THE CIRCULATORY SYSTEM OF LISSEMYS WITH NOTES ON THE COMPOSITION OF BLOOD OF A FEW OTHER CHELONIANS AND SOME SNAKES

THE CIRCULATORY SYSTEM

HEART

Unlike other vertebrate hearts the chelonian heart is broader than long, dorsi-ventrally flattened and the apex of the ventricle almost rounded. In Lissemys (Figs. 80,81) it is shifted from the mid-ventral line towards the right. Besides, it also lies at an angle to the longitudinal axis of the body. The heart of *Testudo elegans* lies along the longitudinal axis of the body and does not show any tilting to the side. The displacement and the tilting of heart of Lissemys to the right seem to have occurred to provide room for the fully retractile head and the neck. In *Geomyda trijuga* also the heart is somewhat shifted to the right, though not to the same extent as in Lissemys. This slight shift of the heart in Geomyda is due to the less dome-shaped carapace than that of Testudo in which there is enough room for the retractile neck and the head.

The heart is covered by a tough, fibrous pericardial membrane which is attached to the apex of the ventricle by a thick strand of connective tissue, the gubernaculum chordis. In Geomyda the gubernaculum chordis is similarly developed, but in Testudo it is completely absent.

The right auricle is larger than the left one. The openings of the two auricles into the ventricle are separated from one another by the inter-auricular septum which projects slightly into the cavity of the ventricle. To this free end of the septum are attached the thin membranous auriculo-ventricular valves, the mesial valve on the right and the lateral valve on the left. These valves are larger than the apertures they guard. There are no chordae tendinae attached to the valves, a feature of the reptilian hearts.

The wall of the ventricle is made up of loosely arranged muscle fibres which gives a spongy texture to that organ. The ventricle is divided into right and left chambers by means of an incomplete septum. The right chamber (cavum pulmonale) is smaller than the left one (cavum dorsale). According to Mathur (1946) the inter-ventricular septum is formed by the union of the dorsal and the ventral septoid processes of the ventricular wall. The right auricle opens into the cavum pulmonale and also into the right side of the cavum *dorsale*. Due to the position of the right auriculo-ventricular aperture the *cavum pulmonale* gets filled first and when it is filled up completely, it flows into the right half of the *cavum dorsale*. From the left auricle the blood enters the left half of the *cavum dorsale* which is comparatively a spacious part. In the *cavum dorsale* a partial mixing of blood seems to take place.

THE ARTERIAL SYSTEM

Three main arteries arise from the right side of the ventricle on its ventral side. Of these the pulmonary trunk is situated on the left of the left systemic which is placed more towards the right side of the heart. These two trunks are visible when viewed from the ventral side. The third trunk, the right systemico-carotid arises almost dorsal to the left systemic. It gives out the right systemic which runs dorsal and after meeting its fellow of the left side, proceeds as the dorsal aorta. The three openings viz., of the pulmonary, left systemic and the right systemico-carotid, into the ventricle, are situated side by side and close to one another.

After issuing from the ventricle the pulmonary trunk runs anteriorly and the later divides into two pulmonary arteries, the right and the left one. The left one is longer than the right one. A pulmonary artery enters the lung with the bronchus of its side and later form fine capillaries round about the alveolar chambers.

The right systemico-carotid trunk gives off the right systemic immediately after its origin and then it branches off into two subclavian arteries of the right and the left sides. The subclavian artery gives off the the common carotid. The common carotid and the subclavian of the left side are longer than those of the right side. This is due to the shifted position of the heart to the right side. The right common carotid artery after arising from the right systemico-carotid trunk runs anteriorly and enters the neck but the left one after its origin runs parallel to the subclavian of its own side at the base of the neck for a considerable distance and then after a short journey through the trunk region it enters the neck from the left lateral side. Each common carotid passes ~~t~~ through a yellowish thymus gland of its respective side. On its way, it gives out small vessels to this gland. Then each carotid proceeds anteriorly along a course parallel to the vago-sympathetic nerve. At its middle each carotid gives off a branch, the vertebral-cervicular artery, which enters the spinal canal through the intervertebral foramen of the third and the fourth cervical vertebrae. This artery soon divides into an anterior and a posterior branch which supply blood to the corresponding regions of the spinal cord in the neck region. From the carotid there are various small branches arising throughout its course upto the base of the skull; these supply blood to neck muscles. The dorsal groups of muscles viz., the *spinalis* *cevico capitis*, *semispinalis* and the *longissimus* are supplied by the dorsal cervicular arteries. The ventral cervicular arteries supply blood to the ventral groups of the neck muscles viz., the *longus colli*, *inter-transversarii colli* and *i.t.c.obliquii* muscles. There are also some arteries arising from the common carotid in the neck region, the tracheal arteries which supply blood to the trachea. Now the common carotid of either side runs farther anteriorly to the base of the skull, and there it gives off a few principal arteries viz., the common hyoidean artery and the occipital artery. The anterior branch of the common hyoidean artery the lingual, supplies blood to the tongue muscles, while the two other vessels supply blood to the hyoid muscles. The occipital artery after arising from the common carotid runs to the dorsal side and supplies blood to the occipital muscles. The occipital artery then gives off a branch, the superficial temporalis artery, which supplies blood to the *depressor mandibulae*, *adductor mandibulae externus*, the *adductor mandibulae medius* and the *adductor mandibulae internus* muscles. After giving off the occipital artery the common carotid divides into the external and internal carotids. The external carotid supplies blood to the mandible, *masseter*, the *adductor mandibulae* muscles and the skin covering the head region. The internal carotid enters the skull through the carotid foramen after passing through the carotid canal of the pterygoid it enters the cranium. Here it gives off two branches, a lateral and a medial one~~s~~. The lateral one after running below the brain passes out anteriorly of the cranium through a foramen in the orbital region and enters the orbit where it supplies blood to the eye, eye muscles and the Harderian gland present in the orbit. The medial branch of one side meets its fellow of the other side forming an anastomosis just behind the hypophysis.

The right subclavian artery (~~Fig.~~) after getting off the carotid artery runs towards the lateral side and gives off a thyroideal artery which supplies blood to the thyroid gland. The right thyroideal artery gives off branches to the oesophagus, the *rectus cervicis* and the *retrachens capitis collique* muscles. Mention should also be made of the coronary artery which arises from the subclavian near the origin of the carotid artery. It runs backwards towards the heart and supplies blood to the heart muscles; a small branch of the coronary artery continues backwards and goes to the pericardial membrane. The continuation of the subclavian artery hereafter known as the axilary artery branches into two main vessels, the anterior and posterior brachials. The anterior brachial artery after its origin immediately gives off the scapular artery which supplies blood to the muscles present on the scapula, the trapezius and the rhomboideus muscles which are responsible for closing the anterior plastral valve. The anterior brachial after supplying blood to these parts runs on the dorsal side of the upper arm and gives off small branches to the triceps muscles. A deltoideus artery arising from the anterior brachial supplies blood to the deltoideus muscles and a branch of the deltoideus artery, the arteria profundus brachii artery, runs on the ventral side of the upper arm and then towards the proximal end of the forearm to supply blood to the forearm muscles at this region. An artery supplying to the biceps muscles also arises from the anterior brachial artery. The anterior brachial artery soon after this enters the forearm and immediately bifurcates into two main vessels, the radial and the ulnar arteries. The radial artery just after its origin gives off a radial recurrent artery which supplies blood to the proximal parts of the forearm muscles of the radial side. After this branch the radial artery proceeds to the dorsal side passing through the space between the radius and the ulna. On its way down the radial artery gives off various branches to the muscles of the forearm at this region. The radial artery after it emerges out on the dorsal side it continues as the *radialis indicus* artery which later divides into four branches to supply the muscles of the first four digits. The ulnar branch of the anterior brachial artery runs along the ulnar side of the forearm and supplies blood to the forearm muscles of this region through various branches. Finally it gives off a small branch to the muscles of the last digit.

