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On the Nerve Supply of the Heart of Flying-Squirrel.

There have been very many histological studies reported on the nerve supply of the heart, but it is universally accepted that the results of such a study with the human heart as object by Prof. H. SETO the Director of this laboratory stand high on a pinnacle above the past works on the subject. For SETO (1936) has succeeded, by his thorough examination of enormous series of preparations of heart tissues stained by his ideal modification of BIELSCHOWSKY's impregnation, in staining out the terminations of vegetative fibres, i.e., the STÖHR's terminal reticula, in the heart muscle tissue with utmost clarity — an achievement regarded hitherto as a well-nigh hopeless task — and beside and above that, in discovering sensory terminations of many varied types in the heart, especially in the myocardium and the endocardium. Besides, it is needless to mention that he described in his report the details concerning the GERLACH's plexus, the ganglia and ganglion cells in them, as well as the general distribution of vegetative fibres. More recently, AIBA (1954), one of his disciples, made a very laudable supplement to the SETO's study on the sensory terminations of the human heart, leading the research on this subject to a level of near perfection. STÖHR and HERMANN has expressed their whole-hearted concurrence with the remarks by SETO on the vegetative innervation of the human heart.

At this laboratory, we are studying the nerve supply of the hearts of various mammals from the viewpoint of comparative histology as a link in the chain of studies on the innervation of the animal circulatory system, and many an interesting observation has been made to date. For example, SATO studied the canine heart and MATSUO the heart of hedgehog from this angle, and pointed out the existence of some specific modes of nerve distribution by genera and species of mammals. They have succeeded in making it clear that, as far the sensory terminations are concerned, those in the human heart are the most complex and the nearest to perfection, while in other mammals, these are much poorer in formation than those in man.

In succession to these predecessors, the present author has been granted the opportunity of studying the innervation of the heart of a flying-squirrel. The materials were fixed for a long time in 10% neutral formol solution, cut into 40 μ frozen sections and stained with the ideal SETO's impregnation in routine use at this laboratory. The large series of the beautifully stained sections thus obtained I subjected to minute microscopic observation, and studying my findings in com-
parison with those concerning the hearts of man and some other mammals, was rewarded with the results reported in the following.

I. Individual Findings.

Since the heart of the flying-squirrel is very small as that of hedgehog (MATSUO), its stained transverse sections could be duly balsam-embedded without further cutting, so that the orientation was very readily determined. I will speak first on some specially mentionable features in the microscopic pictures of my specimens.

The epicardium was found very thin and composed of a loose connective tissue, nearly devoid of fat tissue as found in the human epicardium. Its surface is covered by a prominent one-rowed cubic epithelium. A very specific feature, however, was found in the groupwise presence of numerous blood capillaries here and there in that connective tissue. This epicardium shows a good development around the origins of the aorta and the a. pulmonalis and is somewhat thicker in the auricules but extremely thin in the ventricles.

The myocardium is composed of typical heart muscle fibre units as in the hearts of man and other mammals. Some specific muscle fibres (PURKINJE) seem to be in sporadic existence, but it was very difficult to distinguish such fibres, if any, from the common heart muscle fibres in the flying-squirrel's as in the hedgehog's heart. The endocardium is also rather thick in the auricles but very thin in the ventricles, as was the epicardium above. In the auricular appendages, however, it was considerably thinner than in the other parts of the auricles. The parts of the endocardium lining the auricles consist of a connective tissue rich in cells and the inner surface is covered by one-rowed endothelial cells. In the well-developed endocardium of this animal some smooth muscle fibres, not to be found in the heart of hedgehog, are found here and there. In such thin parts of the endocardium as in the ventricles, however, no such smooth fibres could be found.

As described above, the heart of flying-squirrel shows no essential difference from the human and the canine hearts in structure, but in development, it is much poorer, especially in that of the epicardium and the endocardium, than in man and dog, but somewhat superior to that of hedgehog (MATSUO).

