QUANTITATIVE HISTOLOGY OF WALLERIAN DEGENERATION

I. NUCLEAR POPULATION IN RABBIT SCIATIC NERVE

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INTRODUCTION

The histology of Wallerian degeneration of peripheral nerves has been thoroughly studied in its qualitative aspects (see, for instance, Cajal, 1928; Nageotte, 1932; Weddell & Glees, 1941; Holmes & Young, 1942). In this and subsequent papers our purpose is to provide that more detailed and exact analysis of the process which can only come from quantitative study. We consider this worth attempting not only because Wallerian degeneration is intrinsically an important pathological process. It is also a proliferative phenomenon similar in many respects to the reaction of tissues to local injury. It provides particularly favourable material for experimental analysis of a proliferative process, since it takes place fairly evenly throughout a long stretch of well-demarcated tissue, the peripheral stump of a severed nerve; and the intensity of the reaction is independent of intensity of trauma. Our results may therefore be useful for a comparative survey of proliferation in a variety of tissues.

In this paper we survey the changes in number of nuclei of nerves, excluding the perineurium and epineurium, during Wallerian degeneration, as seen in histological sections. The nuclei of different kinds of cells have, as far as practicable, been analysed separately. We have distinguished between nuclei within the Schwann tubes and nuclei in the endoneurium. The tubal nuclei have been separated into three groups according to size of tube, and the endoneurial nuclei into connective tissue nuclei (fibrocytes and macrophages) and blood vessel nuclei (endothelial and smooth muscle nuclei). The difficult and complicated history of the migratory macrophages cannot be adequately followed in our material. Evidence as to their multiplication and movement can, however, be got from vitally stained animals, and we shall consider this in a subsequent paper. For the present, macrophage and fibrocyte nuclei are not distinguished in the endoneurium, nor macrophage and Schwann nuclei in the Schwann tubes.

MATERIAL AND METHOD

Material

We have used exclusively the peroneal and tibial branches of the sciatic nerve in the thigh of rabbits. For our degenerated material we cut the nerve aseptically with sharp scissors at an initial operation, under nembutal and ether anaesthesia, in the thigh at about 1–2 cm. below the level of the third trochanter. When the period of degeneration was to be longer than 100 days, we brought the central stump outside the muscles and sutured it to the under surface of the skin to minimize reinnervation. In the shorter experiments a gap of 1–3 cm. was left between the two stumps. After the required period of degeneration the rabbit was killed and part of the peripheral stump in the thigh removed and fixed. We have not used the proximal centimetre of the peripheral stump, in the neighbourhood of the trauma, in this work. For our undegenerated material we fixed the corresponding region of nerves in unoperated rabbits.

Our material consisted of sixty-five nerves from thirty-three rabbits, studied intensively by differential counts. In the majority of these animals peroneal and tibial nerves of both sides were used, usually cut at different times and then simultaneously fixed, so that comparison of different periods of degeneration could be made under closely similar conditions. Two tibial or two peroneal nerves prepared in this way will be referred to as a *paired experiment*. We have, in addition to this intensively studied material, used a further forty nerves from thirty rabbits to confirm various points.

Histological technique

Fixation was principally in Susa, though we have also used Bouin, and we found that Bodian's peripheral nerve fixative often gave useful help in differentiating tubal from endoneurial cells because of the severe shrinkage it causes. We used longitudinal and transverse paraffin sections cut at 7 μ. One slide was stained in Heidenhain's haematoxylin and Masson's light green; another slide was usually stained in Mayer's carmalum, which strongly differentiates nuclei from nerve-fibre remnants.

All but four of the nerves of 35 days of degeneration and over, and the majority of nerves of 25 days of degeneration, were fixed and stained by Bodian's method and examined for reinnervation. In almost all of the nerves degenerated 90 days and over a few axons were present, but in no case was reinnervation extensive.

Counting

Transverse sections (referred to as T.S.) proved much superior to longitudinal ones (referred to as L.S.), both for identification of cell types and for

standardization of nerve volume. Changes in nuclear size however affect T.S. much more seriously than L.S., and we had to make special allowances for them. Our usual procedure has been to count differentially, under an oil-immersion objective, a number of fields selected at random in the T.S. of each nerve. Each field contained 20–200 cells, and the number of fields counted, usually 10–20, varied according to the population. Further supplementary counts were often added, including counts of all nuclei in an entire T.S. A different section was used for each field counted, although variation of equivalent fields from section to section was unimportant. Much more important was randomization of position of the fields in the transverse plane: the 'nerve fibre spectrum' (that is, the relative numbers of fibres of different sizes) varies greatly from place to place in a T.S., and the number of nuclei, at least in the early stages of degeneration, varies with it.

Expression of results

Our results are necessarily expressed in highly summarized form. In the tables which follow, the original counts have been standardized for nerve volume and nuclear length (see next section), and the tibial and peroneal results combined after expressing each as a percentage of the mean population of undegenerated tibials or peroneals respectively. Tibials and peroneals are sufficiently similar in composition to permit this, though in absolute number of nuclei tibials, because of their larger diameter, average throughout degeneration 2·15 times the population of peroneals.

Counts of all nuclei have been done on rather a larger sample than have the differential counts (see Tables 2, 4 and 5). The figures have been made consistent as between the various tables by applying the percentage composition derived from the differential counts to the absolute values of all nuclei. The inconsistency arising from the smaller sample of differential counts is in any case small.

The nerves have been formed into eight groups according to period of degeneration, each group designated by its mean period of degeneration. These groups, and (in brackets) the range of period included in each, are as follows: 0 days (i.e. normal undegenerated nerves); 5 days (5 days only); 10 days (10 and 11 days); 15 days (14–16 days); 25 days (23–26 days); 45 days (35–55 days); 110 days (96–160 days); and 225 days (210–250 days). Nerves intermediate between these groups have, in the interests of brevity, been omitted from the tables, though they are sometimes referred to in the text.

STANDARDIZATION FOR NERVE SIZE AND NUCLEAR SIZE

If we suppose that changes in the number of nuclei per microscopic field during the course of degeneration indicate accurately changes in the nuclear population of nerve, we make two assumptions: that the volume of the nerve remains constant during degeneration; and that the size of the nuclei also remains constant. Neither assumption is correct.

Nerve size

It is well known that the diameter of a peripheral stump changes during degeneration, at first increasing, then decreasing. Table 1 shows the magnitude of these changes. In the third column the mean cross-sectional areas of fixed and sectioned tibials and peroneals, excluding both perineurium and epineurium, are expressed together as a percentage of that of undegenerated nerves; they are graphed in Fig. 1. These figures give rather a rough indication of the size changes, because considerable variability has been introduced by histological treatment, and peroneals and tibials do not change proportionately.

Table 1. *Changes in nerve size and nuclear length with time of degeneration*

N = number of nerves used for measurements.

