

A QUANTITATIVE COMPARISON OF THE VASA NERVORUM OF NORMAL AND MAREK'S DISEASE AFFECTED CHICKENS

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A QUANTITATIVE COMPARISON OF THE VASA NERVORUM OF NORMAL AND MAREK'S DISEASE AFFECTED CHICKENS

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INTRODUCTION

A disease of chickens characterized by lameness and variable enlargement of certain peripheral nerves was described by Marek in 1907 under the term polyneuritis (Jungherr and Hughes, 1965). Pappenheimer et al. (1926) introduced the term neurolymphomatosis gallinarum and noted a frequent association of visceral lymphomata with infiltrative lesions of the peripheral nerves. Further investigations which have shown various differences in etiologies and lesions have led to much controversy concerning the proper term to be used in describing this disease. Doyle (1928) stated that the terms neuritis and paralysis were unsatisfactory in describing this disease since neuritis fails to describe the cases in which the pathologic process is limited to the central nervous system, while the term paralysis is too restricted to include the cases in which no paralysis is evident but lesions exist in the nerve. Campbell (1961) suggested a distinction between fowl paralysis granuloma and lymphoid leucosis by showing that the former are reactive lymphogranulomas probably formed as a result of an acute inflammation of yet unproven origin, rather than of a neoplastic nature which is characteristic of leucosis. It has been suggested by Biggs (1961) that Marek's disease should be the term used when describing this disease. He based this on the fact that there are pathological similarities between many of the visceral lesions of fowl

paralysis granuloma and lymphoid leucosis which could lead to a misunderstanding of the term granuloma. Fowl paralysis is also unsatisfactory because it describes a symptom which is not always present. Churchill and Biggs (1967) have described an association of a herpes virus as the etiologic agent of Marek's disease.

The clinical signs of this disease according to Jungherr and Hughes (1965) are usually those of asymmetrical paralysis of the legs, wings, or neck. Initially the clinical manifestation of the affected leg may show inward curving of the toes, weakness and incoordination. As the disease progresses the bird has a tendency to lie on its side with the affected extremity in spastic extention. The gross anatomical features show soft swellings of the peripheral nerve trunks, edema, and the loss of cross striations of the affected nerves. The cross striations are considered by Biggs and Payne (1967) to be caused by contractions of the nerve sheath. The sciatic, vagus and brachial nerves are the nerves most commonly considered in this disease. The swellings may be localized or diffuse, varying with each individual case. Microscopic examination reveals an infiltration of mononuclear cells; the majority of which are identical to the lymphocytes in the circulating blood.

Degeneration of the axons, large amounts of edematous fluid, and the perivascular infiltration of lymphocytes may have some effect on the vascularity of the nerves affected with Marek's disease. The purpose of this study is to compare quantitatively the angioarchitecture of the sciatic, vagus, and brachial nerves in normal chickens with those affected with Marek's disease.

REVIEW OF THE LITERATURE

Considerable literature is available concerning the vasa nervorum of mammals, however very little information is available on the vasa nervorum of the avian class. Peterson et al. (1965) presented a figure which demonstrated the vascular pattern in cutaneous nerves of chickens, however it was not the purpose of their study to present a detailed description of the angioarchitecture.

Adams (1942a) presented a complete historical review of the blood supply to nerves, including the vasa nervorum. It was shown that every nerve contains a rich vascular plexus which is arranged longitudinally. Sunderland (1945), Blunt (1954), Francois and Neetens (1956), Steele and Blunt (1956), and Blunt (1957) studied the vasa nervorum of various human nerves and further demonstrated that the vasa nervorum is longitudinally oriented, with various vascular plexuses within the nerve. A study of the vascular patterns of the canine sympathetic chain by Marcarian and Jenkins (1967a) showed that the vessels were arranged parallel to the long axis with many arteriovenous anastomoses. In 1968 Marcarian and Smith gave a quantitative account of the numbers and internal diameters of the blood vessels in the ulnar nerve of cats. They found that the greatest number of blood vessels were located in the middle portion of the nerve with the greatest percentage of blood vessels having internal diameters of less than 10 microns. They also found that the ratio of blood vessels

to myelinated nerve fibers is a fairly constant figure of 1:25.

