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The Effect of Steroids on the Circulating Lymphocyte Population

III. The size distribution of thoracic duct lymphocytes of the rat and guinea pig after neonatal thymectomy and prednisolone treatment.

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Summary

A comparative study of the rat and guinea pig thoracic duct cell size distribution during the involution and restitution phases after a single steroid treatment was done with a Coulter Counter.

In normal rats it is impossible to separate the different lymphocyte populations by size distribution measurement. After T-lymphocyte depletion (neonatal thymectomy) the distribution curve consists of 2 overlapping cell populations with separated peaks. The peak which includes the larger cells (mean cell diameter about 6.5 μ) apparently represents the B-lymphocyte population and the other peak (mean cell diameter about 5.8 μ) represents the T-cell population.

The steroid sensitive rat has the same lymph cell size distribution before, during, and after steroid treatment. Similar findings were also obtained in thymectomized rats. In the steroid resistant guinea pig, both intact and thymectomized animals kept the same size distribution during the different phases after steroid treatment. The steroid effect seems to be on both T- and B-lymphocytes in rat thoracic duct.

The same size distribution was found in the original and the returning cell population in thoracic duct lymph after steroid treatment, thus supporting the identity of the original and returning lymphocyte population. These findings agree with the hypothesis of lymphocyte trapping and redistribution as a major effect of a single steroid dose in both the rat and the guinea pig.

Key words: Lymphocyte. Thoracic duct. Rat. Guinea pig. Thymectomy. Corticosteroid. Size distribution.

Introduction

Corticosteroids have a more or less pronounced effect on the inflammatory reaction and the immune response in all mammals. With respect to the effect of corticosteroids on lymphoid tissues, especially the thymic cortex, and the dose needed to influence immune response, different species can be divided into steroid sensitive (mouse, rat, and rabbit) and steroid resistant (guinea pig, monkey, and man) (1, 2). The significance of the steroid-induced lymphocytolysis in sensitive species is still unknown and very little is known about the steroid mechanism influencing immune response.

We have earlier reported the effect of a single prednisolone dose on the circulating lymphocyte pool (i. e. in thoracic duct lymph and blood) in both the steroid sensitive rat (3) and the steroid resistant guinea pig (4). The circulating lymphocyte cell level is thymus dependent in the rat as well as in the guinea pig. A single prednisolone injection causes a rapid disappearance of cells from the circulation followed by a restitution to original cell levels within one day both in intact and in neonatally thymectomized animals of both species. Thus, the restitution phase is independent of an intact thymic function and a major effect of steroids in both rats and guinea pigs seems to be a trapping and redistribution of lymphocytes within one or more tissues. Bone marrow may be such a tissue where lymphocytes can be trapped (5, 6, 7). The changes, though principally the same in both species, are less pronounced in the guinea pig with a less accentuated depression in cell level both after neonatal thymectomy and after steroid treatment. It is difficult to exclude some degree of lymphocytolysis in the circulating pool in the rat since it is known that rat thoracic duct lymphocytes are sensitive to steroids *in vitro* (8).

The aim of the present investigation was to analyze the composition of the disappearing, the remaining, and the returning cell popula-

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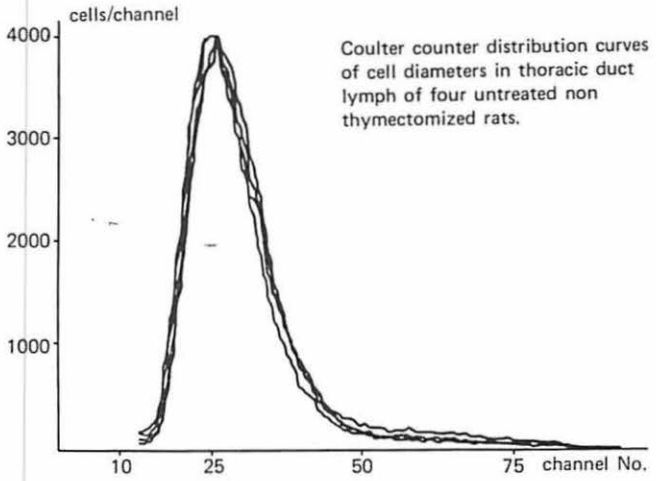

Fig. 1 The distribution X-Y-curves of cell volumes in thoracic duct lymph of nonthymectomized rats.