	Nerve size		Nuclear length	
Days of degeneration	N	Mean cross-sectional area as % of 0-day	N	Mean length in μ
(1)	(2)	(3)	(4)	(5)
0	14	100	9	12·9
5	6	108	7	11·3
10	5	123	7	9·1
15	4	114	6	8·9
25	13	118	9	8·6
45	7	96	9	10·2
110	9	97	8	11·2
225	6	73	6	12·0

In order to allow for these changes in size of nerve, and for the additional variation produced by histological treatment, our index of nuclear population has been the number of nuclei in a complete transverse section of the nerve. In many cases we have directly counted all the nuclei in a section. In others, where a complete count was impracticable, we have estimated the number from sample fields and the area of the section.

Nuclear size

In a section the objects counted as nuclei vary from entire nuclei to minute terminal fragments. It is impracticable to differentiate them, and all must be counted alike. The longer the nuclei the more of them will be represented in each transverse section. Nuclear length certainly shortens during the early cell-division phase of Wallerian degeneration, and subsequently lengthens during the shrinkage in diameter of the nerve. Comparisons of nuclear population at different stages of de-

generation demand therefore a correction of counts for change in nuclear length.

The method we have used for making the necessary correction is to measure nuclear length in longitudinal sections, taking the mean of 50 or 100 random measurements on each nerve. All T.S. nuclear counts of degenerated nerves are then standardized to the value they would have if the nuclei were the same length as those in undegenerated nerves. This is done by multiplying the crude count by $(7+a)/(7+b)$, 7 being the thickness in μ of the T.S. counted, a the mean L.S. nuclear length in μ of undegenerated nerves, and b the mean L.S. nuclear length in μ of the degenerated nerve in question.

Mean nuclear lengths of all nuclei (without consideration of cell-type) obtained from L.S. measurements are given in Table 1, and graphed in Fig. 1.

CHANGES IN TOTAL NUCLEAR POPULATION

Table 2 shows the changes in population of all nuclei with time of degeneration. The mean number of

Table 2. *Changes in total nuclear population with time of degeneration*

Days of degeneration	No. of nerves counted	Mean population as % of 0-day	Standard error
(1)	(2)	(3)	(4)
0	15	100	2·4
5	5	163	8·5
10	5	390	23
15	5	635	35
25	12	840	66
45	8	600	56
110	7	540	41
225	8	500	26

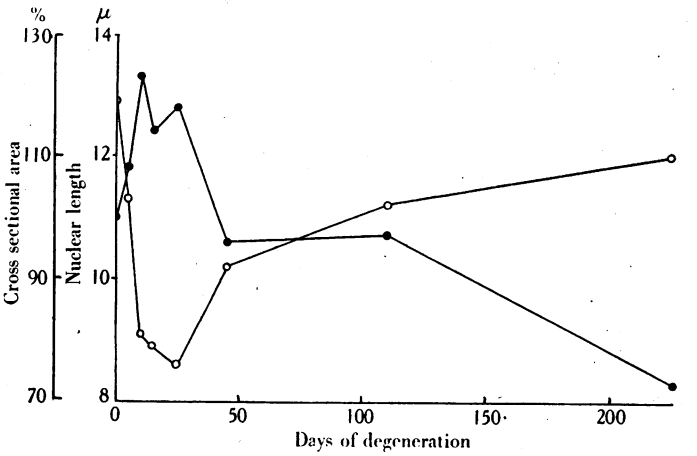

Fig. 1. Mean cross-sectional area of nerves expressed as percentage of that of undegenerated nerve ● and mean nuclear length in μ ○ at different times of degeneration.

Clearly the mean L.S. length will be less than the true nuclear length because we cannot differentiate whole nuclei from shorter nuclear fragments in a section. We estimate the maximum underestimation at 10%, but must defer justification of this statement to a subsequent publication. The method is quite accurate enough for most of our material. But at 225 days of degeneration, and to some extent at 110 days, an error from changing shape of nucleus becomes serious because of the sinuosity of many of the nuclei, which produces a high proportion of short fragments in L.S. The length given in Table 1 for 225 days of degeneration is an estimate based on selection of the straighter nuclei. It is, nevertheless, probably too low. The largest correction for nuclear length occurs at 25 days of degeneration. In these nerves the crude count is 23% less than the population standardized to 0-day nuclear length. The correction is therefore important.

nuclei in a complete T.S. at different times of degeneration, corrected for changes of nuclear length, has been expressed as a percentage of the mean population of undegenerated nerves. The data are shown graphically in Fig. 2.

The nuclear population increases rapidly during the first 25 days, and then falls. We will consider these changes in two sections.

The population increase

There is no significant change in population during the first 3 days of degeneration. The small group of nerves (not shown in the table and figure) at 3 days averages 103% of the undegenerated group; and two paired experiments show the 3-day nerve as 108% of the undegenerated. Rapid multiplication then starts, and by 25 days the population has reached a value averaging 8·4 times that of the undegenerated group. In the case of an average

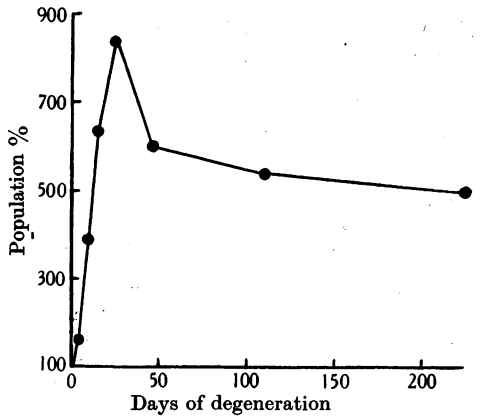

Fig. 2. Mean total population of nuclei of nerves at different times of degeneration, expressed as percentage of that of undegenerated nerve.

average number of nuclei added per day during various periods of degeneration. Since any initial value would be somewhat arbitrary, it is expressed for convenience as the daily increment to an initial 100 nuclei of the undegenerated nerve. It shows that the period of steepest average rise of the growth curve is 10–15 days. At this time an average peroneal is adding nuclei at 10 per day per μ of its length. Before and after this period the daily increment is less, though not very much so. The growth curve is therefore sigmoid.

Presumably the number of nuclei present at a given time in a nerve is of some importance in determining the additive growth rate at that time. The appropriate measure of increase relative to population present is the *specific growth rate*, which is given as an average figure for each of the various periods of degeneration in Table 3, column 3, and shown graphically in Fig. 3. This is the average

Table 3. *Additive and specific population growth rates*

Period of degeneration days	All nuclei		Tubal nuclei		Connective tissue nuclei	
	Additive	Specific	Additive	Specific	Additive	Specific
(1)	(2)	(3)	(4)	(5)	(6)	(7)
3–5	32	24	19	28	13	24
5–10	45	18	35	22	11	12
10–15	49	10	42	11	7	5
15–25	21	3	20	4	0·8	0·8
25–45	−12	−1·7	−10·4	−1·9	−1·6	−1·2
45–110	− 1·0	−0·16	− 0·9	−0·21	−0·05	−0·04
110–225	− 0·35	−0·07	− 0·31	−0·08	−0·02	−0·01

The additive and specific growth rates are defined on p. 40. All additive growth rates are referred to an initial 100 nuclei *of all kinds*. Blood-vessel nuclei growth rates, which are extremely small, are omitted. There is no growth between 0 and 3 days.

peroneal nerve, which in the undegenerated state has nuclei distributed at the rate of 20 per μ of length of the nerve, this means the addition of 150 nuclei per μ of length. (A T.S. count suggests a density of nuclei more than twice as high as these figures. That is because most nuclei are represented in more than one section.)

