>		
before precipitation	7.94	11.85
after precipitation	5.29	7.45
% reduction	33	37

ratios for bilirubin in postnodal lymph are much higher than those for albumin. Thus it is highly unlikely that the bilirubin in post-nodal lymph of the sheep is entirely plasma derived.

Lymph collected from the sheep contains a high percentage of RBC (Table 3). Indeed, the RBC in afferent lymph of the hind limb represent 50% of the total cells present in the lymph. In contrast the RBC in efferent lymph from several regions of the body represent only 20-30% of the total cells present in the lymph, although the actual concentration of RBC in efferent lymph is higher from that observed in afferent lymph.

Table 2. The total bilirubin concentration observed in various sources of lymph and in blood plasma of the sheep and in lymph from the thoracic duct of the rat. The lymph:plasma concentration ratios for both total bilirubin and albumin are also given. Values shown are means  $\pm$  standard errors with the number of animals in each group shown in brackets.

	Concentration ( $\mu\text{g/ml}$ )	L:P Ratio Bilirubin	L:P Ratio Albumin
Plasma (23)	0.81 $\pm$ 0.09		
Afferent popliteal (9)	0.37 $\pm$ 0.06	0.54 $\pm$ 0.12	0.27 $\pm$ 0.02
Efferent popliteal (15)	3.72 $\pm$ 0.57	5.62 $\pm$ 0.89	0.50 $\pm$ 0.03
Efferent intestinal (7)	2.00 $\pm$ 0.38	4.67 $\pm$ 1.74	0.53 $\pm$ 0.02
Efferent prefemoral (2)	3.50 $\pm$ 1.40	4.90 (1)	0.67 (1)
Efferent prescapular (3)	2.30 $\pm$ 0.38	1.70 (1)	0.68 (1)
Afferent renal (2)	0.38 $\pm$ 0.03	0.20 (1)	0.19 (1)
Efferent renal (1)	1.93	2.88	0.68
Rat thoracic duct (4)	3.37 $\pm$ 1.14	2.77 $\pm$ 0.93	0.54 $\pm$ 0.02*

\*Total protein L:P ratio

The results presented in Fig. 1 (a) show that the injection of autologous red cells into a regional site drained by the popliteal lymph node resulted in an increase in the bilirubin concentration in the efferent popliteal lymph. The bilirubin concentration began to rise sharply 2 hr after the injection and had reached a maximum by 5 hr. This rapid change in bilirubin concentration in efferent lymph was not accompanied by a rise in bilirubin concentration in blood plasma or in afferent lymph draining the site of injection. The recovery of a large number of RBC in both efferent and afferent lymph during the first 2 hr after the injection (Fig. 1 b) suggests that capillary damage may have occurred in the area of injection and that most of the RBC in afferent lymph escaped trapping in the popliteal node. Following the injection of RBC, the higher output of red blood cells in efferent lymph relative to afferent lymph was probably due to the failure to recover all the afferent lymph from the site of injection.

Histological examination of the popliteal and mesenteric lymph nodes of 4 sheep revealed the presence of free iron and bilirubin within the reticuloendothelial cells. In Fig. 2a the presence of free iron can be seen in a number of cells located within and adjacent to the lymph sinuses

Table 3. The relative and absolute numbers of red blood cells in various sources of lymph from the sheep and in rat thoracic duct lymph. The lymph flow is also given. The values shown are means  $\pm$  standard errors with the number of animals in each group shown in brackets.

Source of Lymph	Lymph flow (ml/h)	Red blood cells ( $\times 10^6$ /ml)	% Total cells
Afferent popliteal (8)	$2.3 \pm 0.4$	$0.7 \pm 0.2$	$52.1 \pm 6.1$
Efferent popliteal (13)	$5.1 \pm 0.5$	$1.9 \pm 0.5$	$23.1 \pm 5.0$
Afferent renal (1)	6.4	66.6	98.6*
Efferent renal (1)	3.0	4.0	21.2
Efferent intestinal (7)	$42.2 \pm 3.0$	$6.5 \pm 1.4$	$31.0 \pm 3.8$
Rat thoracic duct (2)	$2.3 \pm 1.3$	$5.5 \pm 0.2$	$22.3 \pm 0.2$

