THE THYMUS GLAND IN THREE GENERA OF RHINOCEROS

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(With 4 plates and 3 figures in the text)

The morphology and structure of the thymus gland is described for the first time for single specimens of the Great Indian rhinoceros (*Rhinoceros unicornis*), the Sumatran rhinoceros (*Didermocerus sumatrensis*) and the African Black rhinoceros (*Diceros bicornis*).

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INTRODUCTION

The function of the mammalian thymus gland remains still obscure, despite the considerable attention devoted to thymus embryology and anatomy and to the experimental investigation of that organ. Knowledge of its ontogenetic development, topography and life history is based essentially upon studies made on the human subject and upon the commoner domesticated and laboratory animal species. Little, if anything, is known of thymus morphology in the majority of mammalian forms, including the Rhinocerotidae. This want of detailed information is readily explicable. Material from non-domesticated animals is but seldom available and even when available the small and relatively unimportant pre-pericardial thymus is easily overlooked or destroyed in the physical examination and removal of the thoracic viscera. Unlike the other endocrine organs with which it is usually classed, it has no known function and hence does not invite the particular attention of the pathologist. Moreover, since the organ is known to undergo a more or less complete involution or atrophy about the time of puberty, only its scanty remains are to be expected in the mature animal.

Studies in thymus embryology and veterinarian anatomy would suggest that, in all mammals, the thymus follows pretty closely a common constituent pattern, i.e. that it comprises two more or less elongated, contiguous yet discontinuous, strips of tissue, descendent from the neck into the pre-pericardial region, wherein each strip expands into a relatively large and distinct lobe.

Such bilobate disposition is obtrusively evident during early life, but is less recognizable in the adult animal, following the normal atrophy of the thymus

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at puberty. Between various species minor differences are apparent, both in the original extent and topography of the gland and in the degree of atrophy achieved before the attainment of adult life. Thus in *Equus caballus* the gland manifests a distinct cervical portion in the foal but is absent, totally or subtotally, in the adult animal. The calf of *Bos taurus* presents a relatively larger cervical thymus, but the adult shows the merest remnants of the gland. In *Felis domestica* the thymus in the young occupies the lower neck and ventral thoracic region: in the adult its diminished remnants are confined to the prepericardial region. No cervical portion is encountered in the young of *Canis familiaris* and the thymus appears to be totally wanting in the adult animal.

The literature on the anatomy of the Rhinocerotidae contains no account of either the gross anatomy or the histological structure of the thymus gland in any species of rhinoceros. Concerning the African members of the family, no description has been given to date of any of the major viscera of the Black rhinoceros (*Diceros bicornis*) or of the White rhinoceros (*Ceratotherium simum*). The Asiatic species have been the subject of recurrent anatomical investigation, particularly the Great Indian rhinoceros (*Rhinoceros unicornis*), the form most commonly seen in zoological menageries and one thriving well in captivity. Even so, relatively few morphological accounts (and those imperfect) are available for consultation, and in none is any reference made to the thymus gland.

Thus Owen's (1850) classic monograph on the anatomy of *Rhinoceros unicornis*, a work which took cognizance of all earlier publications on the subject, omits all reference to, and delineation of, the thymus. Yet, presumably, Owen was acquainted with this organ for he states later (1868) that, " in the Rhinoceros ", the thymus is much like that of the foetal elephant, namely, " a flat mass beneath the anterior part of the pericardium " which " encroaches but a little upon the neck ". This statement must be assumed to apply to *R. unicornis*, this being the only species dissected by Owen personally.

Nothing of the thymus is depicted in the 34 unpublished folio plates—listed by Gervais & Gervais (1875)—of *R. unicornis* organs preserved in the Muséum National d'Histoire Naturelle, Paris, prepared by Maréchal and by P. J. Redouté *père et fils*, from the adult male animal dissected by Vicq d'Azyr and Mertrud in 1793 at the Royal Menagerie at Versailles: neither does Vicq d'Azyr's accompanying but unfinished text contain any mention of the thymus.

Burne (1905) published notes on *R. unicornis* histology in an adult male animal, but these do not include any account of the histology of the thymus. Oppel (1896–1914) likewise is silent regarding thymus structure in any rhinoceros species.

For the Javan rhinoceros (*Rhinoceros sondaicus*) nothing is mentioned of thymus anatomy by either Garrod (1877) or Beddard & Treves (1887), nor for the Sumatran rhinoceros (*Didermocerus sumatrensis*) by Garrod (1873) or by Beddard & Treves (1889).

