SOME OBSERVATIONS ON THE DEGENERATION IN THE SYMPATHETIC AND SACRAL AUTONOMIC NERVOUS SYSTEM OF AMPHIBIA FOLLOWING NERVE SECTION.

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THE observations were chiefly made on toads, but a few were made on frogs. The animals were etherised and antiseptic precautions taken. In the case of the 1st, 2nd and 3rd spinal nerves, the nerve roots were cut; in the case of the 6th, 8th and 9th nerves, the nerves themselves were cut outside the vertebral column and centrally of the rami communicantes. The nerves were cut and examined on one side only. After time had been allowed for degeneration, the animal was killed, the sympathetic chain with the rami communicantes and a portion of the spinal nerves were removed and placed in 1 p.c. osmic acid for a day, the sympathetic and attached nerves were then washed, placed in water, and portions teased out in dilute glycerine. The nerves need not be teased out at once. After they have been kept for months in water, the nerve fibres can still be readily isolated and the degenerated fibres counted; the non-medullated fibres however become less distinct.

When the nerves are examined at the proper stage of degeneration there is no great difficulty in counting the number of degenerated fibres in the portions of the sympathetic chain which lie between the ganglia. Not infrequently small groups of nerve cells are present in these portions, but they do not as a rule seriously interfere with the teasing. In the ganglia the counting is more uncertain, especially in the larger ganglia, for if the ganglion be not thoroughly teased, the small degenerated fibres may be overlooked and the number found be too small, whilst if it is thoroughly teased some of the fibres are broken and the number found is apt to be too large.

The sympathetic ganglia of the toad are, as is well known, sessile on the spinal nerves. In separating a ganglion from its spinal nerve care must be taken that none of the fibres of the nerve are detached with the ganglion. It is advisable to examine the isolated ganglion under a low power of a microscope before it is teased so as to make certain that such fibres are not present.

The rami communicantes of the frog present no difficulty, but those of the toad are nearly always too short for satisfactory examination.

Although for our purpose it was not necessary to determine the exact number of degenerated fibres, we have taken some pains with the counting, and when we give definite numbers, they may be taken as accurate, subject to the possibility that the products of degeneration of some fibres had been completely absorbed. It has been mentioned by one of us earlier[1] that the degeneration products are more quickly absorbed in small fibres than in large ones, so that the optimum time for observation is not the same for fibres of 2 μ and of 4 to 8 μ in diameter. At a time when all the latter still have deep black droplets of myelin, the former have no myelin left, and the degeneration is chiefly shown by rather sparse clumps of small yellow brown granules, probably of keratin; the fibres have become spindle shaped (the swellings being at the nuclei) and they easily break on teasing. When this condition is pronounced, counting is untrustworthy.

In a good many of our experiments rather too much time was allowed for degeneration. The rate of degeneration was distinctly slower in the toad than in the frog, and was not quite the same in different animals of the same species.

It is to be borne in mind that the degenerated fibres of a ramus can only be followed upwards or downwards in the sympathetic as far as they remain medullated, and consequently that if any fibres lose their myelin sheath before they reach the ganglion in which they end, the ganglionic connection of the fibres will appear less than it really is. In many of our observations the number of degenerated fibres was very few. It has been shown by Mayer[2] that degenerated fibres are occasionally present in normal cerebro-spinal nerves. The same may be the case in the sympathetic. Consequently when one or two fibres only are found after section of a nerve, it does not necessarily follow that they have degenerated in consequence of the section.

[1] Langley. This *Journal*, XXXVIII. pp. 511, 512. 1909.
[2] S. Mayer. *Sitzb. d. Akad. d. Wiss. in Wien*, LXXVII. Abt. III. 1878.

Section of the roots of the 1*st spinal nerve* (*hypoglossal*).

Six experiments were made, all of them on toads. The time allowed for degeneration was:—

Exps. 1, 2—41 days. Exp. 3—42 days. Exps. 4, 5, 6—46 days.

