yielding conflicting data. If we accept the data in Tables I and II as confirming Metcalf's observations, we must conclude that extracts of L-5178Y grown *in vitro* stimulate lymphocytosis. At present, however, we cannot repeat Metcalf's observations, nor our own data as reported in Table I.

Summary. Attempts have been made to confirm the findings of Metcalf with respect to the lymphocytosis stimulating factor. Extracts of a lymphoblast grown *in vitro* behaved in a similar manner to thymic extracts in the tests used. The test method is subject to fluctuations which yield conflicting data.

Addendum:† The results of Duplan, Foschi and Manson fell into 2 groups. In the first, the control mice showed normal L/P ratios, and injection of thymus extracts induced an elevation of these ratios. In the second group, control L/P ratios were low, indicating the presence of a lymphopenia and/or a polymorph leucocytosis. In the experimental mice of this group, injection of thymus extract produced no elevation of L/P ratios. We have encountered the same phenomenon under 2 different circumstances. When large (greater than 8) litters of mice were used, L/P ratios in individual mice were highly variable. In those mice with initial low L/P ratios, no elevation of the ratios was induced by injected thymus extract. During attempts to foster nurse a second litter of babies on mothers who had already nursed their own litters for one week, the same type of negative results were obtained.

As reported previously (Metcalf, D., *British J. Cancer,* 1956, 10, 431), thymus extracts did not produce a lymphocytosis in mice injected with cortisone.

It seems from the above findings that thymus extracts are unlikely to affect lymphocyte levels in the peripheral blood of baby mice, if these mice are subjected to stress. Such stresses include endemic infections, labelling with marker dyes, overcrowding, poor milk supply and fluctuations in room temperature.

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† Addendum by Dr. Donald Metcalf, Cancer Research Laboratory, Walter and Eliza Hall Inst., Royal Melbourne Hospital, Australia.

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Studies of Cell Source of Macrophages from Human Blood in Slide Chambers.* (27539)

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The formation of macrophages from blood mononuclear cells in tissue cultures has been regularly noted since 1915(1). It is generally agreed that monocytes can transform into macrophages(2,3). Some observers have insisted that lymphocytes can also transform into macrophages(2,3,4) while others have denied this(5).

In this study of blood cells in slide chambers observations are reported in which macrophages are produced from monocytes, but not from lymphocytes.

Methods. Preparation of blood for slide chamber study, descriptions of the chamber and its use for inverted phase microscopy and time-lapse cinemicrography have been detailed by Schrek(6). The culture medium used consisted of 50% fresh human serum of the same blood type as the cells and 50% Parker medium 199 (Difco). Irradiation and

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TABLE I. Number of Macrophages Formed in Suspensions: (A) After X-irradiation to Destroy Lymphocytes, and (B) After Filtration through Glass Beads to Remove Monocytes.

	A. Effect of x-ray				B. Effect of glass filtration			
Day of incubation	Monocytes or macrophages*		Lymphocytes		Monocytes or macrophages*		Lymphocytes	
	Control	1000 r	Control	1000 r	Control	Glass	Control	Glass
0	20†	18	161	172	17	1	178	312‡
1	26	27	181	44	13	1	118	314
2	28	16	144	13	15	0	100	292
4	27	19	133	0	10	1	70	199
6	17	15	114	0	13	0	79	185
7	21	13	35	0	19	1	38	52
9	12	21	11	0	21	1	2	9
11	22	21	0	0	17	1	0	0

* After 6 days only macrophages are present. Before 6 days intermediate forms between monocyte and macrophages are counted.

† No. of viable cells counted in an area of 10 × 0.04 mm.

‡ Lymphocyte counts in glass experiment were deliberately adjusted at a high level in view of absence of competing cells. Note that survival parallels control.

cell counting were performed as previously described(7). Purified lymphocyte suspensions were prepared with glass bead columns by Garvin's method(8) which was modified as follows: heparinized blood was sedimented to remove most of the red blood cells prior to passage over the glass beads and speed of passage through the column was controlled manually rather than by motor driven syringe.