The standard errors of the means at different times of degeneration are given in column 4 of Table 2. All the means covering the increase of populations are significantly different from each other by the *t* test, except that the 15-day mean is not significantly different from the 25-day mean. Nevertheless, we are confident that the increase of population continues between 15 and 25 days, since two paired experiments showed 25-day populations averaging 1·3 times the 15-day population (the grouped data of Table 2 also give a corresponding increase of 1·3 times); and mitoses are frequent beyond 15 days of degeneration.

In Table 3, columns 2 and 3, the increase is presented in terms of growth rates. Column 2 of this table shows the *additive growth rate*, i.e. the

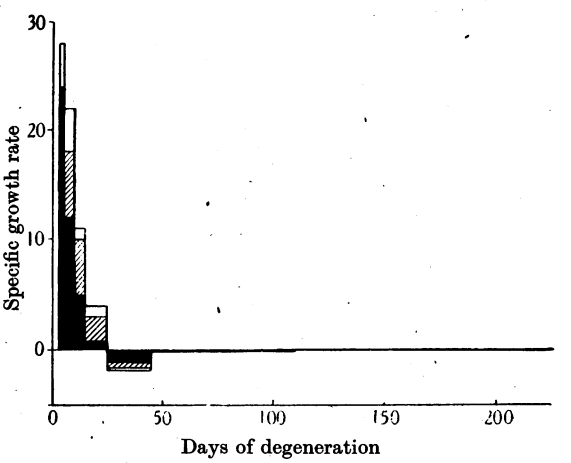

Fig. 3. Specific growth rates of all nuclei ▨, tubal nuclei □, and connective tissue nuclei ■ at different times of degeneration.

number of nuclei added per day per 100 cells present, obtained from the formula

$$100\,(\log_e N_2 - \log_e N_1)/t_2 - t_1 = R,$$

where N_1 and N_2 are nuclear populations at t_1 and t_2 days of degeneration respectively, and R is the specific growth rate. (The specific growth rate is the 'relative growth rate' of Fisher (1938, p. 29); see Medawar (1940) for the change in nomenclature.)

The specific growth rates show that multiplication of nuclear population is most rapid early in degeneration and declines in rate as degeneration proceeds. This conclusion can also be derived from a considerably larger sample of nerves (thirty-nine nerves between 0 and 10 days, including a large 7-day group) for which we have counts of nuclei per field in L.S. Specific growth rates from this data (unstandardized for nerve or nuclear size) show the same decline during degeneration from an initial maximum.

If an initial phase of accelerating specific growth rate exists, it cannot be detected in our data. The specific growth rate at 3–5 days is significantly higher than the rate at 5–10 days by t test. It should be noted, of course, that the *day* of most rapid growth rate, additive or specific, is not necessarily within the 2- or 5-day period of most rapid average growth, which is all that our data give us. We discuss these growth rates further when we consider the different cell-types separately.

The number of nuclei per unit volume of nerve may be physiologically important. It increases by about 7 times during the first 25 days of degeneration. This means an average increase from roughly 43,000 nuclei per mm.^{3} in the undegenerated nerve to 330,000 per mm.^{3} at 25 days of degeneration.

The population decrease

At 25 days, according to our data, the peak of population is reached. We should emphasize, however, that 25 days is not necessarily the true average peak of population, since we have studied nerves at discrete intervals of degeneration and do not accurately know what happens between them. We believe, however, that the curve is likely to be nearly flat between 20 and 30 days, judging roughly by mitosis and nuclear degeneration, and that 25 days is not likely to be far from the average time of maximum population.

The population built up during the first 25 days of degeneration does not persist at that size. We have nerves up to 225 days of degeneration, and during that time the population declines to about 60 % of the peak value. This population is equivalent to that of a nerve of 12 days of degeneration. The extent of the decrease receives additional support from three paired experiments. In these the population of the 225-day peroneal was respectively 66, 55 and 53 %, an average of 58 %, of its contralateral 25-day peroneal. The change in a typical peroneal represents a decline from 170 to 100 nuclei per μ of nerve length.

The loss of cells is statistically significant. Comparing means of peroneals of 25 days of degeneration with those of 100 days and over, a t test shows that the probability that both samples come from the same population is only 0·006 ($t = 3{\cdot}2$, $N = 18$). This is without any correction for nuclear length, which must increase the significance, since the nuclei elongate after 25 days.

The curve in Fig. 2 suggests that the greater part of the decrease of nuclear population occurs between 25 and 45 days. This is also shown in Table 3, which gives additive and specific growth rates (negative during the decrease of population).

It is possible that the extreme concentration of nuclear loss within this period is a sampling error. The decrease is after all a small one compared with the previous increases, and it is too much to expect that our material should be accurate within such narrow limits. The 40–50-day nerves are of unusually small size (see Table 1), and their mean number of nuclei per field in T.S. is actually slightly smaller than that of the 100-day group. All this suggests that the 45-day mean is too low, and therefore that the loss of nuclei is more evenly distributed.

Nevertheless, we believe our data make it very probable that the most important loss does occur soon after the peak of population. This view is supported by estimates of specific growth rates (% per day) from paired experiments, which should not be affected by sampling error in total population (the pairs were 25 and 50, 50 and 100, 100 and 250 days; we had two of each of these pairs). These growth rates are, for the 25–45-day period, −0·8; and for the 45–225-day period, −0·15. Additional support comes from the accurately determined percentage composition of nerves which is also independent of sampling error in total population. Loss of tubal nuclei makes much the biggest contribution to the population decrease, and the percentage which tubal nuclei form of all nuclei declines much more rapidly between 25 and 45 days than between 45 and 225 days (see Table 4, column 4). This is true regardless of the period to which we allocate the small loss of endoneurial nuclei.

We cannot unfortunately give statistical proof that the nuclear loss continues beyond 45 days. But the general trend of all our data suggests that it does. The most likely supposition as to the course of the population curve beyond 25 days of degeneration is as follows: the population falls, as it rises, with a sigmoid curve; the inflexion of the curve is very early in the process; but the fall continues at diminishing rate throughout the period we have studied.

The density of population (number of nuclei per

unit volume) is also maximal at 25 days, but the decline thereafter is slight (density at 225 days is about 90 % of that at 25 days). The large decrease in total population is almost compensated by the shrinkage of the whole nerve.

Variability

The populations of the undegenerated nerves have a relatively low dispersal about their mean (coefficient of variation $9{\cdot}3 \pm 1{\cdot}7$), while at 25 days of degeneration the dispersal is high (coefficient of variation 27 ± 6) and is similar at later stages of degeneration. The proliferative response to degeneration seems therefore to vary widely from rabbit to rabbit.