In each case the severance of the nerve was confirmed by microscopic examination: a large majority of the fibres in the trunk of the nerve was found to be degenerated. The nerve cells of the 1st sympathetic ganglion stretch up amongst the nerve fibres for a short distance from the upper end of the ganglion, and scattered small groups of cells may occur nearly up to the vagus. Between the 1st and 2nd ganglia in the toad, the nerve cells are usually continuous and this portion is difficult to tease satisfactorily.

The sympathetic chain between the vagus ganglion and the 3rd sympathetic ganglion was cut into pieces and each piece teased out.

In Exps. 1, 4, 5, 6 no degenerated fibres were present. In Exp. 2, one large degenerated fibre (6 to 8 μ) was found in the teased out preparation of the 1st sympathetic ganglion; since it was not present in the sympathetic above or below the ganglion, and since fibres of this size do not pass from the anterior roots to the sympathetic, this fibre may safely be taken to have been detached from the spinal nerve. In these five cases then, section of the anterior roots of the 1st spinal nerve did not cause degeneration of a single fibre in the sympathetic. The result confirms the conclusion we reached experimentally in the frog[1]. We found that the 1st spinal nerve had no effect on the iris or on the heart, and concluded that this nerve sends no fibres to the sympathetic.

In one case however—Exp. 3—slight degeneration was present. In the sympathetic a little above the 1st ganglion (in which region many nerve cells were present), there were three obviously degenerated fibres. We estimate that the original diameter of one of these was about 4 μ, and of the two others, 3 to 4 μ; besides these there were three fibres with scanty yellow granules which may have been degenerated fibres of about 2 μ in diameter. In the 1st sympathetic ganglion there were about the same number of degenerated fibres. We did not however succeed in tracing these fibres to the 1st spinal nerve. In the portions of the rami communicantes which remained attached to the 1st

[1] Langley and Orbeli. This *Journal*, XLI. p. 450. 1910.

sympathetic ganglion, there was no degeneration; this fact is not quite conclusive since it is possible that in separating the ganglion from the nerve a small bundle of the ramus containing degenerated fibres may have remained attached to the nerve. Still it suggests that the degeneration was independent of the section of the roots of the 1st spinal nerve; further, two small degenerated fibres were found in the rami of the 2nd spinal nerve, though no degeneration (on a somewhat cursory examination) was seen in the 2nd spinal nerve itself.

Section of the roots of the 2nd spinal nerve, and of these with more or less of the roots of the 3rd nerve.

Two experiments were made on the toad:

Exp. 7. Roots of 2nd nerve cut 45 days.

Exp. 8. Roots of 2nd nerve, and part of roots of 3rd nerve cut 42 days. The injury to the roots of the 3rd nerve was accidental. It was found on teasing out a portion of the 3rd nerve that degenerated fibres, though not in great number, were present.

The number of degenerated fibres found in the sympathetic is given in the following Table.

	Number of degenerated fibres	
	Exp. 7.	Exp. 8.
Between vagus and 1st ganglion	1[1]	0
Between 2nd and 3rd ganglia	22	18 to 20
Between 3rd and 4th ganglia	—	16
Splanchnic nerves	10	4[2]
Between 4th and 5th ganglia	7	12
Between 5th and 6th ganglia	2	10
Between 6th and 7th ganglia	0	6
Between 7th and 8th ganglia	0	1
Between 8th and 9th ganglia	0	0

[1] There was one other slightly granular fibre without myelin drops which may have been a degenerated fibre.

[2] The degenerated fibres were confined to the branches from the region of the 4th ganglion.

The 1st and 2nd ganglia were continuous; in the upper part of the composite ganglion, *i.e.* in the region of the 1st, some degenerated fibres were present. In the 2nd nerve there were many degenerated fibres, but less than a half; in most of the fibres the degeneration was much less advanced than in the sympathetic. The 1st and 4th nerves were normal. The 3rd nerve in Exp. 8 contained as has been said some degenerated fibres. The spinal nerves were only roughly teased out.

