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The Vascular Supply of the Thymus in the Guinea-Pig and Pig

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Summary. A study of the blood supply of the thymus using intravascular carbon or silver shows that the pig and guinea-pig possess a more extensive vascular system than the current model taken from work on the mouse.

INTRODUCTION

During a series of histological studies on the activity of the thymus in both pig and guinea-pig in untreated and antigenically-stimulated animals it became clear that the vascular supply of this tissue differed from the 'model' circulation found in many texts, which appear to be derived from the mouse, and that these differences had important functional implications. This study was therefore undertaken to determine the vascular supply of the thymus in experimental animals and to compare the results with those of previous workers on other species.

MATERIAL AND METHODS

Animals

Twenty-eight guinea-pigs (Dunklin–Hartley strain) weighing between 250 and 1000 g together with twelve pigs (Large White × Wessex) weighing approximately 30 kg were used.

Intravascular silver injection (Olson and Skuse, 1969)

Ilford G5 Nuclear Emulsion was first exposed to strong light, diluted 25 per cent with heparin (Heparin Injection B.P. 5000 units/ml) and warmed to 40° in a water bath before being injected in a similarly warmed syringe. Under Nembutal anaesthesia the thoracic cavity was opened and the emulsion introduced through a No. 1 needle into either the right or left ventricle under direct vision. Care was taken not to distend the ventricle in order to introduce the fluid at a 'physiological' pressure. It was usually possible to inject 1·5 ml of the emulsion before the heart stopped beating and leak-back occurred; this usually happened within 30 seconds after commencing the injection. Tissues were removed immediately after ceasing the injection and fixed in Bouin's or Zenker's fixatives. Serial sections varying in thickness from 2 to 7 μm were mounted on standard glass slides, de-waxed, taken through the alcohols to water and placed in photographic developer for 1 hour or more. After brief washing they were stained with nuclear fast red, haematoxylin and eosin, or Dominici's stain.

After dehydration and clearing, the sections were covered with a glass coverslip, mounted in polystyrene mounting medium and examined using a Leitz Ortholux Ultropak system (as described by Rogers, 1964) which was capable of alternate or combined transmitted and incident light illumination.

Incident light illumination showed the developed grains as shining gold–silver in colour. Photographs were taken with a Leitz Orthomat automatic camera using double exposures from each source in turn.

Fourteen guinea-pigs were studied using this technique.

Carbon injection

Indian ink (Pelican) either undiluted at 20° or in a 50 per cent dilution with gelatine at 40° was injected as described above into either the left or right ventricle of the heart and serial sections of the thymus were prepared as above. In eight guinea-pigs the thymus was carefully exposed, bathed in warm physiological saline, and observed with a Watson dissecting microscope during the injection process.

RESULTS

DIRECT OBSERVATION

Although the interval between starting the injection and observing carbon particles in the thymus varied between 90 and 120 seconds the sequence of events was the same in all eight animals. Firstly, the interior of the gland would slowly darken and this stage would be followed by a sudden filling of the perilobular network of draining veins. These events were never uniform or complete over both lobes of the thymus and some lobules never

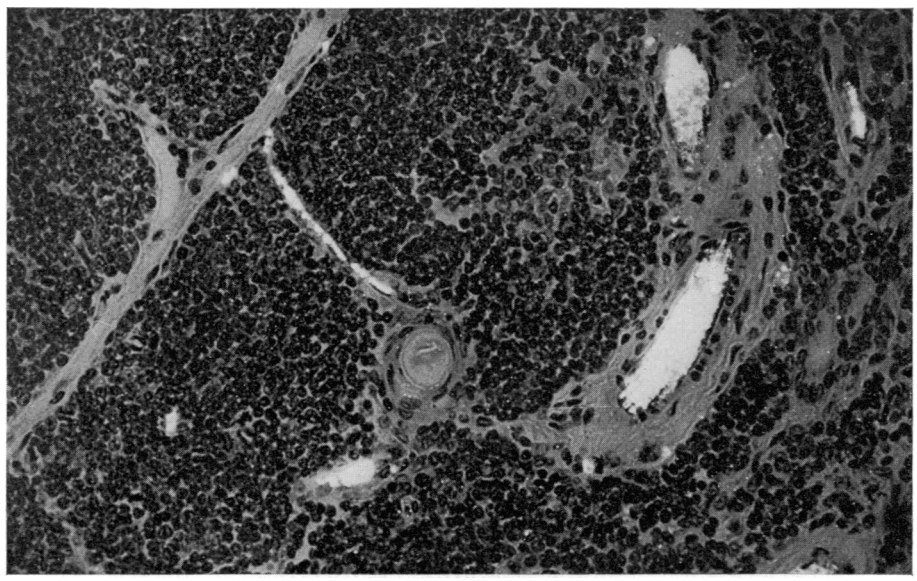

FIG. 1. Guinea-pig thymus after intravascular silver injection. By combined incident and transmitted light the silver is readily seen in a large medullary artery and a fine penetrating cortical capillary. (Nuclear fast red ×200.)

