THE CALCIFEROUS GLANDS OF THE EARTHWORM, WITH APPENDIX ON THE CIRCULATION

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NATHAN RUSSELL HARRINGTON

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NATHAN RUSSELL HARRINGTON.[1]

CONTENTS.

[1] Mr. Harrington died at Atbara in the Soudan, July 26, 1899, while in charge of a zoölogical expedition, without having revised the proofs of the following article.

I. Introduction.

The purpose of this paper is to give a biological treatment of a single organ in the common earthworm. Originally planned as a study of the cellular changes, it has gradually come to include the structure, function, development, glandular action, and blood supply of the calciferous gland. The studies on the development of the gland and neighboring blood vessels were necessary adequately to explain one of the most important of the following observations: *viz.*, the regeneration of glandular cells and nuclei which disintegrate during secretion.

Metabolism, perhaps the most fundamental of the several phenomena classed as vital activities, is often accompanied by structural changes in protoplasm, by which a more or less complete record of the stages of metabolism are recorded. Such changes are especially apparent in actively secreting tissues, which for this reason have been widely studied by those interested in particular problems of cellular metabolism.

These researches have inquired (1) how glandular tissue recuperates after the waste caused by secretion, (2) how the regenerated cell-substance is transformed into specially differentiated material, and (3) how these products of secretion become released from the cell. In some glands, *e.g.*, the pancreas and parotid glands, the processes indicated may be carried on entirely within a single cell, and be continued indefinitely. In others, however, the process is so far intensified that a single cell runs through the cycle but once, death being the necessary resultant of its activity; and in such cases recruiting tissues, independent of the secretory cell, are involved. The sebaceous and milk glands are instances of this kind, as are also the calciferous glands of *Lumbricus terrestris*, which form the subject of this research.

The following paper attempts to show that the cells, which here replace the waste caused by secretion, are derived from the walls of the blood-vascular system. The unusual relations between the glandular and circulatory systems can be interpreted only by regarding the one-layered secretory lamellae bounding the blood spaces as greatly hypertrophied vascular walls representing both the intima and endothelium. Considerable support for this interpretation is found in the structure of similar glands in various other worms, but to establish this fact it was necessary to follow the vascular and glandular tissues during the later larval development.

The present paper therefore has come to cover a wide field.

II. Morphology and Blood Supply.

a. *Morphology.*—Since Julius Leo, in 1820, discovered lime-secreting organs leading into the digestive tract of earthworms, very many workers have examined and reported observations upon these interesting structures. Morren ('26), Lankester ('65), Claparède ('69), and Darwin ('81) have contributed to our knowledge of the glands in *Lumbricus,* and more recently calciferous structures have been minutely described by Beddard ('94) and others in various genera of earthworms. The posterior outlets of the glands near the gizzard, which are explained in the following paper, have escaped observation, however, and the relations of the blood vessels in the anterior pair of glands in *Lumbricus* have never been adequately worked out.

Every one is familiar with the three rounded swellings which come plainly into view when an earthworm is laid open and the hearts and seminal vesicles are removed. They are figured in external profile on Pl. VI, Fig. 1.

In horizontal section through this part of the oesophagus one sees that the anterior pair of glands contain hollow cavities which are not represented in the two posterior pairs. The active secretory cells are all located in the latter, while the anterior pair simply serve as storehouses for the secreted product, which eventually passes through a foramen in the oesophageal wall into the oesophagus. It is necessary to emphasize that at no time in development or in adult life do the second and third pairs of glands communicate directly with the oesophagus. They arise in the embryo (as will be more fully explained below) between the layers of cells lining the oesophagus and the circular muscle layer, and although it is true that they are derived from endodermal tissue, it is by migration, not by evagination, as has been usually stated. The evagination or budding off from the oesophagus, which has been observed by Vejdovský ('88), Wilson ('89), and other embryologists, applies only to the process of formation of the first pair of glands, the cavities of which are derived in this way from the oesophageal cavity.

The blood sinuses, along which the secretory cells are arranged, are radial spaces, each extending longitudinally the entire length of the glandular region (*i.e.*, somites IX to XIV), and reaching radially from the epithelial layer as a center to

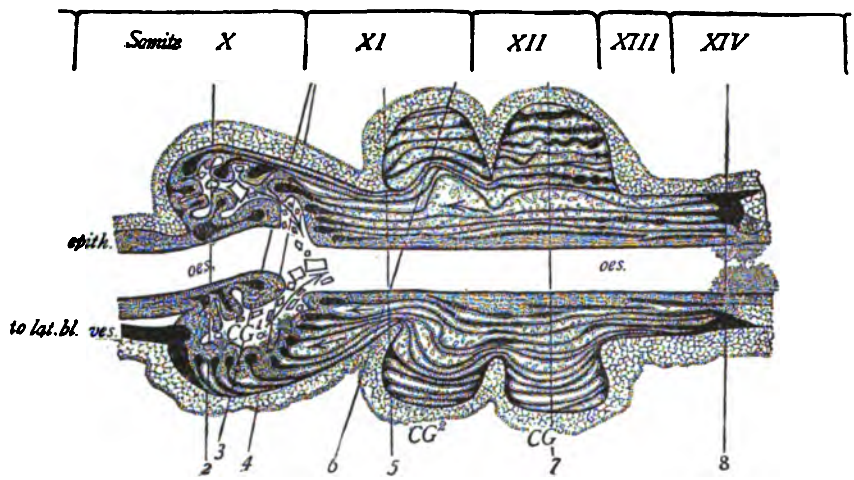


FIG. 1. — A slightly diagrammatic sketch of a longitudinal-horizontal section through the glandular oesophagus.

the circular muscle layer. The structure of the glands might therefore be compared to that of a wide paddle-wheel, the oesophagus being the axle, the radial sinuses the paddles, and the circular muscles the few bands around the outside which hold the parts in place. The arrangement will be best understood by glancing at the successive sections cut in transverse planes, as numbered in Text-fig. 1.

The cavities between two radial planes are the gland cavities into which the lime particles drop from the walls of the partitions. The cavities are also continuous from somites X to XIV, opening at the former into the hollows of the first pair of glands, and at the latter directly into the oesophagus. Inasmuch as the lime is principally formed only in CG^2 and CG^3

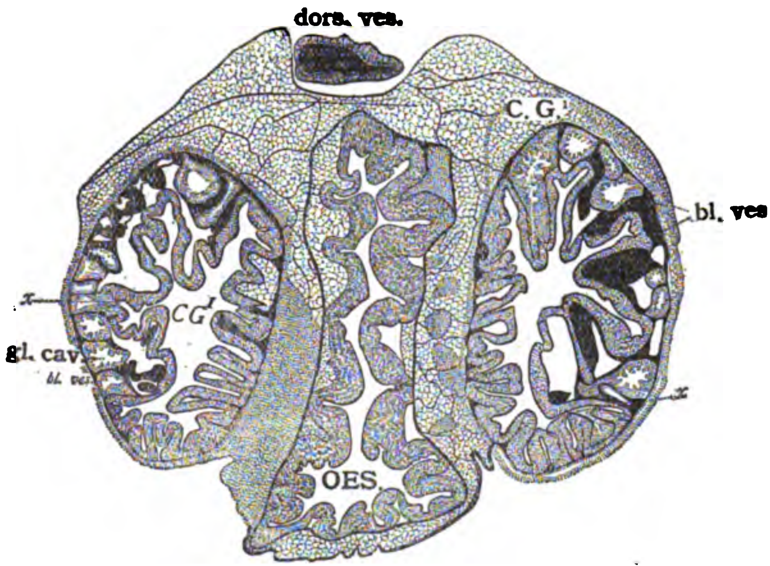


Fig. 2.

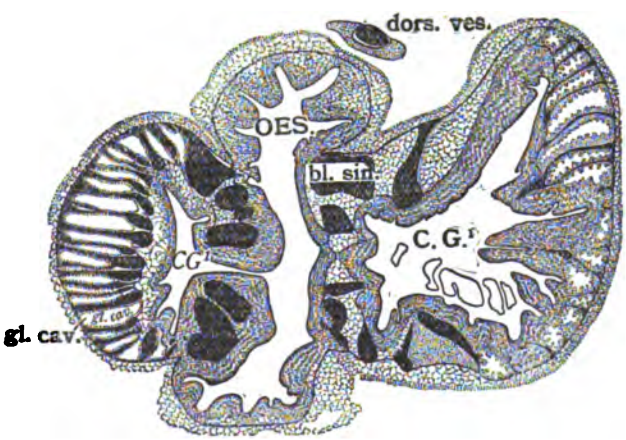


Fig. 3.

Figs. 2 and 3. — Cross-sections cut in planes indicated on Fig. 1.

(the two posterior glands), we have designated as true "gland cavities" only the spaces in these, and have called their continuations or outlets forward to the anterior edge of CG^1 and backward to somite XIV "gland ducts." Where the syncytial cells of the gland ducts meet the epithelial cells of the oesophagus, both in the anterior ventricle and in the middle of the fourteenth somite, there is very often a break in the tissue.

It frequently occurs, however, that the two tissues—the epithelium of the oesophagus and the cells lining the gland ducts—are continuous, showing at the meeting point a rather abrupt transition (see Text-figs. 1 and 8). Ordinarily, the gland cavities are distended with a large number of small white lime globules, which have been formed on the side walls. Seen through the skin these give the familiar white appearance of

FIG. 4.—Cross-section cut in plane indicated in Fig. 1.

the gland. The horizontal red stripes running along this part of the oesophagus are made by the outer edges of the blood sinuses which form the extended longitudinal radial partitions. These partitions will be seen, by comparing Text-figs. 1 and 7, to form walls of gland cavities in CG^2 and CG^3 where they are widest, and to be walls of gland ducts in the ninth, thirteenth, and fourteenth somites where they are narrowest (*cf.* Pl. VI, Fig. 1).

If a worm be opened in saline solution, and the glands well exposed, peristaltic contractions may be seen following each other cephalad, along the glandular part of the oesophagus. During a peristaltic wave the nodule-like swellings are completely leveled, and the red stripes, marking the outer edges of the blood sinuses, completely disappear. In the few moments succeeding contraction, lime crystals may be seen through the oesophageal walls, slowly floating forward, at the region of the septum between IX and X, and backward, in fewer num-

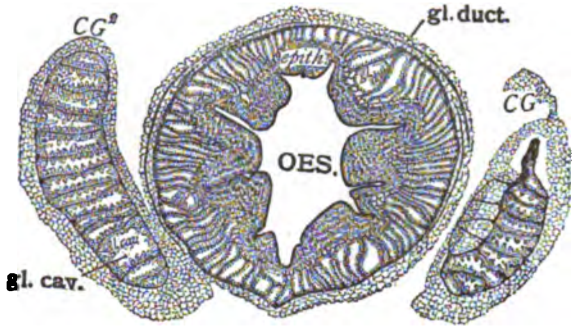


Fig. 5. — Cross-section cut in plane indicated in Fig. 1.

bers, along the ducts in XIII. By the continuous radial partitions they are guided until they reach the anterior edge of *CG¹*, or the posterior extremity of the radial walls in the middle of somite XIV. If the lime has been carried forward, as is generally the case, it issues through irregular openings in

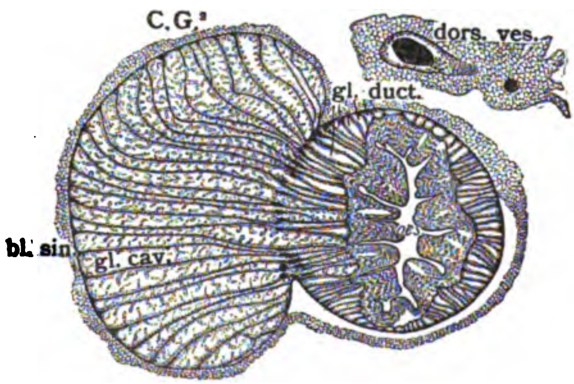


Fig. 6. — Cross-section cut in plane indicated in Fig. 1.

the epithelium at the anterior edge of *CG¹* into the hollow anterior glands, where it is stored up and eventually passed out through the foramen into the oesophagus. If it is passed backward, it breaks through oesophageal epithelium, in this case opening directly into the digestive tract at the middle of XIV (see Text-fig. 8). This second outlet for the secreted product has escaped the notice of previous writers.[1] If the oesophagus be carefully opened along the median dorsal line, the milky fluid which holds the secretion may be seen issuing into the digestive tract from pit-like indentations in the side walls of the oesophagus just in front of the crop.

