from uninfected mice in a volume of 2.0 ml in 60-mm plastic petri dishes at a ratio of normal to leukemic cells of 100:1. The plates were placed in a tissue culture rotator and incubated for 60 min at 37°C. At the conclusion of the experiment the cells were centrifuged at low speed and washed twice with media and fixed with 1% glutaraldehyde. After 60-min fixation, the cells were allowed to settle onto Flotronic silver membranes (Selas Corp., Springhouse, Pa.) or Nucleopore filters (Nucleopore Corp., Pleasanton, Ca.). The cells were processed for scanning electron microscopy (SEM) as previously described[2,3] and examined with an Etec microscope (Etec Corp., Hayward, Ca.) operating at 20 kV.

Results and discussion. Leukemic cells were readily distinguishable from normal splenocytes by their larger size and smoother surface (figure, A). FLV-infected cells treated with anti-FLV or normal rabbit serum alone showed no evidence of morphologic changes. Incubation of normal spleen cells with tumor cells treated with normal rabbit serum also produced few changes in the appearance of the leukemic cells. Occasionally a tumor cell was seen with 1 or 2 lymphocytes adhering to its surface but no evidence of cytolysis was seen. When tumor cells which were preincubated with antiserum were mixed with normal spleen cells, a significant proportion of large smooth cells were observed with villous-covered lymphocytes adhering to their surface (figure, A). Tumor cells were seen in various stages of degradation (figure, B, C, D). Surface blebbing appeared on cells (figure, B) followed by the appearance of punctate lesions (figure, C) and eventual lysis (figure, D). More than 30% of tumor cells had villous covered lymphocytes attached to them, of these 12% showed signs of membrane damage, either by the production of blebs or pores. In addition, tumor cells with no attached lymphocytes were seen undergoing lysis.

The ability of lymphoid cells from normal individuals, who have not been sensitized to target cell antigens, to lyse those cells which have been incubated with antibodies against surface antigens is called antibody-dependent cell-mediated cytotoxicity (ADCC)[4]. In this report, the ability of lymphocytes from normal Balb/c mice to lyse FLV-transformed cells pre-treated with anti-FLV serum is demonstrated by SEM. The attachment of villous-covered lymphocytes results in tumor cell lysis. Similar changes were described in murine mastocytoma cells undergoing T-cell-mediated lysis[5]. These results indicate that the 'spongy' cells seen during the later stages of FLV infection might be the result of immunologic attack. However, this does not exclude the possibility that cytotoxic antibodies or virus release might produce the same morphologic changes. The effector cells for ADCC in other murine tumors has been designated as a K cell because of the absence of markers characteristic of either B or T sells 6. However, recent work by Lamon et al.[7] has suggested that the majority of splenic effector cells in ADCC are T cells. Such cells have receptors for either IgM or IgG and could bind with antibody-treated tumor cells. In humans, T-cells bearing receptors for IgM or IgG have been differentiated by electron microscopic appearance and surface morphology. The T-cell receptors of IgG (T_g) is more highly villous than the IgM receptor-bearing cells (Tm)[8]. Such differences have not been reported for murine T cells.

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Preparation of lymphocytes from small volumes of peripheral mouse blood[1]

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Summary. A method was developed which allowed for the isolation of lymphocytes (95% of leukocytes in the final suspensions) from small volumes of peripheral mouse blood (38% recovery). The method proved of value for studies of murine lymphocytes which required biochemical analysis of samples from individual animals on a serial basis.

The method described herein has proven of value in this laboratory for the study of lymphocytes in the limited volume of peripheral blood available from individual mice on a serial basis. It was developed by modification of previously reported techniques which require larger samples of blood[2-4]. Note that the term 'recovery', as used in this paper, denotes the relative proportion of cells which were present originally.

Materials and methods. Animals. Female SJL/J mice, 13 to 28 weeks old, were obtained from the closed breeding colony maintained in the Animal Services Center, University of Alabama in Birmingham.

Cell counts. Total leukocyte counts were determined in a Spencer hemacytometer chamber. The diluting fluid employed was 0.5% glacial acetic acid containing crystal violet. Differential leukocyte counts were made by examination of Wright-stained preparations. A minimum of 200 cells was counted in each smear.

Glassware. All glassware was siliconized by coating with Sigmacote (Sigma Chemical Corporation, St. Louis, Mo.).

