albumin differed from that of bovine serum γ-globulin. This is in accord with the differences in localization obtained when different proteins are coupled to a particular dye group.

It can easily be argued that the azo-proteins synthesized and used in this study were chemically heterogeneous. The available evidence indicates that this is so(8). Nevertheless, the conclusion that the localization of azo-proteins after parenteral injection may be modified by changes in either the protein or the dye component seems valid.

Summary. The distribution of a series of azo-proteins in mice after parenteral injection was studied by observing the intensity of color in the tissues on gross and microscopic examination. Their distribution appears to be modified either by the azo dye coupled to a particular protein or by the protein to which a particular azo dye is coupled.

8. Gitlin, D., Latta, H., and Janeway, C. A., to be published.

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Development of Reticulum Cells and Lymphocytes in Transplants of Rabbit Lymph Node to Chick Chorioallantois.* (17835)

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The chorioallantoic membrane (CAM) of the developing chick embryo has proven to be a suitable host for various normal and malignant tissues derived from different species(1-4) and it was thought that grafting sections of lymphatic tissue from the rabbit onto the CAM of the developing chick embryo might be feasible. Cervical lymph nodes of guinea pigs were transplanted by Jaffe and Richter(5) into the abdominal wall of rats with regeneration of the lymphatic tissue and with apparent incorporation of the transplanted organ into the economy of the new host.

Technic. The popliteal lymph node of the rabbit was excised and bathed in buffered saline solution containing 300 units of penicillin and 60 μg of streptomycin per ml, then cut into thin slices and incubated in the saline solution at 37° C for ½ to 1 hour. The shell above the natural air sac of 9-day-old embryonated eggs was cut away, and the exposed shell membrane gently teased away from the CAM. A small amount of punctate bleeding of the CAM occurred, which was considered desirable, but eggs which bled profusely were discarded. A slice of lymphatic tissue was placed on the exposed CAM, directly over a surface blood vessel. The egg was then sealed with adequate amounts of Scotch tape and incubated at 37° C in the upright position. After suitable intervals the pieces of lymph node were cut away with the attached CAM and placed in 5% acetic acid Zenker's fixative. Hematoxylin- and eosin-stained paraffin sections were prepared for routine study. Some tissues were also fixed in Serra's solution and stained with methyl-green pyronine.

Results. Embryonated eggs containing slices of rabbit lymphatic tissue were incubated at 37° C for intervals of from 2 to

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1. Goodpasture, E. W., Douglas, B., and Anderson, K., *J. Exp. Med.*, 1938, v68, 891.

2. Goodpasture, E. W., and Anderson, K., *Am. J. Path.*, 1942, v18, 563.

3. Blank, H., Coriell, L. L., and Scott, T. F. McNair, PROC. SOC. EXP. BIOL. AND MED., 1948, v69, 341.

4. Kirber, H. P., Wiener Kirber, M., and Henle, W., PROC. SOC. EXP. BIOL. AND MED., 1950, v73, 481.

5. Jaffe, H. L., and Richter, M. N., *J. Exp. Med.*, 1928, v47, 977.

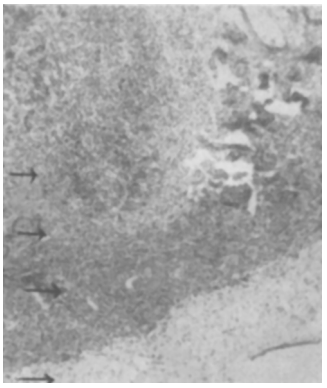

FIG. 1.
Explant of rabbit lymph node on chorioallantoic membrane (CAM) of chick embryo 4 days after implantation. 20 × H and E. Low power view showing from the top downward: two pieces of implanted lymph node; outgrowth of reticulum cells; area of vascularization from CAM (capillaries contain nucleated erythrocytes), with reticulum cells filling the spaces among the capillaries; edematous CAM.

10 days. After 2 days the nuclei of the lymphocytes, while easily discernible, were more dense and pyknotic than normal, and there was little tissue reaction. On the third day the lymphatic tissue was quite adherent to the CAM and the surrounding tissues were swollen. Histologically the CAM was edematous and the lymphocytes in the implanted tissue were undergoing degeneration. Between the implant and the membrane (Fig. 1) there was an area composed of capillaries full of nucleated erythrocytes extending from the CAM. Extending out from the implant toward and among these capillaries were typical reticulum cells. They were large and quite undifferentiated, with structureless eosinophilic cytoplasm and with large oval nuclei also optically empty except for small granules of basophilic material. These are well illustrated in Fig. 4. Where the reticulum cells occurred individualy, the cytoplasm could usually be seen trailing off to a point from each end of the wide oval nucleus. Much more frequently, however, the reticulum cells occurred in groups, without distinct borders of individual cells. On the fourth day the lymphocytes of the implant had undergone further degeneration. No cellular structure was in evidence, and rounded solid masses of material deeply stained with hematoxylin could be seen as the remains of nuclei of lymphocytes. The reticulum cells were present in far greater numbers. These occurred in broad bands set between the slice of lymph node and the CAM, and in many cases this band would expand into a broader mass of such cells beyond the point to which the implanted tissue extended. Many more capillaries filled with nucleated erythrocytes were seen in this area. In these capillaries leukocytes were quite rare. From the fourth to the tenth day not much change could be observed in the structures described, except for apparent further fragmentation of the residual material left from the nuclei of the implanted lymphocytes and deeper staining of the reticulum cells.