The GERLACH's plexus cardiis or primary plexus (SETO) formed in the epicardium in the auricles of flying-squirrel, as that in the hedgehog's heart (MATSUO) is represented by the extension of the nerve plexus formed by intertwining nerve bundles running peripheralwards within the septal connective tissue between the a. pulmonalis and the aorta and some ganglia were found among the bundles. This plexus is best developed on the ventral and dorsa1 sides of the origins of these two large arteries and ganglia are frequently found therein (Figs. 1 and 2). This good development is frankly in parallelism with the development of the epicardium.

Such ganglia are found only in the parts specified above in the heart of hedgehog, but corresponding to the better development of the epicardium in flying-squirrel, they are found in the regions surrounding these parts as well, and small ganglia are also not rarely observable in the myocardium, as found in the human and
the canine counterparts. In the thin parts of the picardium of the ventricle, however, neither the GERLACH's plexus nor any ganglia were ever found.

The development of the GERLACH's plexus is incomparably poorer in hedgehog than in man (SETO) and dog (SATO), and the secondary and the tertiary plexus in the myocardium are only very indefinitely found in formation, but in
flying-squirrel, these plexuses are considerably better developed than in hedgehog, the secondary plexus (Fig. 3) and the tertiary plexus being rather notable in some places of the myocardium. Rather thick nerve bundles are often found running into the well-developed endocardium from these plexuses.

The GERLACH's plexus is formed by non-medullated thin sympathetic and vagal parasympathetic fibres in the main, but contains also some thick medullated sensory fibres, which, to our interest, are found in a somewhat larger number than in the plexus of dog and hedgehog. These sensory fibres may be probably derived from n. vagus.

The ganglia found in the GERLACH's plexus are usually of the small type containing 5—12 ganglion cells per cross-section (Fig. 1), but not rarely, ganglia of rather large type containing 30—40 cells in a section may be seen, as shown in Fig. 2. In this point, the development of heart nerves in this animal is better than in hedgehog. These ganglia, however, generally consist in non-capsulated ganglia alone, as found in hedgehog.

The ganglion cells in the ganglia, in flying-squirrel as well as in hedgehog, are far smaller in size and simpler in construction than those in man and dog; the number of nerve processes emerging from the ganglion cells is very small and cells apparently apolar are not rare, the STÖHR's accessory cell-plasmodium surrounding the ganglion cells is very ill developed and contains only a small number of specific nuclei therein, but the oval-formed nuclei in the ganglion cells are comparatively large, considering the size of the cell bodies, and are generally situated excentrically in the mother cells, as is usual with a sympathetic cells (Fig. 4).

Thus, the intracardiac ganglion cells in flying-squirrel show the multipolarity
characteristic to the sympathetic cells, but their nerve processes as well as the accessory cell-plasmodium around them are very poorly developed, the stage of development being very near to that of the sympathetic cells in human fetus of medium stage (UTSUSHI, TAKAHASHI). The sympathetic ganglion cells seen in the heart of flying-squirrel are thus as little developed as the infantile type cells in the human fetal heart, as are those of hedgehog (MATSUO), showing how little their differentiation is advanced. Such a low development of ganglion cells may be common to all the other small mammals in all probability, for NUMATA and NIIZUMA have reported on the extremely poor differentiation of the ganglion cells in the lung and of those in the AUERBACH's plexus of the rectum and the anus of bat, respectively, adding that the cells were of course beyond all classification into DOGIEL's Type I and Type II cells. I concur in the opinion voiced by NUMATA and MATSUO that it seems possible to determine the level of evolution of an animal by the development of the sympathetic ganglion cells observed in it.

STÖHR (1932) has ascertained that the terminations of vegetative nerve fibres consist in very fine neurofibrillar nets or his so-called terminal reticula, and it was SETO (1936) who succeeded first in clearly proving the existence of such reticula in the human heart. SETO also exploded the doctrine that the sympathetic fibres and the parasympathetic fibres end in respectively different terminations, propounded by DOEKE, LAWRENTJEW and others and generally accepted in the past, and demonstrated that the fibres of both the systems end in their common terminations, the terminal reticula. Subsequently, SATO has obtained the same finding in the heart of dog and MATSUO in that of hedgehog. It seems, however, there are as yet some histologists who cling to the opinion that vegetative fibres

Fig. 4. Ganglion cells of sympathetic nature in a ganglion found in the primary plexus in the right auricle of a flying-squirrel. Details in the text. Same staining. Photo ×800.
end in free endings, as expressed in older works on the terminations of vegetative fibres.

