2. Popper, H., and Chinn, H., PROC. SOC. EXP. BIOL. AND MED., 1942, v49, 202.
3. Oser, B. L., Melnick, D., and Pader, M., *Ind. Eng. Chem., Anal. Ed.,* 1943, v15, 724.
4. Engel, R. W., *J. Biol. Chem.,* 1942, v144, 701.
5. Entenman, C., Taurog, A., and Chaikoff, I. L., *ibid.,* 1944, v155, 13.
6. Beattie, F. J. R., *Biochem. J.,* 1936, v30, 1554.
7. Thorbjarnarson, T., and Drummond, J. C., *ibid.,* 1938, v32, 5.

Received May 24, 1957. P.S.E.B.M., 1957, v95.

## Lymphocytosis Response in Mice and Its Relation to Thymus and Adrenal.* (23296)

DONALD METCALF† AND RITA F. BUFFETT (Introduced by Jacob Furth)
*Children's Cancer Research Foundation, Children's Medical Center, Boston, Mass.*

During the course of observations on the effect of thymectomy on circulating lymphocyte levels in the mouse, it was observed that sham-operated mice did not maintain stable white cell levels as expected, but invariably developed a lymphocytosis. Subsequently, it was found that normal mice developed a similar lymphocytosis when bled daily for the performance of white cell counts. This paper describes this lymphocytosis response in mice, its variation in mice of different strains and experiments throwing light on the mechanism by which the response is produced.

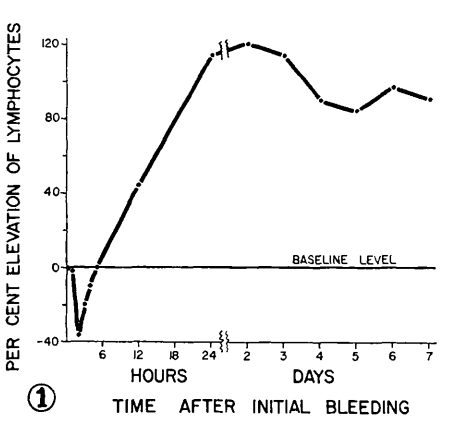
*Materials and methods. Mice.* Mice used throughout these experiments were obtained from the Jackson Memorial Laboratories and were of the following strains: AkR, Rf, $C_{57}Bl$, $LAF_1$, and $C_{58}$. All mice were fed on purina chow and maintained on oral terramycin to prevent intercurrent infections. Male mice were used throughout these experiments to eliminate possible white cell variations with the estrus cycle. However, some groups of female mice were tested to make sure no notable difference was present in the lymphocytosis response of the two sexes. No difference was observed. The age of the mice used was 4-5 weeks. All mice were autopsied at the conclusion of each experiment to check on the adequacy of the operative procedures. Results from any mice showing infections were discarded. *White cell counts.* Standard human white cell diluting pipettes and chambers were used. Differential white cell counts were made, counting 200 cells. Blood was obtained by pricking the tail veins with a sharp No. 13 scalpel blade. "Milking" of the tail to produce a flow of blood was avoided as was making too large a wound with the production of too free a flow of blood. Careful standardization of the bleeding procedure is necessary because of the difference between the absolute numbers of white cells in the peripheral and heart blood in the mouse(1). White cell counts were performed at fixed times during the day to eliminate any possible diurnal variations. Due to individual variations in white cell levels even within inbred strains, groups of 4 to 6 mice were used in each experiment, and the mean value for the group used as a single estimation. *Operations.* When required, thymectomy and/or bilateral adrenalectomy were performed, using I.P. nembutal anesthesia. Adrenalectomized mice were maintained on a single I.M. injection of 0.5 mg of percorten‡ and 0.5% NaCl in the drinking water.

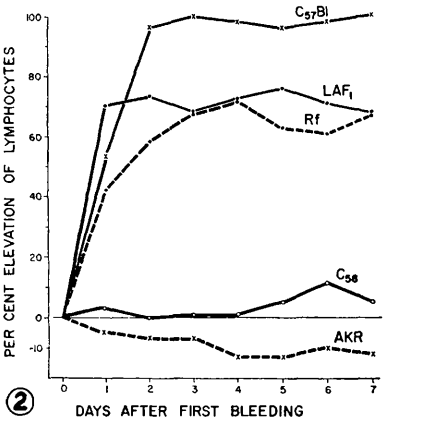
*Results. The lymphocytosis response.* When daily white cell counts were performed on the tail blood of normal mice of $C_{57}Bl$ strain, a lymphocytosis appeared on the second day. This persisted for as long as daily observations were made. The lymphocytosis levels fell towards the baseline levels of randomly bled mice several days after cessation

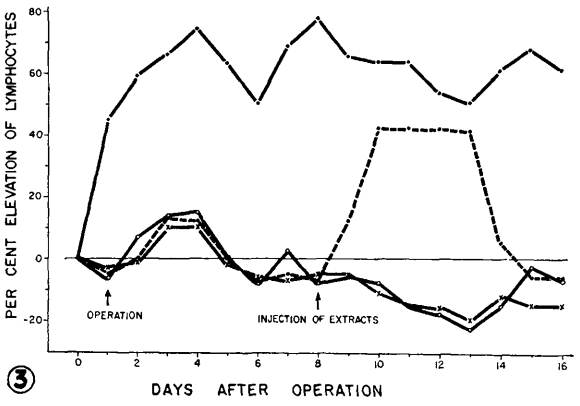
* This investigation was supported by research grant from the N.I.H.