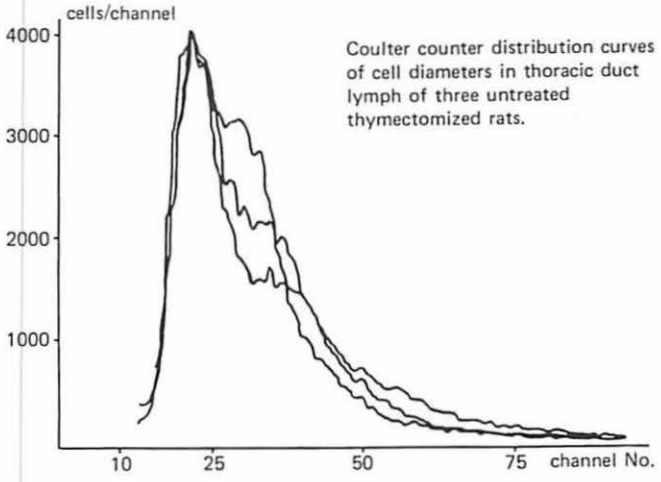

Fig. 2 The distribution X-Y-curves of cell volumes in thoracic duct lymph of thymectomized rats.

tions after a single prednisolone dose in both the rat and the guinea pig. Using Coulter Counter equipment, the cell size distribution was measured in the thoracic duct lymph in both species.

Material and methods

Guinea pigs and Sprague-Dawley rats were thymectomized within 24 hours of birth. Thymectomy was performed under ether anaesthesia using a dissection microscope. A total of 40 rats and 40 guinea pigs of adult age were used. Nonthymectomized animals served as controls. Sex distribution was equal in both species.

A water soluble preparation of prednisolone sodium succinate (Precortalon Aquosum, Organon) was used and given intramuscularly in the thigh in a dose of 10 mg/100 g body weight.

Three, 17, and 40 hours after the steroid injectiona a thoracic duct drainage was started under anaesthesia (Mebumal 60 mg/ml intraperitoneally at 6 mg/100 g body weight). The open-neck-technique of *Reinhardt* (9), was used. The minimum requirements were drainage for 20 minutes and 0.1 cc of lymph collect-

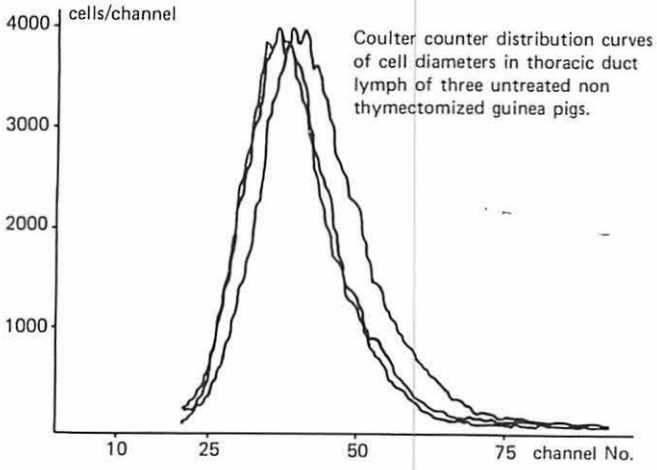

Fig. 3 The distribution X-Y-curves of cell volumes in thoracic duct lymph of nonthymectomized guinea pigs.

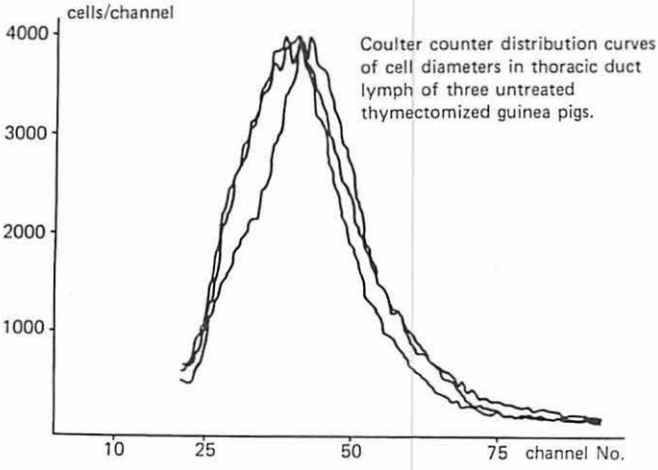

Fig. 4 The distribution X-Y-curves of cell volumes in thoracic duct lymph of thymectomized guinea pigs.

