tension. The question which remains unanswered at present is whether or not immunologically sympathectomized rats develop more severe acute diastolic hypertension than do control animals.

It must be granted that our results were not completely satisfactory from a technical standpoint. In some animals no pressure was recorded, others died during the 6-week observation period, and finally, the level of hypertension achieved in the control animals was not great. Despite these drawbacks, these observations, considered in the light of the work of others, provide support for the postulate that the vasoconstrictor activity of sympathetic nerves is directly related to pathogenesis of the chronic phase of renal hypertension.

Summary. The influence of the sympathetic nervous system upon the course of experimental renal hypertension was examined in normal and immunologically sympathectomized rats. Blood pressures were recorded directly in the unanesthetized animal through indwelling catheters in the abdominal aorta. Immunological sympathectomy did not prevent the development of the acute phase of renal hypertension. Blood pressures of the hypertensive sympathectomized animals returned to normotensive levels 4-5 weeks following renal artery clamping, while untreated animals remained hypertensive at the termination of the experiment. It was concluded that the sympathetic nervous system is not required for development of renal hypertension, but may play a role in maintaining the chronic phase of the hypertensive state.

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Studies on Lymphopoiesis. VII. Size Distribution of Bovine Thoracic Duct Lymphocytes.* (31430)

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Virchow observed lymphocytes within the lymph in 1851(1). Many investigators have since attempted to determine their origin, function, life span and fate. Lymphocytes were classified into categories based upon diameter, morphology and staining characteristics. A common classification separated them into the 3 divisions of small, medium and large. It was difficult, however, to estab-

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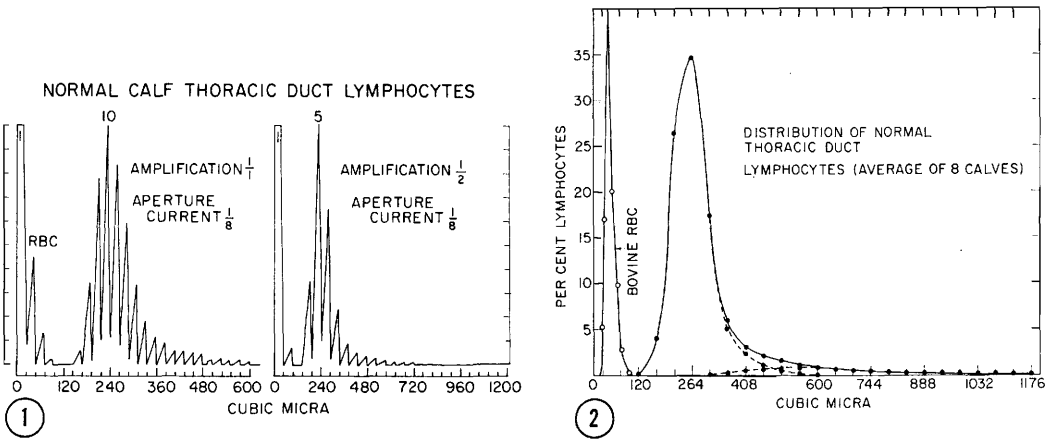

FIG. 1. Copy of plotter tracings showing linear relationship of curves obtained by altering amplification.

FIG. 2. Normal distribution curve of bovine thoracic duct lymphocytes. Dashed lines (.- - - - -.) indicate overlapping populations. Note size relationship of bovine rbc.

lish any reliable criteria of lymphocyte size from diameter measurements(2) or cellular morphology because of the non-reproducible distortion of cells during slide preparation.

Electronic blood cell counters, by measuring cell volumes, have made it practical to perform size distribution studies of mixed cell populations. This report constitutes studies on the size distribution of lymphocytes in the thoracic duct of calves.

Materials and methods. Thoracic ducts were cannulated by the method of Chanana and Cronkite(3). Samples of thoracic duct lymph were collected immediately following cannulation from 8 young calves under identical conditions. Subsequent samples obtained following recovery from anaesthesia verified that the anaesthetic agents thyamylal sodium and halothane had no effect upon size distribution.