Such terminal reticula are in very prominent formation in the heart of flying-squirrel too (Figs. 5 and 6), especially in the heart muscle tissue of its myocardium and in the walls of the blood vessels, in particular, in the arterial walls. The terminal reticula found outside the blood vessels represent the terminations of the proper heart nerves of vegetative nature originating in the GERLACH's plexus, but those in the vascular walls are terminations of the fibres from the perivascular plexus. It is very interesting, however, that the vegetative heart nerves proper and the fibres of the perivascular plexus come into nervous anastomosis here and there (Fig. 3). Ramification and anastomosis are found also among the heart nerves proper, as pointed out by SETO too. Such interconnection is common to the vegetative nerves in all the dodily organs and is an outstanding feature of the vegetative nerve system. The terminal reticula forming the terminations of such nerves, as shown in Figs. 5 and 6, are in the form of individual cords of nets of interwoven finest neurofibrils, but these cords themselves branch out and anastomose with others to form larger net-works almost ad infinitum, indicating that the vegetative nervous system in a body form one large closed reticular system.

In my specimens, the fine structure as well as the manner of control over the supplied cells of the terminal reticula were found to agree with the findings reported in many works from this laboratory. Thus, in my ideally impregnated preparations too, the terminal reticula were observed to come into control over the supplied cells by mere contact, for side-branches cited by many researchers as sent into the bodies of the supplied cells have never been discovered. The transmission of stimuli from the terminal reticula into the cells is probably effected by causing excitation

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Fig. 5. Vegetative terminal reticulum (t) distributed around the arterial wall in the myocardium of the right auricle of a flying-squirrel. Same staining. Photo × 400.
of the supplied cells by the endocrine chemical substances released by the syncytial neuroplasma of the SCHWANN's cells.

As stated above, sensory medullated fibres are found in the auricles of flying-squirrel too, and it is of interest that the number of the fibres here was so large beyond all conjecture — much larger than in the auricles of hedgehog and apparently larger even than in those in dog. E. g. as shown in Fig. 6, several thick sensory fibres were found in one small heart nerve proper running along a blood capillary in the myocardium.

The area of distribution of sensory fibres in the heart of flying-squirrel seems to be limited to the auricles and not to extend into the ventricles, as in man (SETO and AIBA), dog (SATO) and hedgehog (MATSUO). These sensory fibres form their terminations only in the myocardium and the endocardium, but not in the epicardium as in some other mammals. Interestingly enough, the sensory terminations ending in the endocardium are much larger both in number and size.

As the sensory fibres consist in thick myelinated fibres they are very easily distinguishable histologically from the thin nonmyelinated vegetative fibres. For example, as shown in Fig. 7, we can very clearly tell the two thick sensory nerve fibres from the many far finer vegetative fibres running along them in a small
nerve bundle in the heart muscle tissue. Their terminations formed of thick fibres and constituting free endings are just as easily distinguishable from the vegetative terminal reticula.

The sensory terminations formed in the heart of flying-squirrel are incomparably simpler than the same in man and simpler than those in dog, but somewhat more complex than those in hedgehog, in general construction. These terminations, as MATSUO has surmised, seem to be most ingeniously designed in the human heart, and the more simplified in the hearts of the animals the lower in evolution. For example while very typical Type I terminations concerned with the blood-pressure falling reflex (SETO) may be found in the human heart, in dog's heart such terminations are farther simpler made (SATO) and in the heart of flying-squirrel as well as that of hedgehog no such terminations have never been detected. No other terminations of such complex types as found in the human heart can be found in the heart of either the flying-squirrel or the hedgehog, most of the sensory fibres ending in unbranched and branched terminations in these lower animals. Only it is of interest that though in the heart of flying-squirrel Type I terminations
concerned with blood-pressure falling reflex as found in the canine heart are never found, the other terminations are formed somewhat more complex than those in dog and frankly more complex than those in hedgehog.

