ADRENALIN IN ANNELIDS.

A Contribution to the Comparative Study of the Origin of the Sympathetic and the Adrenalin-Secreting Systems and of the Vascular Muscles Which They Regulate.

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The intimate physiological relationship between the sympathetic nervous system and the adrenalin-secreting cells of the medulla of the suprarenal body is now thoroughly established. All the actions of the sympathetic system can be imitated by adrenalin, and failure of the adrenalin supply, such as takes place in Addison's disease, causes failure of the proper action of the sympathetic nerves. The morphological relationship of the two types of cell, the sympathetic nerve cell and the adrenalin-secreting cell, are also equally intimate. The adrenalin-secreting cell is always identifiable in mammals by its chrome-staining reaction. Elliott[1] has brought forward strong evidence that the innervation of the medullary cells of the suprarenal body is a direct one by the medullated connector or tract fibers of the splanchnic nerves. Such an innervation corresponds to the white rami communicantes which connect with the sympathetic nerve cells in their various ganglia.

In the higher mammals the sympathetic ganglia do not usually contain any adrenalin-secreting cells, the latter being concentrated almost entirely in the special tissue of the suprarenal medulla; but in the lower forms of vertebrates the two tissues are both widely distributed and intimately connected. In amphibia, for instance, islets of chrome-staining tissues are to be found in every sympathetic ganglion,

[1] Elliott, T. R., *J. Physiol*, 1913, xlvi, 285.

and Smirnow[2] has shown that such cells are innervated by nerve fibers which are otherwise indistinguishable from those which run to the sympathetic nerve cells lying with them. Further back in the vertebrate kingdom the distribution becomes still more diffuse and the sympathetic nervous system becomes less and less defined and more and more replaced by a diffuse system of chrome-staining cells arranged segmentally throughout the body.

A definite sympathetic nervous system has been long known in the case of the elasmobranch fishes, but it is only comparatively recently that the researches of Giacomini and others have definitely demonstrated its presence in the other groups of fishes. Giacomini finds a definite double sympathetic chain present in the Dipnoi,[3] but in the Ganoidei[4] and Teleostei[5] he was able to find only an irregular system of nerve cells distributed along the cardinal veins. In the cyclostomes[6] the representatives of the sympathetic nervous system are still more scanty, being certain nerve cells which are occasionally to be found around the cardinal veins. In all these groups of fishes there is again an intimate relationship between the sympathetic nerve cells and diffusely distributed chrome-staining cells. The presence of adrenalin in the latter cells was demonstrated by Vincent in the elasmobranchs,[7] and in a previous paper I[8] gave reasons for believing that the chrome-staining tissue of the cyclostome, *Petromyzon fluviatilis,* also contained adrenalin. An extract of such tissue caused a rise of blood pressure in the cat, which was in every way similar to that caused by a small dose of adrenalin. The conclusion may therefore be drawn that chrome-staining tissue, wherever found among vertebrates, secretes adrenalin, and the presence of a sympathetic nervous system and an adrenalin-secreting system may be considered to have been established throughout the vertebrate kingdom. The two systems are in every animal most intimately connected physio-

[2] Smirnow, A., *Arch. mikr. Anat.*, 1890, xxxv, 416.

[3] Giacomini, E., *Atti Accad. Lincei, Rendiconti,* 1906, xv, series 5, 394.

[4] Giacomini, E., *Monitore zool. ital.*, 1904, xv, 20.

[5] Giacomini, E., *Monitore zool. ital.*, 1902, xiii, 183.

[6] Giacomini, E., *Monitore zool. ital.*, 1902, xiii, 143.

[7] Vincent, S., *Proc. Roy. Soc. London*, 1897, lxi, 64.

[8] Gaskell, J. F., *J. Physiol.*, 1912–13, xliv, 59.

logically and anatomically. The work of Kohn[9] has also shown how closely their evolution in the vertebrate is paralleled by their embryological development in the mammal.

A search for the origin of the two systems must therefore be pursued in the invertebrate kingdom. If it is allowed that the presence of a yellow coloration after fixation with a chrome salt in certain cells, which have been called chromaffin cells, occurs among vertebrates only in cells which contain adrenalin, the presence of such a reaction in cells of invertebrates is presumptive evidence that the latter are also adrenalin-secreting cells. The first discovery of chromaffin cells in the invertebrate was made by Poll and Sommer[10] who described the occurrence of the reaction in certain cells of the central nervous system of the leech, *Hirudo medicinalis*. Poll[11] has since extended this observation by finding similar cells in certain other annelids. The only other observation is that of Roaf and Nierenstein[12] who extracted an adrenalin-like body from certain tissues lying in the walls of the branchial chamber of the mollusk, *Purpura lapillus*. Subsequently Roaf[13] located this secretion to certain cells which gave a chromaffin reaction. As the Mollusca are not held to be on the direct line of vertebrate descent attention has been confined to the annelid kingdom.