A few of the fibres counted contained yellow-brown granules but no myelin globules; in Exp. 8 the teasing was less complete than in Exp. 7, so that possibly in Exp. 8 the number was slightly underestimated.

One discrepancy in the results must be pointed out. We should have expected to find more degenerated fibres between the 2nd and 3rd ganglia and in the splanchnic nerves in Exp. 8 than in Exp. 7. Whether the discrepancy was due to some error on our part or to individual variation in the animals we are unable to say. But certain conclusions may fairly be drawn.

We may reasonably infer that in the toad the anterior roots of the 2nd nerve contains nerve fibres which end in the 1st to the 6th sympathetic ganglia inclusive, and also some which end in the ganglia on the course of the splanchnic nerves. The 6th ganglion sends nerve fibres to the upper reno-genital arteries. In our experiments on the frog (*op. cit. supra*) we did not specially investigate the action of the 2nd nerve upon these arteries but found a weak effect on stimulating the 3rd nerve. The fact that in the toad two fibres were found passing from the 2nd nerve to the 6th ganglion suggests that the 2nd nerve sometimes at any rate may have a feeble effect on the upper reno-genital arteries.

We may also reasonably infer that the anterior roots of the 3rd nerve contains nerve fibres which end in all the ganglia of the sympathetic chain with the possible exception of the 9th. It may be recalled that local contraction of some of the arteries of the web of the frog has been observed by one of us[1] on stimulation of the 3rd nerve.

It appears that in general the number of fibres given off by each nerve to the successive ganglia diminishes from the homologous ganglion downwards.

We have seen that in Exp. 8 no degenerated fibres were found in the sympathetic at its junction with the vagus, and in Exp. 7 only one was found. Now degeneration can be traced for a considerable distance in descending fibres, *i.e.* these do not soon lose their myelin sheath; it is probable, then, that if the ascending fibres ended in ganglia outside the sympathetic chain they would retain their myelin in the short course up to the vagus ganglion. Consequently we may take it as probable that practically all the ascending fibres end in the ganglia of the sympathetic chain, as they do in the mammal. As regards the accelerator fibres of the heart, this conclusion was deduced some time ago by Langley and Dickinson[2] from the effects produced by nicotine.

[1] Langley. This *Journal*, XLI. p. 483. 1911.
[2] This *Journal*, XI. p. 278. 1890.

Section of the 6th nerve centrally of its rami.

The chief interest of the observations on the 6th nerve was to determine how far the fibres of its ramus ran upwards in the sympathetic trunk, and whether it sent any fibres to the splanchnic nerve. The 6th nerve was cut outside the vertebral column, centrally of the ramus communicans, so that degeneration was caused in the fibres arising from the spinal ganglia as well as in those arising from the spinal cord. The time allowed for degeneration was as follows:—

Toad.	Exp. 9.	51 days.	Frog.	Exp. 13.	42 days.
	Exp. 10.	52 days.		Exp. 14.	43 days.
	Exp. 11.	52 days.		Exp. 15.	46 days.
	Exp. 12.	52 days.		Exp. 16.	48 days.

In the toad no special attention was paid to the rami communicantes; in the frog they were teased out and were found to have in all cases an appreciable number of degenerated fibres. Except in Exp. 13, however, the normal fibres were in a considerable majority. We may conclude then that as a rule the ramus of the 6th nerve contains considerably more post-ganglionic than pre-ganglionic fibres.

In no case was there any sign of degeneration in the sympathetic just below the 4th ganglion apart from filaments obviously running to the splanchnic nerve, hence in all probability the 6th nerve sends no fibres to the 4th or to any higher ganglion.

The condition elsewhere varied; the results are given in the following Table (p. 119).

It appears then that the 6th nerve sometimes sends no fibres upwards in the sympathetic, sometimes it sends a few, or as many as a dozen or more. The upward running fibres do not as a rule continue their course in the splanchnics as medullated nerves; probably one or two run to the upper reno-genital artery, and most of them end in connection with the cells of the 5th ganglion. The size of a few of the fibres in the reno-genital nerves show that they are afferent fibres.

