

responsible for the development of both proliferations since the occurrence of myelomonocytic leukaemia has been noted by Warner *et al.* (1969) in BALB/c mice after intraperitoneal injection of mineral oil, a stimulus usually leading to plasma-cell tumours. Osserman (1967) stressed the possibility that the chronic stimulation of the reticuloendothelial system may lead to the development of both monocytic and plasmacytic dyscrasias. Though many cases of acute leukaemia supervening on M.M. were labelled as "myelomonocytic" leukaemia, it is worth noting that though lysozyme levels were not studied our two patients apparently had true A.M.L.

Khaleeli *et al.* (1973) recently described four patients with M.M. who developed sideroblastic anaemia several months before the occurrence of acute leukaemia. It is interesting, therefore, to note that a pancytopenia was found in both our patients three to four years before diagnosis. In two other patients with M.M. we found an unusual myeloid hyperplasia with splenomegaly and 30,000-50,000 white blood cells with 90-95% of mature neutrophils. In one of these patients acute leukaemia developed four years later after treatment with melphalan.

The relation between M.M. and A.M.L., which involve distinct cell lines, remains puzzling. Long-term chemotherapy may have only a triggering role in patients with M.M. and some kind of pre-leukaemia state.

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References

- Khaleeli, M., Keane, W. M., and Lee, G. R. (1973). *Blood*, 41, 17.
 Kyle, R. A., Pierre, R. V., and Bayrd, E. D. (1970). *New England Journal of Medicine*, 283, 1121.
Lancet. (1970). 1, 70.
 Osserman, E. F. (1967). In *Gamma Globulins: Structure and Control of Biosynthesis*, ed. J. Killander, p. 573. Stockholm, Almqvist and Wiksell.
 Thijs, L. G., *et al.* (1970). *British Journal of Haematology*, 19, 485.
 Videbaek, A. (1971). *British Medical Journal*, 2, 326.
 Warner, N. L., Moore, M. A. S., and Metcalf, D. (1969). *Journal of the National Cancer Institute*, 43, 963.

PRELIMINARY COMMUNICATION

T-lymphoblastic Leukaemia: A Distinct Variant of Acute Leukaemia

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Summary

Two cases of acute lymphoblastic leukaemia (A.L.L.) of T-cell type are reported. Clinically they were characterized by very high peripheral blood blast cell counts at presentation, the early development of meningeal leukaemia, and relative resistance to treatment with combination chemotherapy. Leukaemic cells from both patients lacked all the B-cell markers investigated, but 60-65% of their cells formed rosettes with sheep red cells. Cytochemical and surface structure studies helped to define additional features of these cells and confirmed their T-cell nature.

It seems that this variant of A.L.L. may not be uncommon and that it can be distinguished on clinical and immunological grounds from the usual type of A.L.L. which runs a less aggressive course and lacks B- or T-cell markers.

Introduction

The improvement in rate of remission and survival in acute ("childhood") lymphoblastic leukaemia (A.L.L.) (Spiers, 1972 a)

is the major factor that has led to a more optimistic approach to A.L.L., which is still considered to be a homogeneous disease. Certain "bad-risk" cases are now recognized, however. For example, patients who have high blast cell counts, organomegaly, or marked thrombocytopenia at presentation fare relatively badly and also have a higher risk of developing meningeal leukaemia (Pavlovsky *et al.*, 1973). It has been suggested that patients with large blast cells (macroblasts) may obtain little benefit from immunotherapy (Mathé *et al.*, 1971), but morphological features are highly subjective and agreement between different centres is poor. A classification based on more objective criteria would be extremely valuable if specific cellular features could be shown to correlate with prognosis. In recent years markers for lymphoid cell differentiation have been used in the study of lymphoproliferative disorders. Though most cells from patients with A.L.L. seem to lack the surface markers for B and T lymphocytes (Lay *et al.*, 1971; Smith *et al.*, 1973 a) evidence is now accumulating that a significant minority may in fact be of T-cell type (Seligmann *et al.*, 1973; Borella and Senn, 1973; Kersey *et al.*, 1973; McCaffrey *et al.*, 1973). Furthermore, it has been suggested that the T-cell form of the disease is more aggressive (Borella and Sen, 1973). We studied two further cases of T-lymphoblastic leukaemia in which its aggressive clinical course, in contrast to the usual type of A.L.L., was clearly shown.