The posterior brachial artery (Fig.81,83) is the longest artery in this region and gives off a number of branches of which the first one is the cervical artery which runs towards the base of the neck and enters the neck to supply the *rectus cervico-plastralis* and the *trapezius* muscles. From this cervical artery a branch, the *arteria intercostalis communis descendence* artery goes to the dorsal side and enters the gap between the neural plates, ribs and the trunk vertebrae and runs posteriorly backwards. From the arteria intercostalis communis descendens, at each vertebral space a thin intercostal artery is given off which runs laterally outwards along the sutural line of the costal plates of the carapace. These arteries supply blood to the inner as well as the outer muscle covering of the lung. These muscles correspond to the intercostal muscles, and the diaphragmaticus and the transverse abdominis muscles respectively. After giving off the cervical artery the posterior brachial artery runs laterally backwards along the margin of the carapace as the margino-costal artery. It runs along with the marginal nerve posteriorly. Dorsal to the pectoralis muscle a branch of the margino-costel artery supplies blood to the sub-coracoideus and the muscles of the scapular region. Another branch of the margino-costal artery pierces through the supra-coracoideus muscle and comes on the ventral side of the muscle along with the supra-coracoideus nerve to supply blood to the distal part of the pectoral muscle and the plastron at this region. The margino-costal artery runs backwards along the margin of the carapace and on its way gives off number of small branches, the arteria intercostalis which supply blood to the inner and the outer muscle coverings of the lung. The margino-costal artery extends posteriorly upto the level of the lateral pubic spine. A number of small branches besides these mentioned above arise from this artery which supply blood to the plastron.

The pectoral artery (Figs.80,83) arises from the subclavian separately and comes on the ventral side of the pectoral girdle piercing through the supra-coracoideus muscle and the acroméo-coracoid ligament to supply blood to the pectoral muscle.

The systemic trunks :- The right systemic trunk is shorter than the left one which after its origin from the right systemico-caratid trunk, immediately runs towards the dorsal side and proceeds posteriorly backwards to meets its fellow of the other side. The left systemic, on the other hand arises independently from the heart and runs parallel to the left pulmonary artery. The left systemic is longer than the right one. Both the systemics show a ligamentous connection with the pulmonary artery of their respective side. This ligamentous structure is the remnant of the defunct ductus of Botallus. The left systemic artery (Figs.80,81) after running for a short distance parallel to the left pulmonary artery goes over the oesophagus and the left bronchus, to the dorsal side. Thereafter it proceeds posteriorly to meet the right systemic to form a single vessel, the dorsal aorta. Before joining with the right systemic, it gives off a number of principal arteries of which the first one is the oesophageal artery which supplies blood to the oesophagus. The second one is the coeliac artery which immediately after its origin gives off three branches, the gastric, the duodeno-pancreatic and the hepatic ones. The gastric supplies the stomach, the duodeno-pancreatic the duodenum and the pancreas and the hepatic the liver. Next to the coeliac artery arises the anterior mesenteric artery from the left systemic. The anterior mesenteric artery branches out profusely supplying the small intestine, large intestine and the spleen.

The dorsal aorta (Fig.52) is shorter than that of the other reptilian groups. It is generally seen in the other groups of reptiles, that the coeliac and the anterior mesenteric arteries arise from the dorsal aorta, while in this animal as it is common in other chelonians also, these arteries arise from the left systemic. Just after its formation the dorsal aorta gives off a pair of renal arteries which run obliquely backwards towards the kidney. The renal arteries of each side on reaching the kidney gives rise to a gonadial artery which supplies blood to the gonads. The renal artery supplies blood to the adrenal gland by a separate branch. Next to the renal arteries a pair of vertebral arteries arise from the dorsal aorta. Each vertebral artery enters the vertebral column through the vertebral space between the sixth trunk vertebra and the corresponding neural plate lying above. After the vertebral arteries a large pair of vessels, the plastral arteries originate from the dorsal aorta. Each one of them runs towards the lateral side and passing on the dorsal side comes to the level of the anterior apex of the femoral flap of its side and here it gives off three branches of which the anterior one supplies blood to the *external* and the *internal oblique* muscles of the abdomen, the second one supplies the posterior marginal region of the carapace and the plastron and the third one the *pubo-ischio-femoralis* and the *obturator internus* muscles. After this the dorsal aorta bifurcates into the two common iliac arteries of the right and the left sides. Each common iliac runs postero-laterally towards the limbs and on its way gives out an artery, the *arteria intercostalis recurrens* which turns towards the dorsal side and enters the gap by the side of the vertebral column and runs anteriorly to give off the intercostal arteries of the eighth, seventh and the sixth intercostal regions. A branch of the *arteria intercostalis recurrens* supplies the tail muscle, the *flexor caudae superficialis* and another branch the median belly of the retrachens capitis collique. The posterior mesentric artery arises from the common iliac and it supplies blood to the gonadial ducts and the posterior part of the rectum. From the common iliac the internal iliac arises and supplies blood to the cloaca, the rectum and to the urinary bladder by a separate branch from the internal iliac. Hereafter the common iliac proceeds as the external iliac and then gives off a pelvic artery which supplies blood to the pelvic muscles. The external iliac enters the thigh region by passing over the hip muscles and supplying blood to them. At this level the artery is known as the femoral artery which runs dorsally on the thigh muscles and supplies them by giving off small branches. The femoral takes a turn to the ventral side and then immediately bifurcates into two arteries, the anterior and the posterior tibial arteries. The anterior tibial artery runs on the dorsal side of the tibia, while the posterior one runs on its ventral side, both supply blood to the muscles of their corresponding regions. At the level of the carpals the anterior tibial artery gives off four branches, while the posterior one gives off two branches. All these branches arising from the tibial artery supply blood to the foot and the toe muscles.

THE VENOUS SYSTEM

The systemic veins could be arranged into three groups, (1) the veins which drain blood from the heart, (2) those which drain blood from the anterior limbs, head, neck and the anterior part of the body all of which culminate in the anterior vena cavae one on each side. (3) The veins draining the blood from the posterior limbs, posterior half of the body and those from the visceral organs excepting the lungs. The third group of veins end in the posterior vena cava. The two anterior vena cavae and the single posterior vena cava open into the sinus venosus which in turn opens into the right auricle. The sinus venosus is a more or less triangular chamber with its base facing anteriorly and the apex posteriorly. At its two anterior corners the superior (anterior) vena cavae open into it, while at its apex the posterior vena cava opens.

The right anterior vena cava (precaval) is shorter than the left one. A precaval is formed by the union of the subclavian and the jugular veins of its side. The subclavian in turn is composed of the following three main veins, the cutaneous, the brachialis and the pectoralis.

The cutaneous vein drains blood from the skin between the carapace and the plastron. Besides this it also drains blood from the trapezius, rhomboideus, and the rectus capitis cervico plastralis muscles. A vein from the thyroid also opens into the cutaneous vein.

The brachial vein is the longest of all the tributaries of the subclavian vein. It drains blood from the fore-limb through various small veins which join together.

The veins draining blood from the pectoral, biceps and the supra coracoideus muscles join together to give rise to the pectoral vein which in turn opens into the subclavian of its side.

The jugular vein drains blood from the head and neck. The jugular veins of the either side are connected with each other by a sinus at the base of the head on the dorsal side. This union has resulted in an anastomosis similar to the one present in the birds which is, however, on the ventral side at the base of the head. This type of anastomosis has been necessitated by the presence of a long versatile neck the movements of which are likely to occlude the vein on certain occasions. In such circumstances, there will be no obstruction in blood flow towards the heart as the other route will still be available for the passage of blood. Another peculiarity of the jugular veins in this animal is that the right jugular is not on the right side of the neck but it is on the mid-dorsal side. Moreover, it is very large and capacious like a sinus. The union of the right and the left jugular occurs at two points on the dorsal side of the neck, one at the base of the head at the level of the axis and the other at the level of the seventh cervical vertebra. The posterior anastomosis is a singularity of the Chelonia which is not found in any other group of vertebrates. Besides the right jugular there is a thin vein which runs parallel to the jugular on the right side of the neck which has been called as the right longitudinal tributary (Mathur,1940) of the right jugular.

The veins of the head and the neck:- The temporal vein drains blood from the adductor mandibulae externus, the a.m.medius, the a.m.internus, the masseter, the palpebralis and the tensor nasalis muscles. The maxillary vein after draining blood from the maxillary region of the head joins with the posterior auricular vein which drains blood from the occipital muscles and also from the depressor mandibulae muscle. After this union the vein opens into the sinus formed by the jugular anastomosis as already mentioned. The blood from the tongue, pharynx and the hypoglossus muscles and also from the hyoid muscles is drained by the respective veins from these regions and they open into the jugular sinus. The mandibular vein which drains blood from the mandibular muscles also opens into the jugular sinus. From the muscles of the neck throughout its length various small veins arise which open into the jugular vein of their respective sides. The longitudinal tributary of the right jugular also drains blood from the neck muscles of its side. It opens into the right jugular at the base of the neck before the latter joins with the left jugular.