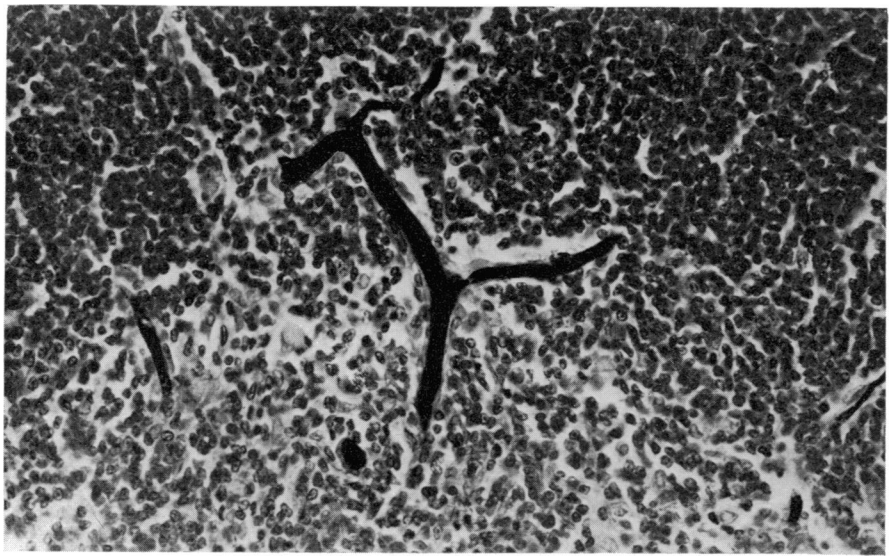

FIG. 2. Pig thymus after intravascular carbon injection. An example of the branching corticomedullary capillary network. (Nuclear fast red × 320.)

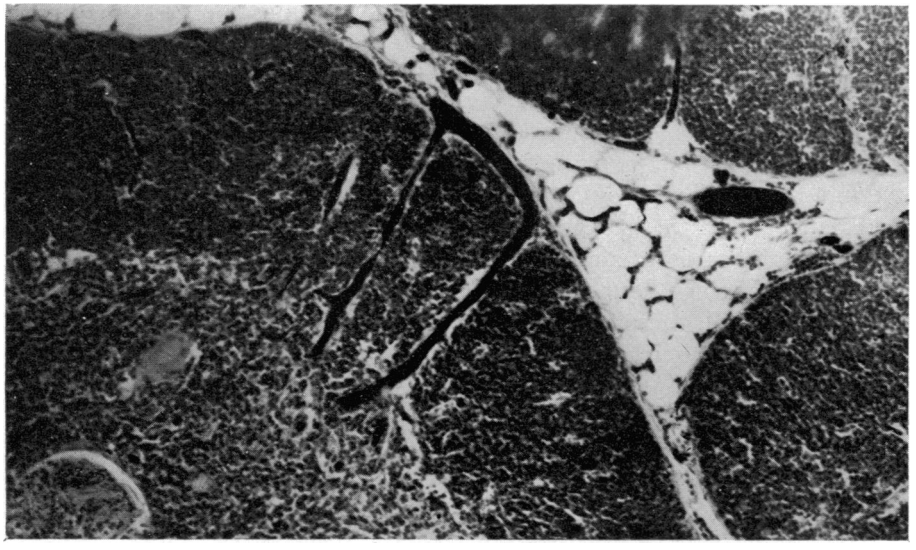

FIG. 3. Guinea-pig thymus after intravascular carbon injection, demonstrating the rows of parallel thin-walled cortical capillaries emptying into the perilobular venous drainage system. (Nuclear fast red × 144.)

darkened during an observation period of 3 minutes. On histological section this variable filling of the lobules was confirmed.

HISTOLOGY

As previously described (Olson and Skuse, 1969) silver emulsion injection has the advantage of displaying the finer blood vessels without obscuring their histological detail. The coarser carbon injection, on the other hand, gives a better picture of the larger vessels, both arterial and venous, especially for low power examination and photography.

A composite description of the vascular circulation of the guinea-pig follows; it does not differ from that found for the pig using carbon injection alone.

On serial section the interlobular arteries were seen to enter the medullary junctions of two or more lobules and sub-divide into a large number of smaller branches. These either traversed the medulla or ran along the corticomedullary junction, Figs 1 and 2. They then angled sharply into the cortex as rows of parallel, thin-walled vessels, Fig. 3. These penetrating vessels sometimes branched and varied in diameter, often becoming large venules before emptying into the system of perilobular veins described above.

In addition, we observed a network of veins along the corticomedullary junction which accompanied the medullary arteries. It was not as prominent as the perilobular venous system.

DISCUSSION

It becomes clear from a study of current texts, whether immunological or histological, e.g. Bellanti, 1971; Passmore and Robson, 1971, that the vascular pattern described for the thymus derives from the extensive studies on the mouse thymus performed by Christianna Smith and her co-workers (1952). Using pressurized injection of India ink she demonstrated that subdivisions of medullary lobar arteries radiated towards the periphery of the cortex where an anastomotic meshwork of capillaries returned the blood to medullary veins. In the discussion of her findings she points out that the mouse thymus differs from that of most other species described in that it has a single medullary venous drainage system and thus lacks a perilobular cortical venous drainage. We feel that the limited blood supply of the mouse thymus is reflected by limitations on its activity and that this state of affairs has resulted in a restricted view of thymic function as a whole. It is apparent from a study of the experimental literature that the theory that the thymus possesses a single subcapsular zone of cell proliferation derives almost exclusively from work on the mouse (Poste and Olson, 1973). Species which have a double vascular circuit such as the rat (Sainte-Marie and Messier, 1969, 1970), the pig (Olson and Hunt, 1969) and the guinea-pig (Poste and Olson, 1968) also have other zones of proliferation even in the unstimulated state. We would suggest, therefore, that the vascular supply of the mouse thymus should be recognized once more as atypical and if any model be required it should be that of the dual circuit system with its additional functional implications.

ACKNOWLEDGMENTS

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