Of the sensory terminations formed in the myocardium of the auricles of flying-squirrel the unbranched terminations are far fewer than in that of dog and hedgehog, the terminations in the former thus consisting mainly in branched ones. Complex branched terminations consisting of a rather large number of terminal fibres are not rare in this animal either. These terminations can be classified by

Fig. 8. An unbranched sensory terminal fibre showing wavy and looping courses here and there found in the myocardium of the right auricle of a flying-squirrel. Same staining. ×320.

Fig. 9. Photograph of Fig. 8. ×400.
the characteristics of the running courses of the fibres forming them into the three subtypes, as follows: Those with fibres showing no marked winding in their courses, those formed of fibres showing rather frank winding and waving and those of which the fibres run peculiarly looped or twisted (coiled like the thread of screw) courses. Of these, the terminations of the second and the third types are found particularly frequently.

Fig. 8 shows an unbranched termination of thick sensory fibre running in a small nerve bundle, which runs a wavy course while forming some loops and showing weak change in size now and then. Fig. 9 is a photograph of the termination shown in Fig. 8.

In Fig. 10 is shown a branched termination Type II, that is, a branched termination composed of fibres running winding courses, found in the myocardium. It is a rather complex branched termination spreading over a comparatively wide area in the myocardium of the left auricle, originating in a comparatively thin sensory fibre, which, after running a gently undulating course accompanying some fine

Fig. 10. A rather complex branched sensory termination belonging to the Type II composed of terminal fibres running winding and waving courses found in the myocardium of the left auricle of a flying-squirrel. A comparatively thin original fibre of this termination. Details in the text. Same staining. Photo ×400.
vegetative fibres, then turning into a very thick fibre showing frequent change in size, parts from the vegetative fibres and singly runs a somewhat weakly winding course before branching out into several fine fibres ending in sharp points. This stem fibre sends out some fine branches in its course accompanying vegetative fibres; these branches again ramify into 2-3 fine terminal fibres each, which end all sharply, after running out gently winding courses.

In Fig. 11 are shown some terminations of rather complex branched type found extending over a considerably large area in the myocardium of the right auricle,

![Fig. 11. Complex branched sensory terminations belonging to the Type II composed of terminal fibres running specific looped or twisted courses found in the myocardium of the right auricle of a flying-squirrel. Details in the text. Same staining. ×320.](image)

formed of fibres running specific looped or twisted courses as detailed below and belonging to the third type above. In the one seen in the left part of the picture, the thick stem fibre, after losing in size, runs for a while a gently winding course, then regains in thickness, proceeds in a very irregular winding and peculiarly looped course while frequently changing its size. In the interim the stem fibre sends out many terminal fibres, more or less long, which are usually thin and show little winding in their courses before ending sharply.

The complex branched terminations seen in the right part of Fig. 11, seemingly two in number, are perhaps formed by one single thick sensory fibre. In these terminations too, we may see similar windings and change in size of the fibres as in the preceding, but since in some parts the fibres are found twisted into screws, these may be put into the category of the third type. Fibres running such twisted courses are often found in the endocardium treated herenuder, too. Possibly, the terminations of this subtype are specific to the heart of flying-squirrel.

The branched terminations of the second and the third subtypes found in the myocardium in flying-squirrel show a considerably close similarity to the complex
branched sensory terminations found in the human heart muscle (AIBA) in their ramification and the wide area of diffusion of their fibres — a very interesting finding. It seems, however, no such helical sensory terminations of sensory fibres entwining the heart muscle fibres spirally, as found in the human heart, can be found in the myocardium of flying-squirrel.

The sensory terminations found in the endocardium of flying-squirrel are generally more simply constructed than those in its myocardium described above, but are less simple than those found in the endocardium of dog or hedgehog. They comprise unbranched and simple branched terminations.