\*Sample taken the day of the operation

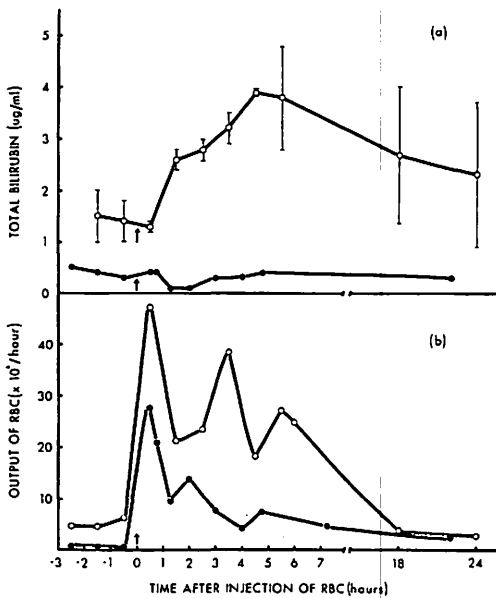


Fig. 1a. Changes in the total bilirubin concentration observed in efferent lymph from the popliteal node (open circles) of 2 sheep and afferent lymph to the popliteal node (closed circles) of 1 sheep following a subcutaneous injection of isologous red blood cells (RBC) below the tarsus.

b. Changes in the output of red blood cells in efferent lymph from the left popliteal node (open circles) and afferent lymph to the right popliteal node (closed circles) of 1 sheep following a subcutaneous injection of isologous red blood cells below the tarsus of each hind leg.

of a popliteal node from one of the sheep. At a higher magnification (Fig. 2b) this free iron can be seen located in granular form within the cytoplasm of large cells. Specific bilirubin staining was observed in a granular form within the cytoplasm of large cells in the sinusoidal areas (Fig. 3). In addition, large numbers of small bilirubin particles were observed in the extracellular space of the node. Fig. 4 shows several large cells in the lymphatic sinusoidal area in which the cytoplasm can be seen to be engorged with RBCs. This section was taken from one popliteal lymph node of a sheep in which the efferent popliteal lymph contained a persistently high concentration of total bilirubin ( $10.8 \mu\text{g/ml}$ ; 27 times the plasma concentration) and large numbers of RBC.

The examination of popliteal and mesenteric lymph nodes from the rat also showed the presence of free iron and bilirubin within reticuloendothelial cells. However, the amount of each catabolism product present in these nodes of the rat was significantly less than that seen in the sheep.

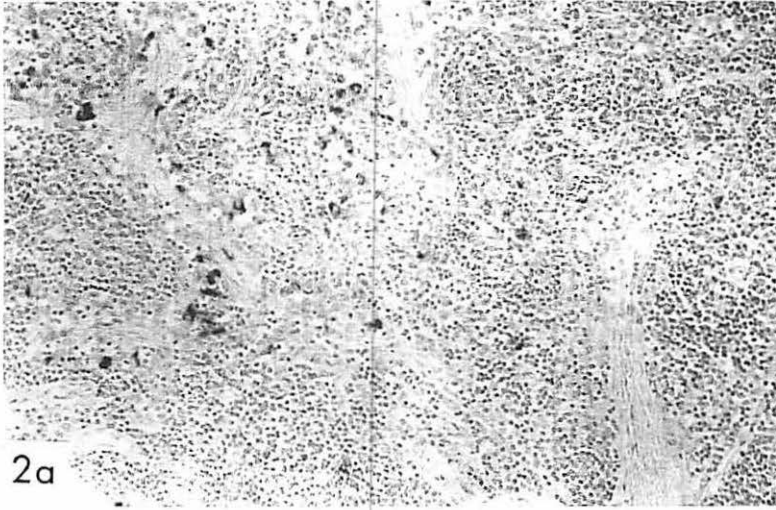


Fig. 2a. A section from a popliteal lymph node stained for ferric iron. Iron stained granules can be seen in the areas adjacent to the lymphatic sinuses. 125X.