We report here the results of these studies because we believe that the immunological tests described should be applied to all new cases of A.L.L. The recognition of the T-lymphocyte origin of a proportion of cases of A.L.L. seems to have important therapeutic implications, but its true incidence cannot be assessed until a much larger series of patients has been studied.

Case 1

A 14-year-old boy was admitted to Hammersmith Hospital in December 1973 with a two-week history of spontaneous bruising. He had a petechial rash distributed symmetrically over the anterior aspect of the trunk. The spleen was palpable 6 cm below the costal margin, but the liver was not enlarged. There was no clinical or radiological evidence of mediastinal enlargement. The peripheral blood showed a haemoglobin of 10.1 g/100 ml, total leucocytes 1,004,000/ μ l and platelets 52,000/ μ l. The differential count showed 95% blast cells with nuclear chromatin aggregation suggestive of lymphocytic differentiation (fig. 1). The bone marrow

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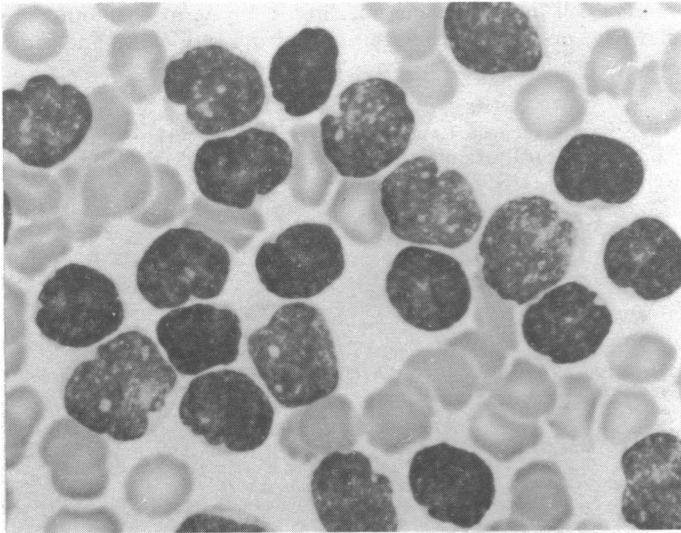


FIG. 1—Case 1. peripheral blood film. (x 1,100.)

was almost totally replaced by small blast cells with one to two nucleoli; some had cleft nuclei. The patient was considered to have A.L.L. and treatment was started with vincristine, prednisolone, and colaspase (L-asparaginase). As his subsequent response was judged to be slow the treatment was changed to doxorubicin, cyclophosphamide, vincristine, and prednisolone. The patient's rash gradually faded and the spleen became impalpable.

After five courses of cytotoxic drugs at weekly intervals the patient was clinically well and his peripheral blood count was normal; 50% of all the bone marrow cells, however, were still lymphoblasts. In February 1974 he complained of blurred vision in the right eye and a lumbar puncture showed 1,400 blasts/ μ l of C.S.F. Methotrexate 10 mg was injected intrathecally on two occasions and he was transferred to the National Hospital for Nervous Diseases where an Ommaya reservoir (Spiers, 1972 a) was inserted into the right lateral cerebral ventricle. He was then treated with cranial irradiation (3,000 rads in 12 fractions over three weeks), and methotrexate administration was continued by injection into the Ommaya reservoir. In March the patient was free of symptoms and his vision was normal. The peripheral blood values were normal and the bone marrow showed active haematopoiesis with only 8% of lymphoblasts.

Case 2

A 30-year-old man was admitted to another hospital in April 1973 with a three-week history of spontaneous bruising. He was pale and had multiple areas of ecchymoses and petechiae distributed over the whole body. There were small firm lymph nodes in both axillae and both inguinal regions. The liver and spleen were not enlarged. The haemoglobin was 13.4 g/100 ml, total leucocytes 170,000/ μ l, and platelets 5,000/ μ l; the differential count showed 93% blasts, 6% neutrophils, and 1% lymphocytes. A bone marrow aspirate showed normal cellularity and almost total replacement by blast cells. A diagnosis of A.L.L. was made.

He received platelet transfusions and was treated with prednisone and vincristine. Within one week blast cells had disappeared from the peripheral blood, and the bone marrow five weeks later showed complete remission. Maintenance treatment was started with cyclical five-day combinations of cytotoxic drugs (Hammer-smith regime; Spiers, 1972 a), but no prophylactic irradiation to the central nervous system was given.

