The Differential Effect of Hydrocortisone on the Short-lived Small Lymphocyte [1]

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ABSTRACT As previously reported, small lymphocytes of the rat comprise two populations of cells with respect to circulating life span, designated as long- and short-lived. By the appropriate injection schedules of H^3-thymidine and by varying the post-injection interval, it is possible to essentially confine the label to one or the other of the populations. Thus two groups of animals were administered H^3-thymidine so that in one group only long-lived small lymphocytes were labeled and in the other, short-lived lymphocytes were labeled. The animals were then injected intraperitoneally with 4 mg doses of hydrocortisone at four-hour intervals. Controls were administered saline. At intervals after receiving from 1 to 12 hydrocortisone injections, the animals were sacrificed and radioautographs were prepared of the thymus, thoracic duct lymph, mesenteric lymph node, bone marrow and spleen. These are tissues known to show significant differences with respect to the percentage distribution of long- and short-lived lymphocytes.

The results from analyzing the percentages of labeled small lymphocytes present in the respective tissues of the two groups are believed to be conclusive in showing that short-lived small lymphocytes are more susceptible to the destructive effects of hydrocortisone than are the long-lived lymphocytes.

Since the experiments of Selye ('36) and those of Dougherty and White ('43, '44, '45a,b, '47), Frank, Kumagai and Dougherty ('53) and Dougherty ('60), the destructive effect of some adrenocortical steroids on lymphoid tissues has been generally recognized. Hydrocortisone was found to be the most potent steroid in this respect. For hydrocortisone to produce its maximum effect, it has to be frequently administered in large doses over a relatively long period of time (Dougherty, '53). After such a regimen, a decrease in the weights of the lymphoid organs (especially the thymus), lymphopenia, and evidences of lymphocyte degeneration followed by phagocytosis of cellular debris by macrophages are observed.

Studies pertaining to the life span of lymphocytes have been made by a number of investigators. Caffrey, Rieke and Everett ('62), using H^3-thymidine, presented evidence that in the rat there are at least two types of small lymphocytes with regard to life span. One type, called "short-lived," had a life span of less than two weeks. The other, designated as "long-lived," recirculated from blood to lymph and had a considerably longer life span. This finding accords with the observations of several other investigators (Ottesen, '54; Christensen and Ottesen, '55; Resegotti, '57; Buckton and Pike, '64; Fitzgerald, '64).

In view of the known effect of adrenocortical steroids on lymphocytes, and since it is now established that there are at least two types of small lymphocytes as far as life span is concerned, the present experiment was designed to determine if there is a differential effect of hydrocortisone on the two small lymphocyte populations.

MATERIALS AND METHODS

Two groups of male Sprague-Dawley rats were used in these studies. Each rat weighed approximately 60 gm at the start of the experiments. Solu-Cortef (hydrocortisone sodium succinate, Upjohn) was administered intraperitoneally in doses of 4 mg every four hours. Control animals were given intraperitoneal injections of saline (volume and frequency equivalent

[1] Completed during the tenure of a two-year Post-doctorate Fellowship from The Rockefeller Foundation and supported in part by research grant GM-06309 from the National Institutes of Health (N. B. Everett, Principal Investigator).

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to hydrocortisone injections to experimental animals).

Group I. Five control and five experimental animals were used. Each animal was given one injection of 1 μc of H^3-thymidine per gram of body weight. By this means, most of the lymphocytes that are labeled are of the short-lived variety (Caffrey et al., '62; Everett, Rieke and Caffrey, '64). Thirty-six hours after the thymidine injection, the five experimental animals were started on the hydrocortisone injection schedule. They were sacrificed at 40, 48, 60, 72 and 84 hours post-H^3-thymidine after receiving 1, 3, 6, 9 and 12 hydrocortisone injections respectively. Controls were sacrificed at corresponding time intervals.

Group II. Again five control and five experimental rats were used. Each rat was given, in a 16-day period, 12 injections of 0.5 μc of H^3-thymidine per gram of body weight. This is in accordance with the injection schedule devised by Everett, Caffrey and Rieke ('64a,b) in this laboratory for labeling an appreciable percentage of the long-lived lymphocyte population. Beginning two weeks after the last injection of H^3-thymidine, when the label had cleared from short-lived lymphocytes and precursor elements (Everett et al., '64a,b; Rieke, Everett and Caffrey, '63), the five experimental rats were started on the hydrocortisone regimen described above. They were sacrificed after receiving 1, 3, 6, 9 and 12 injections of hydrocortisone. At corresponding time intervals, the controls were sacrificed.