All the veins, except the longitudinal tributary of the right jugular vein, are present on the left side of the head and neck.

From each lung two main veins emerge out which join with each other and form the pulmonary vein. The left pulmonary vein is longer than the right one, and runs parallel to the left precaval towards the heart.

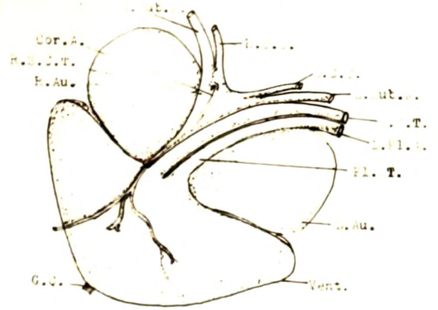
The right pulmonary is smaller and does not run parallel to the precaval of its side but directly runs towards the heart. Both the pulmonary veins go through the pericardial membrane side by side and separately open into the left auricle. The openings of the pulmonary veins into the left auricle are not guarded by any valves.

Veins of the hind limb:- There are five digital veins which receive blood from the muscles of the toes and those of the tarsal region. All these digital veins join to form a dorsal superficial vein comparable to the long saphenous vein of man. There is also a net-work of veins on the ventral side of the foot which ultimately forms a big vein comparable with the short saphenous vein of man. This joins with the deep vein of the leg comparable with the popliteal vein of man and finally joins with the long saphenous vein to form the femoral vein. The femoral vein receives various small vessels from the thigh muscles and finally enters the body cavity where it is recognised as the external iliac vein. The external iliac joins with the internal iliac vein which receives blood from the pelvic muscles and also from the ilio-costalis muscle. The common iliac vein divides into two branches; one of them, the lateral abdominal or the epigastric (Mathur,1940) runs obliquely outwards and anteriorly on the lateral side and enters the liver at its posterior corner. The presence of a pair of lateral abdominal veins is a characteristic of Chelonia in general but Rathke (1848) has observed that only the left abdominal is present in the early stages in Chelone and Dermochelys, Brune (1905), however, mentions that the right abdominal in Dermochelys is present similar to the one of *Testudo gracea* in which it is feebly developed. But as noticed by Mathur (1940), in *Lissemys punctata* the right lateral abdominal is very well-developed and the left one is not so much. The second branch of the external iliac, the pelvic vein runs transversely under the pelvic muscles to meet its fellow of the other side. The transverse pelvic vein is really a sinus and it also receives number of veins from the pelvic and the tail muscles. From this sinus arises a pair of renal portal veins, (vena renalis advehentes posterior, Mathur 1940) which run towards the kidnies of their respective side and then enter it at its middle of the outer border. On its way the renal portal vein receives a vein from the muscles of the ilium and also a branch from the pelvic vein. The branch of the pelvic opening into the renal portal receives a vein, from the marginal vein, the *vena renalis advehentes externa*. There is another vein coming from the vertebral region opening into the renal portal vein. This vein is comparable with the dorso-lumbar vein of the frog. From each kidney a short but broad renal vein~~s~~ starts which meets its fellow of the other side and forms the beginning of the posterior vena cava . The posterior vena cava is also a very short but a broad vessel. It traverses through the right liver lobe and when it emerges out of the liver it receives a pair of large hepatic veins. Finally the post caval opens into the sinus venosus. From the marginal region a pair of veins one on each side enters the anterior corner of the liver lobes of its respective side. The marginal veins drain blood from the carapace and the plastron. The branches of the marginal veins which open into the liver lobes are known as the hepatic tributaries from the shell. On the anterior border of the left liver lobe opens a large and well-developed oesophageal vein. This vein is formed by the union of the two small veins one each from the either side of the oesophagus. Such an extensive drainage of blood from the oesophagus suggests the possibility of the oesophageal respiration being present and or that digestion takes place in this region.

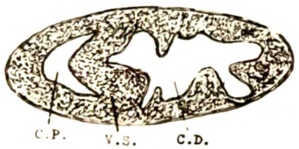
The hepatic portal system in this animal is not so composite as seen in other reptiles since the individual veins from the different parts of the alimentary canal, spleen and pancreas open directly into the liver. The hepatic portal is made up of a pair of intestinal veins draining blood from the intestinal region, a gastric vein from the stomach, a duodenal and a pancreatic veins.

The blood from the shell (carapace and plastron) is drained by various veins of which the following are the principal ones. At the margin of the carapace on each side a longitudinal vein, the marginal, receiving blood from the carapace and the plastron through many small branches. The marginal also receives number of intercostal veins one each from the intercostal region of the carapace. The marginal vein is connected anteriorly with the subclavian vein of its side, and posteriorly with the kidney to which organ it gives off a tributary. This tributary to the kidney from the marginal is the <u>vena renalis advehentes externa</u> (Mathur, 1940) as already mentioned. Into the marginals anteriorly small veins from the posterior cervical region also open. As already mentioned the marginal opens into the liver through a small vessel.

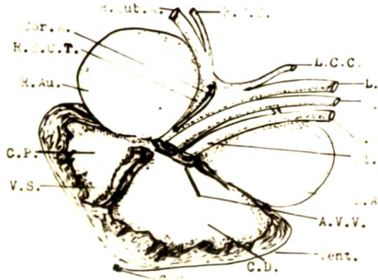
The presence of venous sinuses, like those of the jugular veins (fig 85), the one of the pelvic veins (fig 87) and the drainage of blood from various parts into the large liver lobes are of some significance which perhaps serves as an adaptation to the diving habit of the animal. Such sinuses are present in Testudo too but are too small to be of any positive value to the animal and must have become reduced owing to its terrestrial habit. For diving habit the animal should be able to hold up a large quantity of blood in the sinuses as it is so in the seals and other diving mammals (Harrison, 1955). In Lissemys and the other diving forms this hold-up of the blood in the sinuses is perhaps facilitated by the intaking of the head and the limbs and the animal remaining still in that position under water.