Peroneal-tibial comparison

Throughout degeneration the peroneal nerves have a slightly but constantly higher density of nuclear population per unit volume than the tibial nerves, averaging 13 % more. Further, when the total nuclear populations given in Table 2, column 3, are expressed separately for tibials and peroneals, the 0-day mean population in both cases being taken as 100, the peroneals average about 9 % higher than the tibials, indicating possibly a very slightly greater response to degeneration in the peroneals. Clearly, it is hardly to be supposed that the two nerves are identical in their behaviour, but the differences between them are quite insufficient to necessitate their separate treatment. The slight difference between the means of the two branches exaggerates the standard errors given in Table 2, column 4.

CHANGES IN TUBAL NUCLEAR POPULATION

Identification and standardization

In this section we consider those nuclei which lie inside the *Schwann tubes* of Holmes & Young (1942). Most of these tubes can be easily recognized in transverse section. But a nucleus substantially fills a tube of less than 3–4 μ diameter, swelling it out if it is elsewhere narrower than the nucleus. Such a tightly invested nucleus in the undegenerated and early degenerated nerve is often not easy to distinguish from a longitudinally oriented endoneurial nucleus. As endoneurial oedema increases during degeneration, however, endoneurial nuclei become increasingly distinct, and by 10 days little uncertainty exists. A sure way of establishing whether a nucleus is tubal or not is to trace it through serial sections. This is impracticable in large-scale counting, but samples investigated in this way have given results reasonably concordant with our ordinary counts. Nevertheless, the margin of error here, and particularly with regard to the 0-day nerve, is greater than elsewhere, and we have accordingly been cautious in drawing conclusions about the early stages of degeneration.

As is well known, the largest tubes become invaded by macrophages during degeneration (see Doinikow, 1913). Intravital staining with trypan blue shows that this invasion begins at 5 days of degeneration. The immigration of macrophages, and possibly a later emigration, must be taken into account in assessing the tubal population changes. Morphologically, however, we find it impossible to distinguish tubal macrophages from Schwann cells in T.S., and we can only make general allowance for these migrations until our investigations of trypan blue material are complete.

Table 4. *Changes in population of tubal nuclei with time of degeneration*

		Mean tubal population		% distribution of tubal nuclei		
Days of degeneration	No. of nerves counted	as % of 0-day	as % of all nuclei	Large tube	Medium tube	Small tube
(1)	(2)	(3)	(4)	(5)	(6)	(7)
0	8	100	50	16	21	63
5	5	174	54	8	17	75
10	5	520	66	38	21	41
15	5	940	73	37	29	34
25	9	1340	79	34	36	30
45	7	920	76	25	—	—
110	9	800	74	14	—	—
225	8	730	73	4	—	—

The figures of tubal population have been standardized for nuclear length to make them consistent with the rest of the figures we quote. This standardization is not, however, based on measurements of tubal nuclei, but on the average nuclear length of all cells in the nerve (excluding blood-vessel cells). Such rough standardization is sufficient for our purposes, though it introduces some obvious inaccuracies which will be referred to when necessary.

Table 4, column 3, shows the mean number of tubal nuclei in complete T.S. at different times of degeneration standardized approximately for nuclear length, and expressed as a percentage of their mean population in undegenerated nerves.

The data are shown graphically in Fig. 4. The population of tubal nuclei shows roughly the same changes as the population of all nuclei (Fig. 2)—a rise to a peak at 25 days of degeneration, with subsequent decline.

The increase of population of tubal nuclei

As with all cells combined (see p. 39), there is no detectable change in population during the first 3 days of degeneration. This is followed by a rapid increase which brings the population to a maximum averaging approximately 13 times the original at about 25 days of degeneration. The relation of this increase to that of the other (endoneurial) nuclei of the nerve can be seen in column 4 of Table 4 and in

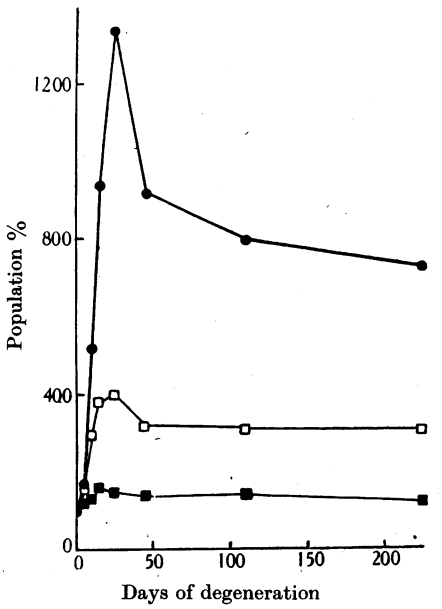

Fig. 4. Mean populations of tubal ●, connective tissue □, and blood-vessel nuclei ■ in nerves at different times of degeneration, expressed as percentages of their respective populations in undegenerated nerve.

Fig. 5, which gives the percentage of all nuclei of the nerve which are intratubal. This percentage increases from 50 in the undegenerated nerve to 79 at 25 days of degeneration. Tubal cells therefore increase much faster than endoneurial cells, though between 3 and 5 days their superiority is not so well marked.

Growth rates of tubal nuclear population are given in Table 3, columns 4 and 5. They show the same trends as those of all nuclei combined. The additive rate given is the daily increment of tubal cells to an initial 100 nuclei *of all types* in the undegenerated nerve. It is thus referred to the same initial population as the additive growth rate of all nuclei, and also that of connective tissue nuclei given in this table. The large proportion of the total increment which is due to the tubal cells is evident by comparing columns 2 and 4. The 3–5-day specific growth rate of tubal nuclei may not really be higher than that of 5–10 days, because of the difficulty over identification; but it is likely to be at least as high. In relation to the whole tubal population, our preliminary information suggests that the macrophage invasion, which is confined to the larger tubes, is so small that it can be safely neglected in assessing growth rates. Correspondingly, any early emigration from the Schwann tubes during this period (Cajal, 1928; Nageotte, 1932) can also be neglected.

The decrease of population of tubal nuclei

The decline in population of tubal cells after 25 days of degeneration is greater than that of all cells. The tubal population at 225 days of degeneration is only 55 % of the tubal population at 25 days

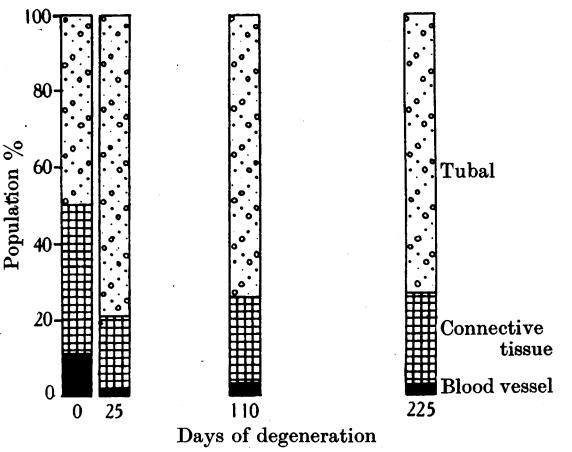

Fig. 5. Percentage composition of nuclear population of nerves at different times of degeneration.

(Table 4, column 3), and during this period the fraction of all nuclei constituted by the tubal nuclei falls from 79 to 73 % (Table 4, column 4, and Fig. 5).

