

## Morphological Changes in the Rabbit Appendix Following Thymic Transplants<sup>1</sup>

The rabbit appendix has attracted the attention of investigators in recent years because it is histologically similar to the thymus but functionally analogous to the bursa of Fabricius<sup>2,3</sup>. The morphogenesis of the appendix is also unusual because it differs from other interstitial lymphoid tissue<sup>4</sup>. Both neonatal appendectomy and thymectomy are necessary to render the rabbit relatively unresponsive to bovine  $\gamma$ -globulin, but either procedure will significantly reduce its antibody response<sup>5</sup>.

Response to antigens, whether soluble, particulate or transplanted, has not been found in thymic tissue, possibly because of its 'blood barrier'. However, reactive changes in the appendix have not been studied. This report describes the finding of reticular cells in the appendix of rabbits that have received transplants of homologous thymic tissue.

Approximately 0.5 cm<sup>3</sup> of fresh homologous thymic tissue was transplanted beneath the kidney capsule of adult New Zealand rabbits. One donor was used per recipient, and the animals were sacrificed 4, 24, 48, 72 and 96 h and 7 days after the procedure. For comparison, tissues were obtained from the donor and sham-operated rabbits, and from animals that had received an i.v. dose of 10 mg, 7S human  $\gamma$ -globulin. The lymphoid tissue in each animal was studied histologically. A total of 106 animals was used for the study, and 2 sections were prepared from each appendix. Reticular cell counts in a 0.0784 mm<sup>2</sup> area were made at random in the cortico-

medullary region of the developed follicle at the luminal end. Analyses of the counts were expressed in terms of the Student *t*-test, and visual evaluations of the number and confluency of reticular cells were tabulated.

The appendix of the adult rabbit is a lymphoid structure composed of closely packed mononuclear cells separated by connective tissue septa containing blood vessels and lymphatics. The luminal portion is dome-shaped and covered with modified intestinal epithelium composed of one to several layers of cuboidal cells. A normal control section of this portion is illustrated in Figure 1. The lymphocyte is the predominant cell, but scattered retic-

<sup>1</sup> In conducting the research reported herein, the investigators adhered to 'Guide for Laboratory Animal Facilities and Care' established by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, NAS-NRC.

<sup>2</sup> O. K. ARCHER, D. E. R. SUTHERLAND and R. A. GOOD, *Nature* 200, 337 (1963).

<sup>3</sup> M. D. COOPER, D. Y. PEREY, M. F. MCKNEALLY, A. E. GABRIELSON, D. E. R. SUTHERLAND and R. A. GOOD, *Lancet* 1, 1388 (1966).

<sup>4</sup> O. K. ARCHER, D. E. R. SUTHERLAND and R. A. GOOD, *Lab. Invest.* 13, 259 (1964).

<sup>5</sup> R. A. GOOD, R. D. A. PETERSON and A. E. GABRIELSON, *Immunological Diseases* (Ed. M. SAMTER; Little Brown and Co., Boston, Mass. 1965), p. 52.

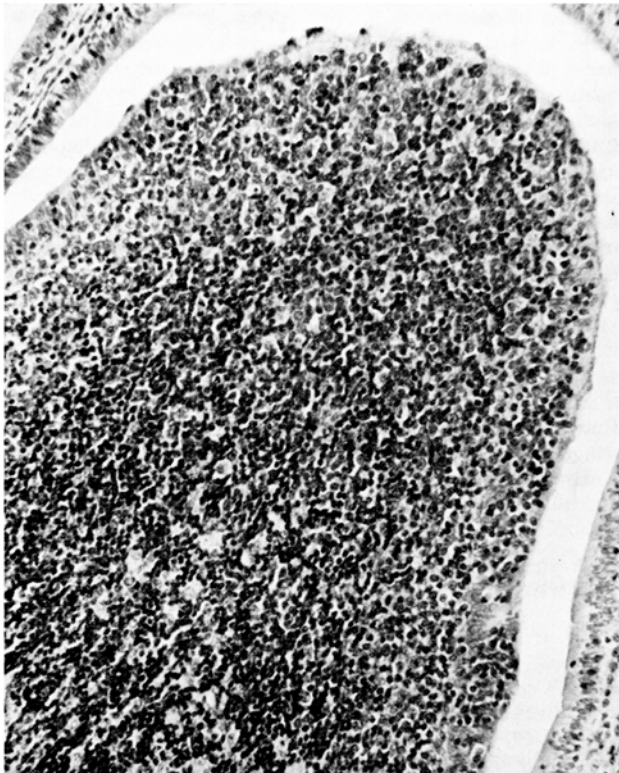


Fig. 1. This section through the apical portion of a normal rabbit appendix illustrated its lymphoid composition. The luminal surface is covered by modified cuboidal epithelium, which is continuous with the intestinal epithelium. At the lower left, adjacent to medulla (not shown), are several large, pale reticular cells. Hematoxylin and eosin,  $\times 210$ .