A representative selection of annelids was examined at the Zoological Station of Naples, and a detailed description of the results has been given in my paper published in 1914.[14] Among the Hirudineæ chromaffin cells have been found in the ganglia of the central nervous system in all species investigated. Seventeen different animals of the polychæte group were examined but in fifteen of these no trace of chromaffin cells could be found; in the remaining two, *Aphrodite aculeata* and *Eunice gigantea,* small chromaffin cells were present. *Lumbricus herculeus*, the only oligochæte investigated, also possessed

[9] Kohn, A., *Arch. mikr. Anat.*, 1903, lxii, 263.

[10] Poll, H., and Sommer, A., *Arch. physiol.*, 1903, 549.

[11] Poll, H., in, Hertwig, O., Handbuch die vergleichende und experimentelle Entwicklungslehre der Wirbeltiere, Jena, 1906, iii, pt. 1, 603.

[12] Roaf, H. E., and Nierenstein, M., *J. Physiol.*, 1907, xxxvi, p. v.

[13] Roaf, H. E., *Quart. J. Exp. Physiol.*, 1911, iv, 89.

[14] Gaskell, J. F., *Phil. Trans. Roy. Soc. London, Series B*, 1914, ccv, 153.

them. Wherever they were found these chromaffin cells were always constant in number and similar in position in the ganglion, six being always present arranged in three groups of two each, a ventral and two lateral groups. The two cells of the ventral group were usually the largest. The size of the cells varied greatly in the different animals, being small in the two polychætes and reaching the largest size in *Hirudo medicinalis*, which was therefore selected for further investigation.

In this animal the two ventral cells are of very large size; they have been called colossal or giant cells by Retzius[15] and others. The lateral group on each side consists of two smaller cells which lie respectively just posterior to the anterior and posterior lateral nerves; their positions are indicated in Fig. 1. These six cells have all the appearance of nerve cells, possessing processes which run out in the lateral nerves and staining similarly to the other nerve cells of the ganglion. It was found that the six cells could be very clearly demonstrated by staining the freshly excised ganglion with methylene blue, and then irrigating the preparation mounted in water under a cover-slip with a dilute bichromate solution; all the nerve cells became bleached by this process with the exception of the six chromaffin cells which retained the blue stain. The probable explanation of this reaction is that the bleaching action of the chrome salt is prevented in the chromaffin cells by its combination with the chrome-staining substance, thus protecting the methylene blue stain. The nerve cells of the ganglion of the leech are divided into groups, shut off from one another by septa, whose arrangement is shown in Fig. 1. The individual nerve cells are unipolar and hang free in their particular compartment lying in a clear nutritive fluid; their relative positions are therefore liable to alterations in the compartment, for instance the two giant cells can be moved about freely by pressure on the cover-slip in a suitable preparation. The nerve cord itself is suspended in a blood space known as the ventral sinus, the thin sheath of the ganglion intervening only between the compartments in which the nerve cells lie and the surrounding blood. It is quite possible that an interchange takes place between the fluid in which the nerve cells are suspended and the blood

[15] Retzius, G., *Biol. Untersuch.*, 1891, ii, 13.