Figs. 1–4
Abscissa: Cell volume (expressed as channel No.).
Ordinate: Frequency of cells per channel (the Channelyzer stops when peak channel reaches 4000 cells).

ed, but usually drainage continued for 60 minutes. The lymph was collected in heparinized glass tubes. Lymph volume was measured and a sample taken for cell counts was diluted in Isoton, a balanced isotone saline solution (from Coulter Electronics Inc.) in order to prevent artificial cell volume changes. Cell volume was in this work measured with a Coulter Counter Model ZBI (with 100 μ diameter orifice), a Coulter Channelyzer Model C-1000 and a X-Y-Recorder (all from Coulter Electronics Inc.). Calibration of the instruments was performed with 7.87 μ and 8.06 μ diameter microspheres (Coulter Electronics Inc.). The Coulter Channelyzer was set to stop registering when the peak channel had registered 4000 impulses.

The theoretical background for using a Coulter Counter to determine cell size are given by *Grover et al.* (10). *Ben-Sasson et al.* (11) found that the lymphocyte satisfied the operational criteria of a rigid non-conducting sphere and was therefore suitable for electrical sizing in a suspension. The registered volume distribution X-Y-curves give only the relative cell size distribution. With the aid of the total number of lymph cells (cells/μl) the distribution curves were transformed to diagrams showing the absolute number of cells in different size classes

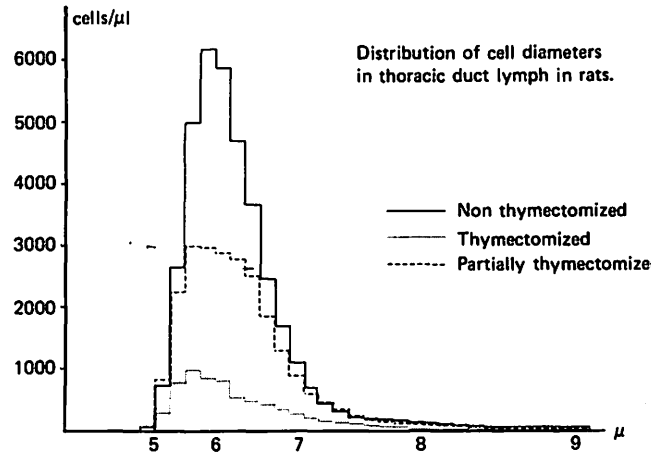

Fig. 5 The total cell size distribution in thoracic duct lymph in nonthymectomized and thymectomized rats.

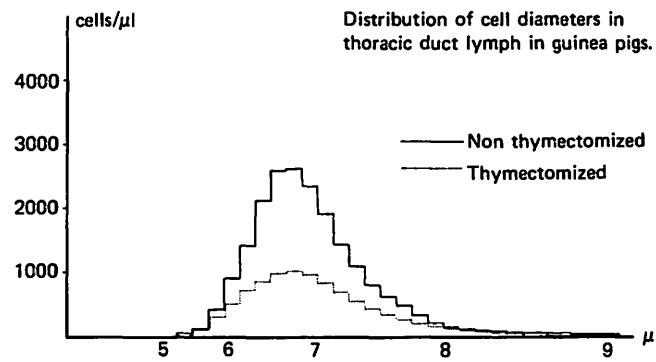

Fig. 6 The total cell size distribution in thoracic duct lymph in nonthymectomized and thymectomized guinea pigs.

expressed as the means of 5 animals. The cell sizes are expressed as cell diameters (μ) because the lymphocytes can be regarded as rigid spheres (11).

A small amount of crystalline heparin was used to prevent lymph clotting in the glass capillaries during the drainage. To exclude cell volume changes induced by heparin, lymph samples from both normal and thymectomized rats were incubated with different heparin concentrations (100, 200, 500, and 1000 IE/ml) at room temperature. Cell volume distribution was recorded immediately before and 5, 15, 30, and 60 minutes after incubation.

Results

Neonatal thymectomy gave in both species a significant reduction of the resting thoracic duct cell count. Further, the lymph showed the same changes as found previously (3, 4) namely, a rapid disappearance of cells with the lowest cell count level about 3 hours after a corticosteroid injection. Within one day the cell level was restored to pretreatment cell count. These changes were principally the same in both normal and neonatally thymectomized animals but in percentage were less pronounced in guinea pigs.