Fig. 2. Several aggregates of the large, pale reticular cells can be seen in this field of the appendix from an animal that had received a homologous thymic transplant 96 h previously. Note the septum separating 2 confluent areas in the upper portion of the photomicrograph. Hematoxylin and eosin,  $\times 155$ .

ulum cells can be seen. In the subserosal portion, a cortex and medulla are more distinct, and numerous cortico-medullary cells can be identified. It is this basilar portion that closely resembles the thymus, particularly when it appears to be separated from the dome.

Striking changes were found in the histological appearance of appendices from rabbits implanted with homologous thymic tissue. The reaction was characterized by a progressive increase in the reticular cells. The earliest

changes were apparent 4 h following surgical transplantation, and islands of these cells were noted by 48 h. The most marked changes, both in reticular cell numbers and confluency, were seen at 96 h (Figure 2). By the seventh day the histological appearance of the appendix approximated that seen in the donor animals. The reactive reticular cells were diffusely scattered throughout the dome, but in the basilar portion, which has a thymic architecture, the reticular cells were frequently aggregated along the septa and cortico-medullary zone. The nuclei of the reticular cells became more vesicular, the area of the cytoplasm was reduced, and mitoses were frequently noted. There was no evidence of a granulomatous reaction, giant cell formation, or an increase in the number of plasma cells. Likewise, thrombosis, necrosis and polymorphonuclear cells, were not seen.

Cell counts confirmed the histological findings, and analysis demonstrated a statistically significant, progressive increase in reticular cells (Table). The animals injected with 7S  $\gamma$ -globulin showed a less marked increase in cell number, and the  $p$  values ranged from 0.9–0.001. The sham-operated animals showed a  $p$  value of  $> 0.9$ . Figure 3 is a histogram of the cell counts from the transplanted and  $\gamma$ -globulin-injected groups.

Histological examination of the transplant site showed the progressive changes in graft rejection.

The most conspicuous component of this histologic reaction was the large reticular cell with faint staining, eosinophilic cytoplasm and a relatively vesicular nucleus. Somewhat similar morphologic changes have been noted on occasion in random sections of rabbits autopsied in this laboratory, but the reticular cells were never so numerous, nor did they occur in aggregates. For this reason a large number of control animals was used for comparison, including sections from all donor animals.

The reaction is apparently non-specific because it was also seen in several of the  $\gamma$ -globulin-injected animals. While the reticular cell counts in these sections exceeded the first standard deviation of control values, the reaction was not comparable in intensity to that in the transplanted animals, although the counts were statistically significant ( $< 0.001$ ) at 3 of the 6 time intervals.

The origin of the increased number of reticular cells is unknown. The time of their appearance does not preclude local proliferation or transformation from another cell type, and the frequent mitoses suggest a contribution, at least, by the former. The reaction is evanescent, and the fate of the reticular cells is unknown. By the seventh day, the sections from transplanted animals could not be distinguished with certainty from those from the control groups. This reaction in the appendix appears to be unique, and the model should be useful in studying the reaction to a variety of antigens.

*Zusammenfassung.* Es wird nach Transplantation von homologem Thymusgewebe in den Blinddarm beim Kaninchen eine Proliferation des retikulären Gewebes erzeugt, was der Reaktion auf Gammaglobulin sehr ähnlich ist.

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Arithmetic and visual grading of reticular cell changes

Time in h	No. of sections	Mean of cell count	$p$	Reaction severity	
				Cell No.	Confluency
Control	56	13.8	-	+	-
<b>Transplant</b>					
4	12	13.8		+	-
24	12	19.9	$< 0.001$	++	-
48	12	33.4	$< 0.001$	+++	+++
72	12	36.9	$< 0.001$	++++	+++
96	12	36.2	$< 0.001$	++++	+++++
188	12	19.7	$< 0.001$	+	-
<b><math>\gamma</math>-Globulin</b>					
4	12	13.9	$> 0.9$	+	-
24	12	18.4	$< 0.001$	++	-
48	12	18.9	$< 0.001$	++	-
72	12	16.0	$< 0.1$	++	-
96	12	21.4	$< 0.001$	+	-
188	12	13.3	$< 0.7$	+	-
Sham	12	13.3	$< 0.9$	+	-

The counts were made in a standard area of 0.0784 mm<sup>2</sup>.

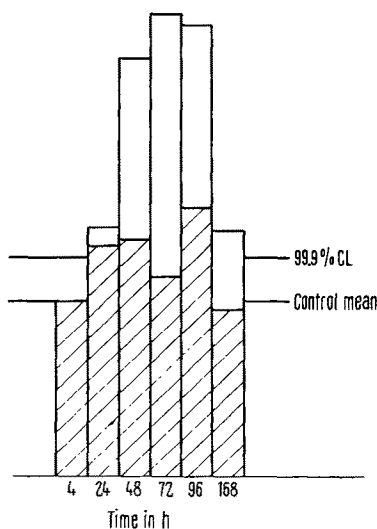


Fig. 3. This histogram illustrates the cell counts/unit area (vertical axis) for specimens at each time interval (horizontal axis). The open bars represent the values for transplanted animals, and the cross-hatched area, those from animals that had received 7S human  $\gamma$ -globulin. C.L., confidence level of 3 standard deviations.