THE DIAGNOSTIC IMPORTANCE OF ABNORMAL URINARY SEDIMENT STAINS IN CANINE RENAL HOMOGRAFTS

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The importance of urinary sediment in the diagnosis of renal disease was recognized first in 1846 by Golding Bird.[1] As urine descends from the glomerular membrane, during its passage down the nephron through collecting tubules and thence along the macroscopical ducts, its composition is altered and its environment changes. While functions of the passage walls, especially of the renal tubular cells, alter its composition, the urine in turn affects the epithelial membrane containing it. The analogy of the study of river water as it flows to the sea is pertinent and many references may be made concerning the mountains in which the stream originates.[2] The urine and its sediment likewise reflect their origin although the changes are more complex, the bed of the urinary stream being composed of living cells. It is these cells that are damaged in the rejection of renal homotransplants and by the reflection of this process in the urine, it is hoped that its onset may be diagnosed.

MATERIAL AND METHODS

Using a standard technique,[3,4] renal transplants were performed in 3 groups of dogs:

1. Autotransplants (6 dogs)
2. Homotransplants without immuno-suppression (14 dogs)
3. Homotransplants with immuno-suppression[5] (10 dogs).

All animals were investigated extensively both pre- and post-operatively with a view to gauging: (a) the advent of threatened rejection, and (b) the cytotoxic effect of the immuno-suppressive drugs on the bone marrow.

The study of stained urinary sediment was used prospectively as a diagnostic index of threatened rejection. Urinary collection and investigation was commenced 3 days before surgery and was continued postoperatively until the animal either died or was sacrificed.

At 12-hourly intervals a fresh specimen of 5 - 10 ml. of urine is collected from the cutaneous ureterostomy. Care is taken to ensure that no contamination from surrounding hair or skin occurs and this is facilitated by a previously carefully constructed muco-cutaneous junction. This specimen is centrifuged for 2 minutes at 1,300 r.p.m. and all but approximately 1 ml. of the supernatent clear urine is discarded. By gentle agitation the sediment is re-suspended, a drop of this placed on a slide and as thin a smear as possible is made. This smear is allowed to dry.

Three different stains were evaluated:

1. 3 parts gentian violet mixed with 97 parts Safranin,[6]
2. a combined Wright's/Giemsa stain,[7] and
3. standard Leishman's stain.

Leishman's Stain

This was the most commonly employed and the method of staining a simplification of that first introduced by Romanowsky,[8] as follows:

The dry film is well covered with the stain. After 1 minute double the quantity of distilled water is added and thoroughly mixed. After 7 minutes this mixture is decanted and the film covered with distilled water for another 2 minutes. This water is washed off with fresh distilled water and the smear blotted dry.

Examination of the smears is performed both under low-power and high-power magnification. The following are the points which should be noted:

1. Presence or absence of lymphocytes.
2. The number of these cells relative to other types of white and red blood cells.
3. Rough estimate of actual numbers of lymphocytes.
4. Whether the lymphocytes are free, clumped or in casts.
5. Haematuria.
6. Polymorphs—index of infection.
7. Tubular casts, ureteric or tubular epithelial cells, hyaline casts, haemoglobin casts, etc.

At least 15 minutes are spent in the examination of each smear.

RESULTS

The lymphocytes, plasma cells and monocytes—although abnormal constituents of urinary sediment—were all of normal size, shape and appearance.

At the time of threatened rejection, lymphocytes appeared clumped (Figs. 1, 2), singly (Figs. 3, 4), and occasionally within the lumen of tubular casts (Fig. 5).[7,9,10] By far the commonest and certainly the most striking of these presentations was multiple clumps of lymphocytes (Fig. 6), easily recognized both under low and high-power magnification. The so-called 'lymphocyte-casts' were found only after prolonged searching and in fact were not evident in all smears.