Immediately before sacrificing, thoracic duct lymph was collected from each animal by the method of Reinhardt ('45). Smears were made from the lymph after centrifugation to obtain a more concentrated suspension of cells. Cell suspensions were made from mesenteric lymph node (MLN), spleen and thymus by mincing pieces of the organs in a few drops of rat serum. Bone marrow cell suspensions were prepared by shaking vigorously bone marrow samples with a small amount of rat serum in a small stoppered vial. All smears were made on slides which had previously been dipped in a solution of 0.5 gm of gelatin and 0.05 gm of chrome alum in 100 cm^3 of water, then fixed in absolute methanol for four minutes and dried in an incubator at 37°C overnight.

Thymus weights were determined for all of the animals to provide a gross baseline for assessing the effectiveness of the hydrocortisone injections in relation to the earlier work of Dougherty ('53) and others. Pieces from the MLN, spleen, thymus and bone marrow were fixed in Zenker's and in Bouin's fixatives. Those fixed in the former were processed for methacrylate embedding, while those fixed in the latter were embedded in paraffin. One micron sections were then made from the methacrylate-embedded tissues and the paraffin-embedded tissues were sectioned at 3 μ.

Radioautographs of tissue smears and sections were made as described previously using Eastman Kodak NTB3 liquid emulsion (Everett, Rieke, Reinhardt and Yoffey, '60). After eight weeks exposure the slides were developed and the smear preparations and 1 μ sections were stained with MacNeal's tetrachrome ('22). The H and E method was used for the 3 μ sections.

The percentage of small lymphocytes labeled was determined from the smear preparations. Depending upon the extent of labeling, 500–1000 cells were counted per slide. The sections were used to evaluate the alterations, if any, in the lymphoid organs of the experimental animals as compared to the controls.

RESULTS

Thymus

The weight of the thymus per unit of body weight decreased as the total dose of hydrocortisone increased (fig. 1). Grossly, after 12 injections of the steroid, only a small amount of thymus remained so that it had to be practically picked piece-meal from the surrounding connective tissue. There was, upon histologic evaluation, an increase in the percentage of reticular cells. Such an increase was probably a relative one due to the loss of lymphocytes. Most of these reticular cells contained debris of cells they had presumably phagocytosed.

The reduction in the percentage of labeled small lymphoctyes in the thymus of

In the A (Group I) animals, short-lived small lymphocytes were labeled. In the B (Group II) animals, long-lived small lymphocytes were labeled (see text).

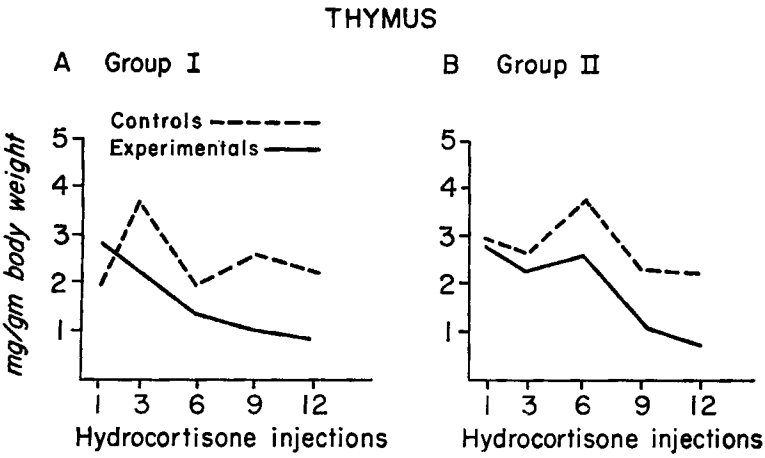
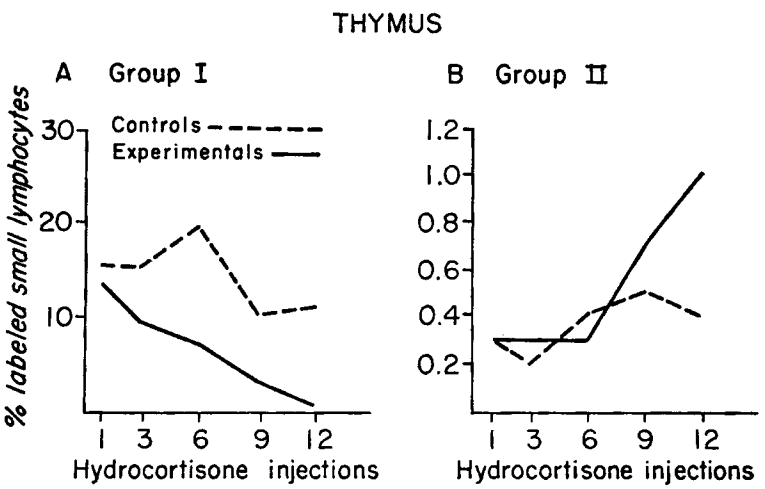


Fig. 1 Effects of hydrocortisone injections upon thymic weights of experimental animals (hydrocortisone recipients) shown in comparison to the thymic weights of controls (saline injected).