The absence from the literature of any explicit reference to the rhinoceros thymus, and the increasing rarity of rhinoceros material (particularly of the Sumatran species) is deemed sufficient justification for the submission of the

present observations, which constitute a description of the gross and microscopic anatomy of the thymus gland in the three rhinoceros forms *Rhinoceros unicornis*, *Didermocerus sumatrensis* and *Diceros bicornis*.

MATERIAL AND METHODS

The thymus gland was carefully dissected free from the ventral aspect of the unopened pericardium after removal of the intact heart in an adult male Great Indian rhinoceros (*Rhinoceros unicornis*) and in a very young female African Black rhinoceros (*Diceros bicornis*). The former animal ("Mohan") died in Whipsnade Zoological Park on 8th March 1961 at a conservatively estimated age of 18 years: its skeleton is preserved (B.M. 1961. 5.10.1) in the British Museum (Natural History). The latter animal died in the Whipsnade Park on 28th December 1960 at an estimated age of 2–3 years: its skeleton is likewise preserved (B.M. 1961.1.31.1) in the British Museum (Natural History).

From the reflected, but otherwise undamaged, pericardial sac of an old female Sumatran rhinoceros (*Didermocerus sumatrensis*), with fibrosed, atrophic ovaries, the thymus was dissected in its entirety. This animal died on 9th September 1961 in the Zoologischer Garten, Basel, and was very thoroughly necropsied by Dr S. Lindt, of the Veterinär-pathologisches Institut of the University of Berne. Its skeleton is preserved in the Basel Naturhistorisches Museum, together with certain of the viscera, which, by courtesy of Dr L. Forcart, Curator of its Department of Zoology, were kindly placed at the writer's disposal.

The three thymus glands were formalin-preserved prior to anatomical and histological study. The unavoidable method of their collection precluded absolute certainty as to the exact limit of their craniad extension, although gross appearances were strongly suggestive of confinement exclusively to the intra-thoracic region. A natural size drawing was made of each gland before its dissection. For microscopical examination tissue from each gland was paraffin-wax blocked, sectioned at 10 μ and stained by haematoxylin and eosin, by van Gieson's stain and by Weigert's elastin stain.

A lymph node situate in the cranial portion of the *Didermocerus* thymus was serially sectioned at 10 μ and the sections stained by haematoxylin and eosin.

OBSERVATIONS

The general appearance and proportions of the thymus gland of the adult male specimen of *Rhinoceros unicornis* are depicted in Pl. 1. The total weight of the organ is 85 gms, of which 50 gms represent the weight of a curious ovoid pseudo-glandular mass of adipose tissue situated at its caudalmost extremity. The thymus comprises two thin paramedian lobes or strips of densely-encapsulated fibro-fatty tissue, confined tc the retrosternal region. In their hinder moieties these paramedian lobes are united across the midline by a bridge of parenchyma, whereafter the gland trifurcates into slender processes which proceed further caudally to become continuous with the ovoid

mass, which proves histologically to be composed entirely of adipose tissue. The thymic lobes are highly vascular and the topographical disposition of their parenchyma appears to be determined by the vascular pattern. Each principal (paramedian) thymic lobe receives, at its cranial extremity, an artery which proceeds caudalwards, branching as it does so, the vessel of the sinistral lobe