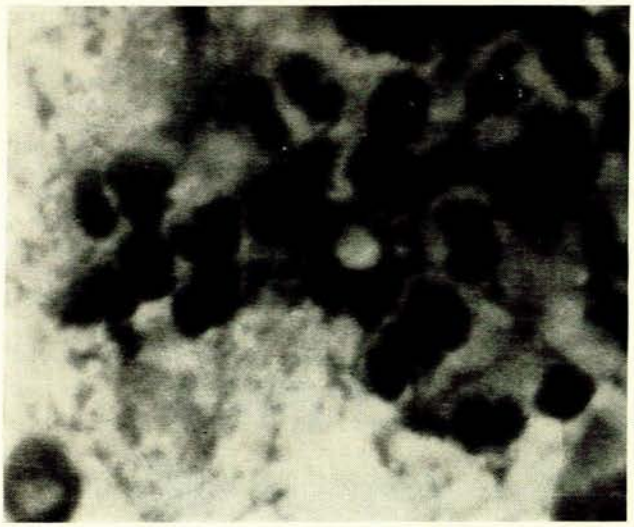

Fig. 1

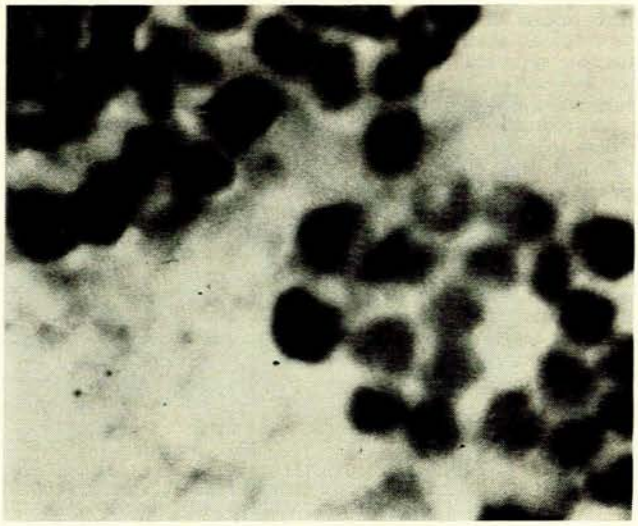

Fig. 2

Figs. 1, 2. Single clumps of lymphocytes.

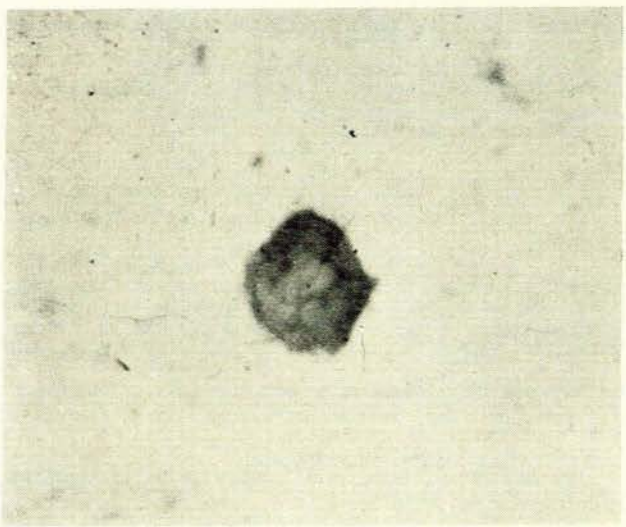

Fig. 3. Large mononuclear cell.

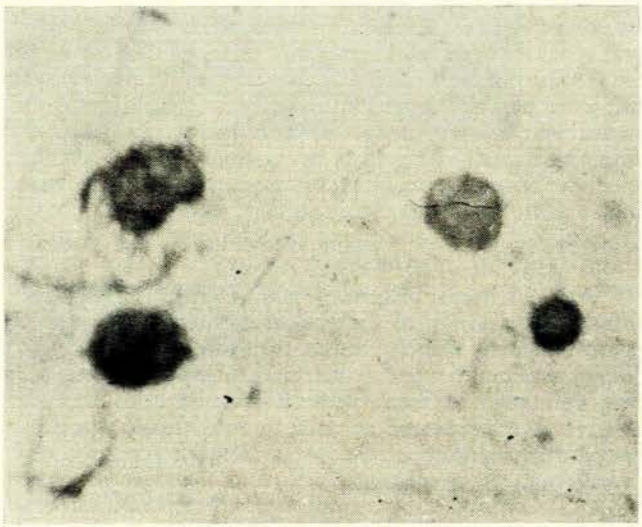

Fig. 4. Solitary lymphocyte.

In the group of autotransplant animals there was at no stage any abnormality of urinary sediment other than for the occasional ureteric epithelial cells. The results therefore relate to homograft animals—either treated or untreated—and these results are translated into different stages before and after transplantation.

(a) *Pre-operatively.* Examination was done to exclude infection and, in addition, provided an adequate baseline for the postoperative findings.