Degenerated fibres were in all cases followed down to the 8th ganglion and to the 9th when this was not fused with the 8th. The number of descending fibres varied; the maximum found between the 6th and 7th ganglia was about 22. The number decreased in passing down, as a rule there were about 5 between the 8th and 9th ganglia.

In most cases the lower reno-genital arteries had no degenerated fibres.

	Exp.	Number of degenerated fibres: Between 5th and 6th sympathetic ganglia	In splanchnic nerves
Toad	9	Some (not counted)	1
	10	4 to 5	0
	11	Small granules in some fibres, degeneration too far advanced for counting. In a branch from the 5th ganglion to a reno-genital artery there was 1 degenerated fibre	0
	12	Some (not counted). In the branches from the 6th ganglion to the reno-genital arteries there were 4 to 6 degenerated fibres	0
Frog	13	1. Also one large fibre (6—8 μ in diameter) degenerated in a branch from the 6th ganglion to the reno-genital arteries	0
	14	A dozen or more; degeneration too far advanced to be certain of number; more seen in the sympathetic than in the rami	4
	15	0	0
	16	A few fibres	One or two above the 5th ganglion which may have gone either to the splanchnic or to the upper reno-genital arteries

Section of the 8th nerve centrally of its rami.

The operation was performed on 8 toads and on 2 frogs, the time allowed for degeneration being as follows:

Toad.	Exp. 17.	43 days.	Toad.	Exps. 22, 23.	52 days.
	Exp. 18.	45 days.		Exp. 24.	53 days.
	Exps. 19, 20.	48 days.	Frog.	Exp. 25.	20½ days.
	Exp. 21.	51 days.		Exp. 26.	46 days.

The 8th sympathetic ganglion and the sympathetic trunk just above and below it were teased out ; in the frogs the ramus was also teased. The number of degenerated fibres in the ganglion could not in most cases be accurately counted.

The results are given in the following Table (p. 120).

We may first consider the experiments upon the frog. It is clear that the 8th nerve differs from the 2nd, 3rd and 6th nerves in its relation to the sympathetic. Even with a considerable posterior shift of the nerves it sends few or no fibres to the sympathetic. The results are such as might have been expected from the results which we obtained in nerve stimulation; they do not however decide the points left doubtful by the stimulation method.

Number of degenerated fibres in the sympathetic.

	Exp.	Between 7th and 8th ganglia	8th ganglion	Between 8th and 9th ganglia	Remarks
Toad	23	1[1]	1[1]	0	
	17	1[1]	3	2	Scattered nerve cells between 8th and 9th ganglia
	24	—	—	1 to 3	Degeneration far advanced
	18	0	some	about 6	
	21	0	a few	a few	
	22	—	—	about 8	
	19	0	some	about 20	The trauma of the 8th nerve stretched down to the ganglion
	20	—	some	about 20	
Frog	25	0	0	0	
	26	0	0	0	

[1] These fibres were about 4 μ in diameter. In the other cases no count was made of the number of fibres of different sizes.

In Exp. 25 the ramus of the 8th nerve contained no degenerated fibres.

In Exp. 26 the piece of nerve cut out of the middle of the ramus contained 1 degenerated fibre of about 4 μ, and 5 smaller degenerated fibres, the latter were together in a small nerve bundle. These degenerated fibres were not traced in either direction. The central end of the 8th nerve with its attached fragment of ramus was teased out, all the fibres of the ramus which were seen were normal, they were readily distinguished from the degenerated fibres of the 8th nerve. The lumbo-sacral plexus was of median *b* type[1], the 10th nerve was larger than the 7th.