The growth rates for tubal nuclei in Table 3 indicate that the most rapid decline is between 25 and 45 days. This we believe to be true, but the extent of the decline is probably exaggerated by sampling error. The arguments put forward for this view with reference to all nuclei on p. 41 hold also for tubal nuclei.

There is no sign that the loss of tubal cells is in any degree due to an emigration of macrophages into the endoneurium, unless such emigrants perish immediately they leave the tubes. The endoneurial population certainly does not rise as the tubal population falls.

The greater part of the loss of tubal cells must be due to degeneration of Schwann cells, even if all the tubal macrophages degenerate in the tubes. This

can be appreciated from Table 6, which is expressed in absolute figures. The loss of all tubal cells (column 3) between 25 and 225 days considerably exceeds the total population of the large tubes at 25 days (column 4); and only a proportion, whose maximum we provisionally place at 25%, of the large tube nuclei can be macrophage nuclei.

Tubal population and tube size

We have attempted a rough analysis of tubal population in terms of size of tube. We counted separately the tubal cells at different stages of degeneration (*a*) in 'large' tubes (larger than 9 μ diameter), (*b*) in 'medium' tubes (4–9 μ diameter), (*c*) in 'small' tubes (smaller than 4 μ diameter). Large tubes, and probably medium tubes, contain only myelinated fibres; small tubes both myelinated and unmyelinated. From the analysis of Gutmann & Sanders (1943) rather less than 20% of myelinated fibres fall in the large group, about 30% in the medium group, and about 50% in the small group. Unmyelinated fibres are more numerous than all myelinated ones put together (Ranson, 1912). Their maximum diameter is roughly 1–2 μ (Duncan, 1934); but while many are single (Nageotte, 1915) others are grouped into bundles with a common sheath (Nageotte, 1932; Ranson, Foley & Alpert, 1933; Weddell & Glees, 1941). These bundles, however, probably rarely exceed 4 μ in diameter, so that few unmyelinated fibres fall into our medium group.

Our analysis into three sizes of tube is rough, first because the number of each of the different kinds of tubes has been assumed to remain constant during the first 25 days of degeneration, though a change does occur. This change is chiefly a loss by shrinkage of large tubes amounting by 25 days to about 20% of that of undegenerated nerves. Secondly, it is rough because considerable differences in change of nuclear length according to tube size have been neglected. The larger the tube the greater appears to be the decrease in nuclear length, and therefore the large tube nuclei are underestimated relative to the small. Because of the approximate nature of the analysis, we have not tabulated our data on tube size in full.

The percentage distribution of the tubal nuclei among these different classes of tube is shown in Table 4, columns 5–7 and Fig. 6. Fig. 7 shows the changes undergone during the first 25 days of degeneration by populations of the nuclei of the three kinds of tube expressed as percentages of their respective populations in undegenerated nerves. These percentages are derived from Table 6.

It is clear that the population changes of the different tubes have different time relations and different magnitudes. Our main conclusions about tube size in relation to nuclear population are:

(i) The small tube nuclei have a very high specific growth rate in the 3–5-day period, and the additive growth rate is maximal during this period. After this initial burst of activity, moderate multiplication continues until 15 days and then dies away.

(ii) The medium tube nuclei show a continuously fairly high specific growth rate between 3 and 15 days. Its maximum is probably a little later than

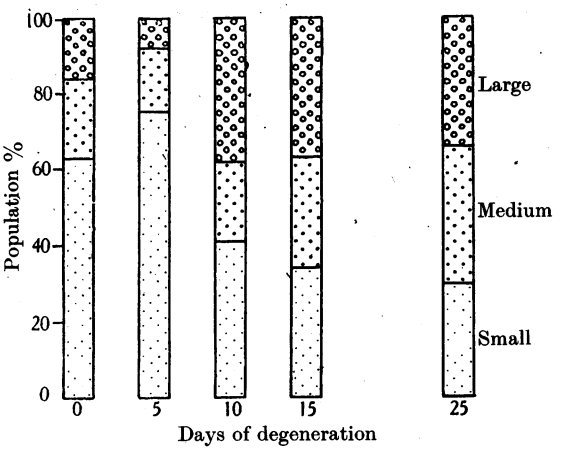

Fig. 6. Percentage composition of tubal nuclear population of nerves at different times of degeneration up to 25 days.

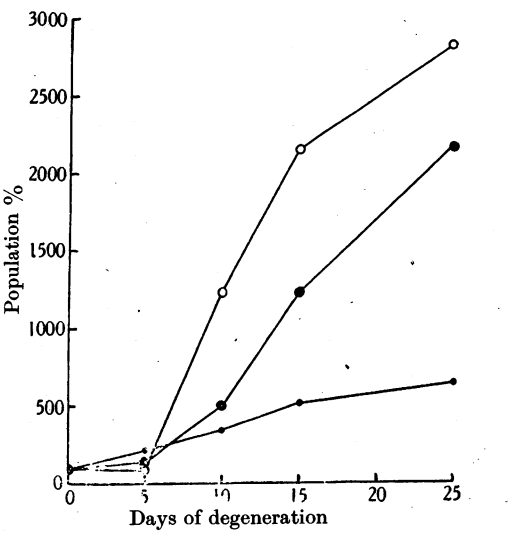

Fig. 7. Mean populations of large ○, medium ●, and small tube • nuclei in nerves at different times of degeneration up to 25 days, expressed as percentages of their respective populations in undegenerated nerve.

that of the small tube nuclei; it falls off after 15 days. The additive growth rate rises to a maximum in the 10–15-day period, and then declines again.

(iii) The large tubes show an initial burst of activity like the small tubes, but it starts about 2 days later. Between 3 and 5 days there is an apparent absolute loss of these nuclei, due to the

extreme shortening they undergo by collapse into the interior of the tubes. Between 5 and 10 days, starting probably on the 6th day, there is an extremely rapid multiplication which causes the large tube nuclei to increase from 8 to 38 % of all tubal nuclei (Fig. 6). Even when allowance is made for immigration of wandering cells by supposing that 25 % of this increase is so caused, the specific growth rate over this period remains the highest for any cell-type at any period of degeneration. The additive growth rate for these nuclei is also probably maximal during this period. After 10 days the specific growth rate falls sharply, even when allowance is made for the loss of large tubes through shrinkage. But, because of the large population built up, the additive growth rate remains almost as high during the 10–15-day period as during the 5–10-day period.

(iv) The larger the tube the greater the multiplication of cells within it. The specific growth rates between 0 and 15 days (which period gives a greater constancy of tube size than 0–25 days, though a loss of large tubes has nevertheless occurred) are, in % per day, 26 for large tubes, 21 for medium tubes and 14 for small tubes. If it is supposed that 25 % of the large tube cells are immigrant macrophages at 15 days of degeneration, the large tube specific growth rate for the period would be 28. The large tubes have the highest additive growth rate during this period (0–15 days), while the medium and small tubes are approximately equal. The result of the relation between tube size and specific growth rate is that the nuclear population, which in the undegenerated nerve is sparse in regions of large tubes and denser in regions of small, becomes much more evenly distributed by the end of the period of proliferation.