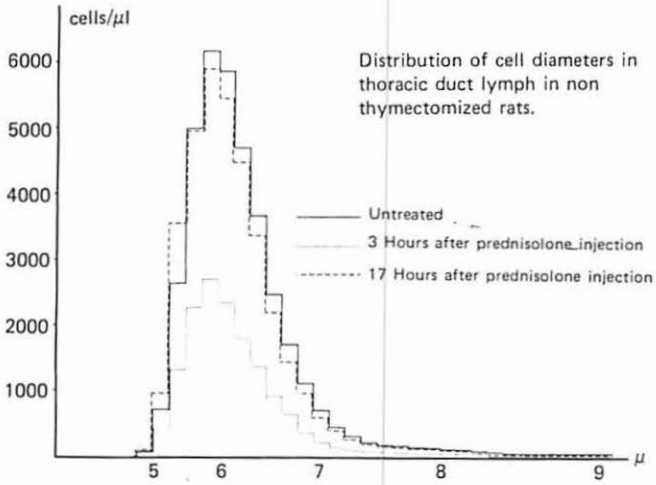

Fig. 7 The total cell size distribution in thoracic duct lymph in nonthymectomized rats at different times after a corticosteroid injection.

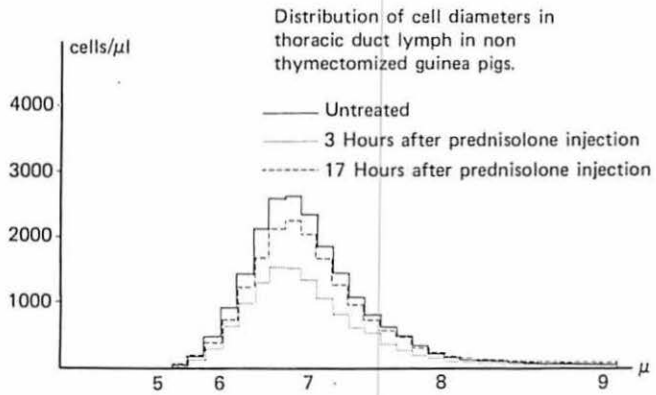

Fig. 8 The total cell size distribution in thoracic duct lymph in nonthymectomized guinea pigs at different times after a corticosteroid injection.

The registered distribution X-Y-curves of some normal and some thymectomized rats are seen in Figs. 1 and 2. In the thymectomized animals the curves have a broader base and, in the descending limb, a second separate peak, which changes in height from animal to animal. This indicates a relative increase of a separate cell population with a larger average cell size. Guinea pigs did not show these changes after thymectomy (Figs. 3 and 4). The rats distribution showed the most frequent cell size to be about 6.0 μ in diameter. Guinea pigs showed a broader and more symmetric cell distribution with the peak ranging from 6.8–7.0 μ in cell diameter.

In neonatally thymectomized animals of both species a reduction was seen in all cell sizes in thoracic duct lymph (Figs. 5 and 6).

The most pronounced reduction was found in the smaller cells with a maximal percentage depression of cells about 6.2 μ in diameter in the rat (Fig. 5) and 6.8 μ in diameter in the guinea pig (Fig. 6).

Some rats were incompletely thymectomized with about 1/10 of the total thymus left. These animals had a reduction of small lymphocytes to a cell level between normal and thymectomized animals (Fig. 5).

During the involution phase following prednisolone treatment, nonthymectomized

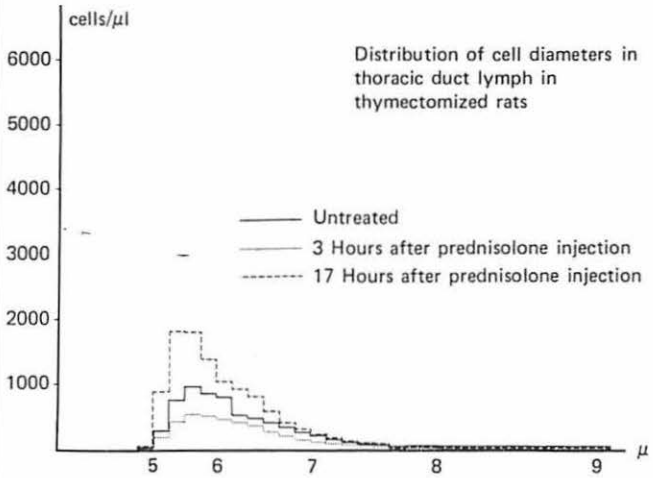

Fig. 9 The total cell size distribution in thoracic duct lymph in thymectomized rats at different times after a corticosteroid injection.