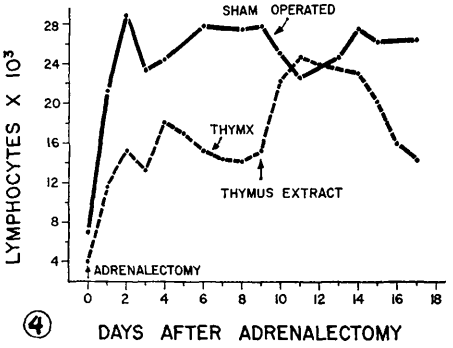
† Carden Fellow in Cancer Research Anti-Cancer Council of Victoria.

‡ This preparation (Percorten trimethylacetate) was generously supplied by Dr. Robert Gaunt, Ciba Pharmaceutical Products, Summit, N. J.



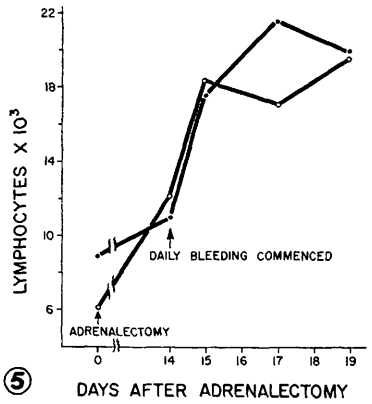




FIG. 1. Lymphocytosis response of repeatedly bled young male mice of Strain $C_{57}$Bl. Each point represents mean lymphocyte count of 6 mice.

FIG. 2. Effect of daily bleeding on circulating lymphocyte levels in mice of various strains. Each point represents mean lymphocyte level in 20 mice of each strain.

FIG. 3. Effect of thymectomy and sham thymectomy on circulating lymphocyte levels. Each point represents mean lymphocyte level in 20 mice. Mice were young male adult Rf strain. Inj. of organ extracts was made on 8th post-operative day. ●——● sham thymectomy; ○——○ thymectomy; ●– – –● thymectomy + thymus extract; ×——× thymectomy + lymph node extract.

FIG. 4. Effect of adrenalectomy and thymus extracts on circulating lymphocyte levels in thymectomized and sham-thymectomized mice. Each point represents mean lymphocyte level of 8 mice.

FIG. 5. Effect of adrenalectomy on lymphocytosis response. Mice were young male adult Rf strain. Graph shows mean results from 2 identical groups of 4 mice each.

of daily bleeding. The level of the lymphocytosis reached varied from group to group of mice, but usually represented a 50-120% increase over baseline values. The polymorphonuclear levels remained relatively constant, with minor daily fluctuations, unless intercurrent infections or fluctuations in room temperature occurred.

A more detailed analysis of the changes following the performance of the first white cell count showed that there was an initial fall in lymphocyte levels lasting for approximately 2 hours (Fig. 1). This lymphopenia probably represents a response to adrenal-mediated acute stress. From this point on the lymphocyte levels rose progressively, reaching a maximum by 20-36 hours.

*Strain differences in lymphocytosis response.* The sequence of events described above was found to hold true for the following strains of mice: $C_{57}Bl$, Rf, and $LAF_1$. Of these, the $C_{57}Bl$ strain tended to show a more pronounced rise than did the other strains.

When AkR and $C_{58}$ strain mice were bled daily, no lymphocytosis occurred, although the initial lymphocyte levels of these mice were consistently higher than for the other strains. With mice of the AkR strain, a definite fall in lymphocyte levels occurred (Fig. 2). It should be noted that the AkR and $C_{58}$ strains are characterized by a high incidence of spontaneous lymphatic leukemia, in contrast to the other strains studied.

*Mechanism of the lymphocytosis response.* The stimulus arousing the lymphocytosis response is associated with wounding of the skin of the tail during the bleeding process. When the initial wound was made through the skin away from the site of a tail vein, with no accompanying blood loss, the lymphocytosis response occurred as before. The effect does not, therefore, appear to depend on either the stimulus of blood loss or stimuli from damaged blood vessels.