Figs. 2–6 Show the percentages of labeled small lymphocytes within the lymphoid tissues after hydrocortisone injections (experimentals) in comparison to controls (saline injected). In rats of Group I, short-lived lymphocytes were labeled and in rats of Group II long-lived lymphocytes were labeled.

animals in Group I after the administration of hydrocortisone was striking (fig. 2A). In contrast, there was an increase in the percentage of labeled cells in the thymuses of the Group II experimental rats as shown in figure 2B. The corresponding controls in both groups had a much more stable percentage of labeled small lymphocytes.

Thoracic duct lymph

In the thoracic duct lymph of the experimental animals in Group I, there was a fall in the percentage of labeled small lymphocytes from 8.2% to 0.8% (fig. 3A). The reduction was less in the corresponding control animals. In Group II, the percentage of labeled cells fluctuated over a wide range from 14% to 25%. However, the first animal, which received one hydrocortisone injection, and the last animal, which received 12 injections, had practically the same percentage of labeled small lymphocytes. The corresponding controls had a relatively stable percentage

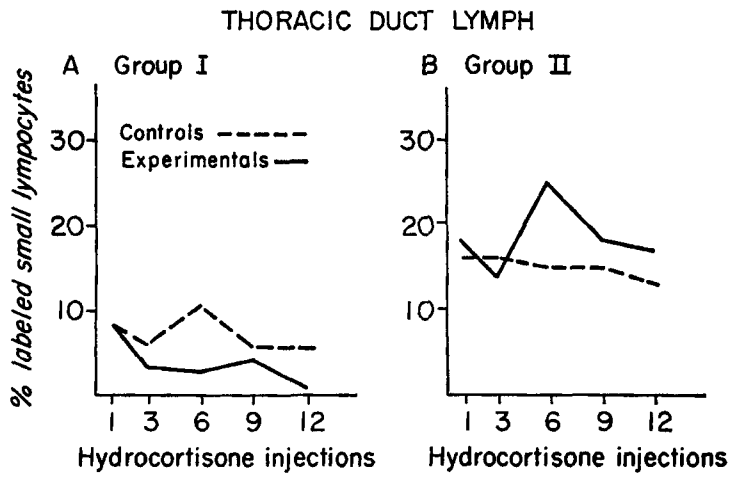


Figure 3

of labeled small cells, although a slight fall was noted more particularly after 12 injections (fig. 3B).

Mesenteric lymph node (MLN)

In the mesenteric lymph nodes of the experimental rats of Group I, there was a gradual decrease in labeled small lymphocytes from 13 to 7% (fig. 4A). The percentage of labeled small cells in the control nodes of this group was relatively constant. In Group II, the MLN of experimental rats evidenced a slight increase, and the controls showed a slight decrease in the percentage of labeled small lymphocytes (fig. 4B). The increase for the experimental rats of Group II could be explained by the destruction of unlabeled (newly-formed) small lymphocytes in the MLN.

The significant drop in the percentage of labeled cells in the experimental rats of Group I was undoubtedly a true decrease in the number of short-lived small lymphocytes. This derives from the fact that essentially all of the labeled cells were of the short-lived variety and the fact that the percentage of labeled cells in the control animals was relatively stable.