Large numbers of the round crystals, after they have left the gland ducts and entered *CG¹* (sometimes also before this), coalesce to make the irregular small pebbles which are always present in this pouch. These are normally emptied into the oesophagus, and may be found anywhere in the digestive tract

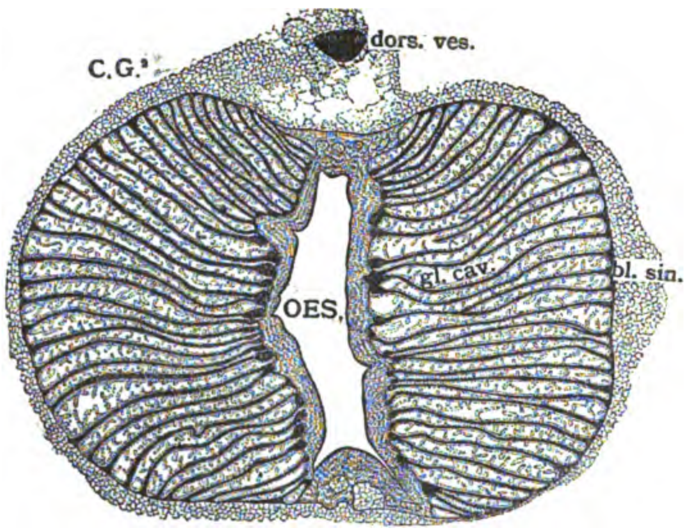


FIG. 7. — Cross-section through *CG³* or ³.

on their way to the anus. Their function in digestion is discussed in the part of this paper dealing with the physiology of the glands. Before these crystals are cast out into the digestive tract, they distend the first pair of glands, making them appear very much larger than the last two pairs.

[1] Claparède ('69) was of the opinion that the lime normally broke through the epithelial layer anywhere, but later observers have shown that lime may be passed forward to the first pair of pouches.

Finally, it must be repeated that the first pair of glands are entirely different morphologically from the two posterior pairs, and are the only portions of the glandular oesophagus which are true epithelial diverticula.[1]

b. *Blood Supply.* — An enormous amount of blood courses through this part of the oesophagus, and a large part of it is

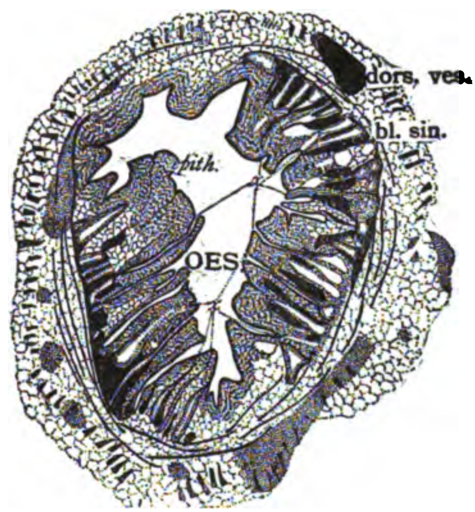


FIG. 8.— Cross-section showing openings of gland ducts into oesophagus

always present in the radial partitions which have already been described as being made up of two secretory lamellae, separated by a broad blood sinus. The blood entering the sinuses by cross-vessels from the dorsal vessel courses along cephalad.[2]

[1] In *Phoenicodrilus taste*, Eisen describes the first pair of glands as non-secretory, and entirely different from the two posterior.

[2] The blood is all derived from the dorsal vessel which gives off the first glandular branch in the middle of the fourteenth somite by an artery defining the posterior limit of the glandular oesophagus (see Pl. VI, Fig. 1). There are two similar vessels from the dorsal, in the thirteenth, which subdivide into smaller vertical branches. Where these encircling supply-vessels cross the longitudinal sinuses, the "palisade" arrangement described by Jacquet ('86) is produced. As will be seen in Pl. VI, Fig. 1, two vessels go from the dorsal in XII to CG^3, three in the eleventh ramify on CG^2 and CG^3, and one in the tenth goes to CG^2. The last is the only vessel mentioned in Jacquet's rather incomplete account as going to any of the glands. This large vessel has a decided cephalad direction, and may serve to drain the glands. At the septum IX–X, a cross-branch connects the

The walls have already been followed under the name of glandular lamellae, so that it is not necessary to describe how the sinuses are broadened out into the two enlargements of this vascular area, which form CG^2 and CG^3, and narrow down again between the glandular projections, as shown in Text-figs. 5 and 6.

When the lime-filled gland ducts open out, one by one, into the ventricle of CG^1, and thus become obliterated, adjoining blood sinuses, previously separated by the gland ducts, coalesce, as is shown in Text-fig. 2 (*bl. ves.*). Finally, when all the lime ducts have opened out and disappeared, the blood sinuses have fused into two collecting ducts (Pl. VI, Fig. 1, *coll. ducts*), which emerge from the anterior end of CG^1, and become the lateral vessel (after receiving the branch which left the dorsal in XIII to supply the reproductive organs).[1]

The lateral vessels run straight forward to the pharynx and head parts, where they divide, one branch following the nerve commissure up to the brain, over which it ramifies. This blood has been freed from elements, partly waste, during its passage through the calciferous glands.

III. Physiology.

1. *Historical.* — Julius Leo ('20), who first described the calciferous glands, says concerning them: "Qui usus corpusculorum fugit me." A number of later authors[2] considered them to be ovaries, or accessory reproductive glands. Morren ('26), by whose name the glands are often called, made out their connection with the oesophagus, but nevertheless held that, since they were very much reduced in winter, they were probably concerned with the reproductive function, and suggested that they might furnish lime for the egg-capsule, which, however, is chitinous.

dorsal with the collecting ducts (Pl. VI, Fig. 1, *coll. ducts*). There is no doubt that these vessels are arteries, for the pulsations from the dorsal may be seen continuing down the vertical vessels in XIII and XIV.

[1] The artery bringing blood from the dorsal in XIV breaks up into small vessels, precisely in the reverse manner (see Text-fig. 8, points *x* being where the gland ducts open).

[2] Williams ('51), Stein ('45), Henle ('42).

Lankester ('65) and Claparède ('69), observing that the calciferous glands opened into the alimentary tract, concluded that they were concerned with the digestive and not with the reproductive function. Lankester formulated no view as to their special function, while Claparède, who was interested in the concretions contained in the anterior pouches, suggested that the special function of the glands was to furnish millstones for the trituration of the food in the gizzard. Perrier ('72) and Darwin ('81) observed that such a function must be of secondary importance, since there are always small stones taken in with the food. Darwin's theory of the function of the glands is that they are primarily organs of excretion, but that some of the lime is used in digestion to neutralize humous acids generated by the decaying vegetable matter in the intestine. Inasmuch as this is the most widely accepted theory of the function of these organs, we wish to dwell somewhat fully upon it.

2. *Darwin's Theory of the Function of the Calciferous Glands.* — Leon Fredericq ('57) had maintained that a digestive fluid like the pancreatic of higher animals is found in the intestine of earthworms. This conclusion was drawn from the following experiment, and therefore was based on insufficient evidence. A large number of whole earthworms, skin, glands, muscles, gonads, alimentary tract and the decaying matter therein were finely chopped up. An extract of the "*haches*" mixture was found to dissolve fibrin and starch. It is, however, impossible to tell from such an experiment what is the source of the ferments. Fredericq further made the bare statement that he had obtained "from the green part" of the intestine of the earthworm an alkaline extract which would dissolve fibrin. But neither this simple fact nor the former experiment proves the intestinal secretion of earthworms comparable to the pancreatic fluid of the higher vertebrates.

Darwin's experiments, however, did clearly prove that the salivary fluid contains proteolytic and diastatic ferments, and is alkaline. Were the lime employed in connection with the saliva, its use as a neutralizer of acid foods would seem very probable. But from the position of the glands this is impossible, and the lime must naturally be associated with digestion either in the gizzard or in the intestine. Since the digestive fluids in the intestine have never been shown to require an alkaline medium for their action, and since there is no reason for believing that they are exactly like the salivary fluid, we are still in the dark as to *how*, if at all, the lime is used to advantage in the digestive processes. Darwin's hypothesis, that it is used in some way in digestion, has neither been explained nor confirmed, and it is hoped that the following experiments on direct digestion may show clearly that some foods act directly as stimulants to the flow of this secretion, although the reason for this, and the way in which they act, must be solved by the physiological chemist.

3. *Experiments.* — (a) *Methods:* Earthworms will burrow in almost any soft material, and in so doing swallow large quantities of the substance in which they are placed. Advantage was taken of this fact to observe what effect would be produced by the presence in the digestive tract of substances different in reaction and composition. It was desired to observe the effect upon the calciferous gland with respect to the disposition of the product already stored in CG^1, and with respect to the action of food upon the secreting activity of CG^2 and CG^3.

More especially the original purpose of the experiments was to ascertain whether (1) acid food in the intestine increased the flow from the gland; (2) whether the same result could be reached (through absorption) by a prolonged presence in the digestive tract of neutral bases which contained the elements of carbonate of lime; and (3) whether the secretory process went on at about the same rate, whatever the substance present in the digestive tract.

The optimum time of exposure to the various foods was determined by experiment.[1]

In the case of worms placed in acid foods, it was desirable to leave them just long enough for the material to have passed some distance into the intestine. By too long absorption of

[1] The substances used and the periods during which they remained in the digestive tract were as follows: starch, 7 days; sawdust, 2–9 days; coffee, 2 days; calcium carbonate, 2–5 days; calcium phosphate, 5–7 days; weak sodium carbonate, 5 days; weak potassium carbonate and water (practically starving), 7 days.

TABULATED RESULTS OF ACID AND NEUTRAL FOODS.

Food.		Effects.					
	Substances and exposures.	1. Lime globules present in oesophagus or intestine.	2. Cells active and lime present in CG^2 and CG^3.	3. Cells not active. Cytoplasm building.	4. Pebbles collected in CG^1.	5. Pebbles in intestine.	6. Concretions.
Acid.	Worm in water, acidulated with lemon juice, 6 hrs. to 3 days.		•		•		
Acid.	Worm for 4 days in $HNaCO_3$.		•				•
Acid.	Acid starch 5 days.		•				
Slightly Acid.	$Ca_3(PO_4)_2$ 7 days.		•		•		
Slightly Acid.	Sawdust 2 to 9 days.	•	•		•		•
Neutral.	Ca_3SO_4 7 days.				•	•	
Neutral.	$CaCO_3$ 2 to 5 days.			•	•		
Neutral.	Coffee 3 days.			•			
Neutral.	Water 9 days.			•		•	

EXPLANATION OF TABLE.—The substance in which the worms were placed is indicated in the first vertical column on the left. The results obtained by the treatment are tabulated in the following vertical columns as their respective effects. The records in the second and third columns were obtained from cross-sections of the secreting tissue and are by far the most significant of all. The presence of pebbles (indicated in the fourth and fifth columns) in either the digestive tract or the anterior pouches depends very largely upon the condition of the worms when it is placed in the food. It was found impossible to remove or control these pebbles by preliminary treatment, and if pouches were filled with them when the experiment began, the results for the fourth and fifth columns were in so far vitiated. The concretions between adjoining lamellae (last column), figured by Claparède and described by Darwin ('81), seem both from histological and physiological considerations to be pathological effects.

acid food, the glands might cease secreting because of exhaustion in the blood of the elements essential to the formation of lime. In alkaline and neutral foods, on the contrary, they were allowed to remain long enough to insure opportunity for the absorption from the blood of salts or carbonates, and their excretion through the calciferous glands.

The digestive processes were stopped, at a desired time, by killing the worm. To accomplish this instantly, mercuric chloride was injected into the oesophagus. The glands were then taken out, hardened still further,[1] and finally sectioned. In order to know the condition of affairs in the lower digestive tract, after removing the glandular oesophagus, every half-inch of the intestine was carefully searched for the lime pebbles manufactured in *CG*¹, and for lime globules. Although these could best be detected by the acid test, pebbles could be readily distinguished by the naked eye, and the lime globules were readily recognized in microscopical examination [2] by their size, brownian movement, and crystalline appearance.

(b) *Discussion.*— It is desirable to describe more fully three of the preceding experiments.

1. *Calcium Carbonate Food.*—In order to distinguish secreted lime from lime taken as food, a form of the latter was used which crystallized in perfect minute rhomboids.[3] These could be readily distinguished by their shape from the ones produced by the last two glands. The worms which were placed in this substance devoured it so greedily that after a day or two their digestive tracts could be seen through the skin, white and distended with the crystalline contents.