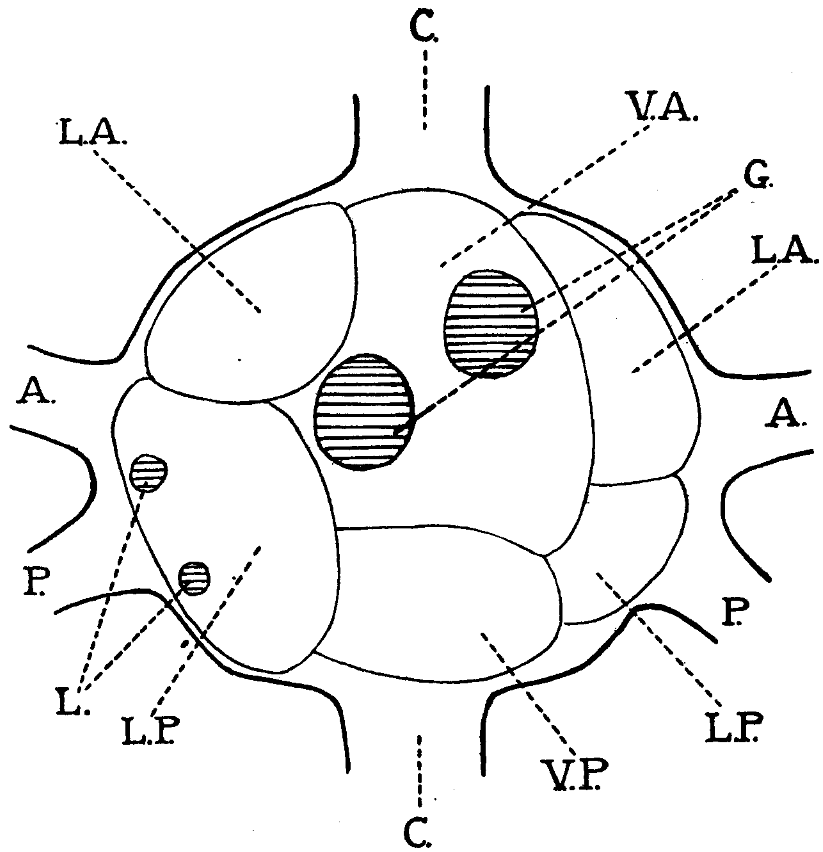

FIG. 1. Diagram of a ganglion of the leech showing the compartments in which the nerve cells are grouped and the position of the chromaffin cells in them. The limits of the various cell groups are shown, with regard to the ventral side of the ganglion on the right and center, with regard to the dorsal side on the left. *G.*, the two giant cells lying in the ventral anterior group. *L.*, the lateral cells lying in the dorsal portion of the posterior lateral groups. *V.A.*, the ventral anterior group. *V.P.*, the ventral posterior group. *L.A.*, the lateral anterior group. *L.P.*, the lateral posterior group. *C.*, the connectives. *A.*, the anterior nerves. *P.*, the posterior nerves.

of the sinus, and that any secretion, such as that of adrenalin in the chromaffin cells, could thus reach the circulation of the segment in which the ganglion lies.

An attempt was made to obtain an extract of the central ganglia in order to see whether any physiological reaction could be obtained similar to that of adrenalin. The nerve cords of a number of leeches were removed under a dissecting microscope by opening up the ventral sinus and cutting through the lateral nerves as near to the ganglia as possible. Each ganglion was then cut away from its connectives and placed immediately in a watch-glass which was kept in a desiccator. About 400 ganglia were thus collected. As the amount of material when dried was extremely small, the physiological test decided upon was the inhibition of the virgin uterus in the cat. I was fortunate in obtaining the assistance of Dr. H. H. Dale in carrying out the test. The uterus was suspended in Ringer's solution and a sufficient amount of histamine was added to the bath to give it a strength of 1 in 3 millions, in order to produce tone and rhythm. The result of the experiment is shown in Fig. 2. An extract of the dried ganglia was made by grinding them up with sand in 2 cc. of Ringer's solution. The bath in which the uterus was suspended contained 50 cc. of Ringer. At *A* the 2 cc. of extract were added to this bath, with the result that a distinct lowering of tone and diminution of rhythm took place. Similar experiments with extremely dilute solutions of adrenalin gave a much stronger inhibition when $\frac{1}{2,000}$ mg. was added to the 50 cc. bath, but a smaller inhibition when $\frac{1}{20,000}$ mg. was added; the extract therefore contained an amount of adrenalin lying between $\frac{1}{5,000}$ and $\frac{1}{10,000}$ mg. This experiment supports the conclusion that the chromaffin reaction of the six nerve cells in the ganglion of the leech is due to the presence of adrenalin in them. It confirms a statement of Biedl that he has been able to obtain the biological tests for adrenalin from these cells. I have, however, been unable to find any detailed description of his experiments.