VENTRAL VIEW OF THE HEART

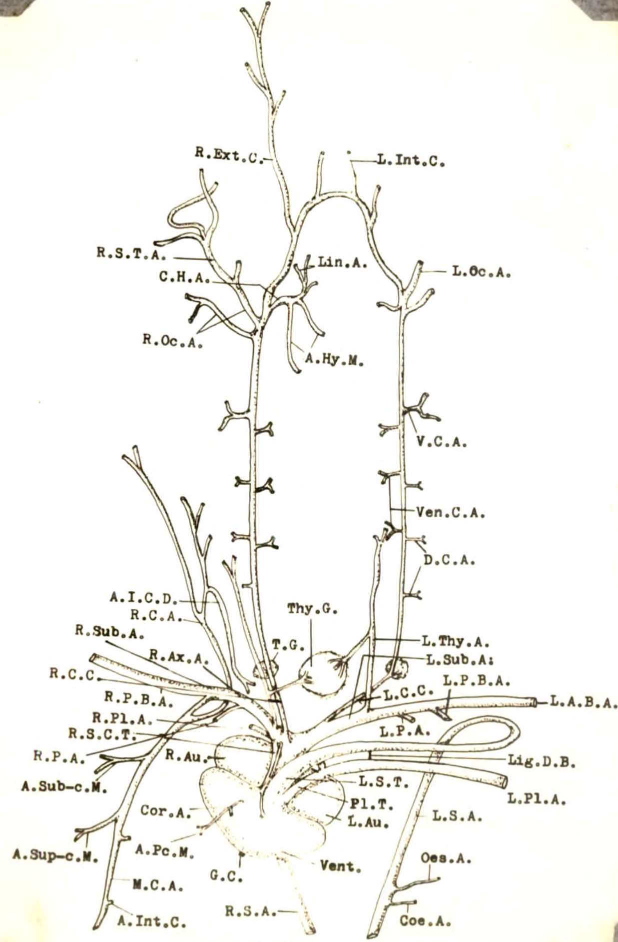


T.S. OF VENTRICLE



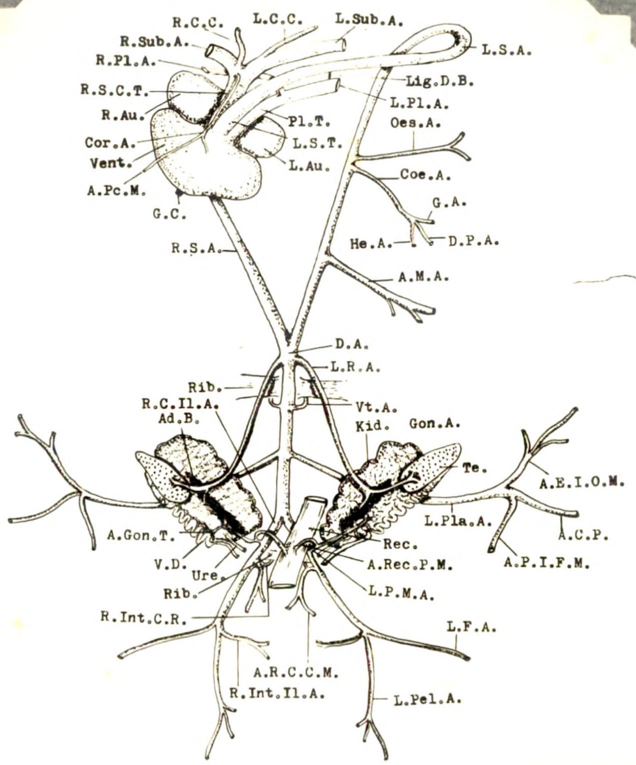
VENTRAL VIEW OF THE HEART WITH VENTRICLE CUT OPEN

FIG. 80



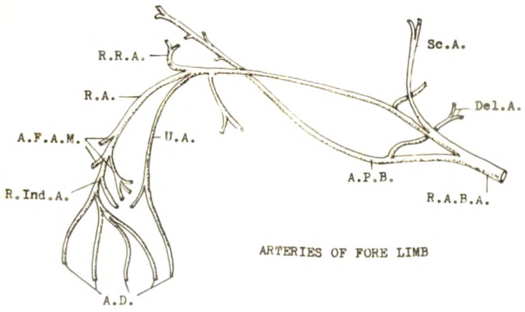
ARTERIES OF ANTERIOR HALF OF THE BODY

FIG. 81

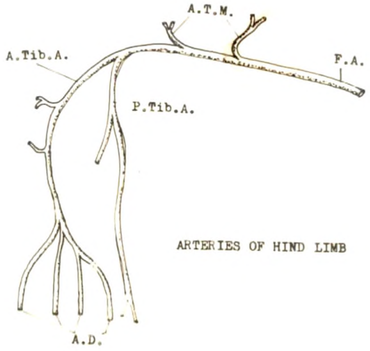


ARTERIES OF POSTERIOR HALF OF THE BODY

FIG. 82

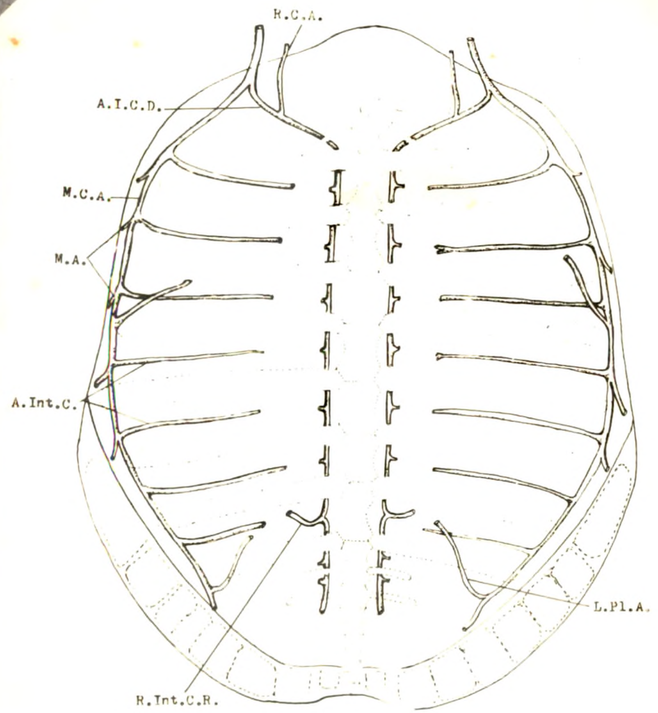


ARTERIES OF FORE LIMB



ARTERIES OF HIND LIMB

FIG. 83



ARTERIES OF THE SHELL (VENTRAL VIEW)

FIG. 84

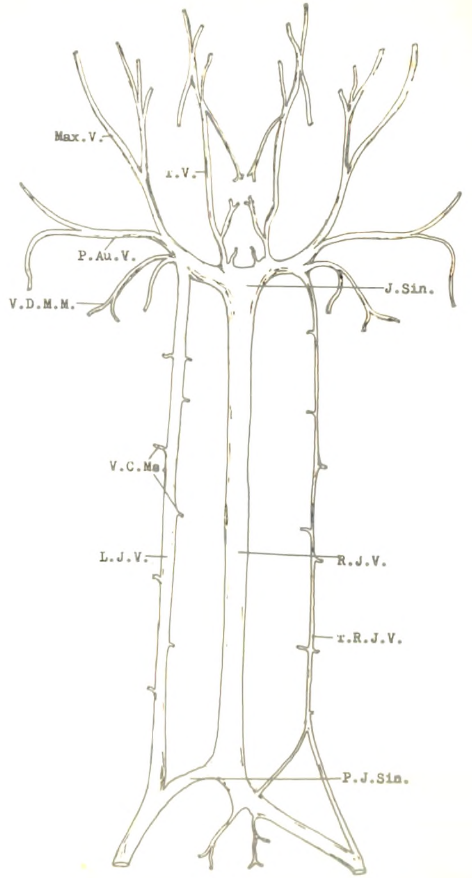


FIG. 85

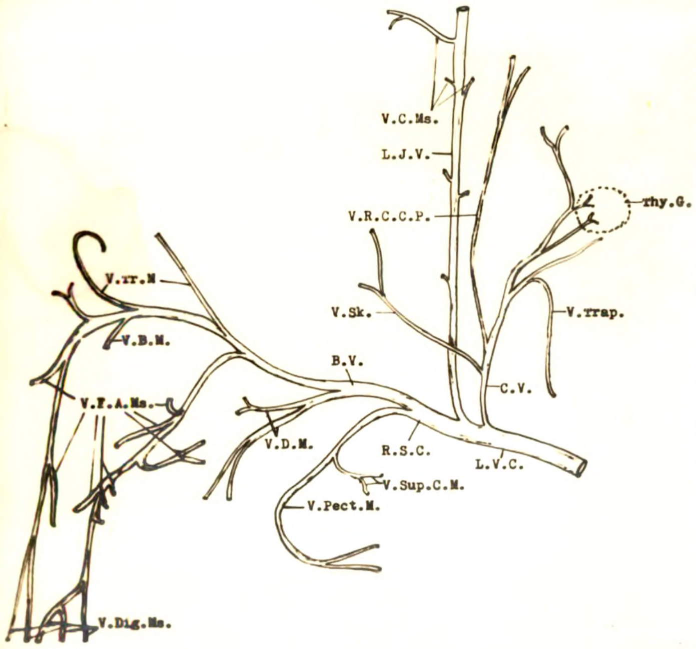
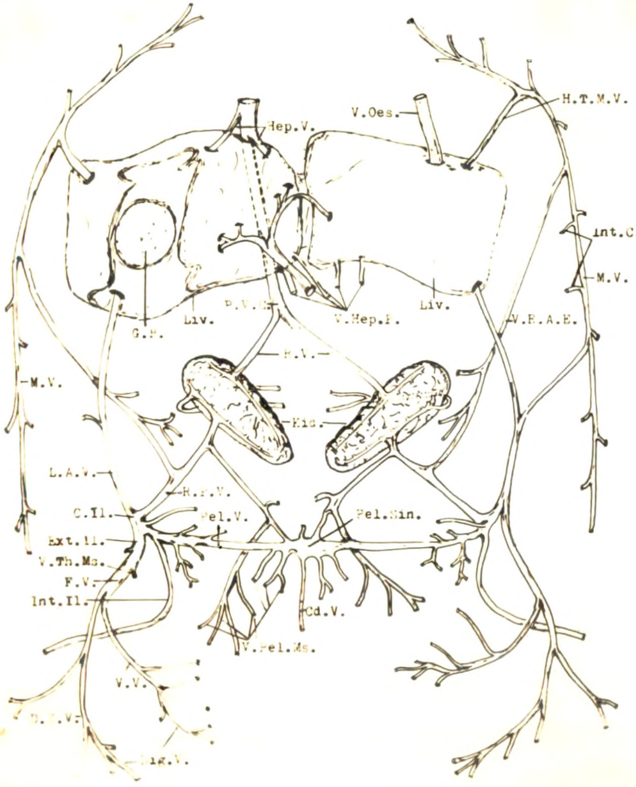

V.C.Ms.
L.J.V.
V.R.C.C.P.
Thy.G.
V.Tr.N
V.Sk.
V.Trap.
V.B.M.
B.V.
C.V.
V.F.A.Ms.
V.D.M.
R.S.C.
L.V.C.
V.Sup.C.M.
V.Pect.M.
V.Dig.Ms.