The unbranched terminations here are formed by single myelinated sensory fibres which usually run rather winding or wavy courses without sending out any side branches and end in sharp points. Often the courses of these fibres are markedly winding and change in their size is sometimes observable (Fig. 12).

In simple branched terminations, the stem fibres, after losing their myelin sheaths, branch out into two or three terminal fibres, which end in so many sharp points. Some of the terminal fibres of these terminations, as those of the complex branched terminations found in the myocardium, run out not much winding courses (the first subtype above), some rather markedly wavy courses (the second subtype above) and some again peculiar looped or twisted courses (third subtype above), while fibres showing marked change in size are not rare either. But none of these terminations may be called complex in type.

Fig. 13 shows a bifurcated termination found in the endocardium of the right auricle. The terminal fibres show no much change in size in their little winding

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Fig. 12. An unbranched sensory termination showing a wavy course and a conspicuous change in size found in the endocardium of the right auricle of a flying-squirrel. e endothelium, m muscle tissue of myocardium. Same staining. Photo ×200.
courses and end sharply just below the endothelium. Thus, this is a branched termination of the first subtype. Another simple branched termination formed of three branches in the endocardium of the right auricle is shown in Fig. 14. Here,
the change in size and the winding in the courses of the fibres are rather notable, so this belongs to the second subtype. In Fig. 15, we see two sensory fibres of

Fig. 15. 2 simple branched sensory terminations composed of fibres running conspicuous wavy courses found in the endocardium of the right auricle of a flying-squirrel. Details in the text. Same staining. ×320.

Fig. 16. Sensory terminations composed of looped or twisted terminal fibres found in the endocardium of the left auricle of a flying-squirrel. s myelinated stem fibre of a simple branched termination, b and c terminal fibres perhaps belonging to an unbranched termination of the same type. Same staining. ×320.
considerable thickness run mutually parallel and distinctly wavy courses, and branch out in their distal reach into two or three short branches which end sharply. A few very fine side branches are sent out in their courses. These also belong to the second subtype.

Terminations of the third subtype with looped or twisted terminal fibres are shown in Figs. 16 and 17. In Fig. 16, a comparatively thin myelinated sensory fibre(s) loses its myelin sheath before reaching the mid-layer of the endocardium, wherein it runs a long-drawn twisted course, then bifurcates into two terminal fibres, both of which end sharply. A short thin side branch also ending sharply is sent out by the stem fibre while it runs its twisted course. Besides, the fibres b and c in the figure perhaps belong to a termination of the same type, but since they are cut mid-ways, we cannot make out the general form of the termination. The simple branched termination of the same subtype shown in Fig. 17 was found in the thin endocardium of the right auricular appendage of a flying-squirrel. Its terminal fibres show conspicuous twisted courses and change in size and end bluntly.

II. Summary.

The GERLACH’s plexus found in the pericardium of flying-squirrel is in best development in the best-developed parts of the pericardium around the origins of the a. pulmonalis and the aorta, containing ganglia, large and small. The development of this plexus is better in the flying-squirrel’s than in the hedgehog’s heart. Small ganglia are also in sporadic existence in the myocardium too, as in the human (SETO) and the canine (SATO) hearts. In the ventricles where the epicardium is thin, neither GERLACH’s plexus nor ganglia are found.

The intramyocardiac secondary and tertiary plexuses originating in the epicardiac GERLACH’s plexus are far better developed than those of hedgehog. In the
well developed endocardium some rather thick nerve bundles originating in these
plexuses are often discovered.

The GERLACH's plexus is composed of non-medullated sympathetic and vagal
parasympathetic fibres in the main, but some thick medullated sensory fibres are
present among them. It is of interest that these sensory fibres are more numerous
in flying-squirrel than in dog or hedgehog.

The ganglia in the heart of flying-squirrel are also better developed than those
of hedgehog. The ganglion cells in them, however, are as poorly developed in
flying-squirrel as in hedgehog and much more poorly than in man and dog, showing
the features of infantile type cells comparable to the sympathetic cells in human
fetus (UTSUSHI, TAKAHASHI).