(v) After 25 days the rapid decrease of percentage of large tube nuclei (Table 4, column 5) is accounted for by the shrinkage of tubes, large tubes becoming medium and medium small. When the large tube population is expressed as nuclei per tube, its decline in numbers is at approximately the same rate as that of all tubal nuclei and is sharpest between 25 and 45 days. The complications of shrinkage during this period are such as to preclude reliable conclusions about small and medium tube nuclei, and they are therefore not treated separately.

(vi) The number of large tube nuclei per unit volume is consistently a little higher (average 14 %) in tibials than in peroneals. This presumably reflects a slight difference in 'fibre spectrum'.

The time relations of nuclear population increase in the different tubes ((i) and (iii) above) will probably be found to be correlated with the time of onset and rate of progress of degeneration in each of the fibre size groups, which is known to differ considerably. Unfortunately, the literature is very confused on these points, owing to lack of definition as to criteria of time and rate, and to absence of statistical treatment. The work of Weddell & Glees (1941) indicates that in general the smaller the fibre the earlier the onset of degeneration. The earliest of all to degenerate are the majority of the non-myelinated fibres (Ranson, 1912). According to our results, specific growth rates of tubal nuclear population have an earlier maximum the smaller the tube size group, and so parallel the time of onset of degeneration.

CHANGES IN NUCLEAR POPULATION OF ENDONEURIAL CONNECTIVE TISSUE

Identification and standardization

In the endoneurium are fibrocytes, macrophages, and blood vessels with their constituent cells. In this section we discuss the nuclei of fibrocytes and macrophages, including those 'adventitial' cells closely attached to the blood vessels.

In an ordinary transverse section we are unable to distinguish macrophages from fibrocytes with sufficient reliability to separate them; and in longitudinal section we are unable to be sure in every case when a cell is intratubal or endoneurial. We have therefore had to undertake a special analysis of the macrophages, using vitally stained rabbits, which will be the subject of a future paper. For the moment we treat fibrocytes and macrophages as a single 'connective tissue' group.

Endoneurial nuclei are not easily distinguished from Schwann cell nuclei of the small tubes in an undegenerated nerve. As degeneration proceeds, endoneurial oedema soon makes identification much easier.

Two further sources of inaccuracy in assessing changes of nuclear population exist for the endoneurium as for tubal cells. They are: (i) migrations of macrophages, and (ii) inadequate standardization for nuclear length, this standardization being based on the average of tubal and endoneurial nuclear length. These will be discussed as necessary.

Table 5, column 3, shows the mean number of endoneurial connective tissue nuclei in a complete T.S., standardized approximately for nuclear length, and expressed as a percentage of that of undegenerated nerve. The data are shown graphically in Fig. 4.

The increase of population of connective tissue nuclei

As usual, there is no detectable change in population during the first 3 days. The increase which follows brings the population by 25 days of degeneration to about 4 times its original figure. This is, of course, a much slower increase than that of the tubal nuclei, and consequently, as shown in Table 5, column 4, and Fig. 5, the percentage of all nuclei of the nerve formed by the endoneurial connective

tissue group diminishes from 39 at 0 days to 19 at 25 days.

Growth rates are given in Table 3 and Fig. 3. Here it is probable, in spite of the difficulty of identification during the early period, that the specific growth rate is maximal in the period 3–5 days, and declines steeply thereafter. The additive growth rate is at least as high in the 3–5-day as in the 5–10-day period, and perhaps declines throughout the first 25 days, unlike that of the tubal nuclei. It will be noted that in the initial period the endoneurial connective tissue nuclei increase almost as fast as do the tubal nuclei, but subsequently lag far behind. It is probable, however, that connective tissue growth is underestimated as compared with tubal during the period 15–25 days. Many of the connective tissue nuclei are at this time shortening by rounding off, while some tubal nuclei are probably elongating. The application of the same correction to both types of cell probably therefore distorts the true picture.

The connective tissue specific growth rates, regarded as a measure of the multiplication within the endoneurium, are underestimated because of the migration of macrophages into the large tubes. The most liberal allowance for this (assuming 25 % of large tube cells are immigrant macrophages) does not alter the conclusion that the growth rate declines from a maximum at 3–5 days, and is at all times less than the tubal growth rate.

The overall multiplication of the connective tissue is in fact less than that of any of the three tubal types analysed above, even when allowance is made for the migration of macrophages into the large tubes.

The decrease of population of connective tissue

The connective tissue population declines after 25 days, but much less than does the tubal. At 225 days the connective tissue population is 75 % of its 25-day population (Table 5, column 3, and Fig. 4), and during this period the fraction of all nuclei constituted by the connective tissue rises from 19 to 24 % (Table 5, column 4, and Fig. 5). The decrease in connective tissue nuclei is, however, probably overestimated, because the average lengthening of all nuclei during this period seems to be mainly due to lengthening of tubal nuclei, while the correction for nuclear length assumes that the connective tissue nuclei lengthen the same amount as the tubal. This source of error is insignificant for the tubal nuclei, since they are in a large majority in the nerve. Supposing that no lengthening of connective tissue nuclei occurred (which is not true), they would by 225 days fall to 82 % of their 25-day value. By t test, pooling the 15–25-day data on the one hand and the 45–225-day data on the other, and assuming nuclear length constant throughout, the fall in connective tissue nuclear population is of borderline significance ($t = 2{\cdot}3$, $N = 31$, $P = 0{\cdot}03$).

Table 5. *Changes in population of endoneurial nuclei with time of degeneration*

		Connective tissue nuclei Mean population		Blood vessel nuclei Mean population		
Days of degeneration	No. of nerves counted	as % of 0-day	as % of all nuclei	as % of 0-day	as % of endoneurial nuclei	as % of all nuclei
(1)	(2)	(3)	(4)	(5)	(6)	(7)
0	8	100	39	100	22	11
5	5	161	38	119	17	8
10	5	297	30	127	11	4
15	5	380	24	157	10	3
25	9	410	19	146	9	2
45	7	320	21	135	11	3
110	9	312	23	140	11	3
225	8	308	24	121	10	3

The fall in connective tissue population makes it very unlikely that all the tubal macrophages leave the tubes and enter the endoneurium again during this period, unless they degenerate as soon as they reach it.

The growth rates given in Table 3 show as usual a relatively pronounced fall in the period 25–45 days, and a very slight fall thereafter. We have previously discussed the probability that the sudden decrease of all nuclei in this period may be exaggerated by, though not entirely produced by, sampling error; and the same arguments applied to tubal nuclei. With reference to the connective tissue nuclei, we cannot be sure that the greater part of the decrease between 25 and 45 days is not due to sampling error, and that the small loss of nuclei which probably occurs between 25 and 225 days is not fairly evenly distributed over that period. Two paired experiments, each of 25 and 45 days, showed no difference between populations of connective tissue nuclei at the two times. The question is one for further study.

CHANGES IN POPULATION OF BLOOD-VESSEL NUCLEI

Identification and standardization

Terminal arterioles, venules and capillaries occur in the endoneurium, in a plexus the meshes of which are chiefly longitudinal. In this section we are only concerned with endothelial and smooth-muscle nuclei, and of these the former far outnumber the latter. The connective tissue cells which occur in association with the blood vessels cannot be sharply distinguished from the rest of the endoneurial connective tissue, and have been counted along with it.