After a week the worms were opened, but neither pebbles

[1] Mercuric chloride was found to be the only killing fluid which did not dissolve all the calciferous matter. Although somewhat acid, it had scarcely any decalcifying property, while ferric ammonio-sulphate, 3 per cent, was a good solvent of lime.

[2] Because of the exclusive use of the latter method, I was for a long time deceived by the globules resulting from the degeneration of the excretophores, or "chlorogogue cells," which exactly resemble the lime globules in size, contour, and brownian movement. These probably got inside the intestine during the opening of the alimentary wall with scissors.

[3] The crystals were made by mixing solutions of calcium chloride and sodium carbonate, and were thoroughly washed before using.

nor *globular* crystals were to be found throughout the entire length of the intestine. Both could have been detected had they been present in any considerable quantity. Sections of the gland of this worm, however, showed that although few globules were forming from the cytoplasm of the cells, constructive metabolism was in progress, and this was indicated by the cell projections, which were well developed, and which contained the large granules figured on Pl. VIII, Fig. 15. Since no calcium carbonate was liberated, however, we may say that this experiment indicates that although the glands may be excretory organs, their action is not increased by the continued presence of calcium carbonate in the digestive tract.[1]

2. *Acid Food.*—With acid food, however, the manufacture and excretion of lime crystals are increased. Aside from finding many small globules on their way to the gizzard, the histological appearance of $CG^{2 \text{ and } 3}$ in acid-fed worms indicates that this gland has been very active, for the cytoplasm is greatly reduced in thickness, so that the entire lamella appears no thicker than a blood vessel, and many crystals and shreds of protoplasm are found in the gland cavities which give evidence of over-secretion.

3. *Starving.*— Finally, the following experiment tends to show that the manufacture of lime is going on, more or less actively, irrespective of the substances present in the digestive tract.

Two worms, taken directly from the ground, were kept for a week or ten days in water, which was changed as often as it became impure from the castings. Small white pebbles, easily recognizable by their shape and reaction as those which occur in CG^1, were found on the bottom of the dish, free or mixed with castings. The following were gathered from the dish containing two worms: On the first day 4 pebbles, on the second day 8 pebbles, on the third day 4 pebbles, on the fourth day 4 pebbles, on the fifth day 5 pebbles, making a total of 25 pebbles in five days.

[1] It would have been an interesting experiment to find the effect upon the gland by the continued feeding of *acacia* leaves, which are said to contain 72 per cent of lime.

When one of these worms was killed and opened, three or four more pebbles were found on their way down the digestive tract. Possibly all these excretions had been stored in CG^1 or were manufactured from the lime already present in $CG^{2 \text{ and } 3}$, although I do not remember ever having found at one time more than eight in any one worm.

✓ 4. *Summary.* — The conclusions as to the function of the calciferous glands are:

(1) Acid foods cause a very decided increase in the secretory activity of the gland.

(2) There is normally, however, a constant output of carbonate-of-lime pebbles into the oesophagus, and during the digestion of starch and cellulose a slight increase is apparent in the secretory activity of the gland. It is suggested that the pebbles emerging from the first pair of glands may be waste matter, while the small crystals coming from the openings in the middle of the fourteenth somite are used in digestion.

(3) The pebbles, which go through the body apparently unchanged, are not increased, nor is the secretory activity increased by an excess of calcareous matter in the digestive tract.

Darwin's theory as to the function of the lime as a neutralizer of acid food is to some extent confirmed by the experiment with acid food, although substances not acid, such as cellulose and starch, also acted as slight stimulants to flow. His suggestion as to the excretory function when the worm lives in a soil which has an excess of calcareous matter does not seem to be supported by the preceding experiments, and the comparison of the digestive fluid of the earthworm with the pancreatic fluid needs confirmatory evidence. The former has been said to contain a proteolytic ferment, but it has not been shown to contain diastatic or steaptic ferments, nor is there any evidence to show that an alkaline medium is more favorable than an acid for its operation. In fact, Darwin's statement that the alimentary tract of earthworms generally gives an acid reaction is entirely confirmed by these experiments.

IV. Cytology.

1. *Introduction and Statement of Problem.*—That the successive phases of cell metabolism in gland cells are recorded up to a certain point by morphological changes was long since pointed out by Heidenhain in the case of the pancreas cell. The present paper was planned to approach, from this point of view, the problem of the processes of metabolism which result in the formation of lime, and more especially the interrelation and special functions of nucleus and cytoplasm.

To this investigation the material chosen seems to be especially well adapted, since: (1) In their highest development the cells are nearly isolated, being separated by a distance greater than their own width. ("Cell," used in this connection, refers to a club-shaped protoplasmic projection and the nucleus which it contains.) (2) The secretion is a solid inorganic substance of known composition. (3) This product makes its appearance as crystals, of constant form and shape, that can be detected as soon as they are formed, since if not exposed to acid reagents the products of secretion are not altered by the processes of preserving and staining. (4) (The principal advantage), A desired stage in the history of the gland cell may be obtained in an entirely normal way of feeding. The earthworm will burrow in and feed upon any substance in which it may be placed, and the alimentary tract being filled by this process, almost any desired effect can be observed.

The cells of the gland are projections from the lamellae described in the preceding account (Text-fig. 7) as radial partitions in the wall of the oesophagus. Arising in irregular fashion from the broad sheet-like lamellae, these cell projections give to the sheet of glandular tissue a very uneven appearance. After an active wave of secretion, the cell projections may almost entirely disappear, being leveled by disintegration to the general surface. The projections too, though separated, are not strictly individuals, for at their bases they are united to form a syncytium without cell boundaries. If we accept Bourne's terms ('95), the present arrangement would be a condition of cells compounded in continuous concrescence. Each nucleus, however, presides over a certain amount of cytoplasm (*cf.* energids of Sachs), and gives rise to a large club-shaped mass of protoplasm projecting from the surrounding secreting surface. Together with the nucleus, this may be conveniently called a cell (see Pl. VII, Fig. 14, or Pl. VIII, Fig. 15).

It is proposed to relate briefly the constructive changes undergone by the cytoplasm during the development of a club-shaped cell like that drawn in Fig. 15, to trace the subsequent destructive changes back to the starting point of the cycle, to follow correlations between nucleus and cytoplasm, and finally to give the complete *nuclear* history.

2. *Secretory Changes in the Cytoplasm.* (a) *Constructive Phases.* — Let us take for the gland cell in its lowest terms the condition found in a worm which has been stimulated to over-secretion by acid food. Here the secreting layer is very thin and may be reduced, as in Pl. IX, Figs. 31 and 32, to the thickness of an ordinary blood vessel scarcely the width of the nuclei which are imbedded in it. The nuclei are all oval, small, with heavily staining granular chromatin and feebly defined nucleolus. The cytoplasm is dense and composed of elements closely knit together. In a later stage the cytoplasm expands a little about each nucleus, a slight projection is raised up, and the secreting surface assumes the appearance shown in Pl. VII, Figs. 3 and 4.

As the action proceeds, the cytoplasm continues to increase in extent. It becomes less dense, and vacuoles appear here and there. A cell projection may continue to grow until it is long and often branched, the outer distal part sending pseudopodial extensions and bridges across to anastomose with adjoining cells, so that in section one sees very many irregular isthmuses. The nucleus becomes decidedly vesicular, the nucleolus large and densely staining (Pl. VII, Figs. 11 and 12). In normal worms this stage represents the height of the constructive activity, and the cell is now ready for the changes which will result in the formation of lime crystals.[1]

[1] In worms fed on certain carbonates, however, another phase is introduced here. Although the nuclear structures remain about the same, this treatment causes large granules to appear at the nodes of the cytoplasmic reticulum in some of the

(b) *Formation of Lime.* — The lime now commences to appear. In the gland under discussion there can be no doubt that the secretion occurs in its final form inside the cell before the latter is broken down. This point has been long disputed, because in the greater number of glands there is no way of telling, by exact chemical tests, when the secreted product has attained its full development. Minute round crystals appear at the nodes in the reticulum and almost fill the spaces, or alveoli, between the loose cytoplasmic network (see Pl. VIII, Fig. 16). Sometimes a single shred of the cytoplasmic substance can be seen in the gland cavity with a few of the crystals attached to it.

In a cell which has passed the height of constructive metabolism these crystals may be seen shooting out, as it were, from the nodes of the disintegrating cytoplasm, apparently having grown in much the way that mineral crystals form in a strong solution. Although I have very distinctly made out the small crystals inside a cell which has not yet broken down, and have seen them dissolved by acid, it is quite impossible to determine whether they are derived from the reticulum, or partly from the cell sap which fills the spaces.

As the small crystals accumulate more and more, growing at the expense of the cytoplasm, or causing the latter to fall away, large vacuoles appear in the club-shaped projections, and through these the lime makes its way into the gland cavities, probably often forced out of the cell by the mechanical contractions of the surrounding tissue. The disintegration of the cytoplasm is in direct ratio to the amount of lime produced. The minute globular crystals often coalesce in CG^2 and CG^3 to make larger ones, which are either round or rhomboidal.

club-shaped cells. These worms have been kept for several days in a weak solution of sodium carbonate, and although the granules might later have been changed into the usual carbonate of lime secretion, they would not, at this stage, dissolve when treated with acid. To test this the cover glass was removed from the preparation from which Fig. 15 was drawn, the balsam dissolved by xylol, and after changing to alcohol, the section was exposed for some time to acid alcohol. It was then restained, remounted, and the same cell found and drawn. The granules had not decreased in number or size, although lime crystals entirely disappear under this treatment. These insoluble structures are not frequent in normal worms.

There is, very probably, an organic matrix surrounding or permeating these small crystals when they are first formed; otherwise one cannot understand why they should be globular rather than rhomboidal in shape. Moreover, a residue is always left after acid treatment. Globules which remain in *CG*[1] for any length of time, however, coalesce into the usual rhomboidal form of lime crystals or into irregular pebbles. Both of these when broken often split into small rhomboids.

(c) *Destructive Phases.* — It remains to trace the cells, after the lime has appeared, back to the reduced condition with which we started the cycle. Nearly all the cytoplasm in the cell projection is used up in the production of lime. If there has been over-secretion, the cytoplasm disappears almost down to the blood sinus. Coincident with the appearance of new nuclear elements from the wall of the sinus or from the blood, the constructive process starts up again and there is a repetition of the process. Practically all the cells in one earthworm are at the same stage of secretion at any given time.

(d) *Summary of Changes in the Cytoplasm.* — The processes of glandular activity may be summarized as follows: (1) Constructive processes during which elements derived from the blood are built up into cytoplasm. (2) Transformation of the latter, in which elements of the cytoplasmic reticulum in the neighborhood of the cytolymph give origin to lime crystals. (3) Gradual accumulation of crystals inside the cell until the walls are broken and the lime globules, with more or less attached cytoplasmic reticulum, are cast out into the gland cavities. (4) Although discussed later, it seems best to mention here the disintegration of the primary nucleus, which shows changes coincident with those of the cytoplasm. With the rise of another cycle of secretion, a younger generation of nuclei appears nearer the blood sinus. Dilapidated nuclei, formerly active, can still be seen at the distal ends of the syncytium.

(e) *Rapid Formation of Lime. Osmosis.* — Although the gradual stages described above include all the different phases in which the lime cells have been found, some of the constructive phases may be omitted when a gland is extremely active. There are often cases in which lime appears to form when cell projections are hardly visible, and this seems to be the case with the glands in *Phoenicodrilus taste*, figured by Eisen ('95). One might be tempted to regard the extremely thin layer of cytoplasm as a membrane through which lime in solution passes, by osmosis, directly from the blood, but no positive evidence whatever can be offered for the truth of this view; while we know that, in the more numerous typical cases, substances from the blood are first connected with the cytoplasm (either the reticulum or the cell lymph), and appear as lime crystals only at the distal end of the cell, after the cytoplasm has undergone considerable differentiation.