The terminations of the vegetative nerve fibres are here too in the form of
terminal reticula (STÜHR). These reticula never send out side branches into the
supplied cells but come into control over these cells by mere contact.

The area of distribution of sensory fibres in the heart of flying-squirrel as well
as in man and other mammals is limited to the auricles and does not extend into the
ventricles. The terminations of these sensory fibres are formed only in the myocardium and the endocardium. It is of interest that the terminations in the endocardium are larger both in quantity and individual size in flying-squirrel than in dog and hedgehog.

The sensory terminations in the myocardium are incomparably simpler than
those in man, but are more complex than the intramyocardiac terminations in the
heart of hedgehog, and also than the branched terminations in dog, excepting the
terminations Type I concerned with blood-pressure falling reflex. That is, unbran-
ched Type terminations are very small in number, so the terminations are mostly
branched ones and these include even rather complex ones in formation.

These intramyocardiac sensory terminations may be classified again into the
three subtypes according to the courses of the fibres as follows: 1. branched termi-
nations composed of fibres showing not much winding in their courses; 2. those
composed of fibres showing perceptible winding and 3. those including fibres run-
ning looped or twisted courses. Of these, the latter two subtypes often comprise
cnsiderably complex terminations and in many cases resembling those in the human
heart (AIBA) — a very interesting finding. Often the fibres in all these termina-
tions show marked change in size and end always sharply.

The sensory terminations found in the endocardium consist in unbranched and
simple branched ones. The latter may be classified into the same three types as in
the above, but here their courses are much simpler in all the subtypes. Change in
size during the courses of the fibres is equally frequent and some of them often end
in close vicinity of the endothelial cells.

内容自抄

ムササとの心房に見られるGerlach氏神経叢は肺静脈と大動脈の起始を取巻く
発達最良の心外膜内で、最も顕著に形成され、中に大小の神経節を含み、発達
は針鼠の場合（松尾）よりも良好である。又心筋層内にも人（瀬戸）及び犬（佐藤）
に於けると同様小神経節の散在を見る。
Gerlach 氏神経叡に由来する心筋層内の第 2 次及び第 3 次神経叡も針鼠に於けるよりも遙かに著明に作られる。尚発達良好な心房心内膜の中にも之等神経叡に由来する小神経束の走行を見る。
Gerlach 氏神経叡は無髓性の交感及び迷走神経性副交感線維から成るが、尚お太い有髓性知覚線維をも可成り多量に含む。神経線内神経細胞は針鼠の場合と同様。人及び犬に於けるよりも発達遙かに劣る。恰も人胎生後期に於ける幼若型交感神経細胞に類似する。植物線維は向々でも Stöhr 氏終端で表わされる。
ムササビの心臓内知覚線維の分布は人及び他動物に於けると同様、心房特に心筋層と心内膜内に見られ、心室には及ばない。
心筋層内に見られる知覚終末は人の場合よりは遙かに単純、然し犬及び針鼠に於けるよりは複雑に構成される。即ち非分岐性のものは甚だ少なく、多くは分岐性終末で表わされ、中には可なり複雑性を示すものもある。この知覚終末は線維の走行状態から略べ 3 型に区別される。1. 原り迂曲走行を示さない線維から成るもの。2. 迂曲走行を著明にするもの。3. 特殊係緒状又は燃糸状走行を示すもの。そして後 2 者は屡々複雑に構成され、面も人心筋層内に見られるものに類似性を示す。尚お終末枝は屡々著明な太さの変化を示す。
心内膜に見られる知覚終末は犬又針鼠の場合に比し、量的にも、規模の点でも、より優勢を示す事は興味深い。知覚終末は非分岐性及び単純性分岐終末に分けられ、後者に於ては前述の心筋層内の終末に類似して、終末枝が余り迂曲を示さないものの、著明な迂曲を示すもの及び特殊係緒状又は燃糸状走行を示す等に類別されるが、然しその規模はより単純である。

References.