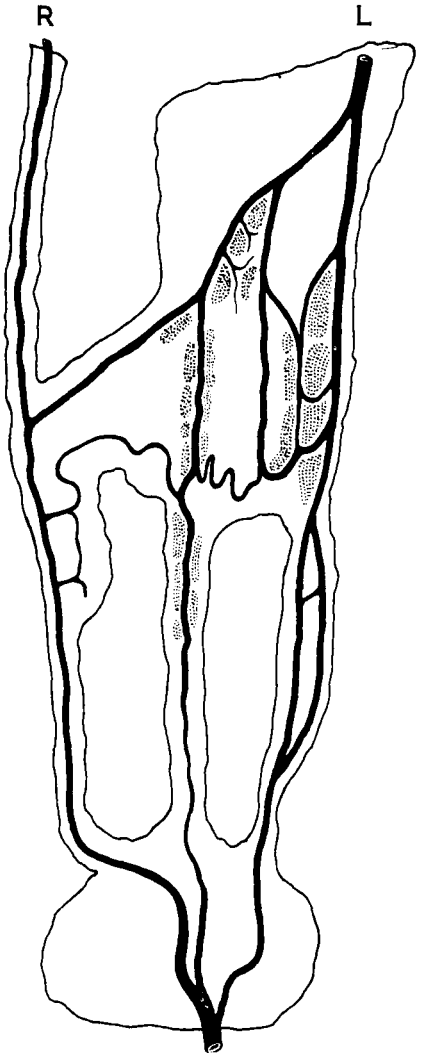

Fig. 1—*Rhinoceros unicornis*. Blood-vessels of thymus (diagrammatic): remnants of parenchyma indicated by stippled areas.

providing three parallel cranio-caudal branches and one large and one small transverse anastomotic branch: these last cross the midline within a bridge of thymic tissue to join the dextral thymic vessel. Within the thymus lobes, the smaller arterial branches of the main vessels form anastomotic loops or arcades, which enclose lobules of thymic parenchyma. The two primary thymic lobar

arteries appear to be reinforced posteriorly by a larger caudal artery, which sends three parenchyma-clothed branches cranialwards from the ovoid fat-mass to anastomose with their principal branches (Fig. 1). All these arteries are provided with relatively large venae comitantes. The precise origins of the right and left lobar arteries and of the caudal artery are not ascertainable.

The histological picture of this specimen is that of complete thymic involution, the organ consisting principally of fat and highly vascular connective tissue. The thick, fibrous capsule encloses numerous, relatively large blood vessels (some containing erythrocytes, others the laked remains of such), much adipose tissue and some extremely sparse lymphoid tissue. This last, in many of the sections studied, is represented by nothing more than a few scattered lymphocytes.

The relatively great number and size of the blood vessels is a striking histological feature. Some pigment inclusions are observable : such elastin as is present is beaded and much fragmented. The available histological evidence is intrinsically so unrepresentative of normal thymic structure that of itself it affords no clue to the provenance of the tissue under examination, the identity of which however is unequivocally established on topographical grounds. Functional activity had clearly long ceased in this particular thymus specimen, which represents the post-involutional organ of adult life.

The general appearance of the undissected thymus gland of the old female specimen of *Didermocerus sumatrensis* is illustrated in Pl. 2. Its weight is 45 gms. The organ consists topographically of two longish, flat paramedian lobes, within an extremely tough fibrous capsule, which become confluent about the beginning of their hinder moiety. A little caudad of this confluence the two lobes reassert their independence and each divides dichotomously towards its free posterior termination. In the angle formed by the divaricating terminal portions of the principal lobes there appears, on the dorsal surface of the organ, an additional linguiform process. A lymph node is embedded in the cranial extremity of each paramedian lobe. The consistency of the gland suggests its actual structure, viz. a mass of adipose tissue containing scattered patches of dense, fibrosed lymphoid tissue, the expected structure of the organ in so old an animal. Microscopically indeed its component tissue is unrecognizable as normal thymus tissue: it consists principally of fat admixed with lymphoid tissue, the whole enclosed within a remarkably thick, tough fibrous capsule. The lymphoid tissue present, scanty in quantity, is extremely dense : it lacks all differentiation into the customary cortex and medulla and reveals no evidence of Hassall's corpuscles. In some sections evidence appears of the attempted formation of secondary lymphoid nodules, such as occur in lymph nodes. Scattered fat cells are present throughout most of the remaining lymphoid tissue and there is everywhere the strongest histological evidence suggestive of the replacement of thymic tissue by fat.

The thymus is extremely vascular, as is apparent upon removal of its fibrous capsule. A right and a left thymic artery (of undetermined origin) supply the gland. Each artery enters the cranial end of the ipsilateral paramedian lobe and proceeds caudally therein, giving off small branches, to about

the middle of the gland, where the two vessels anastomose in the midline. Prior to this, however, they anastomose additionally by an oblique annectant branch which lies within the bridge of thymic tissue uniting the cranial moieties of the paramedian lobes. From the median anastomosis of the right and left thymic arteries two arteries proceed caudalwards, viz. a larger ventral, and a smaller dorsal, vessel.

The former gives off branches dextrally and sinistrally in fairly regular succession to the gland substance, and where the two paramedian lobes again become separate it divides into right and left terminal branches, each of which supplies the terminal forked portion of the ipsilateral lobe.