Further investigations have been concerned with the effects of ischemia upon nerves and their blood supply. Adams (1942b) experimentally excluded the regional blood supply of the sciatic nerve of the rabbit and proved that this had no pronounced effect upon the nerve. but he did not exclude the possibility of a more serious involvement if the occlusion included the intraneural portions of the blood supply. Bacsich and Wyburn (1945a, 1945b) studied the vascular patterns of the regenerating rabbit sciatic nerve after: a) crush injury and b) crush combined with cutting of the nutrient arteries in the thigh region. They found little alteration of the vascularity; inferring that the longitudinal channels are equal to the task of maintaining normal blood flow. When the longitudinal anastamoses as well as the regional nutrient arteries were destroyed, they found restoration of normal blood flow within two weeks. Richards (1951) reviewed the literature describing the anatomical and physiological effects of ischemic lesions on peripheral nerves. The results showed that interference with any source of supply is unlikely to cause serious interruption of the circulation to the nerve, and also that nerves can survive with a blood supply much less than that which they require to function properly. Smith (1966) described the presence of a mesoneurium in human peripheral nerves which contains all the nutrient arteries. He proposed that the mesoneurium possesses a series of arcades which serve as a segmental source of circulation supporting the longitudinal vessels. The mesoneurium of the peripheral nerves is considered

to be similar to the mesentery of the small intestine and all of the blood vessels enter the nerve along a line where the mesoneurium attaches to the nerve.

METHODS AND MATERIALS

In this study 60 normal RPL Line 15 x 7 White Leghorn chickens were used. Upon hatching, the birds were separated into two groups of 30 each and placed in separate modified Horsfall-Bauer Isolators.

One group served as the control while the other group was inoculated intraperitoneally on the day of hatching with 0.2 cc of blood taken from a known JM Marek infected bird. (JM) is a lymphomatosis isolate which, when injected, is 90-100% effective (Sevoian, 1962).

Six birds from each group were taken at one week intervals for four weeks. The remaining number of inoculates died at various intervals during the course of this study; the equivalent number of controls were then unusable. The live birds were injected with undiluted Pelikan biologic ink¹ via the brachial vein using the technic described by Peterson et al. (1965) and Marcarian and Jenkins (1967b). The amount of ink injected varied with the age of the bird; those one week of age (week I) were injected with 2 cc, week II with 3 cc, week III with 6 cc and week IV with 10 cc. The ink was allowed to circulate for 1-2 minutes, the living heart serving as the pumping mechanism, until the sclera and the tongue turned black. The birds were then killed by placing them in a closed chamber filled with dry ice.

¹ John Henschel and Co., Inc., 425 Park Ave. South, New York 16, N.Y.

Segments of the sciatic, vagus, and brachial nerves were removed, each time using identical anatomical landmarks. The sciatic nerve sample was taken from the sciatic foramen to the point where it divides into the tibial and common peroneal nerves; the vagus section was taken from the point where it crosses the common carotid artery dividing into the internal and external carotids to where it entered the thoracic inlet, and the brachial segment was taken from its point of emergence to the tip of the olecranon. The nerves were stretched on a card and fixed in 10% formalin for 24 hours, dehydrated in three changes of acetone (30 minutes each) and then cleared in methyl benzoate, using the technique described by Marcarian et al. (1967b).

The cleared tissues were submerged in methyl benzoate and studied with the aid of a Leitz microscope at magnifications of 16, 32, and 48X. The tissues were submerged in methyl benzoate because they tend to harden when exposed to air.

A segment of the nerve approximately 1 cm in length was then taken from the middle of each cleared nerve, embedded in paraffin, and serially sectioned at 5 microns. The sections were left unstained to achieve greater accuracy when counting and measuring the blood vessels because the ink-filled vessels could be better observed. Only the nerves having the best injection were used for counting and measuring. The injected vessels were counted and their internal diameters were measured using a Bausch and Lomb microscope, fitted with an occular micrometer at magnification of 430X.

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PHOTOGRAPHY

Photomicrographs were taken with a Leica Illg 35 mm camera which was attached to an Ipso 1/3 x intermediate adapter. One eyepiece of a Leitz dissecting microscope was removed and the Ipso adapter and camera were attached to the microscope. The cleared nerves were photographed submerged in a Petri dish filled with methyl benzoate. The nerves were kept stationary by placing a glass histologic slide over them. Kodak high contrast copy film was used to take the photomicrographs.