FIG. 86


Hep.V.
V.Oes.
H.T.M.V.
Int.C.V.
M.V.
G.P.
Liv.
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V.Hep.P.
Liv.
V.R.A.E.
R.V.
M.V.
Kid.
L.A.V.
R.P.V.
C.Il.
Pel.V.
Pel.Sin.
Ext.Il.
V.Th.Ms.
F.V.
Int.Il.
Cd.V.
V.Pel.Ms.
V.V.
D.E.V.
Dig.V.

FIG. 87

NOTES ON THE COMPOSITION OF BLOOD OF LISSEMYS AND A FEW OTHER CHELONIANS AND SOME SNAKES

Blood is essentially the internal environment of the animal body and through the long stretch of the evolutionary history of the vertebrates, it must have undergone dynamic changes in its physico-chemical and physiological constitution necessitated by intrinsic as well as extrinsic factors. Florkin (1949) has developed a biochemical approach to the understanding of the process of evolution and has suggested that the various biochemical changes taking place could be correlated with the morphological and ecological ones. It has therefore become possible for us to visualise a dynamic equilibrium in the animal body, created as a result of the impact of the new changes in the external and the internal environments.

In the light of the new approach to the concept of evolution, physiological studies on the blood of various vertebrates and groups of them should be enlightening. Much work has already been done in the comparative physiology of blood. Erlanger (1921) estimated the blood volume of a number of mammals. Hall and Gray (1929) gave the oxyphoric capacity of the blood of some fishes. Lustig and Ernest (1937) studied the plasma proteins of some vertebrates and invertebrates. Menon (1951) conducted his studies on the plasma proteins, oxyphoric capacity, nutritive capacity and blood volume in a representative series of vertebrates and suggested that there is a progressive increase in the efficiency of the blood as we ascend up the evolutionary ladder. Nair (1955) studied the non-protein nitrogen of the blood of some reptiles and mammals. Rangnekar (1953) studied the invertebrate blood along similar lines. Besides these there are certain useful reviews and treaties viz., Howe's (1925), and Handerson (1928), and a very handy compilation of the standard values in blood (Albritton, 1953).

Of these studies, those on the blood of reptiles particularly of the chelonians and snakes are quite meagre indeed while a lot of information is available on the mammalian blood. Reptiles being the ancestral stock from which higher animals like birds and mammals have evolved it is all the more necessary to have a fuller knowledge of this basic group of vertebrates. Moreover it was observed that the blood of some snakes took a long time to clot after it was shed and that blood of the common pond turtle lakes very readily on trying to prevent clotting with potassium oxalate. So it was found proper to study the calcium content and its significance in the blood of these reptiles in possessing such characteristics.

Again the presence of a striated muscle sheath on the wall of the lung in Lissemys and Geomyda and its absence in other chelonians like Trionyx, Testudo and Eretmochelys seemed to indicate the presence of two different types of respiratory equipment in these animals. Earlier (chapter IV (1)) it was pointed out that there should be an appreciable difference in the oxyphoric capacity of the blood in these two types of chelonians.

The present work on the blood of some chelonians and snakes is therefore an attempt to throw some light on the above mentioned aspects and also to assess the biochemical adaptations in general of the blood in these two groups of reptiles.

Material, Methods and Techniques:

The material chosen for investigations consisted of the following chelonians:- *Lissemys punctata*, *Geomyda trijuga*, *Trionyx gangeticus*, *Testudo elegans* and *Eretmochelys imbricata*; snakes:- *Vipera russelli*, *Naja tripudians*, *Ptyas mucosus* and *Natrix* sp.

Pithing:

The pithing of the chelonians and snakes raised a difficult problem but convenient methods were devised. For pithing the former a pair of crucible tongs was held open with both hands at the head end and as soon as the head came out, the tongs was closed in a sudden snap thus holding the neck between the tongs. The animal could be easily pithed in this position. In the case of the Eretmochelys there was no difficulty at all because of its inability to retract its head inside the shell. It was pithed by a blow on the head. For the snakes a noose device was improvised. At the tip of a long bamboo (5 to 6 ft.) a noose of strong string was made and the long end of the string was held in the right hand and the bamboo in the other. After the animal's head was through the noose the string was pulled in a twinkle. The body of the snake was immediately tied to the bamboo. The animal was thus ready for pithing.

Taking samples of blood for analysis:

Prior to the starting of the work some clean test tubes were prepared in the following manner for collecting the blood. Into the test tubes, 0.1 ml of a stock solution of 10 % potassium oxalate was pipetted and the inner side of the tubes was throughly rinsed with the solution. The test tubes were then dried by heating gently thus ensuring that a thin oxalate film was left on the inner wall of each of the test tubes. About 5 to 10 ml of blood could be taken into each test tube without the danger of its being clotted. In the case of the blood of Lissemys this procedure bacame unsuitable because the oxalate made the blood lake. But using a few crystals of potassium citrate instead of oxalate the blood did not lake but a few grains of heparin was found to be ideal. Heparin was also used as the anticoagulant for all bloods when calcium of blood was to be estimated.

The blood was collected directly from the ventricle by means of a clean, dry syringe and immediately transferred to the test tubes and kept corked in a refrigerator. In the analysis of the plasma, blood was directly transferred from the syringe for centrifuging into centrifuge tubes containing oxalate, citrate or heparin as the case may be.

Methods for the estimation of blood constituents:

The density of blood was estimated by the dehydration method by heating in an electric oven at 103°C. The lipid content of blood was estimated by extraction with ether using the soxhlet apparatus. Blood being a fluid prior to the extraction it was first mixed with some sodium sulphate and dried in an air oven for few minutes and then transferred to the fat extraction thimble which in turn was put into the extraction chamber of the soxhlet apparatus for extraction.

The total proteins, albumen, globulin and fibrinogen in blood were determined colorimetrically. The method followed was the standard micro-Kjeldahl method, as given by Hawk (1949). The non-protein nitrogen and glucose contents of blood were determined colorimetrically according to the methods of Folin and Wu. The calcium, haemoglobin and iron contents in blood were also determined colorimetrically according to the methods of Roe and Kahn; Wong and Kennedy respectively. For all the colorimetric determinations the Klett-Summerson Photoelectric Colorimeter was used. For the determination of clotting time the capillary method was adapted. The cell-volume was measured by centrifuging the blood in a graduated centrifuge tube at a speed of about 3000 r.p.m. and by direct reading. The R.B.C. count was done using the Spencer's Haemocytometer.

Results: Vide table, and histograms 3, 4, 5 and 6.

Discussion:

It is estimated that normal human blood contains about 78 % water and the rest solids (Evans, 1947). The water balance of the blood is a vital matter and is kept more or less constant by nervous and hormonal factors (Prosser, 1950). Among the chelonians studied, the the blood of the fresh water ones, Lissemys and Trionyx, contained the highest amount of water 89.7 %. But in the marine form, Eretmochelys, owing to the high osmotic tension of the sea water it is only 86.8 % which is lower than even the land forms, Geomyda and Testudo, in which it is 88.6 % and 88.9 % respectively. The semi-aquatic Geomyda has a slightly higer figure than the purely terrestrial one, Testudo.

Of the snakes, the Ptyas blood has ~~the~~ least water, 84.3 % while ~~the~~ Vipera blood has the highest, 87.9 % and of the aquatic one, Natrix a close second having 87.1 % with the Naja in between, with 85.9 %. Natrix has a high water content in the blood since it is a water snake. The reason why the viper which is a land form has the highest water content in its blood is doubtful.