It was of interest to determine the effect of thymectomy on this response since it has been shown(2) that the thymus produces a lymphocytosis stimulating factor (LSF). Thymectomies were performed on groups of mice as described above, and serial daily white cell counts performed. The mice failed to show a lymphocytosis but instead showed a slight but definite fall in lymphocyte levels. Sham-operated mice showed a similar lymphocytosis to that occurring in normal animals bled daily. When thymectomized mice were injected with saline extracts of thymus, a temporary lymphocytosis of the same magnitude as that in sham-operated mice resulted. Thymus extracts from strains of mice other than the strain of the thymectomized mice were also used, and the extracts centrifuged at 3000 rpm to minimize introduction of intact lymphocytes. The injection of these thymic extracts was also followed by a lymphocytosis. Injection of similar extracts of lymph node or spleen produced no such effect (Fig. 3). A similar lymphocytosis was elicited by injecting thymus extract immediately following thymectomy. This indicates that the response of thymectomized mice to injections of thymus extract is not dependent on compensatory hyperplasia of remaining lymphoid tissue in the animal. In fact, no such lymph node hyperplasia was observed in thymectomized mice which were killed and examined at the termination of the experiments.

These findings confirm earlier work(2) indicating that the epithelial type cells of the mouse thymus medulla contain the lymphocytosis stimulating factor (LSF), and suggest that in the lymphocytosis response, following tail wounding, thymic release and production of LSF occurs causing a lymphocytosis.

In view of the known lymphopenia-producing effects of adrenal cortex extracts, it was thought that adrenal corticoids might normally suppress thymic LSF activity and that under the conditions producing the lymphocytosis response, a partial or complete release of the thymus from this adrenal suppression might occur. To test these possibilities, bilateral adrenalectomies were performed on groups of thymectomized and sham-operated animals. Normal mice were also adrenalectomized and later tested for their ability to show a lymphocytosis response.

It was found (Fig. 4) that adrenalectomy caused a lymphocytosis both in sham-operated and thymectomized mice. However, the levels reached in thymectomized mice were

consistently lower than in the sham-operated mice. This indicates that the adrenal normally suppresses thymic production of LSF and, consequently, depresses the level of circulating lymphocytes. Adrenalectomies were then performed on normal mice and a period of 2 weeks allowed to elapse to enable wound healing, and stabilization of lymphocyte levels. These mice were then bled daily.

Following the commencement of daily tail bleeding, a lymphocytosis response occurred, superimposed on the pre-existing elevated lymphocyte levels (Fig. 5). These findings suggest that, although the adrenal may normally suppress thymic LSF activity, release from this adrenal suppression plays no part in the development of the lymphocytosis response.

*Discussion.* The lymphocytosis response appears to be a normal response of the mouse to trauma—in the experiments described above, that involved in tail bleeding or in operative wounding. Elevated lymphocyte levels persist for as long as the trauma is repeated and pass off several days after the last injury. Polymorphonuclear levels are not altered significantly during these changes.

Striking strain differences were found in the ability to develop the lymphocytosis response. The high leukemia AkR and $C_{58}$ strains appeared unable to develop a lymphocytosis when stimulated, although the initial lymphocyte levels were higher than all other strains examined. The relation of these findings to the pathogenesis of lymphatic leukemia in these mice is not known, but it has been shown(2) that elevated thymic LSF levels are present in mice with spontaneous lymphatic leukemia.

Thymectomy prevents the development of a lymphocytosis response. This is not the non-specific result of removal of a considerable fraction of the mouse's lymphopoietic tissue. Injections of relatively cell-free saline extracts of thymus tissue from even a foreign strain enable thymectomized mice to develop a lymphocytosis similar to sham-operated mice. This effect does not follow injection of similar preparations of lymph node or spleen, indicating that it is not due to the injection of intact lymphocytes or material from disintegrated lymphocytes.

Adrenal corticoids in the normal mouse appear to suppress the number of circulating lymphocytes both by a direct process and an indirect process via the thymus. In unpublished experiments, cortisone, when injected into mice, has been found to depress the thymic content of LSF. However, removal of this adrenal suppression of thymic LSF production appears to play no part in the lymphocytosis response, as the response occurs in adrenalectomized mice.

The lymphocytosis response, therefore, would seem to be initiated by stimuli arising from damaged tissue and appears to be mediated through thymic LSF. Whether this is a direct stimulation of the thymus or an indirect one, via other organs, is yet to be determined.

*Summary.* (1) Mice of certain inbred strains ($C_{57}$Bl, Rf, and $LAF_1$), when bled daily from tail veins, developed a prompt and persistent lymphocytosis. (2) High leukemia strains AkR and $C_{58}$ did not show this response. (3) Thymectomy prevented this response but lymphocytosis was produced in thymectomized mice by injection of cell-free thymus extracts. Lymph node and spleen extracts were ineffective. (4) Adrenalectomy did not affect the lymphocytosis response.

1. Fekete, E., in *Biology of the Laboratory Mouse,* ed G. D. Snell, New York, Dover Publications, 1956, p94.

2. Metcalf, D., *Brit. J. Cancer,* 1956, v10, 442.

Received May 24, 1957. P.S.E.B.M., 1957, v95.