The structure of these adrenalin-containing cells justifies the conclusion that they are nerve cells, and the question arises whether they innervate some special musculature which is susceptible to the action of adrenalin. That is to say, are they the representatives of the sympathetic cells of the vertebrate as well as the representatives of the adrenalin-secreting cells of the suprarenal medulla? If this is so, in this primitive stage of development, such a nerve cell not only

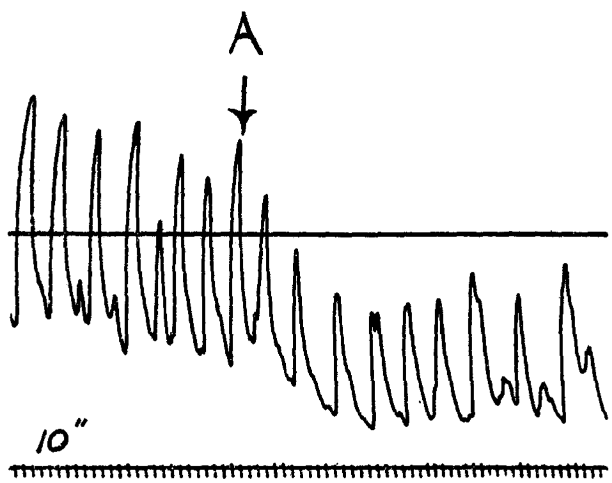

FIG. 2. The action of an extract of leech ganglia upon the virgin uterus of the cat. The isolated uterus was suspended in a bath of 50 cc. of Ringer's solution, to which histamine had been added to produce a strength of 1 in 3 millions; a good tone and rhythm were thus obtained. At *A* the extract of 400 leech ganglia ground up with sand in 2 cc. of Ringer's solution was added to the bath. A diminution in both tone and rhythm was produced. Time markings at intervals of 10 seconds.

regulates its peripheral musculature by direct nervous action but also provides the internal secretion which is necessary for the proper action of its nervous impulses. Later developments have caused a differentiation from this primitive state, so that two separate types of cell have arisen, one of which has become the adrenalin-secreting cell, the other the sympathetic nerve cell. Kohn[9] considers that in the early mammalian embryo the two types of cell arise from a common mother cell, and may develop into either chromaffin cells or sympathetic nerve cells. He has therefore called the chromaffin system the paraganglion system, in order to point out its intimate relationship to the ganglia of the sympathetic chains. There is thus a close parallel between the condition in *Hirudo* and the early embryological development of these tissues in the mammal. The connections of the two types of cell with the central nervous system in the vertebrate, which have already been referred to, also support the theory that they have been derived from a common ancestral cell, for both are supplied by medullated connector or tract fibers which run out from the central nervous system to connect with them.

During the investigation of the nervous systems of the various members of the annelid groups, a vascular system with definite muscular walls was found always to exist when chromaffin nerve cells were present, but to be absent when such nerve cells were also absent. The members of the Hirudineæ investigated all possessed muscular walled vessels, and *Lumbricus* has, as is well known, similar muscular "hearts" which are rhythmically contractile. *Eunice gigantia*, one of the two members of the polychæte group in which chromaffin nerve cells were found, possesses a short portion of vessel at the base of each of the branchiæ, which is suspended on a mesentery and has definite muscular walls. These vessels by their contraction drive blood into the branchiæ to be oxygenated. Contractile muscular walled vessels are therefore present in this animal. The vascular system of *Aphrodite* could not be thoroughly investigated owing to lack of material; the question of the presence of vessels with muscular walls could not therefore be decided. In all the other members of the polychæte group, which possessed any definite vessels at all, no sign of any muscular tissue could be found on their walls. It appears that, wherever vascular muscle is present in the Annelida, chromaffin nerve cells are also to be found in the central nervous system, and that these cells are adrenalin-secreting; on the other hand, if no vascular muscle is present no chromaffin cells exist. These facts support the view that the chromaffin nerve cells innervate the vascular muscles.

Further investigations were made to attempt to discover the nature of the innervation of the vascular muscles in the leech, and also their reaction to adrenalin. The nervous system and vascular system were investigated in detail and the results are described in full in my previous paper.[14] It was found that a small branch of the anterior nerve could be traced directly to the wall of the lateral vessel in each segment, and that this nerve was formed of processes of cells situated in the central segmental ganglion. A branch of the posterior nerve also appeared to run to the same vessel, though its complete continuity was not established. The vascular muscles are therefore innervated by processes of cells situated in the central ganglion, the arrangement being a segmental one.

The lateral vessels were found to have a contractile rhythm with an average beat of about six to eight contractions per minute, and this rhythm was independent of nervous control, for it continued for many hours after complete section of all the lateral nerves. Rhythmical contraction is, in the vessels of the leech, the property of the vascular muscle itself; that is to say, the beat is myogenic not neurogenic.