The lowest figures with regard to density and total solids of the blood of Trionyx, Lissemys, Geomyda and Testudo among chelonians and snakes seem to suggest that this due to the acqusition of the ureotelic type of excretion. But those of the marine turtle Eretmochelys are comparatively high even more than the uricotelic snake Vipera. So the differences in these animals are essentially due to the environment in which the animals live depending on whether in freshwater, sea or on land.

With respect to total proteins in the plasma the chelonians show lower figures than the snakes. Among the snakes, the water snake, Natrix, stands the lowest while among the chelonians also the aquatic ones come lower than the terrestrial ones namely Geomyda and Testudo. There again the completely terrestrial Testudo has a higher figure than Geomyda. Redfield (1933) on studying the total proteins in the plasma of a number of vertebrates from fishes to mammals, concluded that there is an increase in the concentration of plasma proteins as we go up on the evolutionary ladder. He further suggested that this increase is " parallel with the development of a cardio-vascular system capable of containing blood at increasing pressures." This idea appears to justify the presence of a higher concentration in the plasma proteins in snake blood, if we believe that in snakes there exists a cardio-vascular system containing blood at a higher pressure owing to the externally compressed linear shape of the snake body. Again, there seems to be a relation between terrestrialism and the mode of excretion. In the aquatic reptiles the amount of total proteins in the plasma is less than in the terrestrial forms and also, that in the uricotelic ones.higher than in the ureotelic ones. This leads us to visualise two lines of evolution, (1) ureotelic terrestrialism and (2) uricotelic terrestrialism. Those evolving along the former consist of, amphibians and aquatic chelonians and mammals; and those along the latter comprise the lizards, snakes and birds. The terrestrial chelonians e.g. Testudo therefore judging from the concentration of plasma proteins seem to be intermediate between the aquatic chelonians and snakes with respect to ureotelism and uricotelism - Support for this idea is avialable from the recent work of Khalil and Haggag (1955) which as shown that tortoises are to be considered as ureo-uricotelic animals. Support is also avialable from the following statement of Baldwin (1949) " It may be, therefore, that in the tortoises and turtles we have an intermediate group, lying between the ureotelic Amphibia and the uricotelic snakes and lizards: perhaps the uricotelism of the more progressive reptiles is still being evolved in the slow but steady manner usually associated with tortoises." It must however be emphasised that more information from an extensive work on the physiology of these animals which is already in progress under Dr.George in this laboratory, is necessary to arrive at definite conclusions.

An exactly similar pattern of variation in that the values for the snakes are higher than those of the chelonians and that among the chelonians those of the land forms are higher than those of the aquatic ones is seen in the globulin content of the plasma as of its total protein content. The same is true for the albumen content of the plasma also but for the slightly higher figure for the marine turtle among the chelonians.

A more or less similar variation in the non-protein nitrogen (NPN) content of plasma as in the total protein is observed among the reptiles studied. But a lower value of 43.65 NPN and 45.76 NPN (Nair, 1955) in the Vipera is unexplainable as also the very high total protein figure in Ptyas.

The glucose content of blood of the various reptiles show a wide range of variation. The chelonians show higher figures than the snakes. Among the former again, Geomyda and Testudo show the lowest while Trionyx and Eretmochelys the highest. Among the snakes Vipera has the lowest and Naja the highest blood glucose figures. The aquatic chelonians in which the blood glucose value is high are much more active than the terrestrial ones in which this value is low. Among the snakes studied Vipera which has the lowest blood glucose figure is the most inactive amongst these snakes while Naja which has the highest is the most active. The blood fat figures also show a more or less similar variation. So I am inclined to think that the glucose and fat contents of the blood may be with certain limitations indicative of the extent of the activity of the animal.

It is a peculiarity of the snake blood that in many cases it does not clot for a long time. In order to offer a possible explaination for this phenomenon the present study was conducted. On estimating the clotting time it is found that the blood of chelonians clots in about 30 seconds while in the sneke Vipera it takes one and a half to two hours for the blood to clot. Natrix took 25 to 30 minutes, Ptyas 4 to 6 minutes and Naja 2 to 3 minutes.

The calcium content of the whole blood as well as plasma and the fibrinogen content of the blood in these reptiles were studied in order to see if they are the factors responsible for this feature of the snake blood. It is found that the calcium content of both whole blood as well as plasma are much higher in the snakes than in the chelonians. This high calcium content of the snake blood may be due to the fact that they swallow their prey whole together with their bones. But it seems significant that among snakes the blood of Vipera which takes the longest to clot contains the highest amount of calcium while the blood of Naja which clots in the shortest time has the least calcium in blood. Does this show that a very high calcium level in blood prevents clotting? This question does not appear so strange as it does at the first instance, if we take into account what Heilbrunn (1952) has to say on the subject, viz.," Delezenne also noted that whereas dilute solutions of calcium salts favoured the activation of the enzyme, more concentrated solutions had the opposite effect and he drew an analogy to blood in which, as is well known, dilute calcium solutions favour clotting and more concentrated solutions prevent it. This is an interesting statement in view of the fact that modern theories of blood clotting emphasize the important role that a proteolytic enzyme plays in the process."

Taking into consideration another factor namely the fibrinogen content of the blood it is found that snake blood contains only about one-fourth the amount of that in the blood of chelonians. But the blood of Naja which has the least fibrinogen clots sooner than the other snake bloods which contain more ~~of~~ fibrinogen. Again it has been observed that ox blood which clots readily contains only 0.08 gm/100ml. of blood of fibrinogen(Wunderly, 1944)clots readily when shed. So it is most unlikely that low fibrinogen content of the blood is the chief factor in the prolonged clotting time of snake blood.

Another possible factor favouring this feature of snake blood is heparin. But on neutralising heparin of the blood with touledene blue, there is no effect (Nair, 1954). The addition of muscle extract which contains thromboplastin,produced clotting (Nair,1954). So it appears that in snake blood thromboplastin is not readily produced.

Now reviewing the whole problem of delayed clotting of snake blood, it appears that there are more than one factors involved. Those factors may be as follows:-

(1) The slow production of thromboplastin in the blood.

(2) The high calcium level of the blood.

(3) The low fibrinogen content of the blood.

Nair (1954) suggested that a possible explanation for this singularity of the snake blood may be found in " the disproportionate elongation of the body without the necessary high frequency of heart beats to pump blood horizontally to a long distance." He further explained that under such circumstances, there is a possibility of the blood slowing down at the posterior end of the body and producing a thrombus formation.