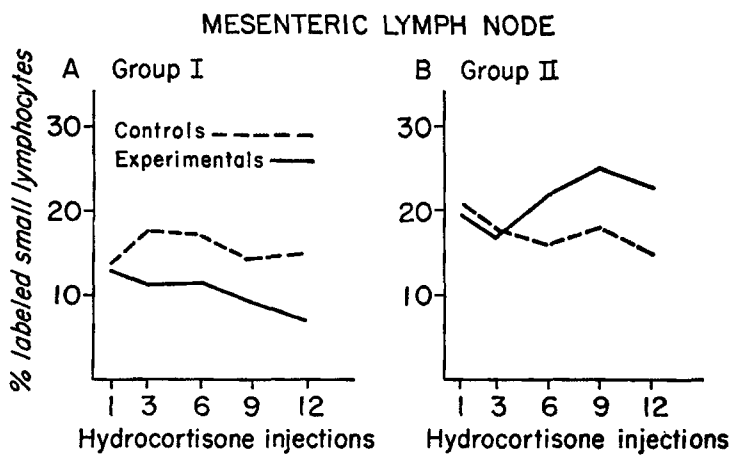


Figure 4

Bone marrow

In the bone marrow, there was a sharp decline in the percentages of labeled small lymphocytes for both control and experimental animals of Group I (fig. 5A). In comparison to the controls, however, the rate of fall for the hycrocortisone recipients was faster, particularly during the first part of the experimental period. Aside from this difference, the animal which received 12 hydrocortisone injections had a lower percentage of labeled cells than the corresponding control animal. In Group II (fig. 5B), there was an increase in the percentage of labeled cells in the bone marrow of the experimental animals. The values for the corresponding controls remained relatively constant. The sharp decline in both the control and experimental animals in Group I may be due to cell migration and/or death of the short-lived cells. The fate of the bone marrow small lymphocytes which are known to be produced *in situ* is not yet clear, although significant numbers enter the peripheral circulation (Everett and Caffrey, '67). The great reduction for experimental animals of this group may be attributed to the effect of hydrocortisone on the small lymphocytes. In Group II, the rather pronounced differences between the controls and experimentals probably reflects a relative increase in the percentages of long-lived cells in the marrow of the hydrocortisone recipients, i.e., these short-lived cells were selectively destroyed.

Spleen

There was a fall in the percentage of labeled small lymphoctyes in the spleen of the experimental rats of Group I (fig. 6A) and a slight increase in Group II (fig. 6B). The short-lived small lymphocytes comprise 75% of the small lymphocyte population of the spleen. Thus, the results are compatible with the proposition that the short-lived small lymphocytes are differentially affected by the steroid. There is no apparent explanation for the rise in Group I at 60 hours post-H^3-thymidine, after six injections in both experimental and control animals. There was a similar increase noted for the experimental animals of Group II at the corresponding interval.

The over-all effects of hydrocortisone on the histology of the lymphoid organs paralleled those which have been described by previous investigators (Antopol, '50; Dougherty, '52; Dougherty and White, '44, '45a,b; Frank et al., '53; Fruhman and Gordon, '55; Selye, '36). In all of the lymphoid tissues, there was evidence of edema, lymphocyte degeneration and destruction and phagocytosis of nuclear debris by macrophages. In the thymus, there were degen-