(f) *Comparison with Other Glands.* — The processes that occur in mucous glands, goblet cells, and pancreas cells, have been recorded by Schiefferdecker ('84), Stöhr, Patzelt ('82), Biedermann ('75), Heidenhain ('93), Roubet ('96), and many others. Most authors agree that the secretion appears first in the form of granules, and Stöhr goes so far as to state that this fact holds for secreting cells generally. The calciferous glands show such striking differences in this and several other regards that there seems to be little common ground between them and unicellular epithelial glands.[1] As will be seen in the further development of this paper, the tissue under consideration comes more nearly under the blood-gland type, since the calciferous glands seem from developmental and histological considerations to be hypertrophied walls of blood vessels and not epithelial structures. Leydig's ('76) description of a deep-lying lime gland in gasteropods recalls in very many particulars the calciferous gland in *Lumbricus*.

[1] In the goblet cells there has been much discussion as to whether in certain stages the cell is filled with secretory products, and at other times is simply protoplasmic. Biedermann, working on living material, and Merk show quite conclusively that the mucus is represented inside the protoplasm by granules, which, when they are expelled from the cell, become clear drops. They both deny that the cell can be changed into secretory products. This position is maintained by Stöhr, who gives the following general characters: A goblet cell grows from an ordinary epithelial cell, and after secretion, during which the nucleus does not go to pieces, may return to the ordinary epithelial type.

Hermann ('88) and others maintain that the secretion which is present in goblet cells goes out by drops when the cell wall breaks, having exerted enough pressure, previously, to alter the shape of the nucleus.

The principal characteristic features in the secretory processes of the calciferous glands are (1) the formation of the product inside the cell; (2) absence of granular or thread-like stages in the normal production of lime; (3) disintegration of the nucleus during secretion after a definite and constant cycle of changes which is correlated with progressive changes in the cytoplasm; (4) replacement of the nucleus. The correlation between nucleus and cytoplasm, and the replacement of the nuclei, will be treated separately and somewhat at length, on account of their far-reaching general significance.

3. *Correlation of Nucleus and Cytoplasm.* — It will be remembered that the cytoplasm near the sinus wall is denser and more closely knit than that at the distal end of the cell, also that the cytoplasm represented in the earlier stages of the constructive phase is denser than the cytoplasm at a later period.

In general it will be noticed that the density of the nuclear reticulum corresponds with that of the cytoplasm surrounding it. The nuclei nearest to the sinus wall are small and oval, with heavily staining membrane and closely crowded chromatic substance (Pl. IX, Figs. 31 and 32, n^1). Nearer the center of the cell, where the meshes in the cytoreticulum are larger and less closely crowded, there are large round nuclei, with thin membranes, prominent nucleoli, and chromatin in large isolated masses. The linin-fibers, like the cytoreticulum just outside, are loosely arranged with large interstices (Pl. VII, Figs. 8 and 12, n^2). Finally, at the very distal ends of some cells, the remains of an old nucleus, which has collapsed and almost disappeared, can be made out, containing vacuoles and scarcely distinguishable from the surrounding cytoplasm by its inconspicuous membrane (Pl. VII, Figs. 9 and 10; Pl. VIII, Fig. 18, n^3). These nuclear and cytoplasmic structures resemble each other very closely in their exhausted appearance. Since all three kinds of protoplasm are to be found at different levels in the same cell, there must be some meaning in this correlation between nucleus and adjacent cytoplasm. There is evidence that the nucleus is the controlling factor in the early constructive phase of the cell's history, and that the cytoplasm may actually grow through the influence, if not at the expense of, the nuclear elements; for when the nuclei first appear at the base of the cell they have merely a thin layer of cytoplasm about them, while after a considerable activity, which is indicated by dense staining, the cytoplasm is greatly increased in amount.

After the cytoplasm has come to project from the lamellar surface as a club-shaped projection, the nuclear activity is concluded; and in the following degeneration stages, when lime appears all through the cytoreticulum, the nucleus probably remains quite passive.

If the substance of the reticulum from which the lime comes was ever an integral part of the nucleus or in structural connection with it, it must have been during the constructive phase when it was generated as cell protoplasm. There are never granules or threads of any kind discernible which arise from the nuclei. The process is here, however, fundamentally different from that described in the pancreas, because in this case the entire outer part of the cell, including the nucleus, falls away.

4. *History of the Nucleus.* — The last-mentioned fact — the disintegration of the cell during secretion — is the essential point in the history of this gland. It is entirely consistent with two other characters which indicate that the lime-producing cells are very much specialized. These are, first, that there is no membrane bounding their irregular contour; and, second, that the cells from which they have descended divide by amitosis.

Whatever the cause, however, or the meaning of it, the primary large nuclei (Pl. VII, Fig. 4, n^2) in the central or distal part of the club-shaped cell productions do eventually collapse or become cast out into the gland cavity during an active secretion. The remnants of degenerate nuclei are shown in Pl. VII, Figs. 3, 9, and 10; Pl. VIII, Figs. 18 and 24, marked n^3.

In many of the cells sectioned axially a third class of structures are seen, which are destined to move outward and, in turn, take on the functions of n^2 and n^3. These nuclei are smaller in size, with regularly distributed chromatin and thick membranes. They are shown in Pl. VII, Figs. 4, 8, 10, etc., marked *n'*, where there is a regular progression outward of these regenerating factors. None of the nuclear elements described in this paper have ever been seen dividing, except the type which have been classed as *n'*, situated near the blood vessel. They frequently occur closely appressed to the sinus wall, as shown in Pl. IX, Fig. 32. There is little doubt that these smaller nuclei are the ultimate source whence the syncytium makes good its waste and is regenerated. They gradually take up the position vacated by the cells of the former generation, and the outward movement may be traced in Pl. VII, Figs. 8–12. So far the history is very plain and there seems to be but one interpretation possible. The affair is much complicated, however, by certain nuclear structures (figured on Pl. VII, Figs. 4–7 and 13, *mig. nucl.*) which are seen (1) loose in the blood; (2) closely appressed to the sinuses; and (3) in every possible position between partial and complete imbedding in the glandular syncytium. It is impossible to draw a sharp dividing line based on position, size, or appearance between these and the smaller nuclei, *n'*, of the glandular syncytium. Repeated observations have demonstrated beyond a doubt that these wandering cells or migratory nuclei are constantly and normally making their entrance into the gland cells at certain periods in the gland's activity. The prevalence of this migration may be illustrated by the fact that in thirty-seven cells, which were counted on one side of a lamella, twenty-two showed protoplasmic processes of this kind making their way into the glandular syncytium.[1]

The gradual gradations between the smaller nuclei (*n'*) and the migratory nuclei (*mig. nucl.*) leave no doubt as to the identity in character of the two structures, leaving only the question as to the direction in which these migratory nuclei are moving. That they are usually progressing from the blood-vessel wall into the cytoplasm is indicated by the relative position of the chromatic elements in the migrating nucleus. Although the latter structure is attended by a thin protoplasmic film, the chromatin, which by its deep staining seems to

[1] The worms, from the glands of which this slide was made, had been fed on calcium carbonate.

be especially active at this time, precedes the cytoplasmic (?) part streaming along behind until the migrating nucleus is well within the syncytium (see Pl. VII, Figs. 6 and 7, *mig. nucl.*).

The most plausible explanation of the process which is going on in the stages (Pl. VII, Figs. 4–11) is that these migratory bodies are pushing their way into the syncytium. I have observed them in most unexpected elongated forms, such as in Pl. VIII, Fig. 19, which had progressed into the syncytium and pushed the wall in front of it for a very considerable distance. Their movement in penetrating the glandular syncytium is precisely like that of free wandering cells; but unless one is sure that the identical structures under observation have at one time been free and have not always been located at the point on the sinus wall whence they start their inward migration, it is better to use the term "migrating nuclei" than "wandering cells." Until the migrating nucleus is completely inside the syncytium, it is surrounded by a vacuole which separates it from the general cytoplasm of the gland.

Amitotic division of the new element is observed quite frequently at this stage, the attachment to the sinus wall in some cases being still visible (see Pl. VII, Fig. 13). The chromatin, meanwhile, is very closely crowded, and is obscured by the deep staining of all the parts of the entering nucleus. After the latter has severed its connection with the membrane at the base, a reticular arrangement of the chromatic substance appears at the advancing end (see Pl. VII, Fig. 7). The thin film of cytoplasm surrounding the nucleus gradually expands, the chromatic part becomes round (although retaining, for a considerable time, traces of its origin by pointing toward the sinus, Pl. VII, Figs. 8 and 9). The cytoplasm which has been brought in by the entering nucleus gradually becomes less dense, and a connection with the general syncytial substance is gradually made across the surrounding vacuole.

To summarize the movements of these migratory nuclei: They appear, as a rule, closely appressed to the syncytial wall either on the side of the sinus or that toward the cytoplasm. After lengthening out they push their way into the glandular layer, where they become round. The thin layer of cytoplasm which has accompanied them gradually increases and expands about the center until it is finally merged into the general glandular tissue. They then become the typical nuclei (n^2 and n^3) of the secretory syncytium, and eventually with the cytoplasm of the latter undergo the degeneration phases which have been described for this gland during the formation of lime.

There are a number of interesting questions at once raised by this somewhat unusual procedure which may best be taken up in their natural order.

1. Are there other recorded cases in which the nucleus goes to pieces during secretion? In the case of the much discussed "goblet" epithelial cells, the weight of evidence now seems to favor the persistence of the nucleus.[1] Nevertheless, in single-celled glands in Protopterus, Kölliker ('89) has shown that there are two nuclei, and that when the cell discharges, one nucleus is cast out and one stays behind. Further, O. Hebold ('79), who tried to settle this single question in the frog, found that the gland cell did not disintegrate during secretion, while in the oesophagus cells may, during over-secretion at least, go to pieces.

Heidenhain ('93) clearly proved, and was confirmed by Vollmer ('93), that in Amphibia, by over-secretion, an entire group of epidermal gland cells may be ejected, nuclei and all, and that these are regenerated from undifferentiated elements, so that a new gland is established in the place of the old. Another well-established case of the disintegration of gland nuclei is the familiar one of the milk gland, where cells and nuclei are both sacrificed in ordinary secretion.

2. A more puzzling problem than the preceding is how structures which do not survive secretion are replaced. And bearing on the present observations, it may be asked whether certain migratory cells can lead, for a short time, a sedentary life. In the case of certain foot-glands in mollusks, it was asserted

[1] The following authorities, Rawitz ('87), Hermann, List ('85), Haller, Paneth ('87–'88), Patzelt ('82), and Stöhr ('82–'87), uphold the latter view. In favor of the expulsion of the nucleus or the death of the cell are Brettauer and Steinach ('57), Eimer ('84), Arnstein ('67), Hebold ('79), Steinhaus ('88), Brock ('84), Vollmer ('93).

(Brock, '84) that connective-tissue cells replace epithelial gland cells. Rawitz ('87) rejected this idea, stating that such a process could not occur without ascribing to fixed cells of a definite morphological and physiological tissue the power of changing themselves into cells of another and not less sharply defined tissue. Instead of these gland cells having finished their career, Rawitz asserted that they simply showed partial degeneration tendencies, and soon would repair.

The recent words of Saxer ('96) and Felix ('97) in this connection carry much weight: "The best work during the last ten years indicates that leucocytes do not in vertebrates change into connective tissue, nor *vice versa*." Nevertheless, the cells replaced in the calciferous glands are of a much less specialized type than those of either epithelial or connective tissue. And the observations previously given assert far less than an impossibility when they are interpreted as meaning *that wandering cells migrate into an enlarged blood-vessel wall and undergo degeneration, during which the accompanying cytoplasm expands and is finally transformed into lime crystals*. The differentiation of wandering cells is not so extreme as to preclude such an interpretation. But another consideration, coupled with this, causes one to weigh the matter very carefully. This consideration is the matter of the germ layers. For what occurs would seem to mean that the cells of calciferous glands presumably endodermal are replaced by wandering cells presumably mesodermal, a procedure which would certainly be somewhat anomalous.

Up to the present time the interpretation of the calciferous glands has been that they originate as epithelial diverticula, while the origin of the blood corpuscles is not definitely known. If the glands have such an origin, the preceding observations on the secretory changes in the calciferous glands would compel us to infer that in the earthworm, cells of one germ layer, of a specialized type, can be replaced by cells of another germ layer of a different type. But it is not right to infer this, until we know the exact morphological standing of the tissues involved; first, the origin and the character of the glandular cells arranged upon the sinus; and, second, the origin and character of the migratory nuclei or wandering cells of the blood. In order definitely to settle this point, it has seemed worth while to go back to the earliest embryological history and follow these structures in

V. Development.

a. *Origin of the Blood.* — The gland does not commence to form until the embryo is about an inch long. The dorsal blood vessel is already filled with blood, as are some of the spaces between the splanchnopleure and the regularly arranged epithelial cells. Previous to this time the yolk-laden endoderm cells appear closely abutted against the circular muscle fibers, with the nuclei collected near the oesophageal lumen (as described in the latest stages given by Wilson ('89), Figs. 91 and 94). No indication of the calciferous glands appears until the solid yolk of the endoderm cells becomes broken up into granules by the active protoplasmic threads extending out into all parts of the cell from the yolk nuclei[1] (see Pl. IX, Figs. 25 and 27).