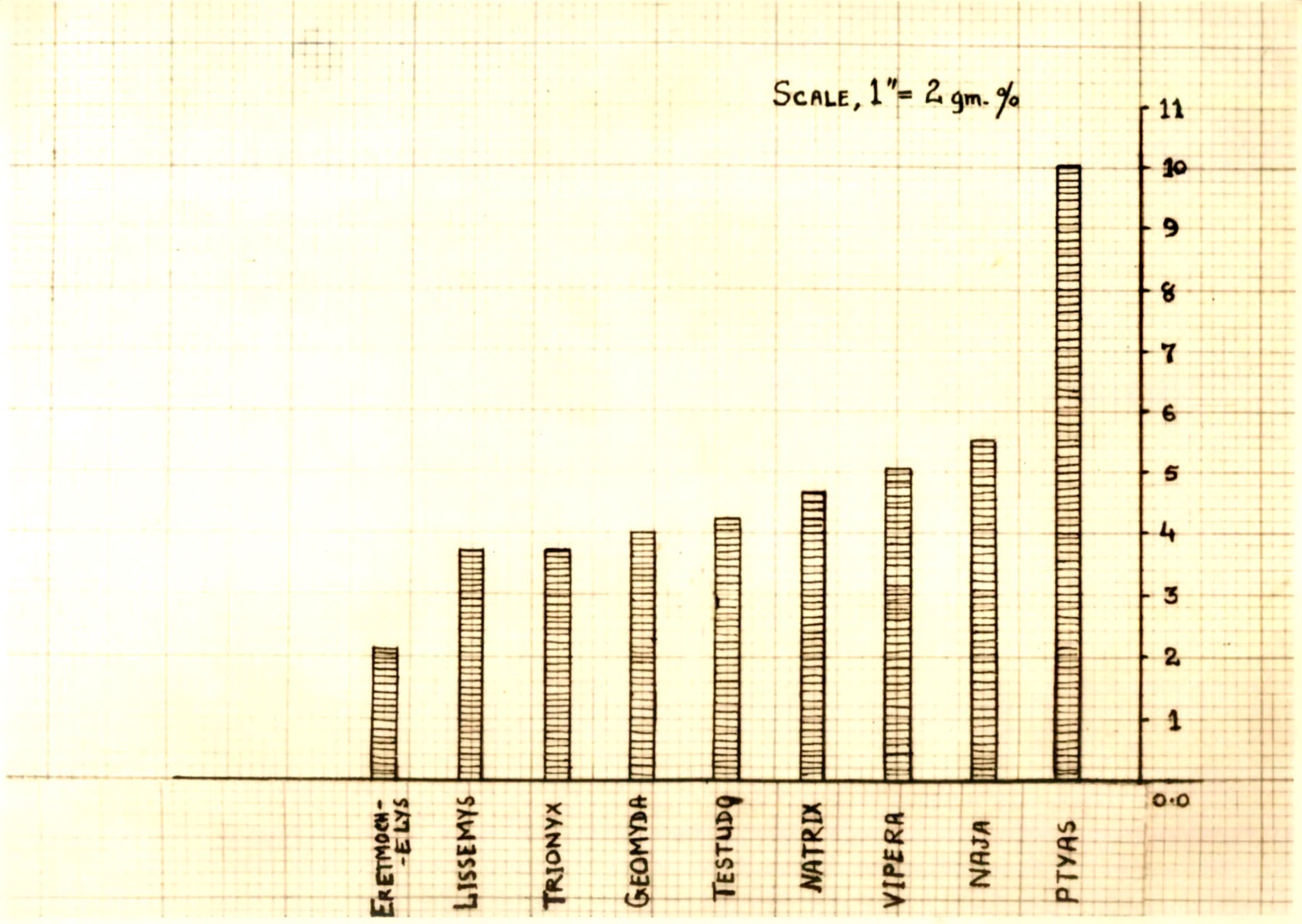
It seems quite reasonable to expect in the light of the above suggestion that the snake blood acquired the three properties I have already suggested above. It also seems possible that such circumstances as those faced by snakes were responsible for the dinodaurs to acquire a bipedal mode of locomotion and a more erect body posture. Among the snakes too the Naja is able to raise itself with its hood up unlike Vipera. It is interesting that in the former the clotting time is only two and a half to three minutes while in the latter it is one and a half to two hours.

The blood of the chelonian Lissemys was found to lake readily with oxalate but not with citrate. It was therefore suspected that the calcium content of its red corpuscles was low. The result obtained show that the calcium content of whole blood, plasma or cells (calculated from the other two values on the bases of the cell to plasma ratio in blood) in Lissemys is not low but on the contrary that of Testudo and Eretmochelys are very low. So the calcium content of whole blood, plasma or cells is not a factor for the ready laking of blood of Lissemys with oxalate. Perhaps the Lissemys red blood cells have a greater permeability to oxalate.

It has already been mentioned in an earlier chapter that with the evolution of larger lungs in the more advanced chelonians viz., Trionyx, Eretmochelys and Testudo, a muscle sheath covering the lungs as seen in Lissemys and to a lesser extent in Geomyda, which helped the lungs to function as a pair of bellows and whose presence gave the early chelonians their survival value, was lost. This loss of the muscle sheath must have been a very severe handicap for the animal and it became necessary to make certain new adjustments in the body. They were (1) greater development and a more active role of the flank cavity muscles in the mechanism of respiration. (2) Developmeat of larger lungs. (3) Acquisition of a greater oxyphoric capacity of the blood. The purpose of the present study on the oxyphoric capacity of the blood of these chelonians is to acertain whether the assumption regarding the acquisition of a greater oxygen storing ability of the blood in the advanced chelonians, is correct or not.

Much work on the oxyphoric capacity of the vertebrate as well as invertebrate bloods has been done. Hall and Gray (1929) estimated the oxygen capacity of the blood of certain fishes and Erlanger (1921) the blood volume of mammals. Prosser (1950) has presented a lot of data on the haemoglobin content of the blood of various vertebrates. Irving's work (1939) on the respiration of diving mammals is also enlightening. In the present investigation the cell volume, the R.B.C. count, haemoglobin and iron contents of the blood of some chelonians and snakes have been determined in order to obtain an index of the oxyphoric capacity of these bloods, which is directly proportional to the iron content. The effeciency of the blood as an oxygen transporting system however is dependent on the cell volume and the haemoglobin concentration.