RESULTS

The vasa nervorum of the chicken is very much like that described for the mammal by Adams (1942a). The results seen agreed so closely with that of Adams (1942a), and Marcarian and Jenkins (1967a) that it is unnecessary to repeat a detailed description of their findings. Figure 1 shows the vasa nervorum of the sciatic nerve of a normal bird. The epineurium contains a large number of blood vessels, running longitudinally, some of which penetrate the nerve. Within the perineurium and endoneurium the vascular channels are longitudinally directed with a great number of anastamoses occurring between them.

The number of blood vessels within the sciatic, vagus, and brachial nerves will be described below for both the control and inoculated birds. The percentage of these blood vessels having internal diameters within the ranges of 0 - 4.54 μ or >4.54 μ - 9.08 μ will then be described as the birds progress in age from week I to week IV.

Sciatic

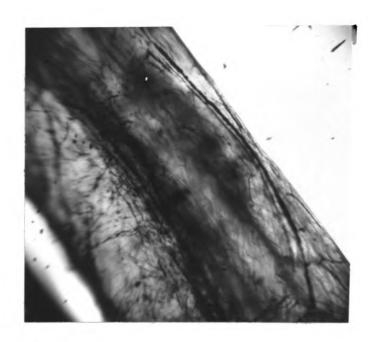
Control: The number of blood vessels present in the sciatic nerve sample from week I to week IV is shown in Figure 2. There is a slight increase in number from week I (148) to week II (150) and also an increase from week III (144) to week IV (168). However, between weeks II (150) and III (144) there is a decrease in the number of blood

FIGURE 1

A surface view of a sciatic nerve taken from a three-week-old control chicken showing the longitudinal arrangement of the blood vessels.

Cleared in methyl benzoate. 64 X.

FIGURE 1

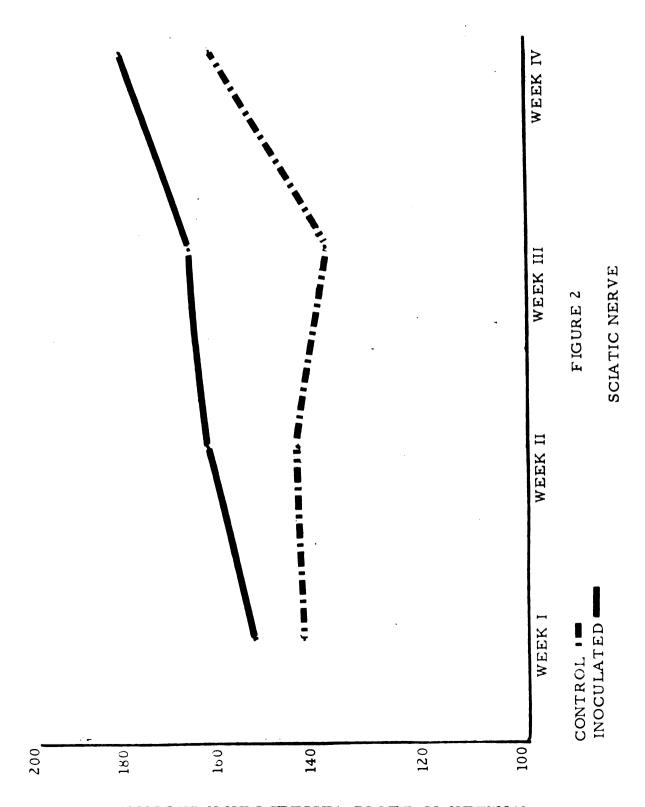


the interval from weeks II to III, for the nerves, is very interesting because it contradicts the normal linear pattern of growth. However, since it does occur in the nerves of both the controls and inoculates, the decrease may have been due to a number of reasons: 1) the result of some unknown and unrecorded environmental change in the incubators, 2) a normal and consistent part of the growth pattern which occurred during this interval, 3) The ratio of the weight of blood compared to the weight of the bird fluctuates as the birds increase in size (Mitchell et al. (1931). This may occur in birds younger than those which were studied by Mitchell, such as the age of the birds used in this study.