Especial attention must be directed to the movements of these yolk nuclei. Originally arranged in large numbers near the lumen (see Eisig, '98), they come to assume positions at various levels in these cells, principally trending, however, toward the splanchnopleure. They finally make their way into the blood-filled spaces between endoderm and the circular muscle (at the points marked *x*, Pl. IX, Fig. 25).

In this as well as in the earlier stages the nuclei contain yellow yolk particles scattered through the karyoplasm, and they can be recognized by means of these wherever they may have migrated. It is a most remarkable fact that they make their way into the blood space between the splanchnopleure and endoderm, or even out into the peritoneal tissue, where

[1] Amoeboid movement similar to those of the endoderm cells in *Lumbricus* have been described and figured in *Capitella* by Eisig ('98), p. 67. They send projections toward the splanchnopleure, but Eisig finds in this no support for his earlier statements ('87) of "epithelial muscle-building." On the contrary, I have observed that, aside from contributing to the vascular system, these same endodermal nuclei do seem not infrequently to be associated with the developing muscle fibers in a very peculiar way.

they generally lie with their axes in the direction of the circular muscle layer (see Pl. IX, Fig. 25). They may also be recognized in the peritoneal tissue.

The cytoplasm surrounding these yolk nuclei makes its way into the blood (as shown in Pl. IX, Fig. 28, at point *X*), and, although some of the yolk nuclei themselves probably persist as a permanent element of the blood, others are shown in Pl. IX, Fig. 30, which stain very feebly and appear in a collapsed condition, closely crowded into the narrow spaces between the epithelial cells and the splanchnopleure. From these observations it seems probable that a very considerable amount of all the blood originating in this region is derived from these yolk-bearing cells which (1) break up the solid yolk masses found next to the oesophageal lumen; (2) increase by mitotic division (Pl. IX, Figs. 26 and 30); (3) migrate with their streaming protoplasm toward the splanchnopleure; and (4) enter themselves into the blood spaces, where a large part of the accompanying cytoplasm at least, and probably some of the nucleoplasm, is transformed into the plasma of the blood.

A most important function of these wandering amoeboid cells of the endoderm remains still undescribed, *viz.*, their grouping in hollow squares around cavities which appear in that part of the endoderm nearest the circular muscles (the peripheral hypoblast of Vejdovský). These cavities become the gland cavities and gland ducts of the calciferous glands, and their mode of origin may be clearly understood by consulting Pl. IX, Figs. 25–30. To trace this process a little more fully, the earliest stage is shown in Fig. 27, where certain of these wandering cells are gathered at the upper part of the endodermal layer. It is evident from this figure that some wandering cells of the endoderm may enter the blood sinuses and migrate through the embryonic tissue, while others collect at the basal part of the endoderm to form the calciferous glands. Some of the yolk nuclei here figured have just emerged from the blood sinus and show unmistakably, by their containing yellow yolk granules, that they have been in the endoderm.

The same yolk nuclei, which have been shown to enter the blood spaces and to contribute to the vascular fluid, may arrange themselves around a space (Pl. IX, Fig. 30) which will later be the cavity of the calciferous gland. These cells themselves become the secretory syncytium, bounding the blood sinuses. A figure of the adult gland (Pl. IX, Fig. 31) is placed beside the developing gland (Fig. 30) to show the relations. The so-called blood spaces broaden out into the blood sinuses. The cavity left by the withdrawing of the endodermal nuclei toward the sinuses becomes the adult gland cavity, and the cells bordering upon this give rise to the glandular syncytium.

The gland cavities are not part of the original archenteron, but are hollowed out anew from the endodermal walls. The description given above of the development of the calciferous glands applies only to the two posterior pairs and the gland ducts found in the anterior pair. The pouches of the first pair are formed as true diverticula from the oesophagus.

b. *Origin of the Blood-Vessel Walls and Gland Cells.* — The blood and glandular cells in this region have been shown to originate from the endoderm. There still remains the wall between the blood sinus and the glandular syncytium. Very many divergent views exist as to the origin of these structures, as also in respect to the embryonic origin of all the elements of the blood (see Felix, '97, for a discussion of this problem).

For our present purpose, however, the origin of the blood-sinus wall separating the blood from the secretory lamella is of especial significance, because imbedded in or closely appressed to this layer are the so-called "migratory nuclei."

It is especially difficult to reach a decision regarding the origin of the sinus walls, because of the clots of blood which fill the sinus spaces, obscuring everything so completely that it is impossible to make out whether the limiting walls are nucleated structures or whether they possess one or two layers. It was found, however, that, after staining in iron haematoxylin, prolonged extraction would remove a part of the heavy blood clots and leave a light stain in the intermediate membrane. Pl. IX, Fig. 29, shows a section of a developing gland which has been treated in this way, in order to see whether or not there was any endothelium in this place. As would naturally be expected, there is a definite boundary line between the endodermal cells and the blood space (Fig. 29, *v. w.*). This boundary was in no sense, however, anything more than a cell membrane, and besides showing no trace whatever of any nuclear structure, it is apparently, from the very beginning, connected only with endodermal tissue. Salensky ('87) asserts that in *Terebella* the blood comes from the endoderm, but that the vascular walls grow down from the splanchnopleure. This does not appear to be true of *Lumbricus terrestris*.

Whatever endothelial wall there is bordering the blood sinus is simply the limiting membrane, which represents both intima and endothelial elements, and which serves at the same time for the wall of the much hypertrophied, but single-layered, syncytial substance. The wall and the cytoplasm resting upon it are not derived from the splanchnopleure but from endodermal elements which group themselves about cavities developed *de novo* at the deeper parts of the endoderm.[1] The conclusions derived from the study of the origin of the vascular elements in the earthworm are: (1) the blood and corpuscles are formed from the endoderm in this part of the oesophagus; (2) the blood vessels do not possess a nucleated endothelial wall, aside from the common syncytial structure bounding the blood sinus. This specialized tissue develops later into a secretory lime-producing tissue. In the light of these facts the replacement of the disintegrating lime-gland cells by cells belonging to the sinus wall becomes less incomprehensible.

c. *Comparative Anatomy.* — A most striking proof of the correctness of the interpretation of the calciferous cells as modified vascular or peritoneal tissue is found in Beddard's ('94) account of the calciferous glands in the Endrilidrae. From adult histological considerations alone, Beddard (p. 260) was driven to the conclusion that these glands could not be considered epithelial structures. He was persuaded of this because (1) they showed unbroken continuity with the peritoneal cells, and (2) because the digestive function had apparently entirely disappeared. Since he accepted the only view then prevalent,

[1] Glands secondarily derived from vascular walls have been observed in the squid, but are best known in connection with lymphoid tissue.

viz., that these structures were derived from endodermal pouches of the oesophagus, he was driven to interpret this change in structure as an instance of substitution of tissue, the epithelial cells having been in some way replaced by elements of the blood, and the function of the organ thereby having been changed to that of a blood gland, like the spleen of higher animals.

On reading this paper for the first time it seemed probable that one could demonstrate how this substitution of mesodermal elements had actually been brought about, even in so typical an earthworm as *Lumbricus*. For the wandering cells of presumably mesoblastic origin had been observed working their way up into the secretory area, and they must eventually replace epithelial tissue which formerly might have been there (see Harrington, *Anat. Anz.*, Bd. XI, 1896, p. 696). The further studies, especially those on development, proved that this interpretation was not the correct one. For the facts from development go to show that blood-sinus wall ("basement membrane" of gland) and gland cells have all a common origin in the endodermal cells which exhibit amoeboid movements during the late larval period. Thus the cells which regenerate the waste caused by secretion are elements which have been associated with the development of these glandular areas from their earliest history.

Lankester and Vejdovský have shown how nuclei from the arterial walls may wander into the blood vessel and lead an independent life as blood corpuscles, while what has preceded goes to show that these same nuclei may migrate into the tissue of an hypertrophied blood-sinus wall (a syncytium of homogeneous elements), and, undergoing a series of changes, give rise to calcium carbonate crystals.

VI. General Summary.

1. The calciferous glands have two openings: (*a*) through the epithelial lining of the first pair of glands into the oesophageal pouch; (*b*) through the epithelium in the middle of the fourteenth somite directly into the oesophagus. The latter opening transmits only the milky fluid in which are suspended great numbers of minute crystals.

2. These small crystals probably act as neutralizers to acid foods, while the pebbles excreted from the anterior pouches pass through the digestive tract unchanged. An excess of calcareous matter in the intestine does not increase the amount of lime produced by the glands, although acid food does.

3. Carbonate of lime is formed and appears as crystals inside the secretory cells.

4. The nucleus and the entire distal part of the cytoplasm disintegrate during active secretion.

5. The nuclei are replaced by smaller nuclei situated near the blood-sinus wall, and around these the general cytoplasm of the syncytium is built up into club-shaped cells.

6. The smaller nuclei are derived in turn from migratory elements which lie closely appressed to the limiting membrane of the secretory lamellae either in the blood or in the glandular tissue.

7. These migratory nuclei, or blood corpuscles, are derived in development from the amoeboid cells of the endoderm. The plasma of the blood is also derived from these same cells of the endoderm.

8. The anterior pair of pouch-like glands are the only ones lined with true epithelium and are developed as diverticula of the oesophagus, while the two posterior pair are formed by migration of the amoeboid endodermal nuclei, which arrange themselves about cavities formed *de novo* near the splanchnopleure and give rise to the secretory elements.

In closing, I refer with gratitude to the friendly assistance and guidance of Professor E. B. Wilson, of Columbia University, by whom this work was suggested, and to the encouragement of my former friend and teacher, Professor J. I. Peck, of Williams College, in which institution part of this work was prosecuted.

Western Reserve University,
January 28, 1899.

VII. APPENDIX.

CIRCULATORY SYSTEM OF THE EARTHWORM.

CONTENTS.

INTRODUCTION.

THE anatomy, histology, regeneration, and development of the calciferous glands each brought us to the conclusion that these glands have a fundamental and intimate connection with the circulatory system — more specifically with the lateral vessels. This relation was suggested by Perrier when he described the lateral vessel, and the part of the digestive tract drained by it, as the "intestino-integumentary" system. Although the lateral vessels have been thoroughly worked out in several of the larger earthworms, the vascular part of the oesophagus, from which they arise, has not usually been discussed as an integral part of this system.

In working up the blood supply of the calciferous glands it seemed necessary to follow the course of the blood from them to the integument at the anterior end of the worm. But a careful dissection of one set of vessels, and more especially the vexed consideration of the course of the flow, involves a thorough understanding of every trunk opposing the system in question.

I have thus been led, step by step, to investigate the entire circulation in *Lumbricus terrestris;* and the attempt will be made to give an accurate account both of the distribution of vessels and course of flow; for, widespread and much dissected as is this species, the greater part of our knowledge of the circulation in earthworms is based upon unfamiliar African forms less widely distributed than *Lumbricus*.

It will be well to consider, first, the typical arrangement which is metamerically repeated in every somite back of the gizzard; second, the circulation in greater detail, in the anterior eighteen somites, where cephalization, as shown in the muscular, digestive, and reproductive systems, has caused a departure from the typical plan, and then the course of flow with reference to each somite and the body considered as a whole.

I. Anatomy.

A. Body Region.

1. *Method of Observation.* — All the vessels below portrayed can be made out in a small living worm if it be held between two watch glasses and viewed with a dissecting lens.

2. *Dorsal Vessel and its Connections.* — The dorsal vessel is the chief pulsatile organ of the body. Its contractions pass forward and can be traced nearly up to the pharynx. It will be seen in Text-fig. 9 that the dorsal has connections, by several vessels in each somite, with the intestine, and by a large vessel which runs out near the septum it is placed in communication with the body wall.

Jacquet ('86), Claparède ('69), Howe, Vejdovský, and others have adequately described the principal vessels ramifying on the intestine. About three branches in each somite connect the dorsal and typhlosolar vessels, and from the latter the highly developed intestinal network is supplied.