In a considerable number of specimens taken 4 or more days after implantation an additional change could be noted. Round or crescentic groups of fresh mature lymphocytes were seen at the periphery of the band of reticulum cells in the direction of the CAM, (Fig. 2) or set, as an island, within a mass of reticulum cells (Fig. 3 and 4). These groups of new lymphocytes never occurred in contiguity with the old, implanted lymphocytes, and in some cases were not near the capillaries from the CAM. The contrast between the appearance of the new and old lymphocytes, always with the band of reticulum cells interposed between them, was

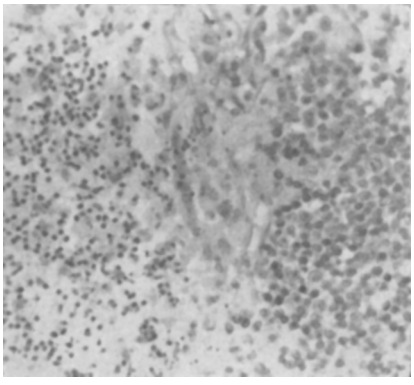

FIG. 2.
Similar preparation 5 days after implantation. 200 × H. and E. From left to right: debris of nuclei of disintegrating lymphocytes from implanted tissue; band of reticulum cells; new lymphocytes.

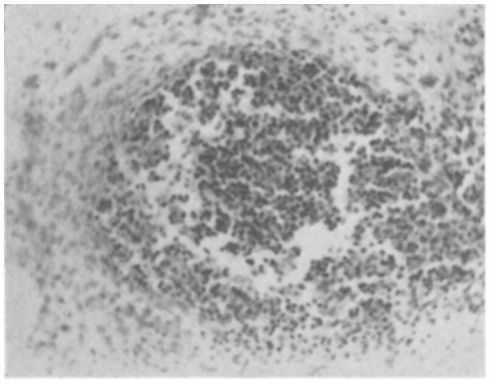

FIG. 3.
Similar preparation 6 days after implantation. 130 × H and E. This preparation shows a nest of new lymphocytes completely surrounded by reticulum cells, which have grown out to a considerable distance from the original implant.

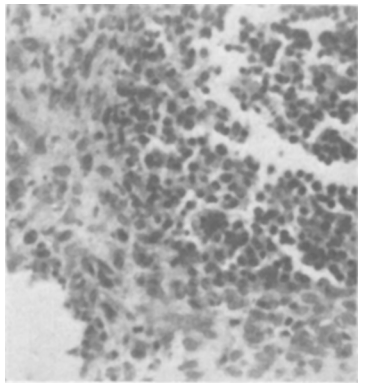

FIG. 4.
Portion of same field as that shown in Fig. 3, under higher power; showing in greater histologic detail the reticulum cells. The new lymphocytes and some transitional forms between them. 200 × H and E.

striking, as is seen in Fig. 5 and also Fig. 2. In many cases the cells at the border between adjacent areas of reticulum cells and new lymphocytes showed gradations between the two cell types with many fine degrees of difference in size and shape of nucleus and cytoplasm, and increasing basophilia and "hardening" of the nucleus. Such cells were not in general much larger than the mature lymphocytes.

All of the above observations were repeated in other experiments in which the tissues were fixed in Serra's solution and stained with hematoxylin and eosin. Other sections were

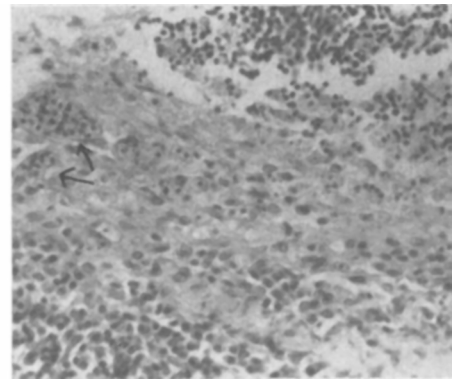

FIG. 5.
Similar preparation 5 days after implantation, showing especially the contrast between the degenerating nuclei of the implanted lymphocytes and the fresh nuclei of the new lymphocytes, with a band of reticulum cells between them. Arrows point to capillaries full of nucleated erythrocytes. 200 × H and E.