The results obtained show that the oxyphoric capacity of the blood of Lissemys is the lowest with its iron as well as haemoglobin contents being as low as 22.98 % and 4.88 % respectively. The figures obtained for Trionyx and Eretmochelys are the highest in the series while those of Geomyda and Testudo which are much less active chelonians, come in between. The figures obtained for Geomyda and Testudo are more or less the same. From the above observations it is clear that in the primitive Lissemys which possesses the muscle sheath on the lung has a low oxyphoric capacity whereas the advanced chelonians that lost this muscle sheath which became more of a hindrance to the full development of the lungs, acquired a greater oxyphoric capacity of the blood. Geomyda, however, is intermediate between Lissemys and Testudo physiologically and as already mentioned in an earlier chapter, anatomically as well.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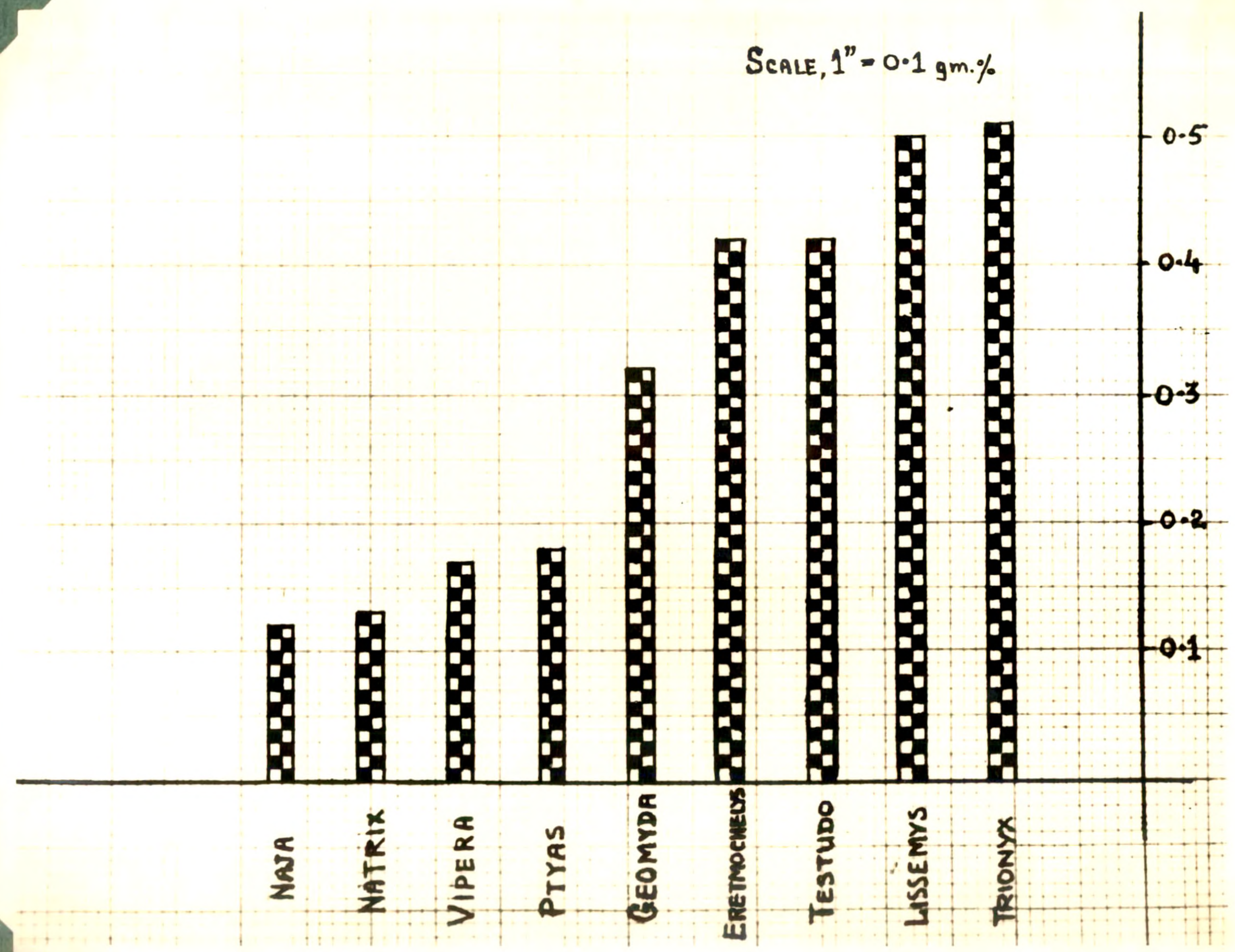
HISTOGRAM 3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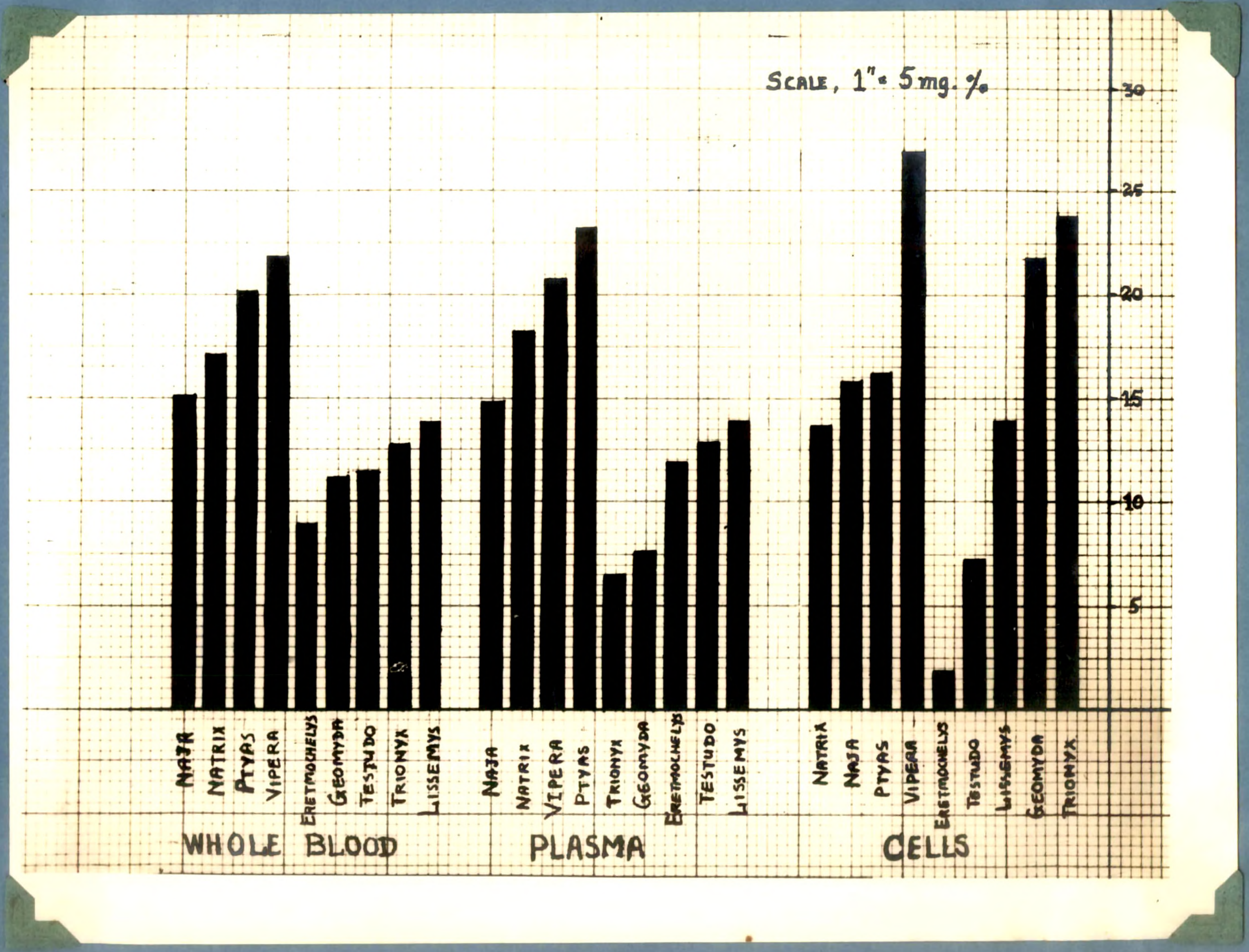
TESTUDO
LISSEMYS
TRIONYX

HISTOGRAM 4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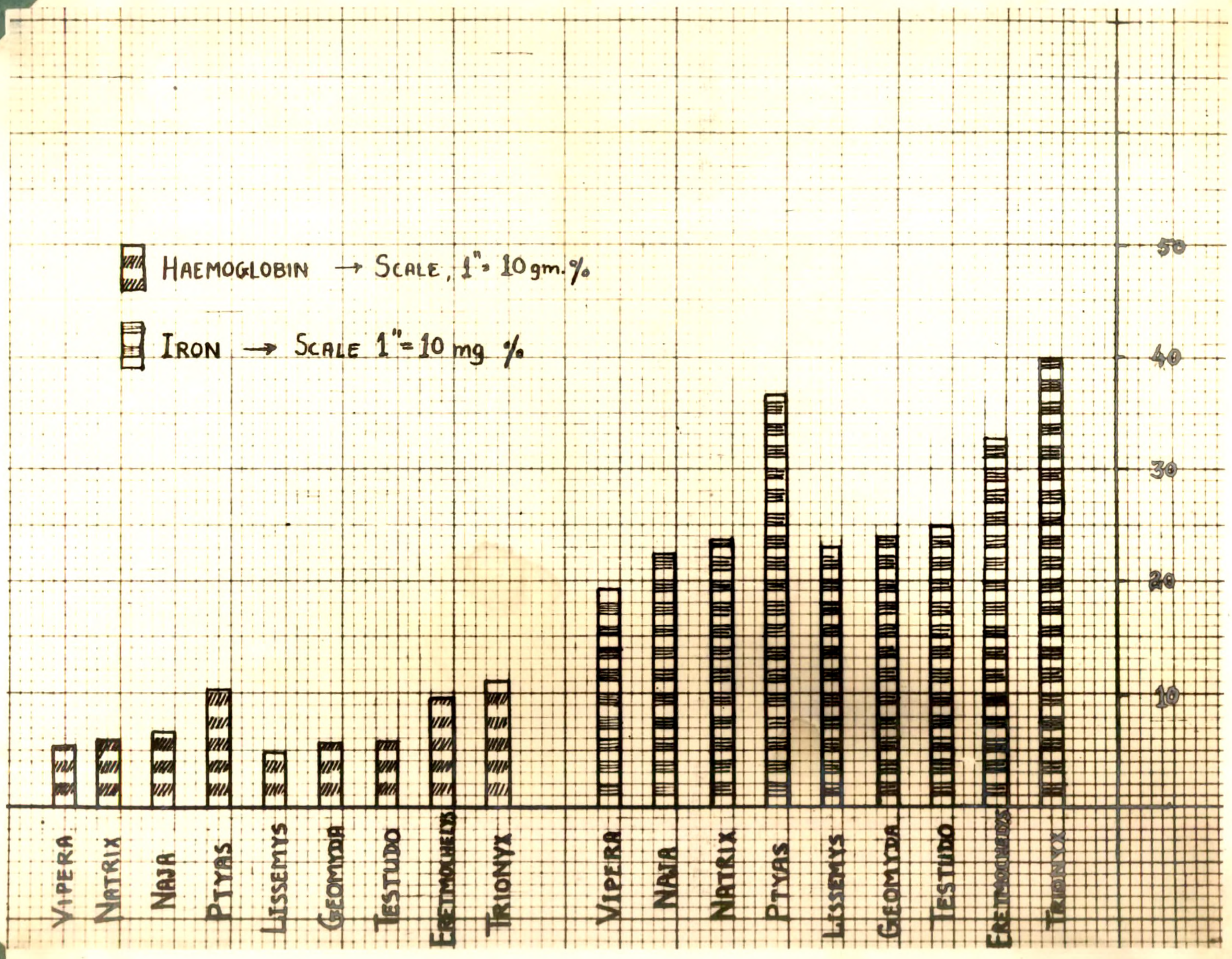
HISTOGRAM 5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HISTOGRAM 6