(b) *Postoperatively, but before rejection.* The findings were dependent to some extent on certain variables of surgical technique—trauma to the kidney, total ischaemic time, ureteric trauma, vascular narrowing. Ureteric epithelial cells and red blood cells were invariably seen, the latter taking origin largely from the ureteric artery and disappearing therefore within 12 - 24 hours after operation. Renal or ureteric trauma and narrowing of the venous anastomosis accounted both for increased numbers of red cells and also for their prolonged appearance. With more severe degrees of technical error, tubular and other casts were seen together with epithelial cells of both tubular and glomerular origin.

(c) *Postoperatively with threatened rejection.* Threatened rejection was heralded by the sudden appearance of lymphocytes, as described above. In addition to their varied presentation, other immunologically competent cells were also seen: large lymphocytes, plasma cells and monocytes. Microscopic haematuria was a constant accompaniment. After some hours, tubular and other casts became evident, at first in small numbers but later, as the rejection process became more florid, there were more—indicative of organic renal damage.

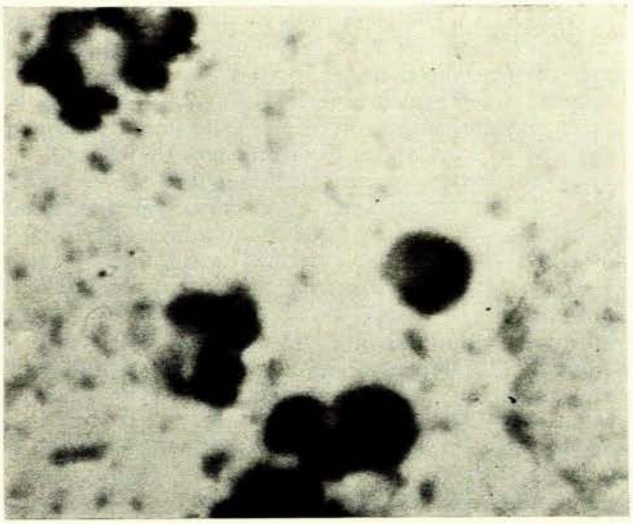

Fig. 5. Solitary lymphocyte together with a lymphocyte cast.

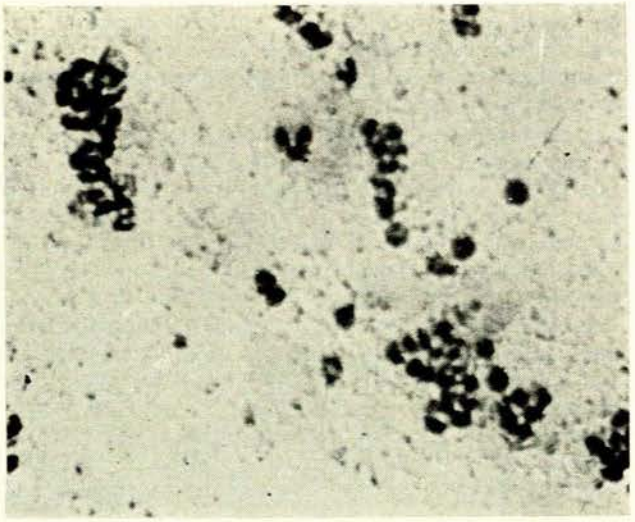

Fig. 6. Multiple clumps of lymphocytes.

A quantitative difference existed between treated and untreated dogs with regard to the numbers of these round cells. Also the progressive urinary signs of renal damage were abrogated by the administration of cytotoxic drugs. In these treated dogs, therefore, both lymphocyturia and the attendant features of renal damage disappeared. A variable period of time was necessary for this to occur—the so-called 'reversal of threatened rejection'.

Untreated animals showed a steady progression of abnormal sediment findings, culminating eventually in massive numbers of lymphocytes and all the various types of casts and epithelial cells before complete anuria ensued. As long as urine was secreted, therefore, lymphocytes persisted—continued rejection.

In some of the treated animals lymphocyturia persisted until immuno-suppressive drugs were again administered in anti-rejection dosages. It is difficult to assess whether this persistence was due to a second episode of rejection, or to inadequate treatment of the primary episode. In these cases the urinary sediment did eventually revert to normal.

(d) *After reversal of threatened rejection.* The sediment was essentially cell-free provided there was no infection present.

(e) *Second episode of threatened rejection.* An exactly similar train of events to that described for the primary rejection episode occurred.

Conclusions

In that clumped lymphocytes appeared most commonly, this provided a simple and sure method of diagnosing rejection, in the presence either of haematuria or of infection. In both, lymphocytes were present but always singly and never in any great number. As in peripheral blood smears with this staining technique, round cells were easily differentiated from polymorphs and red blood cells.