The effect of section and stimulation of the lateral nerves upon the contractile rhythm of the lateral vessels was also investigated. The study of the vascular beat was much facilitated by the use of curare, which completely paralyzes the longitudinal and circular muscles but does not affect the vascular muscles. The dose required is large, being about 0.2 cc. of a 1 per cent solution, but the differentiation of the two types of muscle by the action of curare is a complete one. Attempts were made to obtain some mechanical method of recording the vascular beat but nothing sufficiently delicate could be devised. The beats were therefore observed under a dissecting microscope and recorded by a key signal on a revolving drum. As the animal had to be kept in Ringer's solution throughout the experiments, stimulation was brought about by means of Lucas'[16] electrode applied to the main nerve chain, the only nerve left intact being the one it was desired to stimulate. It was found that section of the anterior nerve with the posterior nerve intact always caused definite slowing of the rate of beat, while stimulation of this nerve with the posterior nerve divided caused acceleration. The anterior nerve thus contains accelerator fibers to the vascular muscle. Section of the posterior nerve with the anterior nerve intact caused quickening of the rate, while stimulation of this nerve with the anterior nerve divided in the cura-

[16] Lucas, K., *J. Physiol.,* 1913, xlvi, p. xxxii.

rized leech had no effect, but in the decapitated animal caused marked slowing of the rate. The posterior nerve therefore contains fibers which are inhibitor to the vascular muscle. The acceleration effect produced by stimulation of the anterior nerve is abolished by the injection of ergotoxin. The vascular system is thus definitely under the control of the central nervous system, the arrangement being a segmental one, and the length of the vessel lying in any segment being controlled by cells in the ganglion of that segment. The accelerator fibers run in the anterior nerve, and their action is abolished by ergotoxin in the same way as is the case with the mammalian sympathetic system. These accelerator fibers have been definitely traced to the muscle and are very probably the processes of the chromaffin nerve cells, which therefore control the vascular muscle in the leech in a similar way to its control in the vertebrate by the sympathetic system. The inhibitor fibers run in the posterior nerve and are also processes of cells in the central ganglion. Their action is abolished by curare. They control the vascular muscle in a manner strictly comparable to the control of the vertebrate heart by the vagus nerve.

The nerve supply of the vascular muscles is therefore a double one which is strictly comparable to the double supply of the vertebrate heart by the sympathetic and vagus systems. The power of rhythmical contraction is an intrinsic property of the vascular muscle itself, to which the two nerves act as regulators. In the primitive form found in annelids, the heart beat is therefore myogenic in origin, but is regulated by the control of the nervous system.

The action of adrenalin was tried both on the longitudinal and circular voluntary muscles and on the vascular muscles. Experiments on the voluntary muscles of the leech were difficult, and were supplemented by similar experiments on *Lumbricus*. No effect could be obtained in these muscles in either animal by the application of adrenalin if it was applied in neutral solution; with the usual acid solution in the form of hydrochloride the acidity of the solution was always sufficient to cause a contraction. Adrenalin borate was found to be a form suitable for the purpose. It was applied directly to a lateral vessel of the leech, after exposing it under Ringer's solution, by injection into the loose tissue lying around the vessel. The effect of the injection of one drop of a solution in Ringer of adrenalin borate of a strength of 1 in 10,000 is shown in Fig. 3. A beat of an average interval of 14.5 seconds was accelerated to a beat of 9.4 seconds. Later this segment stopped in systole, and the neighboring segments increased their rate of beat. Adrenalin therefore causes a marked acceleration and, if in sufficient strength, it further causes complete contraction which abolishes rhythm altogether. The vascular muscle is sensitive to the action of adrenalin while the voluntary muscle is not; and the action on the vascular muscle is strictly comparable to that on the vertebrate heart.

In order to carry still further the close physiological relationship which had become apparent between the muscles of the vessels of the leech and that of the vertebrate heart, the actions of atropine and muscarine were also tried. Atropine was found to cause an accele-

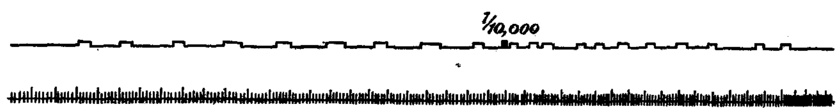

FIG. 3. The action of adrenalin borate on a lateral vessel of the curarized leech. At the signal mark one drop of a solution of adrenalin borate of a strength of 1 in 10,000 was injected into the tissues around the vessel. A beat with an average interval of 14.5 seconds was accelerated to a beat with an average interval of 9.4 seconds. Time marking in seconds.