No account,[1] however, has given in any detail the distribution

[1] Unless it be the paper of Horst, which has not been obtainable.

of vessels in the skin of *Lumbricus*, and it is important to mention that it differs very markedly from the tegumentary capillary arrangement in *Urochaeta*, *Megascolex*, and *Moniligaster*. This difference depends largely upon the disposition of setae, and the setigerous glands and muscles. In *Perichaeta* where the setae entirely encircle the body — imbedded in a protruding ring — the tegumentary circulation is much modified.

In *Lumbricus* a commissural vessel (Text-fig. 9) leaves the dorsal just anterior to the septum, runs dorsad and ventrad along the junction of this with the body wall. The ventral branch anastomoses with the subneural after piercing the

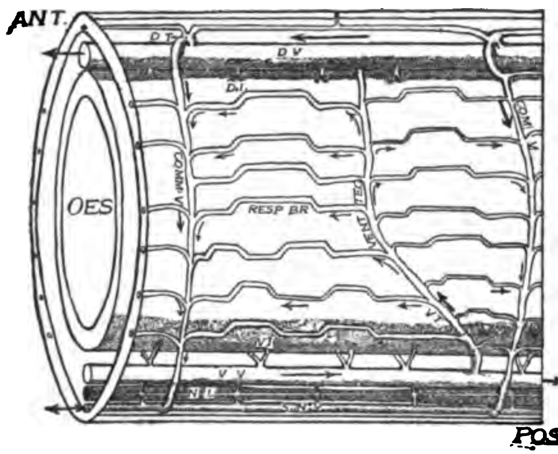


Fig. 9. — To illustrate the circulation in a typical somite (back of gizzard) of *Lumbricus*.

septum near the median ventral line, and this part of the vessel is usually called the commissural. The dorsal branch breaks up in the region of the problematical dorsal pores.

Tegumentary capillaries are given off from the commissural at right angles both backward and forward (Text-fig. 9, *D. T.*). They are directly continuous with capillaries arising in a similar way from the ventro-tegumentary trunk which runs out transversely in the middle of the somite, the commissural vessels being at each end.

It seems hardly necessary to point out that respiration is not the exclusive nor even the principal function of these tegumentary capillaries. For, on *a priori* grounds, it would appear that the entire body musculature must be supplied by them. Structurally the capillaries have two well-differentiated regions which may correspond to the division in function. When the capillaries leave their respective trunks, they are at the level of the inner longitudinal muscle layer. With a sudden turn outward, however, they come to lie immediately beneath the cuticle. The places where the capillaries come nearest to the surface are shown in Text-fig. 9 by the projections *resp. br.*, and it is probably here that the blood gives off the most of its carbon-dioxide and takes on its oxygen. Although but one-third of the capillary area is in this way given over to respiratory functions, this is, relatively to the body surface, a much larger ratio than one will find in any animal having gills or lungs. The respiratory mechanism is necessarily of the very simplest type; a vessel of capillary diameter runs between two trunks, commissural and ventro-tegumentary, which in a general way form the efferent and afferent trunks. This capillary vessel for a part of its course comes in immediate contact with the cuticle, which presumably acts as an osmotic membrane.

This arrangement is fundamentally different from that which occurs in *Moniligaster*, where veins and arteries are parallel and in contiguity, from the place of origin from their respective trunks to their common terminations. An interchange of gases or materials in the blood is supposed by Bourne to take place for a considerable region where these vessels are in contact. In *Lumbricus*, however, as we have just shown, the capillary is a short connecting vessel, and the part of the tegumentary system in *Moniligaster* which corresponds with this respiratory surface of *Lumbricus* would be the small "curl" spoken of by Bourne, where the arteries bend around to become veins.

From observations on a number of worms (*Urochaeta*, *Perichaeta*, *Lumbricus*) I am inclined to think that the parallelism so beautifully worked out by Bourne is more probably concerned with muscular than with respiratory tissue. If respiration were confined to the end-curls in *Moniligaster*, we should have a condition of affairs very similar to that in *Lumbricus*.

Parallelism is shown only in the anterior part of *Lumbricus;* perhaps most perfectly on the seminal vesicles, although Pl. VI, Fig. 2, shows vessels running side by side in many of the muscular walls of the anterior somites. In these cases of parallelism I have not been able to make out final anastomoses.

3. *The Ventral Vessel and its Connections.* — First to form in development and connected with nearly every organ of the body, the ventral is, perhaps, the most stable of all the large vessels. It will be noticed in Pl. VI, Fig. 2, that one of the two vessels going to each of the various organs is always a branch of the ventral (black). Pulsations in the latter are never so well marked as in the dorsal.

Branches from the ventral come off just anterior to the septal wall in each somite. By division the ventro-tegumentary and ventro-intestinal are formed. The ventro-tegumentary, running forward and outward to the middle part of the somite, ascends the body wall and gives off backward and forward the capillaries previously referred to (Text-fig. 9). The ventro-intestinal makes directly for the intestine, where it breaks up, as described by Jacquet and others. Shortly after leaving the ventral, a branch is given off from the ventro-tegumentary to the nephridium.

4. *The Subneural Vessel and its Connections.* — The commissural vessel which connects the dorsal and the subneural has already been described. At the subneural end, branches are given off to the nephridium [1] and to the setigerous glands.

In no somite posterior to the gizzard is there any marked deviation from the plan outlined above. Slight modifications occur at the clitellum, where the blood supply is increased at the breeding season. And in the second somite, back of the clitellum, there is an enlarged commissural vessel, which is especially favorable for observations of the course of flow of the blood.

[1] I have no observations to offer on the distribution of vessels in the nephridium. The subject has been covered by Benham ('91), and inasmuch as the nephridial supply is a closed circuit, off the main track, and does not influence the general circulation, I have accepted Benham's interpretation. The vessels are too small for direct observation.

B. Cephalic Region.

The discussion of this part of the circulatory system may conveniently be taken up as follows:

1. Enumeration and description of each vessel.
2. General morphological considerations.

1. *Enumeration and Description of Each Vessel.*—At septum VII–VIII, dorsal gives off hearts (Pl. VI, Fig. 1, *ht.*[1]).

Subintestinal (ventral) gives off a large vessel on each side having branches: (1) to septum ending in skin (respiratory); (2) to nephridium.

In this somite, VIII, and also in VII, it will be noticed that the nephridium lies posterior to the heart — between the latter and the septal wall.

Subneural gives off a vessel on each side, having (1) anastomosing branch with lateral; (2) branch to setigerous gland (*sg.*); (3) branch to nephridium.

Lateral gives off (1) a branch ventrally which anastomoses with subneural and then supplies setigerous gland; (2) a branch to the oesophagus; (3) a branch running dorsally in septum which after sending branches forward to heart, *vasa vasorum*, and to nephridium VII, then proceeds to dorsal and lateral body wall.

Septum VIII–IX.

Dorsal gives off hearts (Pl. VI, Fig. 1, *ht.*[2]).

Subintestinal gives off on each side (1) branch to skin; (2) branch to nephridium. The latter vessel is queerly enough joined by the branch from subintestinal (ventral) in IX–X, so that nephridium IX has supply from two vessels.

Subneural has branch which supplies setigerous gland, nephridium, and then anastomoses with lateral.

Lateral has (1) anastomosing branch with subneural branch and (2) one running out in septum to supply heart and nephridium VIII and skin VIII.

The septum so tightly encloses the oesophagus that it is hard to tell whether there is a supply to the oesophagus at this point.

There are, however, oesophageal vessels in the middle of somites VII–IX.

Septum IX–X.

Dorsal gives off hearts (*ht.*[3]) and possibly a branch to the nephridium which has been observed to come rather far dorsad in this somite. In somite X there is a branch from this vessel to lateral (*c.v.*).

Subintestinal has branch on each side to skin which gives off divisions both forward to nephridium IX and backward to nephridium X. This vessel encircles the first spermatheca.

Subneural has anastomosing branch which gives off branches to setigerous glands and to nephridium. We have never found the nephridium back of the heart in this somite. It seems probable that these organs change their relative positions during the activities of the worm.

The lateral in this somite assumes reproductive functions, there being an extra vessel which runs out in the body wall (*repr. aff.*[1]) to encircle the first spermatheca. It gives off numerous branches to the body muscles both before and after it reaches the spermatheca, especially on the dorsal body wall. Secondly, the lateral gives off in this somite the regular septal branch (*resp. art.*), which supplies heart and nephridium IX. Also a branch to the oesophagus (?).

Spermatic Arteries. — From the branch of the lateral to the spermatheca, minute blood vessels could be made out which ramified in considerable abundance about the sperm-funnels. From these minute vessels the testis also is supplied. No return vessels for the blood could be made out. The ventral might be expected from analogy to complete the circuit.

In somite X the lateral receives from the dorsal the cross vessel (*c.v.*), which drains the calciferous glands by the collecting ducts (*coll. ducts*) before uniting with the lateral. By this connection with the dorsal there is an equalization and maintenance of pressure in the lateral vessels. According as the first calciferous gland is full or empty of pebbles, this vessel appears well in front of the anterior edge of the first gland, or upon the side of the pouch.

Septum X–XI.

Dorsal gives off fourth pair of hearts, and small vessel to the dorsal pore (?). It receives (?) at about this point a vessel from the second calciferous gland.

Subintestinal gives off branch which divides, one part going to skin and second spermatheca, and the other going to the first seminal vesicle, a second part going to nephridium XI. This region is so very much crowded and complicated that there may also be a branch to nephridium X, which escaped me.

Subneural. — This vessel from this point backward plays an important rôle in the circulation. It is noticeable in this somite on account of the strong anastomosis which it makes with the lateral vessel. This anastomosis is very often asymmetrical, as is also the case with *Moniligaster*. Often the anastomosis is so strong that the subneural vessel proper seems to wane and almost to disappear.

It may be added here that the meaning of this anastomosis is that an important part of the work done by the subneural posterior to this region is thenceforward assumed by the laterals. The subneural in the anterior region still sends branches to the nephridia, but the growth of the systems in red at the expense of those in green on Pl. VI, Fig. 2, anterior to X–XI, illustrates the point very forcibly.

Lateral. — As at IX–X the lateral gives off ventrally an anastomosing branch with the subneural, and dorsally a branch which corresponds to the ordinary respiratory vessel (*resp. art.*, Pl. VI, Fig. 2, represented as cut off), while as in IX–X an accessory reproductive vessel supplies seminal vesicle, testis, sperm-funnels, spermatheca, and dorsal body wall. The branch to the sperm-funnel and testis arises from the vessel which ramifies over the seminal vesicle, and reaches its destination by curling down around and under the latter.

Septum XI–XII.

Dorsal gives off laterally the fifth pair of hearts (*ht.*^{5}, Pl. VI, Fig. 1, folded back), and just posterior to septum three vessels to CG^3 (Fig. 1). These may vary in prominence with the condition of the gland, but they are constant, as is every vessel figured in the lateral view of this region.

Subintestinal gives off an important branch which supplies, first, nephridium; second, seminal vesicle; third, *vas deferens;* fourth, body wall.

Subneural has a branch which supplies nephridium XII, and then anastomoses with lateral. This anastomosis, like that in XI, is very important and carries a great deal of blood from the subneural into the lateral.

Lateral. — Connects with (1) subneural; (2) body wall, and (3) seminal vesicle ($r.a.^3$). On the left side of Pl. VI, Fig. 2, respiratory branch is taken from anastomosis with subneural on right side from reproductive. The more usual mode of origin would be as figured on the right-hand side.

Septum XII–XIII.

The dorsal vessel in this place gives off the lateral on each side. These are the largest vessels given off from any trunk, with the exception of the hearts.

In *Allolobophora mucosa* the hearts are contractile and moniliform, and in *A. chlorotica* their contractions may be seen through the skin. After proceeding ventrad two-thirds of the width of the oesophagus, they turn and run forward parallel to the main trunks. Near this turn two parallel vessels are given off backward, the one running along the sperm duct, the other nearer the nerve cord and just beneath the glandular areas on the thirteenth and fourteenth somites. These both anastomose with the subneurals of XII–XIII, XIII–XIV, XIV–XV.

The ventral gives off a branch which divides into a vessel going out in the septum to the skin and another supplying (1) the ovary; (2) the nephridium; (3) the skin just parallel to the branch above mentioned, which follows along the glandular surfaces near the nerve cord.