The latter proceeds caudalwards in the median plane without any branches of appreciable size, to terminate within the linguiform process (Fig. 2).

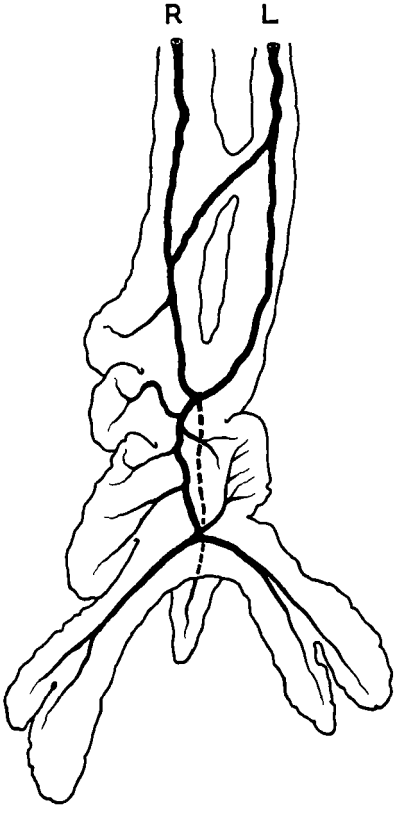

Fig. 2—*Didermocerus sumatrensis*. Blood-vessels of thymus (diagrammatic).

The lymph nodes within the gland's cranial portions show evidence of fibrosis, possibly of pathological origin. Their trabeculae are thicker and coarser than their stout fibrous capsules, and among the trabeculae a wide range of cell types is distinguishable which includes numerous young lymphocytes, older lymphocytes and many fibroblasts. Polymorpho-nuclear lymphocytes are absent. A fairly eosinophilic precipitate represents laked erythrocytes. The nodes are intensely vascular, with relatively large arteries and veins.

The overall anatomical picture, therefore, of this particular specimen is that of the involuted thymus gland of old age, with its lymphoid tissue reduced to a mere remnant and largely replaced by fat and with its vascular framework rendered consequently the more obtrusively prominent.

The macroscopic appearance and proportions of the undissected thymus gland of the young female *Diceros* specimen are illustrated to scale in Pl. 3. The organ is bilobed and weighs 60 gms. Each of its component lobes presents an elongated, flat and narrowish cranial portion, contiguous and partly adherent to its fellow in the median plane, and a widely-expanded caudal portion, of roughly triangular outline, clearly separated from its contralateral fellow. The cranio-lateral aspect of the expanded caudal portion of each lobe is joined by a narrow, highly vascular strip of glandular tissue, whose free cranial extremity is situated somewhat caudal to the cranial extremities of the principal lobes. This lateral strip (composed largely of blood-vessels within a fibrous tissue envelope) would appear to represent a lateral thyro-thymic ligament.

The whole thymus is enclosed within a tough fibrous tissue capsule. It is strikingly vascular in naked eye appearance, numerous and relatively large arteries and veins ramifying upon its surface, entering or leaving its parenchyma and uniting the two moieties of the organ. Evidence of adipose tissue is notably wanting.

Removal of the thick fibrous capsule, followed by careful dissection of the *Diceros* thymus, reveals in greater detail the macroscopic constitution of the gland. Its two principal lobes are seen to be compounded of numerous discrete subsidiary lobules, embedded in annectant connective tissue and each composed of a variable number of minor lobules, the basic units of the whole gland.

Thus the fully-dissected thymus has an appearance not unlike that of a vascular tree bearing minor glandular lobules for leaves, so that Galen's term *θύμος* for this organ is demonstrated to be not inapt (Fig. 3). Each minor lobule is a flattish, ovo-lanceolate mass of thymic tissue, dependent from its blood-vessels as a leaf from its stalk, and in outline much resembling that of the short-stalked, ovate, entire leaf of the thyme plant (*Thymus vulgaris*). (Galen's deliberate application of the botanical term to the nomenclature of the mammalian organ on the grounds of such close superficial similarity constitutes indirect, but cogent, evidence that he himself was familiar with the dissected thymus gland of some non-human (probably ungulate) mammal).

The intense vascularity of the thymus is more fully manifested by its dissection. Each major and each minor lobule is provided with its own vascular pedicle, the principal vessels thereof being relatively large. (Unfortunately the origin of the thymic arteries and the termination of the thymic veins are indeterminable in the excised specimen.)