In the toad the case was somewhat different. Here there was not complete absence of degeneration in the 8th ganglion in any experiment, though in one experiment only one degenerated fibre was found and in another case only three. This suggests that in the toad the 8th nerve commonly sends a few fibres to the sympathetic. Some of the degenerated fibres were certainly afferent since they were 5 to 7 μ in diameter, whether the others were afferent or efferent the experiments do not satisfactorily decide. A more frequent presence of pre-ganglionic sympathetic fibres in the 8th nerve of the toad than in that of the frog might be expected from the difference which exists in the lumbo-sacral plexus of the two animals. In the toad the 7th nerve is relatively smaller than in the frog and the 8th relatively larger. But although the experiments suggest that the 8th nerve in the toad usually sends a few more fibres to the sympathetic, there are two facts to be borne in mind. The 8th ganglion has sometimes aberrant nerve cells stretching along the rami a short way up and down the 8th nerve. If in cutting the 8th nerve, the section passed peripherally of any such nerve cells

[1] Cf. Langley and Orbeli. *Op. cit.* p. 452.

and their post-ganglionic fibres were medullated, some degeneration would be caused in the sympathetic. The nerve was usually cut 3 to 4 mms. from the sympathetic ganglion, but in the cases (Exps. 19 and 20) in which most degeneration was found in the sympathetic, the section was nearer the ganglion and the traumatic degeneration extended to the level of its upper end.

So far we have been considering the sympathetic system. In the case of the 8th nerve the question arises whether it sends nerve fibres to the bladder and rectum not by the sympathetic but by the sacral autonomic nerves. By the stimulation method we found (*op. cit.*) that in the frog, efferent sacral autonomic fibres were commonly, at any rate, absent. By the degeneration method we have only attempted to arrive at a first approximation to the answer of the question. In removing the nerves from the body, the branches given off by the lumbo-sacral plexus were cut a few millimetres from the plexus, but the peripheral distribution of the branches was not determined, so that in most cases it was uncertain whether they ran to the bladder or rectum (pelvic nerves) or to the compressor cloacæ and external sphincter of the anus (pudic nerves). Under the microscope the former can usually be distinguished by their containing a smaller proportion of nerve fibres more than 4 μ in diameter, but since one nerve branch may contain both pelvic and pudic fibres, we think it better to give the number of degenerated fibres found in all the branches together.

Number of degenerated fibres in pelvic and pudic nerves.

Exp.	Fibres 5 to 8μ in diameter	Fibres about 4μ in diameter	Fibres about 2μ in diameter
18, 21, 22, 23	0	0	0
25 (frog)	0	?[1]	0
26 (frog)	1	0	0
24	1	0	0
17	2	1	?[1]
20	4	0	0
19	2[1]	1	0

[1] A lower small branch given off by the sciatic which may have belonged to the ischio-coccygeal plexus had four degenerated fibres all above 4μ in diameter.

In several cases the first branch given off by the sciatic below those forming the pelvic and pudic nerves were examined, it contained degenerated fibres, varying in number from about 30 to 50.

It will be seen from the Table that in the 10 experiments there was no case in which there was certain degeneration in either pelvic or pudic nerves of any fibre of about 2 μ in diameter, *i.e.* of the size of

the majority of efferent[1] autonomic fibres. In five cases however and possibly in six, there were one or more fibres of larger diameter than 2 μ, most of these were undoubtedly not efferent autonomic fibres; they may have been either afferent fibres of pelvic or pudic nerves, or efferent fibres for the striated muscle supplied by the pudic nerves. We did not find any relation between the presence or absence of degenerated fibres and the type of the lumbo-sacral plexus.

Section of the 9th nerve centrally of its rami.

The operation was performed in two toads (Exps. 27, 28). The time allowed for degeneration was in each case 44 days. The effect on the nerve fibres of the ischio-coccygeal plexus was in striking contrast to that of the section of 8th nerve. The great majority of the fibres of the ischio-coccygeal plexus were degenerated. The number of sound fibres differed in the two experiments in correspondence with the size of the 10th nerve.

In Exp. 27 the lumbo-sacral plexus did not receive a branch from the 10th nerve; in the pudic and pelvic nerves only 10 normal fibres were found, seven of them being in the filaments given off by the 9th nerve before its junction with the 8th and five of these running close together; possibly these arose from the sympathetic ganglia.