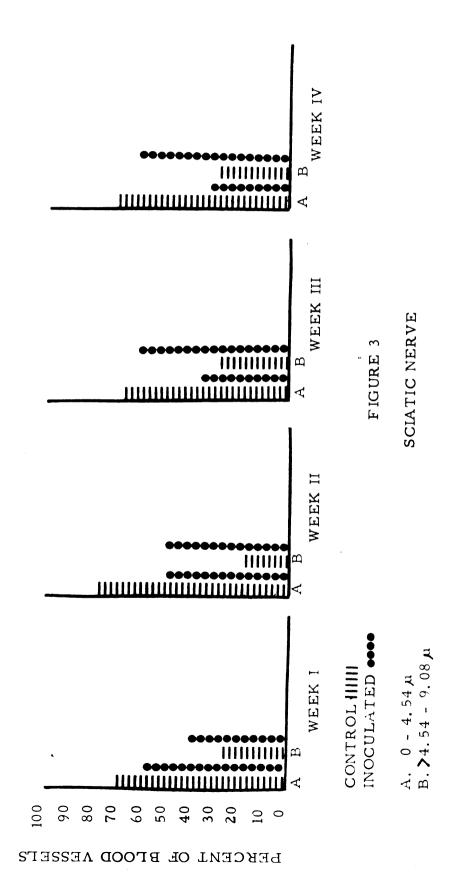
Shown in Figure 3 is the percentage of blood vessels having diameters of $0 - 4.54 \,\mu$ or $>4.54 - 9.08 \,\mu$. On the average 67% of the blood vessels in each week had internal diameters of $0 - 4.54 \,\mu$, 27% had diameters within the range $>4.54 - 9.08 \,\mu$, and 5% had diameters larger than 9.08 u. The percentages within the ranges of $>9.08 - 13.62 \,\mu$, $>13.62 - 18.16 \,\mu$, or $>18.16 \,\mu$ fluctuated inconsistently by week.

Inoculated: It is demonstrated in Figure 2 that there was a gradual increase in the number of blood vessels in the sciatic nerve as the birds progressed from week I (158) through week IV (186).

It is shown in Figure 3 that from week I to week IV there was a decrease in the percentage of blood vessels having internal diameters in the range $0 - 4.54 \mu$ with a steady increase in the percentage of those in the range $>4.54 - 9.08 \mu$. Week I had 56% in the range $0 - 4.54 \mu$



NUMBER OF BLOOD VESSELS PER X-SECTION



and 40% from 74.54 - 9.08 μ , in week II 47% of the blood vessels had internal diameters in the range 0 - 4.54 μ and 48% >4.54 - 9.08 μ , week III had 36% in the range 0 - 4.54 μ and 60% >4.54 - 9.08 μ , and week IV had 33% in the range 0 - 4.54 μ with 61% >4.54 - 9.08 μ . The remaining percentages were in the range >9.08 μ .

Control and inoculated compared: It is demonstrated in Figure 2 that the nerves of the inoculated birds had a greater number of blood vessels each week when compared to the controls of the corresponding week. There was, in the inoculated birds, a difference of 6.7% in week I, 12% in week II, 19.4% in week III, and a 10.7% difference in week IV of the number of blood vessels.

When a comparison is made of the internal diameters it can be seen from Figure 3 that the greatest percentage of blood vessels of the nerves from the inoculated birds in weeks I and II were in the range of $0 - 4.54 \,\mu$, while in weeks III and IV the greatest percentage were in the range >4.54 - 9.08 μ . The control birds, on the other hand, had the greatest percentage in the range $0 - 4.54 \,\mu$, consistently from week I through week IV.

Vagus

Control: Shown in Figure 4 is an increase in the number of blood vessels from week I (28) to week II (34) and an increase from week III (30) to week IV (50). As in the sciatic nerve there was also a decrease in the number of blood vessels from week II (34) to week III (30).