Blood-vessel nuclei occur in much smaller numbers than the nuclei we have so far dealt with; and instead of being fairly evenly distributed, they are arranged in discrete groups, which leads to great variability in the number per field. Correspondingly the counts are less reliable than those of other cell-types.

Unlike all other cells, endothelial nuclei do not shorten during the early stages of degeneration, but, on the contrary, lengthen a little. They have therefore required separate standardization for length, which has been done by measuring them in longitudinal section. Mean lengths in μ (in brackets the number of nerves measured) were: 0 days, 13·5 (6); 5–25 days, 14·5 (12) (no significant change during this period); 100 days, 14·1 (5). 'Lengths' of smooth muscle nuclei (which are mainly extended transversely to the nerve) have been neglected.

Number of blood vessels

There is no significant change in the number of blood vessels throughout degeneration, although there is considerable random variation. There are about 100 blood vessels, almost all cut transversely, per mm.^{2} of section.

In the undegenerated nerve each μ of blood-vessel length supplies about 0·4 cell, at 25 days of degeneration about 3·5 cells, and at 225 days about 2 cells.

Changes of nuclear population of blood vessels

In Table 5, column 5, we give the population of blood-vessel nuclei at different times of degeneration, standardized for nuclear length. They are expressed as a percentage of the mean number in undegenerated nerves, and are shown graphically in Fig. 4. The data suggest an increase to about 1½ times the initial value by 25 days of degeneration (though the maximum may be reached earlier than this), and there is apparently a subsequent decline. The increase is very much smaller than the average for all nuclei, so that the percentage of all nuclei formed by blood-vessel nuclei drops from 11 in the undegenerated nerve to 2 at 25 days (Table 5, column 7, Fig. 5). Similarly, the rest of the endoneurial nuclei increase much faster than the blood-vessel nuclei (Table 5, column 6). The decrease after 25 days is smaller, but not much so, than the average of all cells and of the endoneurium.

The growth rates of blood-vessel nuclei are not tabulated, since they are not very reliable.

The increase in blood-vessel nuclei in early degeneration is substantiated by numerous paired experiments. For instance, a 0- and 23-day pair showed an increase of 46%. The later decline receives some support from four paired experiments, each of 25 and 225 days. Three of these show considerably fewer nuclei at 225 days, while the fourth shows no significant difference between the pairs. These four 225-day nerves average 80% of their 25-day contralaterals.

The evidence seems to us satisfactory that an increase in the number of blood-vessel nuclei occurs. Doubt must, however, remain about the subsequent loss of some of these nuclei, since our measurements of endothelial nuclear length are not really adequate in late degeneration, and the changes of populations are too small to be substantiated by our available material.

SYNTHESIS OF NUCLEAR POPULATION CHANGES

In previous sections we have described the population changes of each cell-type, in each case taking the population of this particular cell in the undegenerated nerve as 100. The relative increases of the different cell-types on this basis are shown in Figs. 4 and 7 (taken from Tables 4–6). We have also given the percentage which each cell-type constitutes of all cells at different stages of degeneration, and this is shown in Figs. 5 and 6 (taken from Tables 4 and 5). Table 6 gives the composition by cell-type of a representative sample of 100 nuclei in the undegenerated nerve, and what this group of nuclei becomes as degeneration proceeds. Some of the stages from this table are illustrated in Fig. 8.

DISCUSSION

We defer a general theoretical discussion of our findings until our quantitative data on Wallerian degeneration are more complete. In this section we consider a few points which call for comment.

Specificity of material

We have limited this study to the tibial and peroneal branches of the sciatic nerve in the thigh of the rabbit. The changes of population during degeneration are extremely similar in these two branches. It is, however, unwise to attempt to generalize our conclusions to other nerves until we have made comparisons, since the literature suggests that the time relations of degeneration may vary considerably. We mention two points in this connexion: (*a*) the tibial branch is rather more than twice the size of the peroneal, which indicates that

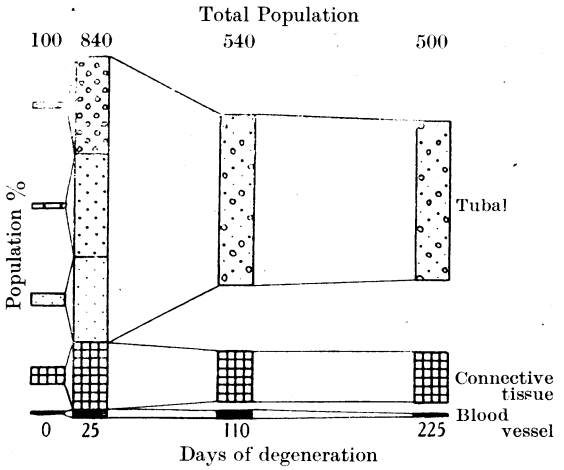

Fig. 8. Changes undergone by a representative sample of 100 nuclei of undegenerated nerve during subsequent degeneration. Large ○, medium •, and small · tube nuclei are shown separately at 0 and 25 days and subsequently combined.

hypothesis of chemical activation can obviously be extended to cover the vascular changes and macrophage mobilization in a degenerating nerve, and a close analogy with the processes of inflammation and repair of other tissues suggests itself.

We have given specific growth rates for the total population of nuclei, and they show a rather regular decline with time of degeneration (Table 3 and Fig. 3). Since we have suggested that autolysis of nerve fibres produces nuclear multiplication, it might be supposed that these growth rates give a measure of the concentration of autolytic substances. The nerve is, however, according to our analysis, highly heterogeneous with respect to growth and of changing heterogeneity. Further, the several distinct subpopulations of the nerve, especially those in different sizes of Schwann tube, clearly grow with a considerable degree of independence. In the circumstances we cannot infer from changes in the overall specific growth rates changes in the overall concentration of products of autolysis, or in activity of any controlling agent (see Gray, 1929). The specific growth rates of the

Table 6. *The nuclear population changes which occur in a region of nerve containing* 100 *nuclei when undegenerated, analysed according to cell-type*

		Tubal				Endoneurial		
Days of degeneration	All nuclei	Total	Large tube	Medium tube	Small tube	Total	Fibroblast and macrophage	Blood-vessel nuclei
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
0	100	50	8	11	31	50	39	11
5	163	87	7	15	65	76	63	13
10	390	260	99	55	106	130	116	14
15	635	469	173	136	160	166	149	17
25	840	667	227	240	200	173	157	16
45	600	460	—	—	—	140	125	15
110	540	403	—	—	—	137	122	15
225	500	367	—	—	—	133	120	13

a considerable size range does not affect the nuclear population changes; (*b*) the nuclear population changes of any nerve will presumably depend at least in part on its 'fibre spectrum'.