Results. 1. *General observations.* During studies of living white blood cells in slide chambers, monocytes have been regularly observed to transform gradually over a period of 5 to 7 days into mature macrophages(10). The mature macrophage was a large phagocytic cell, 40-80 μ in diameter when rounded, and up to 120 μ long when extended. Change of rounded into elongated, spindle form and vice versa was often observed. The cytoplasm was sharply divided into a very granular endoplasm and an extensive clear ectoplasmic veil which encircled the cell. The nucleus was large with a light nucleoplasm and usually contained one or two sharply outlined nucleoli. Ameboid motility was minimal, although there was considerable waving of the ectoplasmic veils. Lymphocytes have never been seen to change into macrophages nor have intermediate forms between lymphocytes and macrophages been observed. Mature macrophages, moreover, developed in the chambers while lymphocyte counts remained high. Fully developed macrophages and lymphocytes were regularly observed in the same microscopic field. The control columns in Table IA and IB show counts of typical experiments. It is evident that the number of macrophages formed corresponded closely to the number of monocytes initially present. At least under the conditions present in the slide chambers, lymphocytes did not appear to change into macrophages. Further experiments were designed to check this impression.

2. Development of macrophages after irradiation. Previous studies by Schrek(7) showed that a dose of 1000 r applied to cells in the slide chamber killed nearly all lymphocytes after 2 days. Irradiated and non-irradiated neutrophilic granulocytes are also all dead in 2 to 3 days. The development of macrophages in an irradiated chamber would thus indicate their origin from monocytes.

Cells from normal blood were accordingly exposed in slide chambers to 1000 r. The precipitous fall in viable lymphocytes after irradiation in a typical experiment is shown in Table IA. It is also apparent that the number of viable monocytes was well maintained.

Daily observation showed progressive transformation of the surviving monocytes into macrophages. The monocytes were, however, evidently not completely unaffected by this dose of radiation. Mature macrophage transformation was slower by a day or 2 than in unirradiated chambers with prolonged persistence of the monocyte appearance. The macrophages formed were generally smaller

and less well developed than those in the control chambers with fewer numbers of the dark granules which are so numerous in, and characteristic of, the fully matured macrophage. The irradiated monocytes and the macrophages formed from them showed much less tendency to flatten and spread out than did the control cells.

In 5-7 days, nevertheless, all the monocytes had changed into mature macrophages, albeit these were smaller, more rounded, less flattened with fewer projections and less dark granules than normal.

3. *Non-development of macrophages from lymphocytes in absence of monocytes.* A suspension of lymphocytes free of monocytes should not produce macrophages. To demonstrate this, suspensions of white blood cells were passed through Garvin's columns of glass beads. Practically all the monocytes and granulocytes were removed and resulted in suspensions of 97-100% lymphocytes. These lymphocyte preparations were studied in the slide chambers. Table IB shows lymphocyte and monocyte/macrophage counts in a sample experiment. Lymphocyte counts remained high for 7 days, yet the numbers of macrophages produced were very few and corresponded to the small numbers of monocytes in the chambers at the start.

Discussion. We have found, as have others, reviewed in(9), that identification of the mononuclear blood cells on stained smears after they have been cultured even briefly is very difficult. In slide chambers using inverted phase microscopy under good optical conditions very accurate identification of living cells has been possible based on such morphological criteria as: differences in nuclear detail, presence of ectoplasmic veils in monocytes and macrophages, but not in lymphocytes; and on such physiologic criteria as differences in motility and spreading. Accurate cell identification is obviously essential to such studies as are here presented.

The present studies seem to indicate that macrophages, at least under the experimental conditions, are formed only from monocytes. The possibility remains that under other conditions, or under the influence of special stimuli, lymphocytes might be induced to undergo transformation. Thus, a transformation stimulating factor related to the inflammatory source of the lymphocytes used may have operated in Rebuck's(3) experiments.

Summary. In slide chambers the number of macrophages formed closely paralleled the number of monocytes present in the initial suspension. When most monocytes were removed by glass beads very few macrophages formed. When lymphocytes were removed by irradiation the number of macrophages formed corresponded to the number of monocytes at the start. Intermediate stages between lymphocytes and macrophages were never observed, whereas cells intermediate between monocyte and macrophage were regularly seen.

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