Histologically the *Diceros* thymus presents an extremely thick capsule enclosing a little fat, relatively plentiful lymphoid tissue and a preponderance of highly vascular supportive connective tissue. Relatively little intracapsular fat is present. The lymphoid tissue, abundantly provided with small blood-vessels, tends to be somewhat diffusely disposed, with a particular aggregation

around and between the vascular bundles which permeate the entire organ. Embedded in connective tissue, it shows many plasma cells and lacks all differentiation into cortex and medulla. It is nowhere invaded by fat cells. Hassall's corpuscles are not recognizable with certainty. The connective tissue (stroma) which accounts for the bulk of the gland is extremely vascular : its contained blood-vessels are noticeably larger than those in the lymphoid tissue and many of them are lagged by perivascular condensations of dense fibrous tissue. The blood-vessels dominate the histological picture of all parts of the organ and constitute its most obtrusive histological feature.

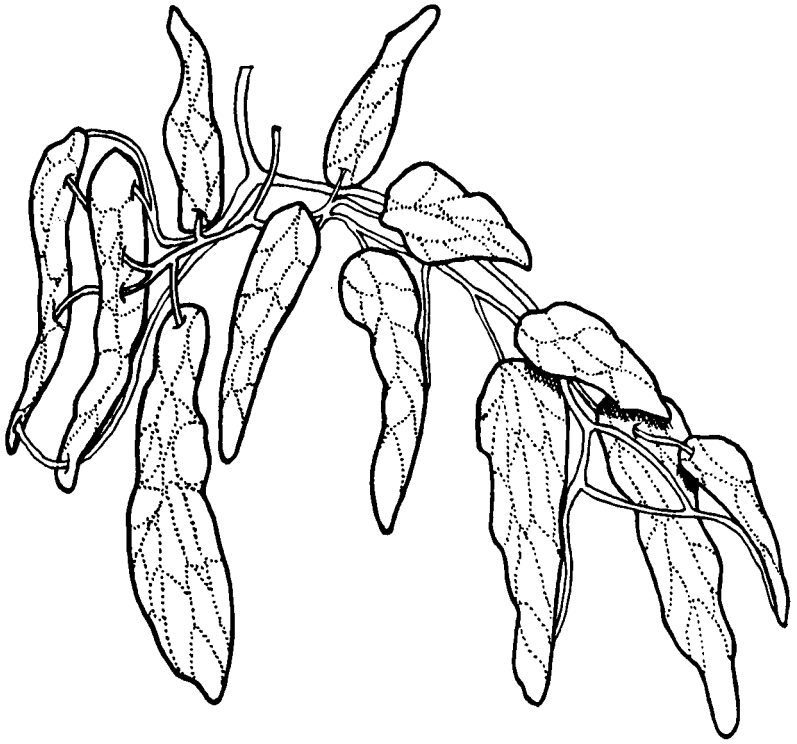

Fig. 3—*Diceros bicornis*. Portion of young thymus, fully dissected, showing constituent lobules and related blood-vessels.

The general microscopical evidence suggests an actively functioning organ which has possibly not yet attained its maximal physiological development. Such functioning activity is indicated by the elaborate intrinsic vasculature (reflecting a copious arterial supply to, and an adequate venous return from, each anatomical unit of the gland), by the relative abundance of normal lymphoid tissue and by the total absence of any evidence of its fibrosis or of its infiltration and replacement by fat.

DISCUSSION

Histological study of the present limited range of material necessarily provides impressions, rather than proof, of thymus gland function in the

rhinoceros. The available evidence, however, unequivocally indicates that the rhinoceros thymus, like that of other mammals, is essentially a lymphatic organ, an integral and significant component of the general lymphatic system, with which it maintains intimate physiological association. During its period of activity in early life it undoubtedly manufactures lymphocytes in quantity and it may well constitute the principal source of such cells pending the functional establishment of the general lymph nodes. Its physiological regression at, or following, puberty, conforms to the general mammalian pattern and is probably effected by the action of the steroid hormones of the adrenal cortex.

CONCLUSIONS

1. In *Rhinoceros*, *Diceros* and *Didermocerus* the thymus gland has the customary mammalian disposition of an intrathoracic (epipericardial) bilobed organ. Each principal lobe is composed of discrete lobules, dependent from the blood-vessels and embedded in supportive connective tissue.