ration which gave the fastest rhythm ever observed, the average interval between beats dropping to 4.2 seconds, a rate of over fourteen beats per minute. The efficiency of the beat also became maximal. Muscarine in strong solution caused complete cessation of beat in diastole; in dilute solution it caused weakening and slowing of the beat. The complete cessation of the beat caused by muscarine can be removed by a subsequent injection of atropine, if the atropine is injected soon after the beat has ceased. The vessel gradually resumes a rhythmical contraction. If, however, the muscarine has been allowed to act for some time, atropine has no longer the power to recover the beat. These actions of atropine and muscarine are strictly comparable to their actions on the vertebrate heart.

The leech *Hirudo medicinalis* thus possesses a very definite type of vascular muscle clothing certain of its vessels which is rhythmically contractile and has physiological actions in every way comparable to those of the vertebrate heart. It is controlled by similar nerves and reacts to the drugs adrenalin, atropine, muscarine, and curare in an identical way. The two main vessels, which lie in the extreme lateral position in the body just under the longitudinal muscle layers, are also similar in function. Their chief purpose is to drive blood into an extensive capillary network which lies in the skin and is respiratory in function. The condition in *Eunice gigantea* is probably a primitive form of that in the leech. The segmentally arranged musculature which surrounds a short portion of the vessel lying at the base of each branchia has become more diffusely spread and has fused to form one continuous contractile vessel on each side. In *Eunice* again the function of these "hearts" is entirely branchial. The growing around of the lateral folds of the invertebrate to form the ventral surface of the vertebrate, which is hypothecated in the theory of the origin of vertebrates brought forward by W. H. Gaskell,[17] would carry with them the two lateral vessels. They would thus become midventral and would lie in the position of the two vessels from which embryologically the vertebrate heart is formed. The physiological function of such a heart would be always branchial from its earliest origin in the annelid kingdom.

The formation of a specialized vascular muscle immediately demanded the formation of special nervous and chemical regulators to this muscle; we therefore find a segmental nervous control already established in annelids for the vascular muscle of each segment, and a specialized cell which secretes the necessary internal secretion; namely, adrenalin. In this primitive condition the secretion of adrenalin is a function of nerve cells, constant in number, which are situated in the segmental ganglion and are also in all probability the nervous regulators of the vascular muscle. These cells therefore represent the common ancestors of the sympathetic and of the adrenalin-secreting systems of the mammal. In the course of evolution the two functions have become separated, and two distinct types of cell have arisen, one of which is purely secretory and the other purely nervous. In the earliest vertebrates the secretory system is chiefly

[17] Gaskell, W. H., The origin of vertebrates, London, 1908.

in evidence, but it is here most intimately connected with the ganglia and trunks of the posterior nerves, an arrangement strictly comparable with Ónodi's[18] description of the early development of the mammalian sympathetic system. The evolution in the vertebrate kingdom of the two now separate systems takes the form of a steadily increasing development of the nervous or sympathetic type of cell and a relative diminution and concentration of the secretory type. The final condition reached in the mammal is a widely distributed complex sympathetic nervous system, with a complete concentration of the secretory system into the medullary tissue of the suprarenal capsules.

The emigration from the central nervous system of the cells which secrete adrenalin took place at the same time as that of the nerve cells of the sympathetic system; their close association and similarity of nervous control is still clearly seen in animals as high in the vertebrate scale as the amphibia, where chromaffin cells are incorporated in every sympathetic ganglion.

CONCLUSIONS.

1. The sympathetic nervous system and the adjuvant adrenalin-secreting system are found in their earliest form in the annelid kingdom, and consist of cells situated in the central nervous system which are the common ancestors of both, and which are both secretory and nervous in function.

2. These cells are developed in the annelid kingdom parallel with the development of a contractile vascular system, which possesses muscles comparable in physiological actions with the muscle of the vertebrate heart.

3. This vascular muscle is regulated by the processes of the common ancestral cells as well as by their secretory activity.

4. In the primitive form contractile rhythm is an intrinsic property of cardiac muscle; its nerve supply regulates the rhythm, it does not initiate it. The beat is therefore myogenic, not neurogenic.

5. The contractile vascular system of annelids is mainly branchial in function. The vertebrate heart has been derived from it by the growing around of the lateral body folds to form a new ventral surface.

[18] Ónodi, A. D., *Arch. mikr. Anat.*, 1885–86, xxvi, 553.