From the branch running out in the septum to the skin a vessel is given off following the sperm duct and the branch from the lateral. The parallel course of these two vessels was observed long before it was noticed that their especial function was to supply the sperm duct which lies buried in the body wall.

Subneural has branch with two parts. The first supplies nephridium (Pl. VI, Fig. 2, right-hand side); the second receives the anastomoses from the lateral vessels, and is continued dorsad to coöperate with the ventral in respiration. As mentioned above, the vessels from the lateral are carried backward for two somites, everywhere anastomosing with subneurals given off at the septa.

Lateral has been described under dorsal.

Septum XIII–XIV.

Dorsal. — The respiratory function should here appear in connection with the dorsal, since the lateral, which up to this point has given off the respiratory arteries, now has disappeared. However, the vessel which ramifies on the body wall of this somite is continued forward from the posterior somites (XIV or XV).

The homologous branch to the respiratories of posterior somites in this case runs out in the septum to the oviducal gland, almost encircling the oesophagus, as do the dorso-tegumentaries posteriorly.

Ventral has branches (1) to nephridium, and (2) along septum to skin. The latter anastomoses with the two parallel vessels of this system, which run along the sperm duct and glandular body wall (3) to oesophagus. From the vessels of the *vas deferens* little ducts anastomose with the latero-neurals.

Subneural has branches (1) to nephridium; (2) to skin, and anastomoses with vessels of *vas deferens.* From the latter in this somite there is a branch to the oviducal gland.

Septum XIV–XV.

Dorsal has respiratory branch which runs forward over XIV and XIII.

Ventral has branches (1) to nephridium; (2) to *vas deferens* and setigerous gland vessels; (3) out in septum to skin.

Subneural has branches (1) to nephridium; (2) to skin. Latter has anastomosis with *v.d.v.* and *s.g.v.*

Septum XV–XVI.

The circulation here is nearly like that of the typical posterior somites, differing in the respiratory branch of the dorsal.

Septum XVI–XVII.

The typical arrangement is found, the dorsal anastomosing with the subneural.

2. *General Morphological Considerations.* — (*a*) It seems proper to consider each trunk and its connections as a separate system, for they are sharply defined by their beginnings and endings in capillaries. The continuity and distinctness of these systems are proved only where anastomoses occur. And the difficulty which is encountered by this mingling of systems is only apparent, since there is a fundamental connection between the dorsal, lateral, and subneural systems by means of the dorso-tegumentaries, and when enumerated all the anastomoses — with one exception (namely, the hearts) — are of three kinds: (1) dorsal with subneural; (2) dorsal with lateral; (3) subneural with lateral.

The several systems have been figured, therefore, in different colors. Certain trunks have centrifugal branches, the blood flowing out in such to the skin and the various organs. These vessels have been referred to as afferent or arterial; while the branches from a centripetal system have been referred to as efferent or venous.

(*b*) In the preceding detailed account it will be seen that the circulation in the region anterior to the gizzard has undergone profound modification. This has been due to the presence of the various reproductive organs and the specialization of the muscular, sensory, and respiratory systems at the anterior end of the body.

Changes in Dorso-tegumentaries.—In Pl. VI, Figs. 1 and 2, one readily sees by changes in the color from blue to red that the dorsal vessel anterior to somite XIV gives up its respiratory functions and up to IX has relations only with the oesophagus and the contractile hearts.

As an interesting example of the principle of *substitution of function*, it is well to trace the gradual transformation of the "commissural" vessel, running from dorsal to subneural, into an enlarged contractile heart.

At XV–XVI (Pl. VI, Fig. 2) occurs the last (most anterior) typical commissural vessel.

At XIV–XV the lower ramification (dorso-sous-nervien of Jacquet) is normal, entering as usual the subneural vessel after giving branches to nephridium, etc. The "tegumentaire" portion, however (that supplying the muscles of the body wall), is enlarged and extends forward over the dorsal wall of two somites (XIV and XIII).

At the next septum the commissural vessel as such has disappeared, since there is no tegumentary branch to the vessel arising here (XIII–XIV, Pl. VI, Fig. 1) and no anastomosis with the subneural. This vessel, which is the representative of the commissural, runs out in the septum nearly to the mid-ventral line, ending in a network upon the oviducal gland and making the latter quite a noticeable organ.

c. The modification of the commissural vessel is carried still farther in the next somite, XIII–XII, where the lateral vessel is given off. This runs out in the septum and sends down two anastomosing branches to the subneural.

Although the problem is not an easy one, it seems probable that the hearts are the last step in this series. The evidence for this statement is (1) similarity of position; (2) variable number of hearts in different genera; (3) the fact that in at least two species of *Allolobophora* the *lateral vessels*, like the hearts, have valves, a moniliform appearance, and, especially, are contractile. The difficulty with considering the hearts to be modified commissurals lies in the fact that they connect the dorsal and subintestinal, instead of the dorsal and subneural. This must of a necessity, however, be so, if they are to serve as the propelling force for the whole body. And if the hearts are homologous with either set of vessels connecting with the dorsal, it must be with the peripheral vessels rather than with the intestinal, since the latter are represented in three somites in which there are also hearts (XII–XI–X).

d. In the three somites last mentioned the lateral has taken on the work of respiration, which behind this point was assumed by the dorso-tegumentaries. In addition, from X forward, it also supplies the oesophageal wall. From this standpoint alone one might consider the lateral-longitudinal vessels to be a part of the dorsal system bearing the tegumentary and intestinal vessels which had been *split off from the main trunk*. A reason for this lies in the fact that the latter is specialized for a propelling organ in this region.

This conception is possible in the case of *Lumbricus*, but in other *Oligochaeta* the relations are not so simple, because the propelling force may be located in the lateral ("intestino-tegumentaire") system itself (*Allolobophora*). In nearly all earthworms, however, the lateral arises from (or empties into) a glandular network on the oesophagus (the oesophageal glands when such occur); *e.g.*, *Microchaeta*, *Megascolex*, *Urochaeta*, *Perichaeta*. And if this part of the lateral system in *Lumbricus* be considered alone, we have the typical intestino-tegumentary system of *Megascolex*, with which the relations to the main trunks are not concerned. Such an arrangement is simply a set of vessels which connect central and peripheral capillaries.

I venture the suggestion, therefore, that the lateral-longitudinal system of *Lumbricus* and *Allolobophora* is composed of two elements, the anterior part from X forward being homologous with the entire intestino-tegumentary of *Megascolex* and *Microchaeta;* while the part rising from the dorsal and supplying body wall and reproductive organs is a separate element split off from the dorsal, and possibly homologous with the longitudinal vessel of *Moniligaster*.[1]

[1] The element coming from the dorsal and supplying ovaries, oviducts, seminal vesicles, seminal receptacles, and the body wall may be compared with the branch from the posterior heart which Bourne has figured in *Moniligaster*. Of this he says: "Intestino-integumentary vessels, *i.e.*, vessels carrying blood between the peripheral capillary networks and the intestinal capillary networks, are not present in any region of the body. 'Latero-longitudinal' vessels are obvious in the anterior segments, from Segment X forwards to Segment V. They are the main

Summary. — The more important conclusions reached above are as follows:

a. Conception of each trunk as a separate system.

b. Disappearance of dorso-tegumentaries in front of somite XIV, and

c. Substitution for these by (1) lateral vessels, by (2) hearts.

d. Conception of caudal part of lateral as a split-off portion of dorsal, which carries respiratory vessels and evidence of this from the serial arrangement, finally terminating in the lateral system.

e. More plausible conception of lateral as made up of two elements, one of which is branch from dorsal going to supply gonads, body wall, etc., the other of which is a carrier of the blood coming from the digestive tract laden with food and deprived of digestive fluids, which after taking on aërated blood from subneural goes to supply head parts (true portal).

f. Supply of sperm duct and testis, oviducal gland, hearts (*vasa vasorum*), and setigerous glands.

II. Course of Flow.

1. *Statement of Work of Previous Authors.*

The direction of the blood flow has been verified by direct observation in two of the large longitudinal trunks many times. It is now generally agreed that in all earthworms the blood flows forward in the dorsal vessel, ventrad in the aortic arches, and backward in the subintestinal.

Beyond this short statement there is still dispute, especially in respect to the direction of flow in the dorso-tegumentary, the

trunks, in the anterior region of the body, of a system of vessels of which the subneural vessel is the main trunk in the posterior region of the body. This system must be dealt with as a whole. This system is connected on the one hand with capillary networks, and on the other with the hearts of Segment IX" (*Quart. Journ.*, vol. xxxvi, p. 324).

Jacquet, who did not work upon living specimens, interpreted this second part of the lateral which arises from the dorsal as being a reproductive branch arising at the cross vessel (*c.v.*) and going to supply the vesicles. That he erred is shown by the fact mentioned above, that pulsations really start from the dorsal at XII–XIII and travel downward and forward.

subneural, and the lateral vessels. One is inclined at first to think that in different genera of worms the flow in homologous vessels may be different, and probably in many cases, where the direction of flow is disputed, the vessels are not morphologically equivalent. For this reason it is questionable whether generalizations as to the course of flow which will apply to all so-called lateral, subneural, or portal vessels can be made, although our observations on *Lumbricus*, *Urochaeta*, *Perichaeta*, and *Allolobophora* show that the circulation in these four genera can be reduced to a common type (Text-fig. 10, *Lumbricus*, etc.).

The points under discussion relate to the commissural vessels, laterals, and intestinals. Perrier ('72), whose interpretation was accepted by Benham ('91), held that the blood flowed forward in the dorsal, outward from the dorsal into the intestinal capillaries and hearts, and was returned to the dorsal from the subneurals *via* the dorso-tegumentaries. He also said that the blood flowed forward in the laterals [1] which were arterial in function (centrifugal).

Bourne ('91, '94) pointed out that branches from the ventral vessels were the same from an anatomical point of view all through the body, and he took it as granted that these elements were arterial (afferent) for the whole course of the ventral. As this system is opposed in one part of the body by the subneural and in another by the lateral branches, he held that both these lateral and subneural systems were venous in nature. He therefore considers that in the intestinal capillaries the flow is from the ventral toward the dorsal, while in the peripheral capillaries the flow is from the dorsal into the subneural. He states that the course of flow in the lateral is caudad and that it is a venous (centripetal) system.

The two views are diametrically opposed, and it is evident that new data on this subject are essential, although it is true that the genera under discussion are different.

Aside from the facts of comparative anatomy, Bourne used the methods (1) of holding the vessels with fine forceps; (2) of cutting the vessels and observing flow; (3) study of valves. The first method was found by me applicable only to the larger

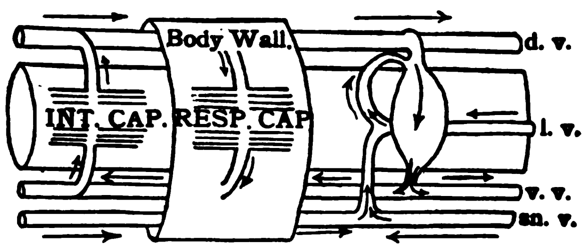
[1] See the masterly argument for this, *l. c.*

vessels (of *Lumbricus*) and not to those imbedded in the body wall or intestine. The second method assumes that the pressure and supply of blood governing flow are the same from both cut ends (central and peripheral).

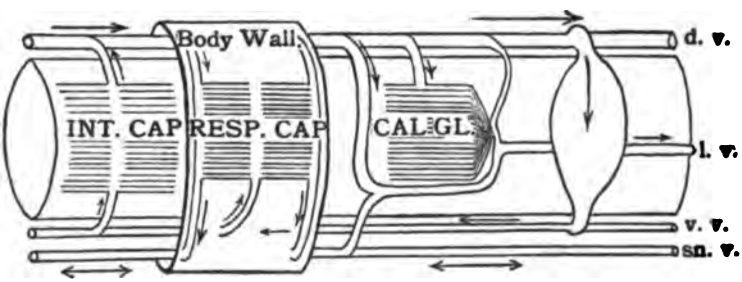
The third method of studying the valves does not seem conclusive, since Benham and Bourne both find valves leading out from the dorsal vessel, but they entirely disagree as to the direction in which these dorsal valves cause the blood to flow.

2. *Observations.*

Although a difficult task, the course of flow in most of the small vessels may be determined under favorable conditions by



MONILIGASTER, after BOURNE.



LUMBRICUS, ETC.