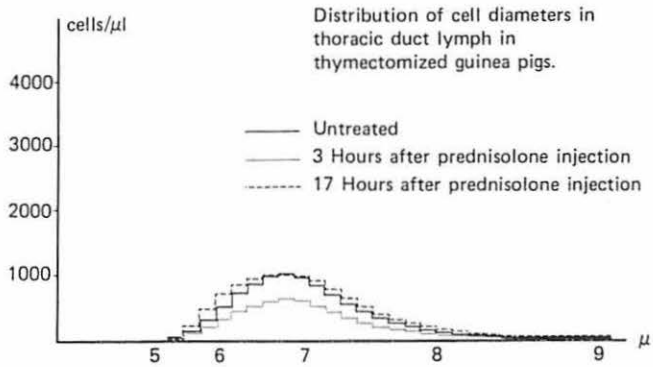

Fig. 10 The total cell size distribution in thoracic duct lymph in thymectomized guinea pigs at different times after a corticosteroid injection.

Figs. 5–10
Abscissa: Cell size expressed as cell diameter (µ).
Ordinate: Total number of cells in different cell size intervals expressed as cells per µl of lymph. Each curve represents the mean of 5 animals.

animals of both species kept the same size distribution. The restitution phase showed a return to pretreatment cell levels with unchanged size distribution (Figs. 7 and 8). Forty hours after the injection the distribution was the same as before treatment.

The neonatally thymectomized animals of both species showed the same changes in size distribution with only a slight reduction of all cell sizes during the involution phase (Figs. 9 and 10). For the guinea pigs there was a return to pretreatment cell levels with unchanged cell size distribution during the restitution phase (Fig. 10). In this material based on only 5 animals there was a tendency to a cell count overcompensation in the rat lymph during the restitution phase (Fig. 9). However, in our total material (3) such an overcompensation has not been observed.

Lymph samples from both normal and thymectomized rats, incubated in series with increasing concentrations of heparin for up to one hour, did not show any changes in cell size distribution (Figs. 11 and 12).

Discussion

Lymphocytes, both circulating in blood and lymph and stationary in lymphoid tissues, have often been divided according to size into small, medium, and large (12, 13). The limits between the different sizes have varied not only according to various authors' definitions but also with respect to whether total cell or

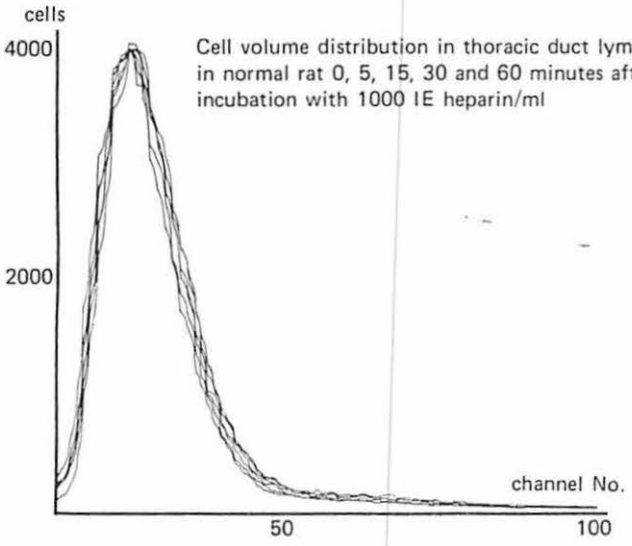

Fig. 11 The distribution X-Y-curves of cell volumes in thoracic duct lymph of a nonthymectomized rat 0, 5, 15, 30, and 60 minutes after incubation with 1000 IE heparin per ml.

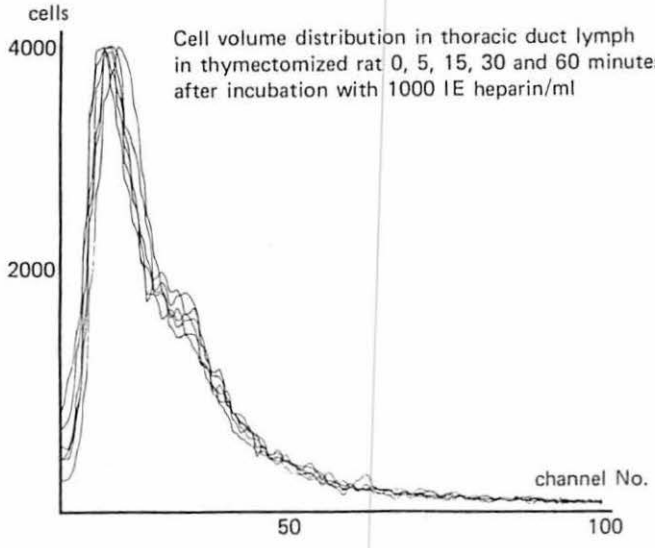

Fig. 12 The distribution X-Y-curves of cell volumes in thoracic duct lymph of a thymectomized rat 0, 5, 15, 30, and 60 minutes after incubation with 1000 IE heparin per ml.