In October he developed headaches, loss of vision in the right eye, and weakness of the muscles of the left side of the face. The C.S.F. was not examined at this time. He was treated with prednisone 60 mg daily with some improvement. In November he was readmitted to hospital with further deterioration of vision in the right eye, left sided tinnitus, headache, and vomiting. The C.S.F. contained 2,000 blasts/ μ l. He was treated with methotrexate 12.5 mg intrathecally and transferred to Hammersmith Hospital. An

Ommaya reservoir was inserted and he was treated for his neuro-leukaemia with methotrexate injected twice weekly into the lateral ventricle, and cranial irradiation was given (3,000 rads in 12 fractions over three weeks). In December a bone marrow specimen showed 15% blasts and he was judged to be in marrow relapse. Systemic treatment was therefore continued with courses of a combination of eight cytotoxic drugs (TRAMPCOL; Spiers, 1972 b). By the end of January 1974 he was feeling well and his vision had returned to normal; his blood and bone marrow were again consistent with complete remission.

He was readmitted to hospital four weeks later acutely ill. There was marked enlargement of his liver and spleen. His haemoglobin was 7.7 g/100 ml, total leucocytes 450,000/ μ l, and platelets 24,000/ μ l. Most of the leucocytes were blast cells which appeared less differentiated than those originally present in the blood and bone marrow. He was treated with cyclophosphamide, doxorubicin, vincristine, and prednisolone with immediate clinical improvement. A third complete remission of his disease has not been obtained.

Methods

Peripheral blood from both patients was studied at the time of the maximal leucocyte counts (1000 x 10³/ μ l in case 1 and 450 x 10³/ μ l in case 2). Blast cells were separated from heparinized blood by simple centrifugation and subjected to the following studies.

Immunological Techniques.—For B-cell markers surface-bound immunoglobulins were shown by direct immunofluorescence by means of antisera against the heavy chains of IgM, IgG, IgA, and IgD (Papamichail *et al.*, 1971). Receptors for Fc and C3 were sought by using ox red cells coated with IgG (EA rosettes; McConnell, unpublished) or with IgM and C3 (EAC rosettes; Pepys and Butterworth, 1974). For T-cell markers the methods used were spontaneous sheep red cell rosette formation (Wybran *et al.*, 1973) and transformation with phytohaemagglutinin (PHA) in a three-day suspension culture.

Cytochemical Stains.—The periodic-acid Schiff (P.A.S.), myeloperoxidase, Sudan black B, and acid phosphatase (Goldberg and Barka, 1962) reactions were studied on freshly made peripheral blood films after adequate fixation.

Scanning Electronmicroscopy.—Cells from the buffy coats were fixed in 1% glutaraldehyde and processed on silver membranes (Polliack *et al.*, 1973) for critical point drying (Anderson, 1951) in carbon dioxide. They were then shadowed with gold palladium to a thickness of 50 nm and examined in the scanning electron microscope (Cambridge S4).

Results

All the investigations for B-cell markers (surface-bound immunoglobulins and Fc and C3 receptors) were negative. The sheep red cell rosette test was positive in both cases; 65% of the blast cells formed rosettes in case 1 and 60% in case 2. They were easily identified in cytocentrifuge preparations (fig. 2). There was no transformation with PHA in the one patient studied (case 1).

The cytochemical reactions for myeloperoxidase and Sudan black B were negative in the blast cells of both cases. The P.A.S. reaction showed fine granules in 30% of the blasts of case 1, and in less than 5% of case 2. In the slides made at the time of presentation in case 2, however, 30% of the leukaemic cells showed large single blocks of P.A.S. positive material as is often seen in A.L.L., and a few cells showed several coarse blocks. The acid phosphatase reaction was moderately to strongly positive in more than 90% of the cells in both cases.

The appearance with the scanning electron microscope (fig. 3) was similar in both cases. The surface morphology showed a spectrum of cells ranging from those with smooth surfaces to those with numerous digitations and microvilli of variable length. Blast cells with numerous digitations were more frequent in case 2 than in case 1. A curious feature in case 1

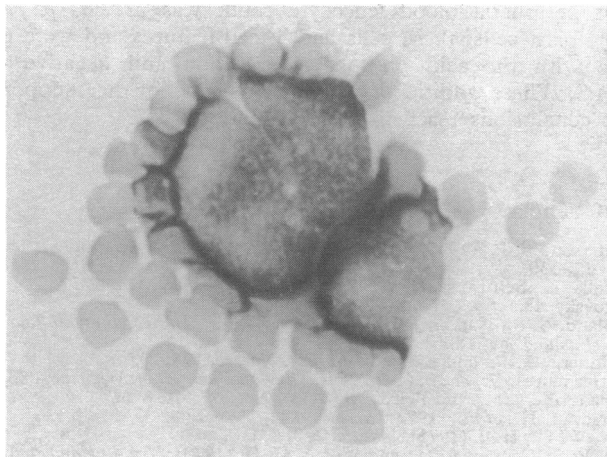


FIG. 2—Case 2. Sheep red cell rosette with two blast cells. (x 1,400.)