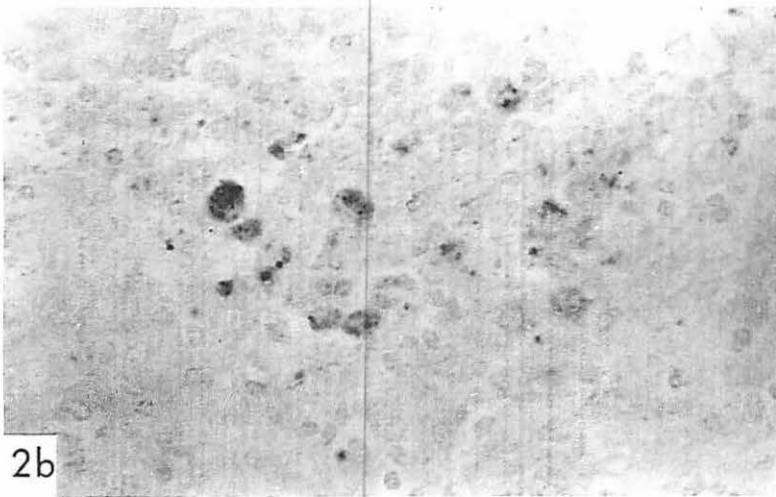


Fig. 2b. A higher power magnification from the same section as above showing the location of the iron stained granules within the cytoplasm of several large cells. 500X.

### Discussion

The peripheral lymph of most species contains a substantial number of RBC (30). Indeed, afferent popliteal lymph of the sheep contains approximately  $10^5$ - $10^6$  RBC/ml (Table 3). The popliteal node of the anaesthetised dog and rabbit have been shown to be capable of removing autologous RBC when they are infused into the afferent lymphatic duct (10, 11, 12). In these two species, the number of RBC recovered in efferent lymph depended on the number of RBC infused; recoveries varied from 3% in lymph when  $10^6$  were infused to 27% when  $10^8$  were infused. It is also noteworthy that massage of the infused node in rabbits caused a significant release of RBC (10, 11). In contrast, similar experiments carried out in the conscious sheep suggest that under normal circumstances the popliteal node does not remove large numbers of isologous RBC from afferent lymph. *Trevella and Morris* (unpublished observations) recovered up to 68% of  $^{51}\text{Cr}$ -labelled autologous RBC in efferent lymph of sheep in the first

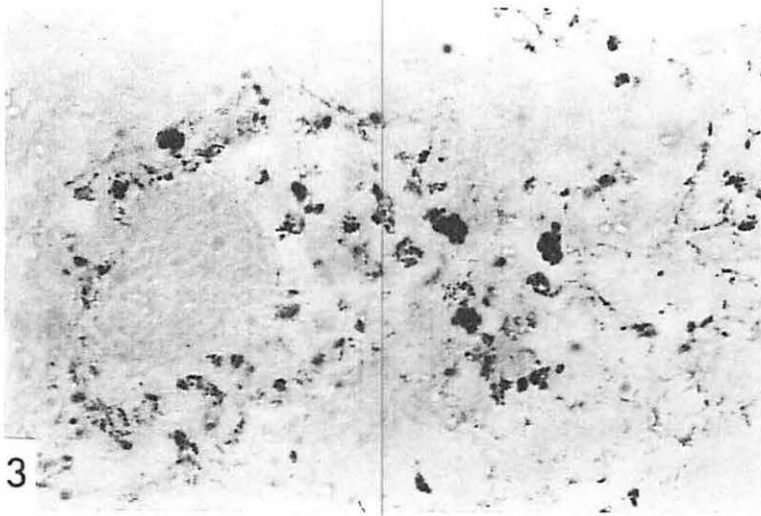


Fig. 3. A section from a mesenteric lymph node stained for bilirubin. Stained granules can be seen in the cytoplasm of several large cells and additional smaller granules can be seen in the extracellular space of the node. 325X.