2. Its intensely vascular lymphoid tissue content is not essentially dissimilar to lymphoid tissue elsewhere, save that it manifests no cortico-medullary differentiation and but little attempted formation of secondary nodules. Hassall's corpuscles are not certainly identifiable in the present material.

3. The relatively large thymic blood-vessels are strikingly abundant: their disposition suggests the copious discharge into the general circulation of some important product of thymus activity—most probably newly formed lymphocytes.

4. The histological structure of the thymus gland is strongly suggestive of the closest functional relationship with the lymphatic system.

5. The rhinoceros thymus undergoes the usual mammalian involution of puberty, whereby its lymphoid content is first infiltrated and ultimately replaced by fibrous and adipose tissue.

ACKNOWLEDGMENTS

Grateful acknowledgment is tendered to the Zoological Society of London for the gift of the *Rhinoceros* and *Diceros* specimens, to Dr L. Forcart and Dr S. Lindt for the loan of the *Didermocerus* material, to Dr F. J. Aumonier for authoritative histological opinion, to Mr Peter Cull and Mr W. Serumaga for the accompanying illustrations and to Mr T. J. McElwain, Mr S. Campbell-Smith, Miss Wendy Sanders and Miss Carol Martin, for valued prosectorial assistance.

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ADDENDUM

Since the above notice was written an additional thymus gland of *Diceros bicornis* has become available for examination by the death of a 4-years-old male animal at Whipsnade Park on 16th November 1962. The organ was removed entire and undamaged with the heart and pericardium : it was notably " lumpy " and adherent to the retrosternal aspect of the fibrous pericardium. It shows no cervical extensions nor anything in the nature of a possible thyro-thymic ligament, but consists simply of two intrathoracic, paramedian, asymmetrical moieties (the right being the larger), mutually contiguous, but not continuous, in the ventral midline. Its parenchyma, in section, is of a distinctly pinkish-red colour. The whole gland is embedded in a thick envelope of very dense and very tough fibrous tissue: dissected free from this, its weight is 85 gms. Two or three lymph nodes are intimately associated with the cranial portion of the right thymic lobe.

In its general morphology and in its abundant vasculature this young male *Diceros* thymus closely resembles that of the young female described above : its histology is suggestive of a relatively greater functional activity.

Each thymic lobe is descriptively divisible into subsidiary lobules more or less isolated from their fellows by an ensheathment of thick fibrous tissue, and each of these lobules is compounded of a number of minor lobules, the essential structural units of the gland. The completely dissected gland has the thyme-plant general appearance shown in Pl. 4, the lobular gland units depending leaf-fashion from their vascular pedicles.