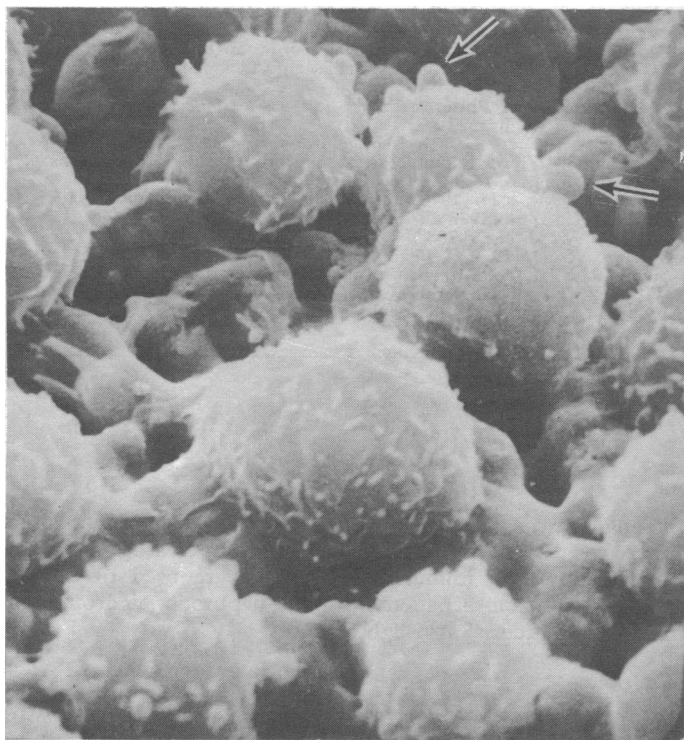


FIG. 3—Case 1. Buffy coat cells seen with scanning electron microscope showing blast cells with either smooth or irregular surface and some with blebs (arrowed). (x 4,500.)

was the presence of surface blebs (see fig. 3). Two to 10 such blebs could be found in some cells, but they were not seen in cells with a smooth surface without digitations.

Discussion

In early studies on blast cells from patients with A.L.L. immunological markers for the identification of B lymphocytes only were used and these gave consistently negative results (Wilson and Nossal, 1971; Frøland *et al.*, 1972; Shevach *et al.*, 1972; Gutterman *et al.*, 1973). Thus, unlike, chronic lymphocytic leukaemia (C.L.L.) A.L.L. seemed not to be a B-cell malignancy. More recently both B and T markers have been used, and a clearer picture of the findings in A.L.L. has emerged (Selig-

mann *et al.*, 1973; Borella and Sen, 1973; Kersey *et al.*, 1973). Seligmann *et al.* reported positive sheep red cell rosettes in leukaemic cells in eight out of 34 cases, Kersey *et al.* reported positive findings in four out of nine cases, but one of the cases in which rosettes did not form gave a positive result with an anti-T antiserum, and Borella and Sen found T features in one of their four cases. Others did not find T-cell markers in small numbers of cases (Lay *et al.*, 1971; Smith *et al.*, 1973 a). It should be noted that thymic involvement was not present in most of these positive cases nor was it present in our two cases, which suggests that they do not represent the leukaemic phase of the so-called "Sternberg sarcoma" (Smith *et al.*, 1973 a).

The clinical implications of these earlier findings were not immediately obvious. Only Borella and Sen (1973) suggested a more aggressive or extensive disease in their patient with T-lymphoblastic leukaemia. Our two cases exemplified dramatically the aggressive nature of this variant of A.L.L. Both presented with extremely high blast cell counts (1000×10^3 and $170 \times 10^3/\mu\text{l}$), and their subsequent course, with early manifestations of meningeal leukaemia and some refractoriness to the intensive combination chemotherapy currently used in A.L.L., confirmed that they corresponded to a "bad-risk" group (Pavlovsky *et al.*, 1973). Interestingly three out of the five T-lymphoblastic patients reported by Kersey *et al.* (1973) had high leucocyte counts, while this was so in only one out of four of their non-T cases. It seems therefore from the 60 cases studied with B- and T-cell markers that T-lymphoblastic leukaemia may represent 20% or more of all cases of A.L.L., and it is associated with clinical and haematological features reflecting a more aggressive course. It has in the past been assumed that the "bad risk" in the "high leucocyte" group of patients was due to the relatively advanced stage at which the disease was diagnosed, but these patients may, in fact, have this distinct variant of A.L.L. Further studies in larger numbers of cases are essential to confirm these findings.