DISCUSSION

Interpretation of urinary sediment smears did not present any difficulty and on each occasion staining and reading were performed by the same individual.[10] As already discussed, a variety of different staining techniques were evaluated. Our eventual method (using Leishman's stain) proved not only simple but also highly efficient.

The basic essentials of any stain should be that (i) lymphocytes are recognized easily, and (ii) other formed elements should not stain similarly—lymphocytes should be differentiated easily.

With this procedure both these criteria were fulfilled with the result that the claim of 100% accurate diagnosis is felt to be justifiable. In addition, we were able to confirm the presence of lymphocytes in the urine of humans with acute glomerulonephritis. Their presence is also noted in disseminated lupus erythematosus.[7]

In homotransplant surgery it is important to diagnose rejection early, since not only is its pathological nature progressive[11] but it is also reversible.[12] The earlier adequate suppressive measures can be instituted, the better must be the ultimate prognosis for that kidney as a result of the prevention of further renal damage. The diagnostic criteria (Table I) are based on subjective and objective clinical signs, together with both routine and highly specialized laboratory investigative procedures.

TABLE I. DIAGNOSTIC CRITERIA OF RENAL HOMOGRAFT REJECTION

1. Abnormal urinary sediment stain.
2. Decreased total urine output.
3. Unexplained temperature elevation.
4. Increase in size of homograft with pronounced tenderness.
5. Increase in proteinuria.
6. Rise in blood urea.
7. Decrease in renal clearance values.
8. Positive urinary catalase.
9. Increased total white cell count with marked polymorph preponderance.

Lymphocyturia

The presence of lymphocyturia is diagnostic of threatened rejection, this being confirmed in each group of animals for the following reasons.

1. Lymphocyturia was never seen in autotransplants. From this it can be deduced firstly that these cells are not

the result of surgical trauma in transplantation and, secondly, that their appearance is dependent on the kidney being a homograft and must therefore be the consequence of an immunity response.

2. In untreated homotransplants, lymphocytes appeared at the expected time of threatened rejection (2 - 4 days), and 24 - 48 hours later full-blown rejection was present with its attendant gross impairment of renal function. Lymphocyturia appeared uniformly, therefore at a stage when repudiation could be described as threatened, before any serious dysfunction or irreversible damage.

3. In the treated animals, immuno-suppressive drugs were administered on the diagnosis of threatened rejection and in all of these dogs rejection was reversed. With the disappearance of the other signs of threatened rejection, lymphocytes in the urine also diminished and eventually disappeared.[7] In addition, a second episode of threatened rejection was heralded by the reappearance of these same cells in the urine.

The diagnostic value of lymphocyturia is far from undisputed. There is considerable controversy among workers in this field.[13] However, the majority recognize it as both a dependable and an early sign of threatened rejection.

Local Complications

Among the local complications which may affect the graft are:[14]

1. Vascular occlusion of either artery or vein,
2. Obstructive uropathy,
3. Infection,
4. Acute tubular necrosis based on the total ischaemic time,
5. Haemorrhage from the ureter, and
6. In human transplant surgery the graft may acquire the fundamental renal disease.[13]

In any of these, impairment of renal function will result. In none, however, will lymphocytes alone appear in the sediment. The corollary is even more important: with impaired renal function in the presence of lymphocyturia, rejection must be presumed.

Observations

Although no wholly satisfactory theory can be propounded for the mechanism by which these cells appear in the urine, some interesting observations may be made:

(*a*) These are the immunologically competent cells responsible for this cellular type of immunity.[15,16] It is hardly surprising, therefore, that they make their appearance at the time of threatened rejection.

(*b*) Pathologically, such a round cell infiltrate coincides with the flushing of lymphocytes into the urine. Initially present in the periglomerular, peri-arteriolar and to a lesser extent in the peritubular areas of the kidney, they are eventually found throughout the interstitium.[17] By means of radioactive labelling it has been shown that their cell population is of both host and graft origin, the greater number arising in the lymphoid tissues of the host.[18,19]

Exactly how these cells gain access to the tubules is not understood. Renal biopsies confirm their presence within the tubular lumen, but whether their entry is from the interstitium via the glomerulus or directly from the capillary loops has never been shown.

(*c*) When rejection has been reversed the abnormal cellular elements of the urine disappear, implying both their immunological competency and their precise and finely-balanced relationship to threatened rejection.