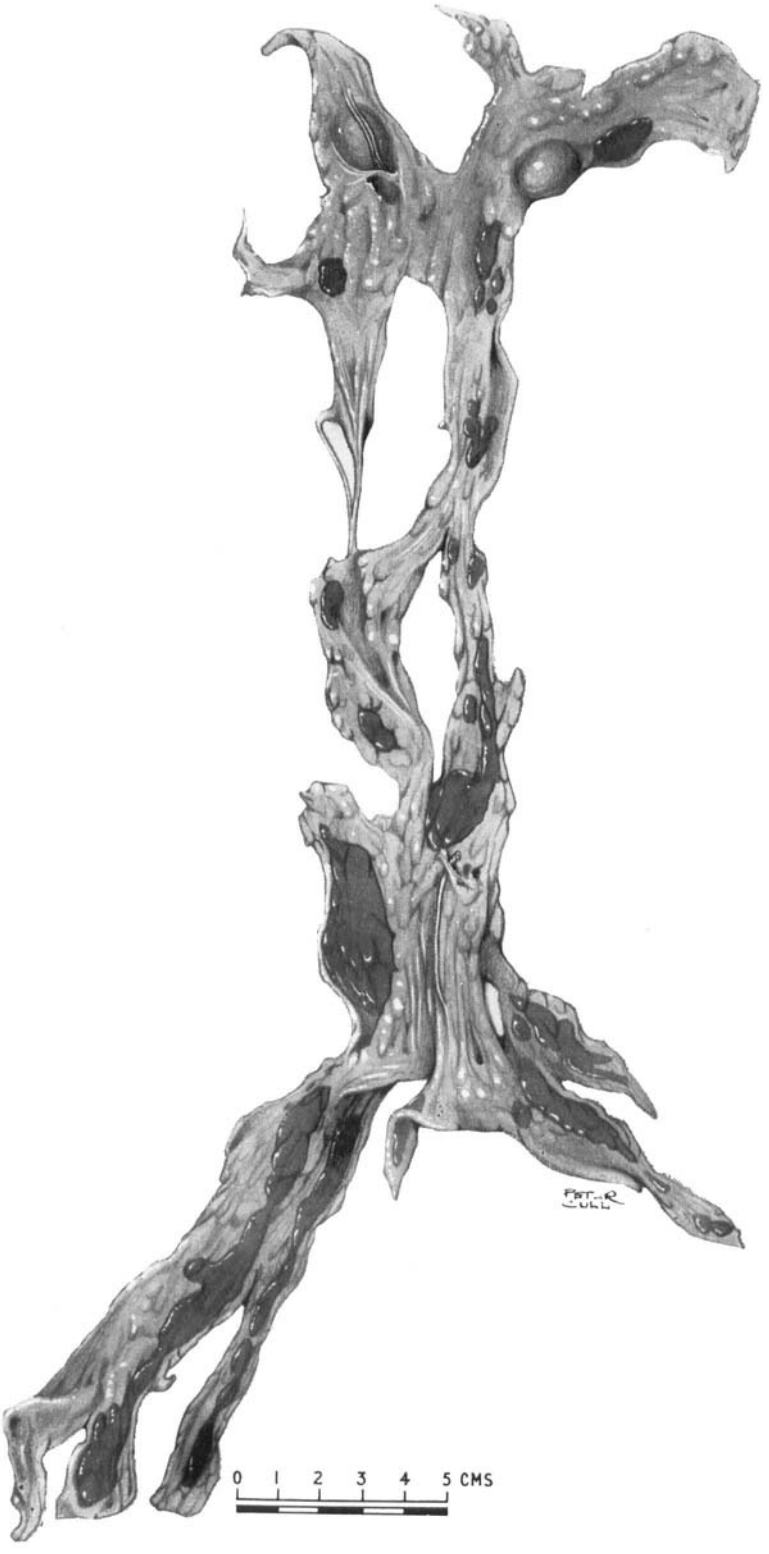
A specific thymic artery (given off presumably from the internal mammary artery) supplies each principal lobe of the thymus gland, wherein it proceeds cranio-caudally, branching and rebranching in dichotomous fashion. The larger branches of either thymic artery are an anterior (cranial), a middle and a pair of posterior (caudal) vessels, which supply the corresponding parts of the thymic lobe. No cross-anastomotic arterial vessel is recognizable. The finest of the arterial branches are the terminal twigs, which supply the individual minor lobules of the gland, and are the " stems " to its glandular " leaves ". The arteries lie wholly dorsal to the vein, and the pattern of venous drainage follows closely that of the arterial supply. Small peripheral veins from the minor lobules unite, usually in pairs (occasionally in threes), to form a set of larger veins, which are the tributaries of the principal thymic vein. This vessel is, in each thymic moiety, constituted in the caudal part of the gland lobe by the union of two pretty equal inferior (caudal) veins : it proceeds cranialwards within the lobe, receiving in its course distinct middle and anterior (cranial) major tributaries. Each principal thymic vein emerges medialwards from the cranial third of its lobe and unites with its contralateral

fellow in the median plane to form a single emergent vein for the entire gland. Close to this union, the dissectable veins from two lymph nodes embedded in the gland substance drain into the right thymic vein. The main or terminal thymic vein would appear to drain into the internal mammary system.

Histologically a minor lobule of the thymus gland shows a capsule of collagen, which sends inwards through the parenchyma a numerous series of stout collagenous trabeculae, dividing the gland substance very considerably. These trabeculae are accompanied by many, relatively large blood-vessels, surrounded by a highly cellular variety of areolar tissue in which extravascular macrophages (loaded with haemosiderin) occur in abundance, together with small lymphocytes. This palely-staining tissue represents such " medulla " as may be distinguishable : it has a curiously branched and diffused distribution throughout the gland tissue so as to isolate islands of " cortex ", i.e. darkly-staining areas, packed with small lymphocytes, in which pigment-containing macrophages also occur, having presumably infiltrated into these " cortical " areas along tbe blood-vessels. Thus the cortical and medullary portions of the parenchyma are not discrete, but considerably intermingled. No certainly recognizable Hassall's corpuscles occur in any of the sections. The parenchymal blood-vessels are relatively very large and everywhere abundant, in conformity with the macroscopic vascular appearance of the entire gland. Lymphatic vessels, in the shape of narrow, endothelial-walled tubes, are recognizable in many of the sections.