Two characteristics of the cells in our cases deserve further comment: their cytochemical profile and their surface structure. The P.A.S. reaction at the time of our study was positive in the form of small granules in only a few cells, while the acid phosphatase reaction was positive in most. These findings, unlike those commonly seen in other A.L.L. cases, were also observed in one case of T-prolymphocytic leukaemia, while the complete reverse, P.A.S. positivity in most of the leukaemic cells and a weak or negative acid phosphatase reaction, was observed in nine cases of leukaemia of B-cell type (B-prolymphocytic and C.L.L.; Catovsky *et al.*, 1974). These findings, which were not related to the degree of cellular differentiation, suggest basic metabolic differences between B and T lymphocytes which may be helpful in identifying these cells when classifying lymphoproliferative disorders.

Changes in leukaemic cells, as seen under the scanning electron microscope, have not been reported since the work of Polliack *et al.* (1973) on the different surface structures of B and T lymphocytes. Normal B lymphocytes and those from patients with C.L.L. have a complex villous appearance while T lymphocytes have a generally smooth surface (Polliack *et al.*, 1973; Lin *et al.*, 1973) when the critical point drying technique is used (Anderson, 1951). The findings in the T lymphoblasts of our two patients resembled those of T-derived lymphoid cells. Some differences should be noted, however. Firstly, some cells tended to have more microvilli than normal T lymphocytes; this was more obvious in case 2, where some features intermediate between those of normal B and T lymphocytes were seen. Secondly, prominent blebs were present in case 1 and we have not found this structure described in lymphoid cells; their significance remains uncertain. Microvilli on the surface of B and T lymphocytes seem to represent differentiated structures and, in the case of T lymphocytes, appear to be the sole point of contact with the sheep red cell when forming rosettes (Lin *et al.*, 1973). Further studies on the surface structure of blast cells in A.L.L. may help to determine whether the appearance of microvilli correlates with T-cell differentiation.

It is clearly important to examine the blast cells from A.L.L. patients by the methods described to determine the proportion of T-cell cases. The use of specific anti-T sera may be necessary to identify some cases that would be missed by using only the sheep red cell rosette test (Kersey *et al.*, 1973; Smith *et al.*, 1973 b). Other possible methods for establishing the T-cell nature of the cells include the establishment of permanent cell lines (Minowada *et al.*, 1972) and the demonstration of a thymus-specific enzyme (McCaffrey *et al.*, 1973).

Finally, the extension of these studies may give further information on the possible viral aetiology of A.L.L. In intact mice virus-induced leukaemias have been shown to be of T-cell type while those induced by chemical carcinogens are of B-cell type (Haran-Ghera and Peled, 1973). It is thus of great interest to establish whether the blast cells will prove to be of T-cell origin in all patients with A.L.L. or only in the minority as now seems to be the case. In the meantime the features noted in T-lymphoblastic leukaemia deserve further attention for they suggest that both clinically and haematologically it is a distinct variant of A.L.L.

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ADDENDUM

Since our paper was submitted for publication we have had the opportunity to study five further patients with A.L.L. Four of these had neither remarkable physical signs nor very high peripheral blood leucocyte counts at presentation. Their blast cells were negative for B and T cell markers and for acid phosphatase, and positive with the periodic acid Schiff reaction. The fifth patient (referred for the study by Dr. P.

Roberts, West Middlesex Hospital) was an 8-year-old girl who presented with a large spleen and an anterior mediastinal mass. Her peripheral blood leucocyte count was 221,000/ μ l., with 99% blast cells; these cells had T-cell features and were positive with the acid phosphatase reaction and negative with P.A.S. These additional observations give further support to the conclusions reached in our paper.