The nuclear population increase

It is difficult to interpret the population increase in the endoneurium during Wallerian degeneration except as due to a chemical stimulus coming by diffusion from the autolysing nerve fibres. The interruption of the nerve fibres more than a centimetre away from the region we studied can hardly lead to this multiplication of nuclei by any other means. It is a natural assumption that the same stimulus produces multiplication in tubal nuclei. In a study in tissue culture of nerves undergoing Wallerian degeneration (Abercrombie & Johnson, 1942) we were likewise led to infer a diffusing activator to explain the similar outwandering activity of Schwann cells and fibrocytes. Such a whole population must therefore be regarded at present as purely descriptive. It is also safest to assume that the same limitation applies to the specific growth rates of the different cell-types, until the populations they refer to have been shown to be reasonably homogeneous.

Our hypothesis of a diffusing activator requires that the tubal contents should influence endoneurial population growth. The general trend of connective tissue specific growth rates actually follows fairly closely that of tubal nuclei as a whole (Table 3 and Fig. 3), and is therefore apparently consistent with our hypothesis. The *close* correlation of endoneurial specific growth rates with those of tubal nuclei as a whole is, however, not a necessary consequence of our hypothesis, in view of the heterogeneity of tubal nuclei. For instance, maximal specific growth rate in the connective tissue might well be correlated with the intense multiplication in the large tubes; but our data

suggest that it coincides rather with that of the small tubes.

The present survey of nuclear population cannot hope to do more than raise these problems of the relations of the various cell-types. Their solution must await much more extensive data.

The nuclear population decrease

At present we have not sufficient data to put forward a reasonable hypothesis as to the population decrease. We must, however, comment on its relation to reinnervation. It is not, unfortunately, possible to prevent entirely reinnervation in long-degenerated nerves, and it may be supposed that reinnervation leads to some loss of cells as the nerve returns towards normal. Actually there is no detectable correlation in our material, none of which is heavily reinnervated, between amount of reinnervation and size of population. Further, the major loss of population seems to follow closely the peak of population at 25 days; and this is not a period when reinnervation presents any problem. While therefore reinnervation may produce some of the slow loss of cells late in degeneration, it cannot explain the more important loss immediately after 25 days of degeneration.

Relation of population changes to outwandering activity of Schwann cells in tissue culture

In our investigations of the outwandering activity in tissue culture of Schwann cells at different times of degeneration (Abercrombie & Johnson, 1942), we found that this activity rose from zero in the undegenerated nerve to a high peak at about 25 days of degeneration, then fell steeply until about 60 days and subsequently declined very slowly until up to a year. Clearly the outwandering activity closely follows the population cheangs of tubal cells (Table 4, column 3, and Fig. 4), and it is tempting to consider the outwandering curve as explained by population changes. There are, however, important differences, which suggest that the relation is more complicated, such as that the outwandering curve starts from zero, and shows a much more extreme rise and fall than the population curve.

SUMMARY

1. Changes of nuclear population during Wallerian degeneration of rabbit peroneal and tibial nerves (excluding perineurium and epineurium) in the thigh region are recorded up to 225 days of degeneration. The population has been standardized for changes in nerve size and nuclear size and partly analysed according to cell-type.

2. The total nuclear population shows no change during the first 3 days of Wallerian degeneration; the growth curve then shows a sigmoid rise to a peak population about 8 times the initial population, reached at about 25 days of degeneration; and finally a fall, on a much smaller scale, so that at 225 days of degeneration the population is about 0·6 time that of the peak, and is equivalent to that of a nerve of about 12 days of degeneration (Table 2, Fig. 2).

3. The daily increment of nuclei (the additive growth rate) has its highest average value in the 10–15-day period of degeneration. It averages a little lower in the 3–10-day period, and considerably lower in the 15–25-day period. It reaches zero at about 25 days of degeneration. The negative additive growth rate of the population decrease has the highest average value near the beginning of the decrease (between 25 and 45 days), and falls thereafter. The trend of our figures suggests that it may not reach zero during the 225 days of degeneration that we have investigated (Table 3).

4. The growth of population relative to the number of cells present (specific growth rate) is highest at or near the beginning of the period of population increase, and declines to zero at about 25 days of degeneration. The negative specific growth rate of the period of population decrease has the same trend as the additive growth rate for that period (Table 3, Fig. 3).

5. The cells inside the Schwann tubes (Schwann cells, together with a relatively small number of macrophages at some stages of degeneration) account for most of these population changes. They increase in number about 13 times during the first 25 days of degeneration, and by 225 days have fallen again to about half of this peak value. They start at 50 % of all nuclei in the undegenerated nerve, rise to 79 % at 25 days, and fall to 73 % at 225 days of degeneration. The additive and specific growth rates of tubal nuclei show the same trends of change as those of all nuclei described above (Table 4, Figs. 3–5).

6. The tubal nuclear population has been analysed into three groups, according to diameter of the Schwann tube, over the period of population increase (Table 4, Figs. 6, 7). The average specific growth rate over the whole of this period is higher the larger the tube diameter. The additive growth rate is more nearly alike in the different sizes of tube, but is highest in the large tubes.

At the beginning of the population increase (3–5 days of degeneration) the nuclei of the small tubes (less than 4 μ diameter) have the highest specific growth rate of the tubal population. The nuclei of the large tubes (more than 9 μ diameter) are quite inactive at this time. The initial wave of multiplication in the small tubes is short-lived, and it is succeeded (starting at 6–7 days of degeneration) by a short wave of intense multiplication in the large tubes. The medium tubes (4–9 μ diameter) have a more diffuse period of high specific growth rate, with maximum probably situated in time be-

tween that of large and of small tubes. The nuclei of all three sizes of tube show a decline in specific growth rate after 10 days, reaching zero between 15 and 25 days. The maximum additive growth rate of small tube nuclei occurs at 3–5 days, of large tube nuclei at 5–10 days, and of medium tube nuclei at 10–15 days.

7. In the endoneurium fibrocytes and macrophages have not been distinguished but are treated as a single connective tissue group. They increase in number about 4 times during the first 25 days of degeneration, and by 225 days have fallen again to about 0·8 of this peak value. They start at 39 % of all nuclei in the undegenerated nerve, fall to 19 % at 25 days, and rise to 24 % at 225 days of degeneration. During the period of total population increase the endoneurial connective tissue nuclei increase in general like the tubal nuclei, with highest specific growth rate near the beginning of multiplication, declining to zero between 15 and 25 days. Unlike the tubal population, the highest additive growth rate of connective tissue nuclei is near the beginning of the period of population increase. The average specific growth rate for the period of increase is lower for connective tissue than for any of the three groups of tubal cells analysed (Tables 3, 5 and Figs. 3–5).

8. The number of blood vessels in the endoneurium shows no change during degeneration. Each μ length of blood vessels supplies in the undegenerated nerve about 0·4 cell; at 25 days of degeneration about 3·5 cells; and at 225 days about 2 cells.

9. The number of blood-vessel nuclei (endothelial and smooth muscle) increases a little during the period of general population increase, reaching about $1\frac{1}{2}$ times its initial value by 15–25 days of degeneration; and perhaps decreases again during the period of population decrease. Blood-vessel nuclei start at 11 % of all nuclei in the undegenerated nerve, fall to 2 % at 25 days, and rise to 3 % at 225 days of degeneration (Table 5, Figs. 4, 5).

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