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The Lymphocytosis Stimulating Factor (L.S.F.) (27538)

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Metcalf(1) has described a factor which can be isolated from the thymus of normal mice and is able to induce lymphocytosis when injected into newborn mice. In leukemic mice, a factor is found in the plasma which has the same effect as the thymic factor. The first has been named the lymphocytosis stimulating factor (L.S.F.); the second, the lymphocytosis stimulating hormone (L.S.H.); but there is no clear evidence that they differ from one another.

The main interest in this problem is the possible function of LSF and LSH in either spontaneous or radiation-induced leukemogenesis. It could be that endogenous leukemia-inducing viruses are able to promote leukemia only if LSF is present in excess. On the other hand, it is likely that injection of a large excess of virus can induce leukemia even in animals in which LSF is limited. In many respects the mechanism of leukemogenesis would therefore be similar to the one admitted for carcinogenesis of the mammary gland.

It seems necessary then to repeat the experiment of Metcalf which seems to deal with the most fundamental aspects of leukemogenesis.

Materials and methods. Extracts were tested by being injected into newborn ICR mice; they belong to the Wistar breeding colony and are not inbred. The C57Bl, DBA/2, BRVR and AkR mice used were from inbred lines. Lewis rats were also used as a source of material. *Tissue culture.* Two strains of cells grown *in vitro* have been used: mouse fibroblast, strain L; and mouse lymphoblast, strain L-5178Y(2). *Preparation of extract.* Extracts were made according to Metcalf's procedure. Organs or cells grown *in vitro* were ground in the cold in a mortar with sterile sand, phosphate buffered saline (PBS) with penicillin added to obtain a 10% suspension (instead of a 5% suspension as used by Metcalf). The suspension was centrifuged in cold for 15 minutes (3000 rpm), the supernatant fluid harvested and stored at -60°C.

Heparinized plasma was prepared from blood obtained by heart or eye puncture. The plasma obtained after centrifugation was injected undiluted.

Testing of extract. Newborn ICR less than 48 hours old were injected intracerebrally with 0.03 ml of extract or plasma. Six days later the tail was cut and the first drop of blood used to make a smear. After staining by May-Grunwald-Giesma the ratio of lymphocyte to polymorphonuclear leucocyte (L/P) was determined. In our first experiments control smears had been obtained from untreated litters of the same age; later we thought it more accurate to split each litter, inject one-half with the extract and keep the other half either untreated or injected with PBS.

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Results. Our results must be divided into 2 groups according to the technic used. During the first period of our work we were injecting complete litters and using separate litters as controls.

Our first results are summarized in Table I and are in good agreement with Metcalf's findings. In addition we found a positive test after injecting an extract of leukemic lymphoblast grown *in vitro*. If the tumor was grafted in the isologous host (DBA/2), extracts of the cells grown *in vivo* showed no activity.

Soon after we had obtained the results described above, we began to notice that we had large discrepancies between the L/P ratio of control and injected litters and that we were no longer able to confirm our earlier findings. For this reason, we thought that it would be more accurate to split each litter, using half of it as control and injecting the other half with the material to be tested. Tables II and III show the results obtained with this second technic. From Table II it is clear that the only positive results that we were able to find were that thymic extract and *in vitro* L-5178Y extract gave a positive test.

This was followed by a 6-month period during which we obtained the data found in Table III. During this period the L/P ratio of all the mice had dropped from 1.7 to around 1.0, and no extract was found to be active. Extracts that had previously shown consistently positive results were now completely negative.

Discussion. In Table I we have concluded that all the L/P ratios above 2.0 were positive, for the reason that in control groups, only 4 mice out of 25 had an L/P ratio over 2.0, two of which had ratios between 2.0 and 2.5, and 2 between 2.5 and 3.0. On the other hand, the animals injected with a material expected to be active showed a shift to higher values in their L/P ratios. An example of this shift is shown in Fig. 1 for L-5178Y *in vitro* extract and C57Bl thymic extract.

In Table II we have concluded that a 50% increase in L/P ratio of injected animals above the L/P ratio of control animals was significant. The data shown in Table III indicate that none of the injected materials stimulated lymphocytosis. Since a change occurred in L/P ratios of control mice at this time, it is possible that this change had induced the mice to be refractory to stimulation.