References

- Anderson, T. F., (1951). *Transactions of the New York Academy of Sciences*, 13, 130.
- Borella, L., Sen Luisa, (1973). *Journal of Immunology*, 111, 1257.
- Catovsky, D., *et al.*, (1974). To be published.
- Frøland, S. S., Natvig, J. B., and Stavem, P. (1972). *Scandinavian Journal of Immunology*, 1, 351.
- Goldberg, A. F., and Barka, T. (1962). *Nature*, 195, 297.
- Gutterman, J. V., *et al.*, (1973). *New England Journal of Medicine*, 288, 169.
- Haran-Ghera, N., and Peled, A. (1973). *Nature*, 241, 396.
- Kersey, J. H., *et al.*, (1973). *Science*, 182, 1355.
- Lay, W. H., *et al.* (1971). *Nature*, 230, 531.
- Lin, P. S., Cooper, A. G., and Wortis, H. H. (1973). *New England Journal of Medicine*, 289, 548.
- Mathé, G., *et al.* (1971). *Revue Européenne D'Etudes Cliniques et Biologiques*, 16, 554.
- McCaffrey, R., Smoler, D. F., and Baltimore, D. (1973). *Workshop on Prognostic Factors in Human Acute Leukaemia*, Schloss Reisensburg, Ulm.
- McConnell, I. Unpublished
- Minowada, T., Ohnuma, T., and Moore, G. E., (1972). *Journal of the National Cancer Institute*, 49, 891.
- Papamichail, M., Brown, J. C., and Holborow, E. J. (1971). *Lancet*, 2, 850.
- Pavlovsky, S., Eppinger-Helft, Mariana, and Sackmann Muriel, F. (1973). *Blood*, 42, 935.
- Pepys, M. B., and Butterworth, A. E. (1974). *Clinical and Experimental Immunology*, In press.
- Polliack, A., *et al.*, (1973). *Journal of Experimental Medicine*, 138, 607.
- Seligmann, M., Preud'homme, J. L., and Brouet, J. C. (1973). *Transplantation Reviews*, 16, 85.
- Shevach, E. M., *et al.* (1972). *Journal of Clinical Investigation*, 51, 1933.
- Smith, J. L., *et al.* (1973 a). *Lancet*, 1, 74.
- Smith, R. W., *et al.* (1973 b). *Journal of Immunology*, 110, 884.
- Spiers, A. S. D. (1972 a). *Lancet*, 2, 473.
- Spiers, A. S. D. (1972 b). *British Journal of Haematology*, 23, 262.
- Wilson, J. D., and Nossal, G. J. V. (1971). *Lancet*, 2, 788.
- Wybran, J., Chantler, S., Fudenberg, H. H. (1973). *Lancet*, 1, 126.

MEDICAL MEMORANDA

Membranous Nephropathy due to Skin-lightening Cream

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The high incidence of nephrotic syndrome in some African countries is thought to be due to its association with *Plasmodium malariae* infection which produces an immune-complex glomerulonephritis (Gilles and Hendrickse, 1963; Kibukamusoke *et al.*, 1967). This is not the case in Nairobi (Rees *et al.*, 1972) but there does seem to be an association between the nephrotic syndrome and the use, by more sophisticated

young African women, of skin-lightening creams containing mercury (Barr *et al.*, 1972). Light microscopy, electron microscopy, and immunofluorescence were used to examine renal tissue from a patient with the nephrotic syndrome who had used mercurial skin-lighteners.

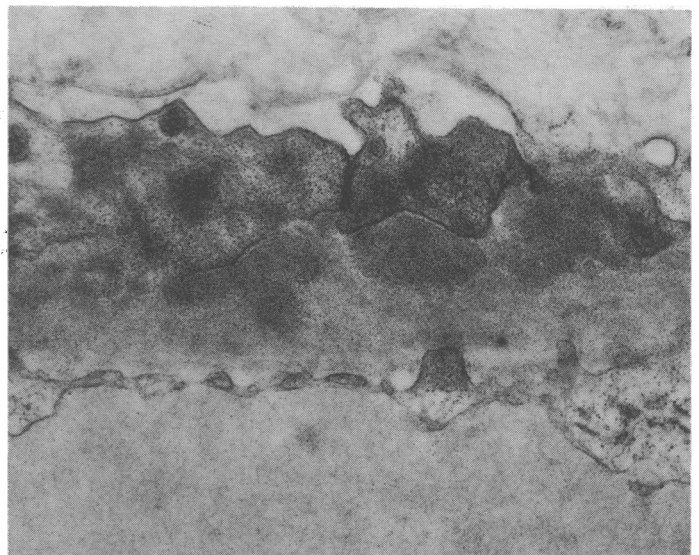


FIG. 1—Electron-dense deposits in epimembranous position and fusion of epithelial cell foot processes. (X25,000.)

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