Separation of lymphocytes. Blood samples (0.4 ml/mouse) were collected by tail-bleeding into heparinized tubes. Each sample was diluted with an equal volume of cold Hanks' balanced salt solution (H-BSS) and layered carefully with a

Characteristics of mouse leukocyte preparations before and after separation of cell types in small volumes of peripheral blood*

	Leukocytes (10^3 cells/mm^3)	Cell types (% total leukocyte count) Lymphocytes	Monocytes	Granulocytes
Before separation	9.3 (5.9–12.0)	85.8 (80.0–91.3)	3.7 (1.3–5.6)	10.5 (6.2–15.5)
After separation**	3.2 (1.9–4.3)	94.9 (92.3–97.2)	2.3 (1.5–3.5)	2.8 (1.1–4.6)
Recovery (%)	34.4 (26.0–46.0)	38.1 (27.6–52.9)	21.4 (12.5–28.4)	9.2 (4.3–13.4)

* Each value represents the mean obtained from 18 mice with ranges in parentheses. ** Contamination with erythrocytes and platelets was negligible in most samples.

Pasteur pipette over 0.6 ml of Lymphoprep (Accurate Chemical & Scientific Corporation, Hicksville, New York) in a 12×75 mm culture tube. The tube was centrifuged (international clinical centrifuge with a horizontal head) at 100× g for 25 min at 20 °C. Then, the upper layer of plasma and platelets was siphoned off and discarded by use of a Pasteur pipette, and the layer of enriched lymphocytes at the interface was collected carefully with another Pasteur pipette. The cells were transferred to a clean tube and, after the addition of 6–10 volumes of cold H-BSS, the tube was centrifuged at 160× g for 5 min. Subsequently, the supernatant was discarded, and the pellet of cells resuspended in 0.4 ml of H-BSS.

Results and discussion. The results are summarized in the table. It can be seen that 26–46% of the total leukocytes in the 18 blood samples were recovered after separation, and that the mean percent of lymphocytes was increased from 85.8 to 94.9% of the total cell count. Thus, the average number of lymphocytes obtained from 0.4 ml of blood was 38%. This level of recovery proved sufficient for selected biochemical studies.

The recovery of monocytes in the final suspensions (21%) was higher than expected. Presumably, it has its basis in the small volumes of blood which were employed and in the tendency of these cells to follow the lymphocytes during separation. Finally, the marked reduction in granulocytes from 11 to 3% of the total cell count (9% recovery) was considered as additional evidence for the ability to separate cell types by this method.

It should be noted that variations in the method with respect to the dimensions of the tube, the dilution of the blood, the volume of Lymphoprep, the period of centrifugation and the relative centrifugal force were found to have an adverse effect on the composition of the lymphocyte preparations. However, the care in collection of cells at the interface proved the most important variable since the removal of excess fluid was accompanied by the highest levels of contamination with erythrocytes and granulocytes.

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Autoradiography and differential hemoglobin staining as aids to the study of fish hematology

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Summary. The confused state of the existing knowledge regarding the cell types in peripheral blood and hematopoiesis of fishes has been highlighted. Inadequacies of techniques presently used have been pointed out and the advantages of using Graham-Knoll's method for haemoglobin staining when counter stained with Giemsa together with autoradiography using tritiated thymidine have been demonstrated.

Jakowska[2] commenting on the state of our knowledge of the hematology of fishes stated: '... The spirit which till now dominates the literature on the hematology of fishes seems to lack soundness. Several authors have expressed this very opinion on the confused state, the contradictory opinions, the inadequate descriptions, the unsatisfactory illustrations and the disagreements which arise from a highly varied nomenclature ... This paper has been prepared with the hope of helping newcomers to the study of hematology of fishes and to put an end to the "confused state" which still obtains in this branch of biology ...'

Inspite of a commendable effort made by her in recording original observations on a number of freshwater and marine fishes, and reviewing the existing literature with the stated end in view, the situation even after 2 decades of unprecedented technological progress since the publication of the above report, does not seem to have improved in any appreciable manner even though there has been considerable accretion to literature. For example, more than 900 references have been listed up to the end of the last decade[3] and a few hundreds would have been added in the seventies. That the 'confused state' continues is testified by a statement recently in a review[4] on the leucocytes of fish that '... The state of the literature concerning the eosinophils, and mast cells in fish is so confused that an entirely new approach to a study of these cells is warranted ...'

Part of the 'confused state' referred to above is due to the fact that, despite the ever-growing interest in fish blood and consequential number of research publications, very few workers have thought of using improved techniques. An example of the confusion is illustrated by the observation[5] that 'the nucleus of thrombocytes resembled the erythrocyte nucleus and the cytoplasm took on the same shade of pink as that of erythrocytes suggesting that the thrombocytes may also contain hemoglobin ...'. To our knowledge no one so far has unambiguously contradicted the state-