(*d*) Somewhat surprisingly, the first appearance of lymphocyturia is attended by a marked peripheral-polymorph response.[4,10] From this it is hardly feasible that the urinary findings can be explained on the basis of simple filtration of these cells from the glomerular tufts. An origin from the interstitium or budding of vascular endothelium would appear more likely.

In stressing the value of routine examination of stained preparations of urinary sediment, it must nevertheless be emphasized that the final diagnosis of graft repudiation is never made on this one solitary positive finding.[7,20] This latter statement is universally accepted. In this series of experiments lymphocyturia was invariably accompanied by other confirmatory signs, thus making graft rejection a certainty. None of these other positive features were sufficiently reliable to be diagnostic in their own right, being misleading in the face either of infection, haematuria or both. In all probability, utilizing finer parameters of renal function such as more elaborate enzyme assays[21,22] and radioactive renograms,[23,24] and even more definite diagnosis of rejection may be possible. We do not feel at this stage, however, that any of these will diagnose threatened rejection at an earlier stage than urinary sediment.

We should like to thank Prof. J. H. Louw, of the Department of Surgery, University of Cape Town, for continued encouragement. Grateful thanks are also due to Drs. A. J. G. Fisher and R. Maartens, Messrs. L. Aitken and C. C. Goosen and Mrs. I. du Toit, for technical advice and material assistance, and to Mr. G. McManus for photography. For financial support we are indebted to the Staff Research Fund and the J. S. Marais Bequest, University of Cape Town.

REFERENCES

1. Bird, G. (1846): *Urinary Deposits*, 2nd ed., p. 263. London: Churchill.
2. Lippman, R. W. (1962): *Urine and the Urinary Sediment*, 2nd ed. Springfield, Ill.: Charles C. Thomas.
3. Pierce, J. C. and Varco, R. L. (1964): J. Surg. Res., **4**, 275.
4. Ackermann, J. R. W., Terblanche, J., De Villiers, D. R. and Barnard, C. N. (1965): S. Afr. Med. J., **39**, 295.
5. Varco, R. L. (1964): Personal communication.
6. Sternheimer, R. and Malbin, B. (1951): Amer. J. Med., **11**, 312.
7. Kauffman, H. M., Clark, R. F., Magee, J. H., Rittenbury, M. S., Goldsmith, C. M., Prout, G. R. and Hume, D. M. (1964): Surg. Gynec. Obstet., **119**, 25.
8. Hunter, D. and Bomford, R. R. (1956): *Hutchison's Clinical Methods*, 13th ed. London: Cassell.
9. Goodwin, W. E., Kaufman, J. J., Mims, M. M., Turner, R. D., Glassock, R., Goldman, R. and Maxwell, M. M. (1963): J. Urol. (Baltimore), **89**, 13.
10. Calne, R. Y. (1964): Brit. J. Surg., **51**, 282.
11. Woodruff, M. F. A. (1960): *Transplantation of Tissues and Organs*. Springfield, Ill.: Charles C. Thomas.
12. Calne, R. Y. and Murray, J. E. (1961): Surg. Forum, **12**, 118.
13. Human Kidney Transplant Conference (1964): Transplant. Bull., **2**, 147.
14. Starzl, T. E., Marchioro, T. L., Brittain, R. S., Holmes, J. H. and Waddell, W. R. (1964): J. Amer. Med. Assoc., **187**, 734.
15. Simonsen, M. (1960): In *Ciba Foundation: Cellular Aspects of Immunity*, p. 122. London: Churchill.
16. Medawar, P. B. (1960): *Ibid.*, p. 134.
17. Calne, R. Y. and Porter, K. A. (1964): Brit. J. Surg., **51**, 469.
18. Dempster, W. J. and Williams, M. A. (1963): Brit. Med. J., **1**, 18.
19. Yoshii, T., Jolley, W. B. and Hinshaw, D. B. (1963): Surg. Forum, **14**, 212.
20. Murray, J. E. and Harrison, J. H. (1963): Amer. J. Surg., **105**, 205.
21. Prout, G. R., Macalalag, E. V. and Hume, D. M. (1964): Surgery, **56**, 283.
22. Wacker, W. E. C. and Dorfman, L. E. (1962): J. Amer. Med. Assoc., **181**, 972.
23. Collins, J. J., Pizak, L. F., Tamvakopoulos, S. K. and Wilson, R. E. (1963): Surg. Forum, **14**, 217.
24. Reemtsma, K., McCracken, B. H., Schlegel, J. U. and Pearl, M. A. (1964): J. Amer. Med. Assoc., **187**, 691.