FIG. 10.

direct observation of the *pulsatile contractions*. On young or transparent individuals of *Perichaeta*, *Urochaeta*, *Allolobophora*, and *Lumbricus* held between two large watch crystals and viewed with a dissecting lens, the following direct observations were made:

a. *Dorso-tegumentaries.*—At the points in the middle of the body where the commissural vessels leave the dorsal, the pulsations of the latter can be seen to be continued along the former vessels, and the blood current very plainly proceeds ventrad. (*Urochaeta* and *Perichaeta*.)

There is in *Allolobophora chlorotica* a rather large dorso-tegumentary vessel about two somites back of the clitellum. The blood could be made out coming down from the dorsal in this by regular pulsations, and when it came to the subneural it was seen a number of times to flow both backward and forward in the latter. It flowed backward *only* a number of times.

In the region of the gizzard the dorso-tegumentaries may be well observed against the clear background. Here the blood flows toward the subneural as before and, on reaching it, divides, some going backward, some forward. (*Allolobophora el.*)

b. *Subneural.*—This vessel may or may not be filled. A number of times, in *Urochaeta*, it was observed distended with blood of distinctly lighter color than the ventral, and the regularly occurring pulsations herein followed in time those of the ventral. As said above, the blood may flow either cephalad or caudad in this vessel.[1]

Ventro-tegumentaries.—In spite of careful and diligent study, the contractions in these deeply lying vessels have not been made out. It will be noticed that these are the only vessels in which the flow has not been observed.

c. *Dorsal.*—Back of the gizzard, the dorsal vessel may be seen to fill from the typhlosolar sinus. Anterior to the gizzard, on the other hand, the dorsal gives off blood to the digestive tract (Pl. VI, Fig. 1, vessels in red XI–XIV). These afferent branches supply the longitudinal sinuses of the glandular oesophagus, which run between the lamellar secreting surfaces. When peristalsis occurs, blood passes forward through the calciferous glands, gathers into the anterior pouch, then passes by the collecting ducts into the lateral vessel.

d. *Lateral.*—The pulsations of this vessel, both where it leaves the dorsal and where the calciferous gland vessels fuse (*coll. ducts*, Pl. VI, Fig. 1), have been seen through the skin

[1] The difference in color of invertebrate venous and arterial blood has already been pointed out by Graf ('91) in the leeches.

(*Allolobophora*). The course of flow can best be demonstrated in worms opened in salt solution, the blood passing forward to the pharynx, where it splits into an upper and a lower branch, the latter following the nerve commissure to the brain.

3. *Summary of the Circulation in the Longitudinal Trunks.*

	Anterior to Gizzard.		Posterior to Gizzard.	
	Receives blood from	*Gives blood to*	*Receives blood from*	*Gives blood to*
Dorsal	Posterior region.	Dorso-tegumentaries 14 and 15. Oesophagus. Hearts.	Dorso-intestinals	Dorso-tegumentaries.
Ventral	Hearts and ventro-intestinals.	Ventro-tegumentaries.	Anterior ventral and latero-neurals.	Ventro { intestin. tegum.
Subneural	Posterior subneural.	Laterals and skin (?).	Dorso-tegum.	Latero-neurals.
Lateral	Dorsal, subneural, and calc. glands.	Lateral-tegumentaries.	Represented by subneural.	

4. *Summary of the Circulation in the Integument.*

In the anterior region the *ventro-tegumentaries* and *ventro-intestinals* are afferent (running to the trunks); in the posterior region both sets of capillaries are efferent (arterial).

In anterior region, laterals, dorsals, and subneurals are centrifugal systems (arterial); in posterior region, the subneural is a centripetal system, while the dorsal both receives and gives off blood.

5. *General Discussion of the Circuit of the Blood.*

The shortest circuit which a blood corpuscle could make in a journey from the dorsal back to dorsal again, would be out through the dorso-tegumentaries to the subneural, through the anastomoses with the latero-neural to the ventral, back in this to the first ventro-intestinal, given off to the central (intestinal) capillaries, through these to the dorso-intestinals and then by way of the typhlosolar sinus to the dorsal.

On the other hand, a corpuscle may pass forward the whole extent of the dorsal, then down into the lateral, by way of the dorso-intestinals in somites XV to X, through these to the body wall, back thence by the (anterior) ventro-tegumentaries to the ventral (to which it could have come directly from the dorsal by the hearts), back in the ventral to the extreme posterior region, where both ventro-intestinals and commissural vessels are flowing dorsad, and by either the ventro-intestinals or caudal ventro-tegumentaries, it would be brought again to the dorsal.

It will be noticed that anterior to a certain point — the region of the gizzard—there seems to be a change from the normal direction of flow (which is centripetal) in the dorso-intestinals and the commissural subneural branches; and also a change from the normal centrifugal direction in the ventro-intestinals and ventro-tegumentaries to the opposite directions.

If the short circuit for a corpuscle given above was the normal path in every somite from head to tail, there need be no variation from the typical arrangement in any part of the body. But since there is a continuous flow in the dorsal and ventral vessels which is not metameric, there must be a piling up of blood at the extremities of the body, unless the vertical channels operate to keep up the circuit. The hearts do so operate at the anterior end. At the posterior end the intestinal capillaries naturally flowing dorsad will operate to reduce the pressure, and it seems probable that there is also a reversal in the ordinary course of the tegumentaries, or that the amount of blood which they take out of the dorsal is decreased.

Bourne considers that when the commissural vessels disappear, the laterals take up the *venous* functions of the subneural ends of the commissurals. This is a possible interpretation if one believes that the laterals flow caudad. Aside from positive evidence that the laterals do not flow caudad, this interpretation necessitates our assuming a change in the direction of flow in the main ventral trunk (if, as Bourne suggests, the blood may flow forward the anterior ventral, lest there should be too little blood going to the anterior part of the body, and too much coming away therefrom). In *Moniligaster* a considerable part of the worm lies in front of the hearts, and possibly in such a case this is true *if the laterals are venous trunks*. In *Lumbricus* and *Allolobophora*, however, there is still a weakly developed subneural to act with the ventral in taking away blood from the head parts; but aside from all this, the lateral may be seen flowing forward in these genera, both from its point of inception on the dorsal[1] and where it leaves the calciferous glands.

All familiar with the waves of pulsations which pass along the dorsal vessel in *Lumbricus* have probably noticed that they send forward a column of blood which extends over six or eight somites. These columns of blood alternate with intervals in which the vessels are collapsed, so that when peristalsis occurs, some of the blood is forced out into those dorso-tegumentary capillaries which open opposite to the column of blood. After peristalsis has propelled the column of blood forward the vessel collapses (in systole). Then follows a diastole, in which the dorsal is filled from the dorso-intestinals (in addition to the forward pulsatile flow from behind).

It will, therefore, be readily seen that peristalsis in the dorsal vessel of the earthworm is not like that in the inferior *vena cava* of vertebrates, but each somite has a potential heart which both fills and empties in diastole and systole.

[1] Bourne suggested (*Megascolex*, *Quart. Journ.*, vol. xxxii, p. 62) that the origin of the lateral from the dorsal in *Lumbricus* might have some effect on his theory of circulation.

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DESCRIPTION OF PLATE VI.

FIGS. 1 and 2 show lateral and dorsal views of the circulation so arranged that the corresponding somites are exactly opposite in both figures.

a.	anastomosis.
$CG^{1, 2, 3}$.	First, second, and third calciferous glands.
c. v.	cross vessel from dorsal to lateral.
coll. ducts	collecting ducts.
dors. v.	dorsal vessel.
en. s. g.	enlarged setigerous glands.
$ht.^{1, 2, 3, 4, 5}$.	the five hearts.
int. v.	intestinal vessel.
i. t.	intestino-tegumentary vessel.
lat. v.	lateral vessel.
n.	nephridial branch.
n. c.	nerve cord.
neph. aff.	artery to nephridium.
neph. eff.	vein from nephridium.
ov.	ovary.
ovid. gl.	oviduct gland.
ovid.	oviduct.
ov. vessel.	vessel to ovary.

$r. a.^{1, 2, 3, 4}$.	four arteries supplying reproductive organs.
$r. e.^{1, 2, 3}$.	three veins draining seminal vesicles.
repr. aff.	same as $r. a.^{1, 2, 3, 4}$.
repr. eff.	same as $r. e.^{1, 2, 3}$.
resp. art.	respiratory artery.
resp. vein.	respiratory vein.
sg.	setigerous glands.
sp. a.	spermatic artery.
spthc.	spermatheca.
subn. v.	subneural.
s. v.	seminal vesicles.
vas. def. eff.[1]	vas deferens.
vas. def. aff.	afferent vessel of vas deferens.
vas. d.	vessels to the vas deferens.
vasa vas.	vasa vasorum.
v. d.	vas deferens.
v. d. v.	vas deferens vessel.
vent. ves.	ventral vessel.
v. v.	ventral vessel.

[1] The reference lines from this and from *vas. def. aff.* are wrong in the plate. The explanation applies to the lettering as it stands.

LETTERING OF PLATES VII–IX.

bld. corp.	blood corpuscle.
bl. s.	blood sinus.
c. g.	gland cavity.
c. gl.	gland cavity.
circ. m.	circular muscles of gut.
en.	entoderm.
en. ylk. c.	entodermic yolk cells.
gld. lam.	gland lamella.
ht.	heart.
l. v.	blood sinus.
m.	muscle fibres.
mig. nuc.	migratory nuclei.
n^1	youngest gland nuclei derived from wandering cells.
n^2	older gland nuclei.
n^3	degenerating gland nuclei.
per.	peritoneal tissue.
s. p.	calcareous concretions.
w. c.	wandering cells.
ylk.	yolk granules.
ylk. n.	yolk nuclei.

DESCRIPTION OF PLATE VII.

FIG. 3. Section of calciferous gland drawn with camera lucida and 1.5 mm. Zeiss immersion, as are all the figures on these plates unless otherwise stated. Representing an early stage in the secretory process.

FIG. 4. Stage showing the cell in constructive phase of metabolism, with wandering cell or blood corpuscle in sinus.

FIG. 5. Showing increase in amount of cytoplasm and correlated changes in the nucleus. A wandering cell is shown starting on its way into the syncytium, as well as one of the younger generation of nuclei.

FIG. 6. Stages in the entrance of the migratory nucleus.

FIG. 7. The migratory nucleus almost entirely within the cell.

FIG. 8. The resumption of the resting form of the nucleus.

FIG. 9. Three generations of nuclei, in the same cell projection.

FIG. 10. Three generations of nuclei, in the same cell projection.

FIG. 11. Showing a nucleus (n^1) which has entered in the stage preceding division.

FIG. 12. The stage succeeding amitotic division of the entering nucleus.

FIG. 13. Entering nucleus, still having connection with sinus wall, which is undergoing amitotic division.

FIG. 14. Cell showing cytoplasm in readiness for the formation of lime crystals.

DESCRIPTION OF PLATE VIII.

FIG. 15. Showing concretions which may collect in the cytoplasm prior to the formation of lime. This section was taken from a worm which had been fed on calcium carbonate for several days.

FIG. 16. Stage showing the breaking down of the cytoplasm to form lime. Disintegration of nucleus and lime crystals.

FIG. 17. Stages in the amitotic division of epithelial nuclei.

FIG. 18. Low power magnification of a blood sinus, all the various stages in the nuclear history.

FIG. 19. Movement of a wandering cell making its way through the syncytium from the blood sinus.

FIGS. 20–23. Showing thread-like structures which travel through the cytoplasm from one disintegrating nucleus to another. Bacteria (?) or chromatin structures.

FIG. 24. Thread-like structures attending the wandering cells.

DESCRIPTION OF PLATE IX.

FIG. 25. Development of *Allolobophora foetida*. Gland cavities and blood vessels are shown in process of formation. At points marked x, the amoeboid nuclei may be seen making their way into the blood. The gland cavities are also shown around which the endodermal nuclei group themselves.

FIG. 26. The same enlarged. The origin of the blood is also indicated here.

FIG. 27. Enlarged view of a very young earthworm (*A. mucosa* or *A. foetida?*), in which the striated arrangement of blood vessels has already appeared and in which the endodermal cells are arranging themselves in hollow squares to form the gland cavities.

FIG. 28. A somewhat later stage in *L. terrestris*.

FIG. 29. *A. foetida* showing the relation of the blood clots and the developing "basement membrane" of the gland.

FIG. 30. Karyokinesis in the endodermal cells.

FIG. 31. A part of adult gland *L. terrestris* for comparison.

FIG. 32. Primary nucleus and blood corpuscle in adult gland.