cut from the latter blocks and stained with methyl green and pyronine, for desoxyribonucleic acid (DNA) and ribonucleic acid (RNA) respectively. In these sections the reticulum cells took essentially no stain. The nuclei of the new lymphocytes were well stained with methyl green, indicating a considerabe degree of deposition of DNA in these cells, in contrast with the reticulum cells. As in the case of the other stain fine gradations in the amount of methyl-green stained nuclear material could be seen at the periphery of the groups of new lymphocytes. In contrast to these new cells, the remains of the nuclei of old lymphocytes were of a rather pale but distinct pink color in such sections. Other tissues were implanted from the rabbit onto the CAM of the chick for comparison with the observations made with the lymph node. In the case of rabbit spleen similar changes were observed to those described above. As control material, muscle, liver and kidney were similarly explanted. In each case there was outgrowth of various cell types, but none of these showed the development of reticulum cells or of lymphocytes. Of a total of over 100 graftings of lymphatic tissue undertaken thus far, more than 95% have been successful. Attempts to transplant explants to the CAM of other eggs have not thus far been successful.

Discussion. Several observations of those recorded above offer evidence of the successful grafting of the slices of rabbit lymph node on the CAM of the chick embryo. Among these is the intense vascularization of the area between CAM and implanted tissue, (with the nucleated erythrocytes in the capillaries indicating the origin of these blood vessels), and the position of the new reticulum cells and lymphocytes at the junction of CAM and implanted lymphatic tissue (indicating that elements from the explanted tissue were undergoing proliferation). The possibility was, of course, considered that the new lymphocytes found in later days might have emerged from the capillaries of chick blood, rather than have developed from the rabbit tissue, but several considerations seemed to make this possibility extremely remote. First, few leukocytes were seen within the capillaries of chick blood, and these were rarer outside the capillaries. Of those seen in the capillaries the great majority were neutrophils. Second, new lymphocytes were not found in all sections, although vascularization was in evidence in every case. Third, the distinct groups of lymphocytes mentioned above were not at all necessarily near capillaries but were always bordering on, or in immediate proximity to, the bands or groups of reticulum cells. In some cases, as shown in Fig. 3, the nest of lymphocytes was completely surrounded by reticulum cells. Finally, very subtle gradations in histologic form could often be seen by both stains between reticulum cells and lymphocytes at the junction of the two cell types. It would then appear to be entirely likely that these new lymphocytes were derived from the rabbit tissue, probably by some process of conversion from the reticulum cells which appeared first.

The chronologic appearance of reticulum cells and then, in close proximity, lymphocytes, is consistent with the findings of Jaffe and Richter on whole-organ transplants(5), and with results previously reported from this laboratory(6). In the latter study ribonucleic-acid-staining granules appeared in reticulum cells of the popliteal lymph node of rabbits 3 days after the injection of antigen into the foot pad. Five days after the injection of antigen these granules and crescents were seen in lymphocytes. The histologic characteristics of the ribonucleic-acid granule-containing cells changed in a manner which suggested that the lymphocytic hyperplasia known to occur in the lymph node after a local injection of antigen and during the period of antibody formation(6-8) was primarily a diffuse reticulum-cell hyperplasia, with gradual conversion of reticulum cells into lymphocytes. The observations made in the current study suggested in another way that the reticulum cells may be the immediate precursor of the lymphocyte, and that some observations which might be taken to imply growth of lymphocytes may in actuality imply multiplication of reticulum cells with subsequent conversion or maturation of these cells into lymphocytes.

Summary. Rabbit lymphatic tissue was placed on the CAM of the developing chick embryo and after incubation of 3 or more days evidences of successful grafting were obtained. Histologic sections of the tissues involved showed:

1. Appearance of many capillaries filled with nucleated erythrocytes.
2. Degeneration of the lymphocytes in the implanted tissue.
3. Appearance of bands of reticulum cells in a layer between the implanted lymph node and the CAM.
4. On occasion fresh mature lymphocytes appeared, always in proximity to the reticulum cells in the direction of the CAM. These cells occurred either in clusters at the periphery of the band of reticulum cells or in nests among the cells.

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6. Harris, T. N., and Harris, S., *J. Exp. Med.*, 1949, v90, 169.

7. Ehrich, W. E., and Harris, T. N., *J. Exp. Med.*, 1942, v76, 335.

8. Harris, S., and Harris, T. N., *J. Immunol.*, 1949, v61, 193.

Received February 14, 1950. P.S.E.B.M., 1950, v74.