The Coulter Model B Blood Cell Counter with an automatic recording distribution plotter† was used. The principle and operation of this instrument has been thoroughly reviewed by Brecher *et al*(4).

For this report each window was assigned the cell volume midway between the upper and lower limits of the excursion of the plotter. A 50 μ aperture was used to minimize coincidence error. The instrument was calibrated by suspending red blood cells of known mean corpuscular volume in a relatively particle-free standard physiologic saline solution‡ so that each excursion of the plotter represented 6 μ^3. Subsequent calibration with a commercially available stabilized suspension of fixed human red blood cells, assayed for size distribution,§ confirmed the accuracy of the first method. By altering the aperture current and amplification of the instrument, the range of each excursion was altered proportionally as shown in Fig. 1.

One mg of heparin per ml of lymph was used as an anticoagulant in each sample. The cell sizing was completed within 20-25 minutes following dilution in the saline solution. Generally there was a constant time between collection and dilution, and between dilution and sizing. Within the time limits of 0-45 minutes there were no changes in size. The concentration of cells was adjusted to maintain a flow rate of approximately 2000 cells per second, or a total count of about 8000 cells per window. Coincidence error under these conditions was approximately 1.25%.

Since the automatic distribution plotter graphs a succession of 25 windows of equal proportions, the total volume range covered

† Coulter Electronics, Hialeah, Fla.

‡ Unopettes, prepared by Becton-Dickenson and Co., Rutherford, N. J.

§ Erythro-trol, prepared by Dade Reagents, Inc., Miami, Fla.

divided by 25 gives the volume of each window. If a range of 6 μ^3 between excursions is desired the total range is limited to 150 μ^3. Likewise if a total range of 600 μ^3 is desired the increment of each excursion is 24 μ^3. The only alternative is to plot each excursion manually, but this would exceed the time limits imposed to preserve the integrity of the cell size. Although it was first felt that a range of 600 μ^3 would be adequate, it soon became apparent that larger cells existed. In order to avoid duplicate plotting, a range of 0-1200 μ^3 with increments of 48 μ^3 was used. A few red cells appeared in the lymph of each of the 8 animals studied. The largest bovine erythrocytes are almost as large as the smallest lymphocytes so a separate plot of 0-300 μ^3 was also made to facilitate separation of the red cells from the lymphocytes.

Results. In Fig. 2 the total distribution of cells is shown. The mode is between 240-288 μ^3. The large cells are considered lymphocytes on the basis of exclusion of coincidence error and by elimination of granulocytes and monocytes on the basis of examination of stained concentrates of the lymph. The range is from 120 μ^3 to 1200 μ^3.

Discussion. It has been shown that multiple peaks represent multiple populations of cells(4). This is illustrated in Fig. 2 where the red cell and lymphocyte peaks are plotted. The absence of a peak does not necessarily exclude multiple populations. A curve that appears as a continuum in Fig. 2 between 120 and 1200 μ^3 may represent overlapping cell populations. This has been demonstrated by Winter and Sheard(5) for red cells. A similar analysis was made in the case of our data. In Fig. 3 the data of size distribution are plotted on logarithmic probability paper. Note the sharp divergence from linearity at 312 μ^3. By assuming that both populations are distributed normally with logarithms of the volume, the separate volume distributions are shown in Fig. 3 and placed in Fig. 2 by dotted lines to show the degree of overlapping. The larger sized population represents about 5.5% of the total overlap.

Earlier work(6) on extracorporeal irradiation of the blood showed that the smaller lymphocytes disappeared more rapidly than