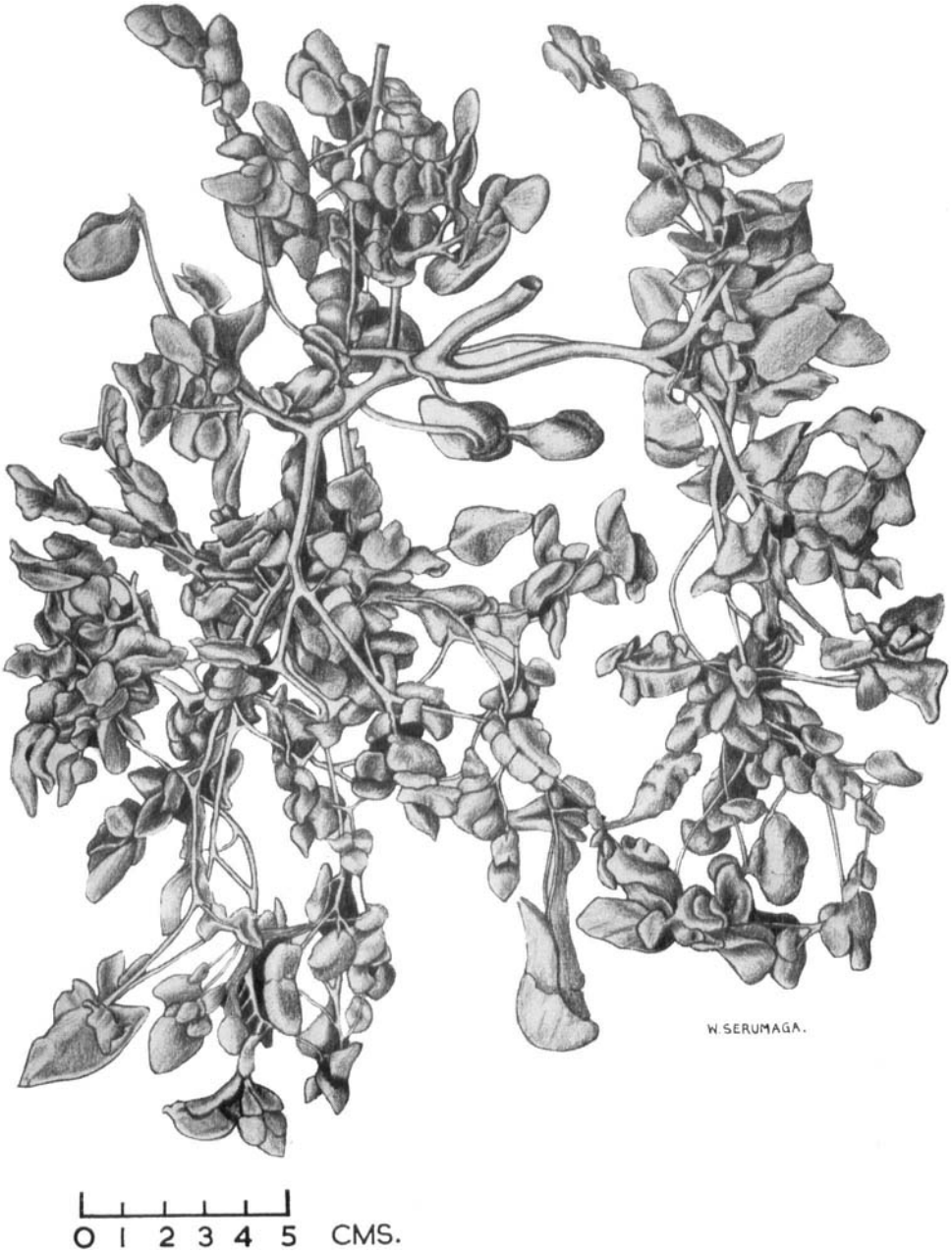
In essentials, therefore, the thymus of this additional *Diceros* specimen agrees histologically with that of the female specimen detailed above.

Thymus gland of *Rhinoceros unicornis*, ad. ♂



Thymus gland of *Didermocerus sumatrensis*, old ♀

Thymus gland of *Diceros bicornis*, juv. ♀



Thymus gland of *Diceros bicornis*, ♂, 4 years, fully dissected.