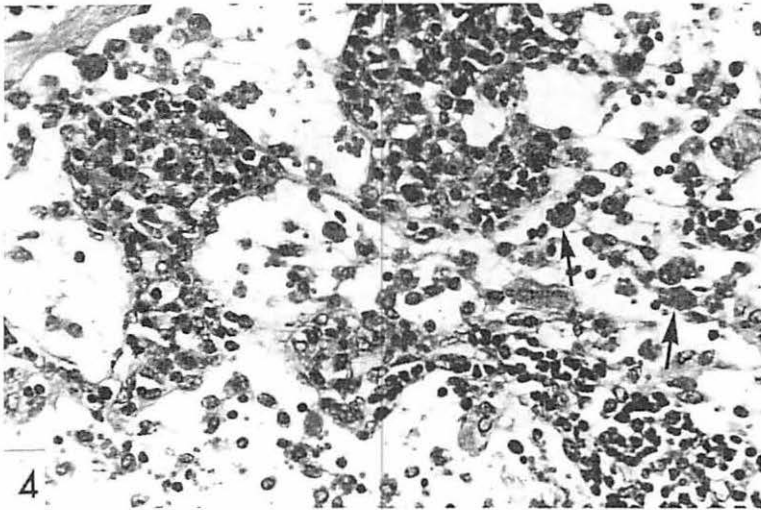


Fig. 4. A section of a popliteal lymph node showing several large macrophages (arrowed) engorged with red blood cells. Haematoxylin and eosin. 325X.

6 hr after their infusion into the popliteal node by way of an afferent lymphatic duct. In the present experiments, a comparison between the numbers of RBC in efferent and afferent lymph of the popliteal node following a peripheral injection of isologous RBC (Fig. 1) is also suggestive that the popliteal node of the conscious sheep does not remove large numbers of isologous RBC from afferent lymph. However, the increases in total bilirubin following a rise in the number of RBC reaching the node indicates that the reticuloendothelial cells within the node are capable of removing and catabolising some of RBC in afferent lymph.

It is suggested that the high concentration of bilirubin present in post-nodal lymph of the sheep is derived from the catabolism of senescent RBC within the node. The presence of whole RBC and of iron and bilirubin stained granules within the cytoplasm of large cells surrounding the lymph sinuses would tend to support this hypothesis. The senescent RBC may reach the reticuloendothelial cells of the node via the afferent lymph, or from capillary leakage within the node. In this connection, the finding that the number of RBC in ef-



ferent popliteal lymph was significantly higher than in afferent popliteal lymph (Table 3) would point to a significant addition of RBC from the blood stream at the level of the local lymph node in the sheep. The significant correlation between the concentration of RBC and total bilirubin in efferent popliteal lymph, together with the increase in total bilirubin in efferent popliteal lymph following an induced increase in the number of RBC reaching the node, are evidence that the total bilirubin concentration in post-nodal lymph may be related to the number of RBC reaching the node.

It is of interest that the bilirubin levels in post-nodal lymph of the sheep are higher than those reported for thoracic duct lymph in humans (31) and cats (32). However, in common with the present results, both of these papers reported lymph:plasma ratios for bilirubin significantly higher than would be expected for albumin, suggesting that the bilirubin in lymph in these species also could not be accounted for by capillary filtration alone.

The metabolic pathways for the conversion of haeme to bilirubin in reticuloendothelial cells have been reviewed by *Fleishner and Arias* (33) and *Lathe* (3). In the present study the bilirubin formed from RBC catabolism in the lymph node has been shown to be bound to plasma albumin and the complex released into the post-nodal lymph. It is apparent that bilirubin reaching the plasma is quickly and irreversibly removed from the plasma since the half life of  $^{14}\text{C}$ -labelled bilirubin-albumin complex in sheep is about 6.4 minutes (34). This complex is removed from the plasma by the liver (35). Within the liver the bilirubin is dissociated from albumin (36, 37), transported to its sites of metabolism by cytoplasmic proteins (38, 39), conjugated with various sugars, principally glucuronic acid, and excreted in the bile (40). This system allows for the rapid removal of bilirubin from blood plasma and would thus account for the low levels of total bilirubin observed in sheep plasma, despite the continual addition of bilirubin to the plasma pool by post-nodal lymph.