Figs. 11–12
Abscissa: Cell volume (expressed as channel No.).
Ordinate: Frequency of cells per channel (the Channelyzer stops when peak channel reaches 4000 cells).

nuclear diameter have been measured. Using a "nuclear index" *Rieke et al.* (12) found that most of the thoracic duct cells were small lymphocytes (nuclear diameter less than 7.5 μ) and *Everett et al.* (14) approximate about 90% of the lymph cells to be small lymphocytes. This division into small, medium, and large cells can be done for practical reasons, but there is no support that it really represents three different sized populations of cells. The development of Coulter Counter with the ability to determine the size of particles suspended in an electrolytic medium gave a new approach to this field (10, 11).

The distribution curves in this work show that the most common cell in the thoracic duct lymph of normal rats is a small lymphocyte about 6 μ in diameter. This agrees with results using smears and a micrometer method (12).

Earlier work (15) has described a preferential depletion of lymphocytes with a diameter of 8 μ or less in the rat thoracic duct after thymectomy. After neonatal thymectomy, the most pronounced lymph cell reduction in this work was found in the 5.5–6.5 μ diameter range while a relative increase of cells was also found in the 6.5–7.0 μ diameter range. For the guinea pig the reduction was most pronounced for cells 6.5–7.5 μ in diameter.

In normal animals the lymph cells are dominated by T-lymphocytes. According to *Goldschneider* (16), 90% of rat thoracic duct lymph cells respond to anti-T-cell serum. In thymectomized animals the number of T-cells in lymph depends on the method used to produce T-lymphocyte depletion. After adult thymectomy combined with irradiation and bone marrow reconstitution, 14% of the rat lymph is made up of T-cells (16). In the mouse, 10% of the lymph cells are theta-positive after neonatal thymectomy, irradiation and bone marrow reconstitution (17). With neonatal thymectomy alone, 20% of the remaining lymph cells in mice are T-lymphocytes (17). Thus, the curves in Fig. 1 will represent strongly T-cell dominated distributions where the B-cell populations (about 10% of total cell count) can not be detected, but may represent the assymetric lower parts of the descending limbs. The distribution curves in Fig. 2 have reduced T-cell populations and the peaks in the descending limbs probably represent the B-lymphocyte populations. The different levels of the peaks in different animals might reflect the animals maturation when thymectomized and/or the completeness of the thymectomy. The B-cell populations are magnified relatively to the reduced T-cell populations. Thus, the animals with high peak in the descending limb have the greatest T-cell depletion. This means that the B-cell population in the rat thoracic duct lymph has a larger mean cell diameter (about 6.5 μ in diameter) than the T-cell population (about 5.8 μ in diameter). This agrees quite well with the results of *Ruhenstroth-Bauer et al* (18). By combining electrophoretic mobility and size distribution, they could separate the small lymphocytes in the rat thoracic duct lymph into two subpopulations. In the subpopulation with larger mean cell diameters, they found B-cell characteristics.

In the guinea pig (Figs. 3 and 4) the T- and B-cell populations can not be separated after thymectomy in the same way as they can in the rat. Here, the distribution is more symmetric, perhaps reflecting a greater overlap between T- and B-cell populations.

This work shows that after a subtotal thymectomy the circulating cell level will decrease. Thus, a certain volume of thymus tissue is necessary to keep the circulating lymphocyte level intact (Fig. 5).

Earlier it has been reported that heparin induces cell volume changes in both peripheral blood cells and tumour cells *in vivo* and in tumour cells *in vitro* (19). In this work heparin-induced changes of the lymph cell volume can be excluded since the size distribution present after incubating thoracic duct lymphocytes with heparin did not show any changes (Figs. 11 and 12).

Thus, the involution and restitution phases studied 3 and 17 hours after steroid injection seem to reflect a loss of circulating thoracic duct lymphocytes affecting all cell sizes and a returning of cells with the same size distribution. Als judged by the unchanged cell size distribution both T- and B-cells seem to be influenced by the steroid mechanism. This would support the hypothesis of trapping and redistribution as a major effect of a single short acting corticosteroid dose, in both a steroid sensitive and a steroid resistant species.

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