In Exp. 28 the 10th nerve sent a branch to the lumbo-sacral plexus and the normal nerve fibres in the ischio-coccygeal plexus were more numerous. Anatomically the plexus arose from the 9th and 10th nerves, so that it is unlikely that any of the normal fibres arose from the 8th nerve.

As regards the sympathetic, in one case no degeneration was found in the 9th ganglion; in the other the 9th ganglion contained one degenerated fibre 6 to 8 μ in diameter, one of 2 to 3 μ, and two or three fibres with yellow granules which may have been degenerated fibres of 2 μ. The rami could not be adequately teased out, but a few normal fibres were followed from the rami into the central end of the 9th nerve, the great majority of them running peripherally. Apart from the fibres of the rami, no normal fibres were seen in the 9th nerve.

[1] We speak of 'efferent' autonomic fibres to avoid misunderstanding, although afferent fibres cannot at present be divided with any advantage in 'autonomic' and 'somatic.'

Summary and Remarks.

The degeneration following nerve section was chiefly observed in toads. In comparing the results obtained in these observations with the experimental results obtained in frogs, it is to be remembered that the spinal nerves though similar in the two animals, do not exactly correspond.

Section of the roots of the 1st spinal nerve in five out of six cases caused no degeneration in the sympathetic. In the 6th case there was slight degeneration, but the central connection of the degenerated fibres was not traced.

Section of the roots of the 2nd spinal nerve (one case) caused degeneration up to the 1st sympathetic ganglion and of one nerve fibre above this; it caused degeneration downwards as far as the 6th ganglion, but only of two fibres between the 5th and 6th ganglia.

Section of the roots of the 2nd and of more or less of the 3rd spinal nerves (one case) caused degeneration upwards to the 1st sympathetic ganglion but of none above; the downward degeneration could be traced to the 8th ganglion, but only one degenerated fibre was found between the 7th and 8th ganglia.

We conclude that the upward running fibres end in nerve cells of the sympathetic chain, and that the fibres running to the cranial nerves are post-ganglionic.

Section of the 6th nerve centrally of its ramus communicans did not cause degeneration above the 5th sympathetic ganglion; in the toad (four cases) there were a few fibres degenerating upwards as far as the 5th ganglion; in the frog, there was no degeneration upwards in one case, and some, varying in amount, in four cases. A few fibres (one to four) were in some cases found degenerated in the splanchnic nerves. The downward degeneration extended as far as the 9th sympathetic ganglion.

Section of the 8th nerve in the toad caused some degeneration in the 8th sympathetic ganglion and below, the number of degenerated fibres varied in different cases. In the frog (two cases) no degenerated fibres were found in the sympathetic. The results suggest that the 8th nerve more commonly has pre-ganglionic sympathetic fibres in the toad than in the frog, but the experiments are not quite conclusive.

Section of the 8th nerve caused no certain degeneration of the small medullated fibres in the ischio-coccygeal plexus, *i.e.* of those about

2 μ in diameter, but in several experiments a few larger fibres were found degenerated. We conclude that in these cases the 8th nerve had no sacral autonomic fibres, the few fibres sometimes found degenerated were probably fibres for the pudic branches of the plexus.

Section of the 9th nerve caused no degeneration in the sympathetic in one experiment; in the other three or four degenerated fibres were present. It caused nearly complete degeneration in the ischio-coccygeal plexus, except of such strands as obviously arose from the 10th nerve.

The results given above support in the main the conclusions which we deduced from our observations on the effects of nerve stimulation (*op. cit. supra*). They show that there is a gap between the origin from the central nervous system of the cranial autonomic and of the sympathetic nerves and give evidence though less clear that there is a gap between the origin of the sympathetic and of the sacral autonomic nerve fibres. The course taken by the nerve fibres running from the several spinal nerves to the sympathetic is very much what would be expected from the experimental evidence. There is however a lack of correspondence in some details, and further observations are required to show whether these are due to differences between the frog and the toad, or to slight inaccuracies in one or other method.