#### Acknowledgement

We wish to thank Dr H.R.P. Miller for his guidance and assistance with some of the surgical procedures, Mr. S.D. Tudey for preparing and running the T.L.C. plates and Professor F.C. Courtice for his helpful comments on the preparation of the manuscript.

#### References

- 1 *London, I.M., R. West, D. Shemin, D. Rittenberg*: On the origin of bile pigment in normal man. *J.biol.Chem.* 184 (1950) 351
- 2 *Gray, C.H., A. Neuberger, P.H.A. Sneath*: Studies in congenital porphyria: incorporation of  $^{15}\text{N}$  in the stercobilin in the normal and in the porphyric. *Biochem.J.* 47 (1950) 87
- 3 *Lathe, G.H.*: The degradation of haem by mammals and its excretion as conjugated bilirubin. *Essays Biochem.* 8 (1972) 107
- 4 *Harris, I.M., J.M. McAlister, T.A.J. Prankerd*: The relationship of abnormal red cells to the normal spleen. *Clin.Sci.* 16 (1957) 223
- 5 *Jandl, J.H., A.R. Jones, W.B. Castle*: The destruction of red cells by antibodies in man. I. Observations on the sequestration and lysis of red cells altered by immune mechanisms. *J.clin.Invest.* 36 (1957) 1428
- 6 *Jandl, J.H., M.E. Kaplan*: The destruction of red cells by antibodies in man. *J.clin.Invest.* 39 (1960) 1145
- 7 *Kaplan, M.E., J.H. Jandl*: Inhibition of red cell sequestration by cortisone. *J.exp.Med.* 114 (1961) 921
- 8 *Jandl, J.H., N.M. Files, S.B. Barnett, R.A. MacDonald*: Proliferative response of the spleen and liver to hemolysis. *J.exp.Med.* 122 (1965) 299
- 9 *Murray, R.K., G.E. Connell, J.M. Pert*: The role of haptoglobin in the clearance and distribution of extracorporeal hemoglobin. *Blood* 17 (1961) 45
- 10 *Drinker, C.K., M.E. Field, M.K. Ward*: The filtering capacity of lymph nodes. *J.exp.Med.* 59 (1934) 393
- 11 *Widdicombe, J.G., R. Hughes, A.J. May*: The efficiency of filtration by the popliteal lymph node of the rabbit. *Brit.J.exp.Path.* 36 (1955) 473
- 12 *Fisher, B., E.R. Fisher*: Barrier function of lymph node to tumor cells and erythrocytes. *Cancer* 20 (1967) 1907
- 13 *Courtice, F.C., J. Harding, A.W. Steinbeck*: The removal of free red blood cells from the peritoneal cavity of animals. *Aust.J.exp.Biol.Med.Sci.* 31 (1953) 215

Permission granted for single print for individual use.

Reproduction not permitted without permission of Journal LYMPHOLOGY.