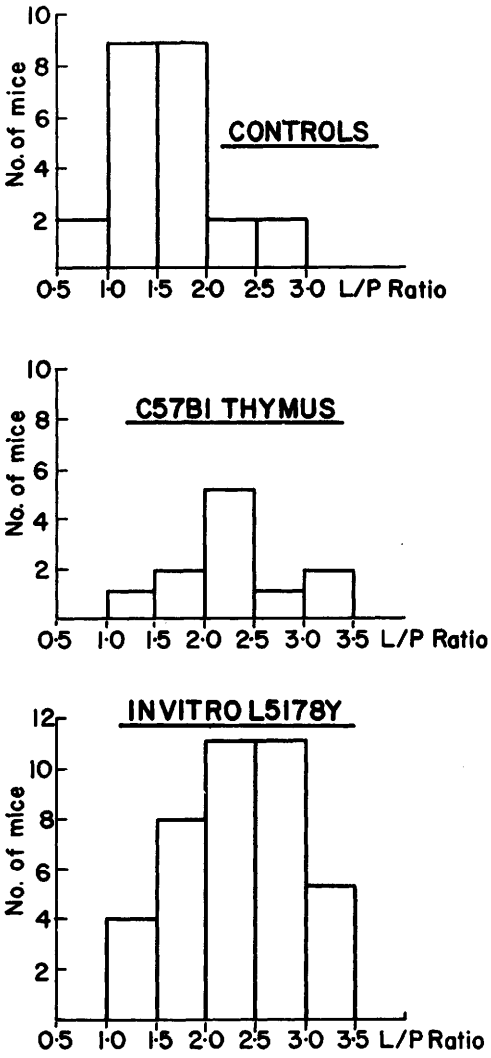

FIG. 1. Distribution of L/P ratios among individuals of a complete litter.

If our initial results seem to agree with those of Metcalf, it must be pointed out that we found a much smaller increase in L/P ratio than did Metcalf. The only data we obtained that confirmed Metcalf's findings was the activity of thymic extract. To obtain this result we have been obliged to use extracts whose concentrations were twice those made by Metcalf; if we used a 5% suspension of our material we did not get any positive re-

TABLE I. L/P Ratios Using Single Litter Technic.

Material inj.	No. of mice	Expected results	Ratio L/P	Experimental results
0 or PBS	25		1.7	—
Plasma C57Bl	9	—	1.7	—
Spleen C57Bl	10	—	1.7	—
Thymus C57Bl	11	+	2.3	+
Plasma AkR	17	+	2.2	+
Rat thymus	8	+	2.5	+
Rat plasma	23	—	1.7	—
Leukemia ICR plasma	8	+	3.0	+
L-5178Y extract *(in vitro)*	38	?	2.4	+
L-5178Y medium*	14	?	1.9	—
Medium control†	16	?	1.9	—
L cells	14	?	1.6	—
L-5178Y extract *(in vivo)*	14	?	1.4	—

* Tissue culture fluid obtained after harvesting an adult culture of L-5178Y cells.

† Fresh, unused tissue culture growth medium.

sult. We agree that the ICR strain that we have been using may not be as sensitive as Metcalf's original strain, but none of the other strains tested (C57Bl, C3H, BRVR) have given better results.

We wish to point out that extracts of lymphoblast L-5178Y grown *in vitro* gave a significant increase in L/P ratio (Tables I and II). This is a new finding. We do not know if this activity is related to the presence of LSF; we only know that it is to some extent specific for L-5178Y cells because extracts of L cells were inactive. If the L-5178Y cells were grown *in vivo* either as a solid neoplasm or as an ascitic tumor, no activity was found in the tumor extract, the ascitic fluid, or in the plasma of the host. These last findings confirm previous work by Metcalf. Our interpretation of these facts in terms of LSF production would be that L-5178Y lymphoblasts are capable of producing a factor having a similar end result to LSF. There is no regulatory mechanism *in vitro* and the factor is inhibited by the normal mechanism of regulation of the host. However, no direct evidence exists supporting the preceding statements. We think that a hormonal regulation of lymphopoiesis exists and that thymus probably plays a role in this mechanism; but from our experiments it seems that in using the present technic, fluctuations of an unknown nature in the responsiveness of mice to stimulation may occur,

TABLE II. L/P Ratios Using Split Litter Technic—Early Results.

Material inj.	No. of mice	Expected results	Ratio L/P	Experimental results
ICR thymus extr.	4	+	1.5	+
Control	4	--	.9	—
ICR spleen extr.	4	—	.6	—
Control	3	—	1.0	—
AkR	8	+	2.5	—
Control	6	—	2.5	—
Rat thymus extr.	4	+	1.7	—
Control	4	—	1.7	—
L-5178Y *(in vitro)*	6	+	3.1	+
Control	4	--	2.1	—
L-5178Y extr. *(in vivo)*	12	—	1.5	?
Control	9	—	1.1	—

TABLE III. L/P Ratios Using Split Litter Technic—Later Results.

Material inj.	No. of mice	Expected results	Ratio L/P	Experimental results
0	199	—	1.2	—
ICR thymus	4	+	1.7	—
Control	3	—	1.7	—
AkR leukemic spleen	5	—	1.4	—
Control	5	—	1.3	—
L-5178Y extract *(in vivo)*	5	—	.8	—
Control	4	—	.6	—
L-5178Y lymphosarcoma	3	—	.9	—
Control	3	—	1.2	—
L-5178Y lymphosarcoma	4	—	1.4	—
Control	4	—	1.2	—
AkR thymus	3	+	1.2	—
Control	3	—	1.1	—
L-5178Y *(in vitro)*	5	+	.7	—
Control	5	—	.8	—
DBA/2 plasma from mouse with lymphosarcoma	5	?	1.1	—
Control	5	—	1.0	—
L-5178L extract *(in vitro)*	7	+	.8	—
Control	6	—	.5	—
BRVR leukemic thymus	4	?	.9	—
Control	3	—	1.1	—

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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