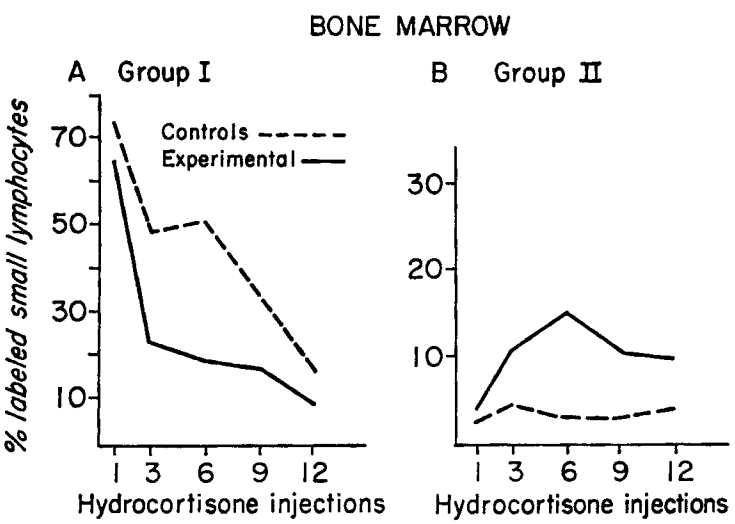


Figure 5

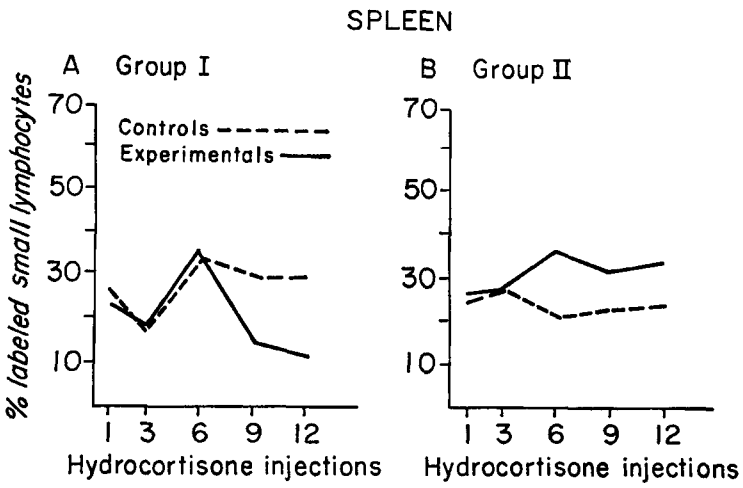


Figure 6

erative changes in the form of nuclear pycnosis of the small lymphocytes. There was extensive phagocytosis by large reticular cells. In the lymph nodes, there was depletion of lymphocytes from the cortex and many of the germinal centers became indistinct especially in those animals which received the greatest number of hydrocortisone injections. Sections of the spleen showed evidences of edema and lymphocyte destruction in both the white and red pulp. The bone marrow also evidenced some lymphocyte degeneration and destruction.

DISCUSSION

The early work of Dougherty ('60) emphasized that cortisone did not produce destruction of all lymphocytes and that the thymus and bone marrow small lymphocytes appeared to be the most sensitive. The results reported here confirm and extend these earlier observations. Significantly, it is now possible to relate the differences in the responses of small lymphocytes of various tissue compartments of the rat to quantitative differences that have been estabilshed for the percentages of long- and short-lived small lymphocytes within the respective tissues.

Previous studies from this laboratory (Everett et al., '64b) have shown that in the rat about 95% of the small lymphocytes in the thymus and essentially 100% of those in marrow are short-lived. At the other extreme, only about 10% of the small lymphocytes in thoracic duct lymph are short-lived the other 90% being long-lived. The lymph nodes have the next highest percentage of long-lived cells, approximately 75%. The spleen, however, has approximately 75% short-lived and 25% long-lived cells.

The present experiments were not designed to label all the lymphocytes of either the short-lived or long-lived variety. It is clear, however, that the ratios of the two labeled varieties within the respective tissues were at zero time comparable to those reported earlier. Moreover, it is quite apparent that the extent of lymphocyte destruction, or disappearance in tissues following hydrocortisone treatment may be directly and positively correlated with the percentage content of labeled short-lived cells. Reciprocally, there is a negative correlation between small lymphocyte destruction and the percentage of labeled long-lived lymphocytes in the tissue.

The observation that short-lived lymphocytes are more susceptible to the destructive effects of hydrocortisone than are the long-lived cells is in accord with the recent observations of Craddock et al. ('67) and with those of Miller and Cole ('67).

At present it is difficult to relate the limited available information relative to functional roles of the two varieties of

small lymphocytes to the results of this study. With respect to the long-lived small lymphocytes, it has been well established that they are involved in immune response mechanisms (Everett and Tyler, '67) and that they recirculate from blood to lymph by way of the lymph nodes and Peyer's patches. The functional role of the short-lived lymphoctyes, however, remain obscure. Moreover, it is now known what possible developmental interrelations may exist between the long- and short-lived cells. It does not appear, however, that short-lived small lymphocytes recirculate to any extent, if at all.

The question remains relative to a possible relationship between the observed effect of hydrocortisone upon short-lived lymphocytes and the known immunosuppressive effect of the drug. The effectiveness of hydrocortisone is very dependent upon the time and duration of administration in relation to the antigen injection (Berglund, '56; Fagraeus, '59). Cortisone does not affect established antibody production and administration of the drug must be started at least two days prior to antigen and continued for some time after antigen administration to be effective in suppressing the antibody response. Homograft rejection is elicited shortly after cessation of treatment. In contrast, it has been shown that animals made deficient in long-lived small lymphocytes remain immunologically incompetent for prolonged periods of time without further treatment whereas immunological suppression with cortisone requires continued treatment (Everett and Tyler, '67).

With respect to the immunosuppressive activity of adrenocortical steroids on the graft reaction, the studies of Gowans and others (see review papers; Gowans and McGregor, '65; Everett and Tyler, '67) provide for postulating that the effect is at some level along the pathway from the immunologically competent lymphocytes (small long-lived variety) → large pyroninophilic cells → to the immunologically active lymphocytes capable of destroying the graft. The relationship of short-lived lymphocytes, if any, to the derivative cells of immunologically competent lymphocytes is not apparent. It could be that the relationship exists through some mechanism such as that suggested by Craddock et al. ('67). These investigators postulated, without objective evidence, that short-lived lymphocytes may enhance antibody production by processing antigen in some way and thus expediting phagocytosis and not be producing cells which are involved in antibody production.

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