- 14 Hall, J.G., B. Morris: The output of cells in lymph from the popliteal node of sheep. *Q.Jl exp. Physiol.* 47 (1962) 360
- 15 Lascelles, A.K., B. Morris: Surgical techniques for the collection of lymph from unanaesthetised sheep. *Q. Jl exp.Physiol.* 46 (1961) 199
- 16 Hall, J.G., B. Morris: The lymph-borne cells of the immune response. *Q. Jl exp.Physiol.* 48 (1963) 235
- 17 Pedersen, N.C., B. Morris: The role of the lymphatic system in the rejection of homografts: a study of lymph from renal transplants. *J.exp.Med.* 131 (1970) 936
- 18 McIntosh, G.H.: Ph.D. Thesis, Lymphatics of the urogenital system of the sheep. The Australian National University, Canberra 1969
- 19 Bollman, J.L., J.C. Cain, J.H. Grindlay: Techniques for collection of lymph from the liver, small intestine or thoracic duct of the rat. *J.Lab.clin.Med.* 33 (1948) 1349
- 20 Brodersen, R., J. Jacobsen: Separation and determination of bile pigments. *Meth.biochem. Analysis* 17 (1969) 31
- 21 Michaelsson, M.: Bilirubin determination in serum and urine. *Scand.J.clin.Lab.Invest.* 13 (1961) Suppl. 56
- 22 Henry, R., F. Ibbot, A. Mather, W. Faulkner, S. Meites: Recommendation on a uniform bilirubin standard. *Clin.Chem.* 8 (1962) 405
- 23 Thompson, B.P.M., A.F. Hoffman: Separation of bilirubin and its conjugates by thin layer chromatography. *Clinica chim. Acta* 35 (1971) 517
- 24 Mancini, G., A.O. Carbonara, J.F. Heremans: Immunochemical quantitation of antigens by single radial immunodiffusion. *Immunochemistry* 2 (1965) 235
- 25 Alsever, J.B., R.B. Ainslie: A new method for the preparation of dilute blood plasma and the operation of a complete transfusion service. *N.Y. St.J.Med. (State)* 41 (1941) 126
- 26 Bunting, M.: The histochemical detection of iron in tissues. *Stain.Technol.* 24 (1949) 109
- 27 Kuteitz, I.E.: A histochemical method for bilirubin and biliverdin. *Acta histochem.* 4 (1957) 145
- 28 Van den Berg, A.A.H., P. Muller: A method for direct and indirect diazo-reaction for bilirubin. *Biochem.Z.* 77 (1916) 90. Cited in: *The Extra Pharmacopoeia* (1955), vol. II, ed. Capper, K.R. and S.C. Jolly, London: Pharmaceutical Press
- 29 Blauer, G., D. Harmatz, J. Snir: Optical properties of bilirubin-serum albumin complexes in aqueous solution. *Biochim.biophys.Acta* 278 (1972) 68
- 30 Yoffey, J.M., C.K. Drinker: The cell content of peripheral lymph and its bearing on the problem of the circulation of the lymphocyte. *Anat.Rec.* 73 (1939) 417
- 31 Werner, B.: The biochemical composition of human thoracic duct lymph. *Acta Chir.Scand.* 132 (1966) 63
- 32 Carlsten, A., Y. Englund, O. Thulesius: Bilirubin, alkaline phosphatase and transaminases in blood and lymph during biliary obstruction in the cat. *Acta physiol.scand.* 53 (1961) 58
- 33 Fleishner, G., I.M. Arias: Recent advances in bilirubin formation, transport, metabolism and excretion. *Am.J.Med.* 49 (1970) 576
- 34 Mia, A.S., R.R. Gronwall, C.E. Cornelius: Unconjugated and conjugated bilirubin transport in normal and mutant Corriedale sheep with Dubin-Johnson syndrome. *Proc.Soc.exp.Biol.Med.* 135 (1970) 33
- 35 Mann, F.C., J.L. Bollman, T.B. Magatu: Studies on the physiology of the liver. IX. The formation of bile pigment after total removal of liver. *Am.J. Physiol.* 69 (1924) 393
- 36 Brown, W.R., G.M. Grodsky, J.V. Carbone: Intracellular distribution of tritiated bilirubin during hepatic uptake and excretion. *Am.J.Physiol.* 207 (1964) 1237
- 37 Bernstein, L.H., J. Ben Ezzer, L. Gartner, J.M. Arias: Hepatic intracellular distribution of tritium labelled unconjugated and conjugated bilirubin in normal and Gunn rats. *J.Clin.Invest.* 45 (1966) 1194
- 38 Levi, A.J., Z. Gatmaitan, I.M. Arias: Two hepatic cytoplasmic protein fractions Y and Z and their possible role in hepatic uptake of bilirubin, sulfobromophthalein and other anions. *J.Clin.Invest.* 48 (1969) 2156
- 39 Litwak, G., B. Ketterer, I.M. Arias: Ligandin: an abundant liver protein which binds steroids, bilirubin, carcinogens and a number of exogenous anions. *Nature (London)* 234 (1971) 466
- 40 Heirwergh, K.P.M., J.A.T.P. Meuwissen, J. Fevery: Critique of the assay and significance of bilirubin conjugation. *Adv.Clin.Chem.* 16 (1973) 239

*J.W. Quin, Department of Experimental Pathology, The John Curtin School of Medical Research, The Australian National University, P.O. Box 334, Canberra City, A.C.T., 2601, Australia*