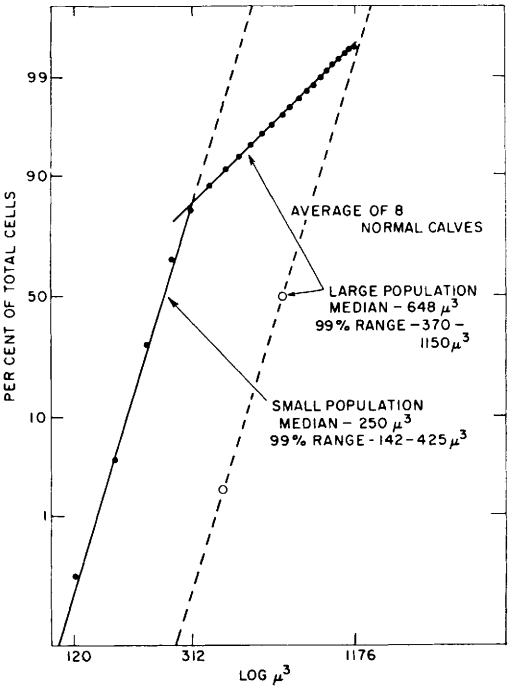

FIG. 3. Cumulative distribution of 48 μ^3 size increments. Note sharp divergence at 312 μ^3.

the larger ones. Labeling studies with tritiated thymidine have shown that the larger cells are shorter lived(7). Janett, *et al*(8) have shown that the large and small cells of the thoracic duct lymph have different generation times. In unpublished work we have demonstrated that output of small lymphocytes in the thoracic duct lymph is rapidly diminished by extracorporeal irradiation of the blood and lymph. The diminution of output of the larger cells is much less prominent. Within each size distribution there are further subdivisions that may be separated on the basis of basophilia (RNA content) and generation time.

We feel, on the basis of the preceding results, that thoracic duct lymphocytes can reasonably be separated into two groups on the basis of cell volume. If specific functions can be attributed to one or both populations, this could be a valuable tool in the area of lymphocyte physiology.

Summary. Size distribution studies based upon cell volumes of pure thoracic duct lymphocytes of normal young calves indicate the existence of two populations of cells and

delineate the size and range of each.

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Failure of L-Phenylalanine to Reverse Growth Inhibitory Effects of Chloramphenicol on McCoy Cell Cultures. (31431)

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In a recent report(1) it was suggested that the non-fatal toxic effects of chloramphenicol on the bone marrow(2) might be reversed by administering L-phenylalanine to patients receiving this antibiotic. If this agent could prevent bone marrow toxicity it is conceivable that it might also prevent the rare, fatal aplastic anemia caused by chloramphenicol (3).

The present report describes the inability of L-phenylalanine to reverse the growth inhibitory and toxic effects of chloramphenicol on a strain of cultured cells of human origin.

Materials and methods. The McCoy strain (4) of cells derived from human synovial fluid was used. Since the cells had been maintained for some time in a penicillin-streptomycin-containing medium, they were propagated for 6 passages in the presence of kanamycin sulfate (100 μg/ml) to minimize the possibility of latent mycoplasma (PPLO) contamination(5). Thereafter, the cells were maintained in an antibiotic-free medium. Periodic culturing of cells and fluids, both before and after kanamycin treatment, on Difco PPLO agar and in a modified PPLO medium(6) has disclosed no PPLO.

The cells were grown at 37°C in screw capped tubes containing 1 ml amounts of Eagle's basal medium supplemented with 10% calf serum. The medium was changed every 3-4 days and a pH of 6.8-7.4 was maintained by addition of sodium bicarbonate solution. Chloramphenicol* and L-phenylalanine were sterilized by filtration and incorporated in the medium to give the final concentrations noted. Test cultures were prepared in triplicate by inoculating 15,000-25,000 cells/tube of a 7- to 10-day culture contained in 0.05 ml of Hanks' basic salt solution. Morphologic observations were made directly of the living cells and also by examination of Wright stained cover slips on which the cells had grown in a replicate series of culture tubes. Direct cell counts were determined by counting at least 400 cells in a haemocytometer after removal of the cells with 0.05% trypsin. Each experiment was repeated 2-3 times.

Results. Chloramphenicol was employed at 10^{-5}M (3.23 μg/ml), 3×10^{-5}M (9.69 μg/ml) and 10^{-4}M (32.3 μg/ml) because these concentrations approximate the usual serum levels in patients treated with this drug. It is evident from the results shown in Fig. 1 that inhibition of cell growth was directly related to the concentration of chloramphenicol. Morphologic changes, indicative of cell toxicity, were produced by higher

* Kindly provided by Parke-Davis & Co.