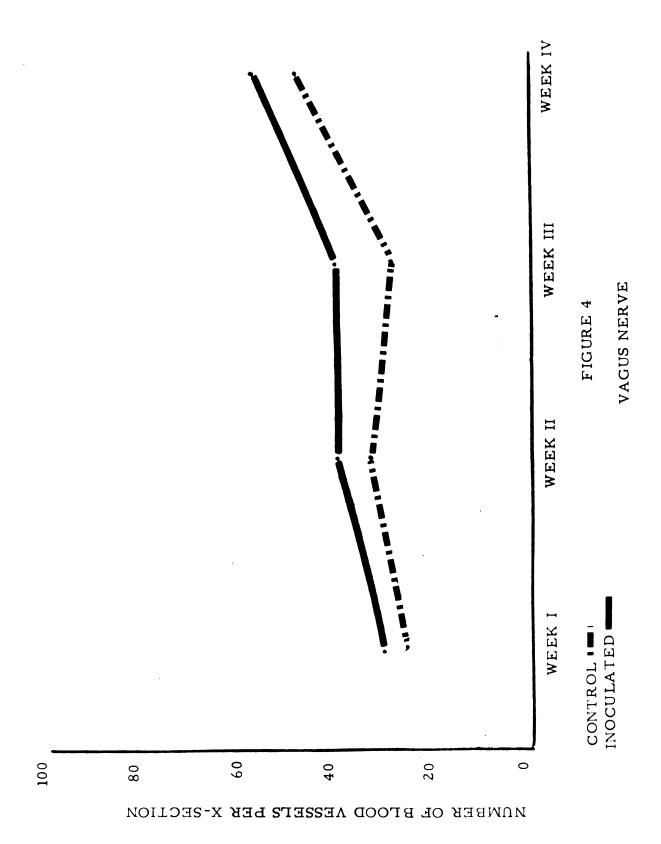
The diameters of these blood vessels are comparable to those in the sciatic, with the largest percentage being in the range of 0 - 4.54 \mu, and a much smaller percentage from >4.54 - 9.08 \mu. This is fairly consistent in the vagus nerves from week I to week IV as shown in Figure 5.

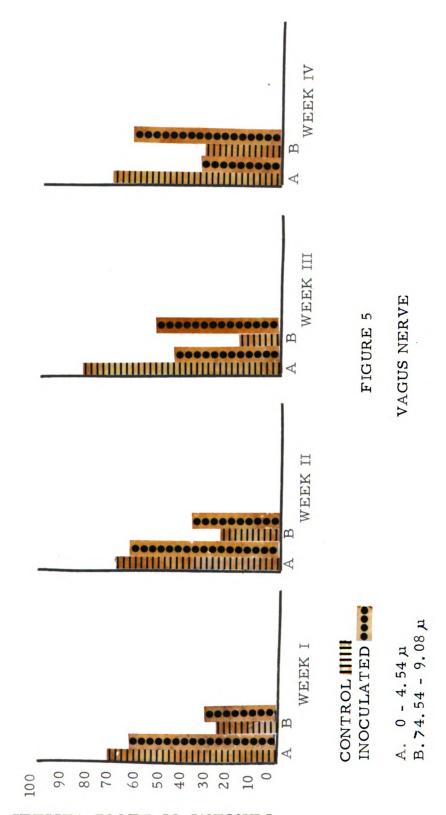
Inoculated: Figure 4 shows an increase in the number of blood vessels from week I (32) through week IV (58) with a leveling between week II (42) and week III (42).

It is demonstrated in Figure 5 a decrease in the percentage of blood vessels having internal diameters in the range 0 - 4.54 μ with an increase in the percentage of those having diameters 74.54 - 9.08 μ . Week I had 61% in the range 0 - 4.54 μ with 29% 74.54 - 9.08 μ , week II had 60% in the range 0 - 4.54 μ and 40% 74.54 - 9.08 μ , week III had 45% in the range 0 - 4.54 μ and 48% were 74.54 - 9.08 μ , and in week IV 30% of the blood vessels had internal diameters in the range 0 - 4.54 μ and 60% 74.54 μ - 9.08 μ .

Control and inoculated compared: It is shown in Figure 4 that the nerves of the inoculated birds had a greater number of blood vessels for each week. There was a 14.2% difference in week I, a 23.5% difference in week II, a 40% difference in week III, and a 16% difference in week IV.

The greatest percentage of blood vessels of the control had internal diameters in the range of 0 - 4.54 μ in each week; while in the nerves of the inoculates the greatest percentages were in the range





DEFCENT OF BLOOD VESSELS

0 - 4.54 μ in weeks I and II with the greatest percentage in the range
74.54 - 9.08 μ in weeks III and IV as shown in Figure 5.

Brachial

Control: Figure 6 has no values for the number of blood vessels in the brachial nerve of week I. This was due to the small size of the chicks and also since the brachial vein was used as the site of injection, large hematomas developed which proved to be prohibitive to locating the nerve.

There was a decrease in the number of blood vessels from week II (56) to week IV (48), with a leveling effect between weeks II (56) and III (56).

It is shown in Figure 7 that in week II to week IV the major percentage of blood vessels had internal diameters in the range of 0 - 4.54 \mu.

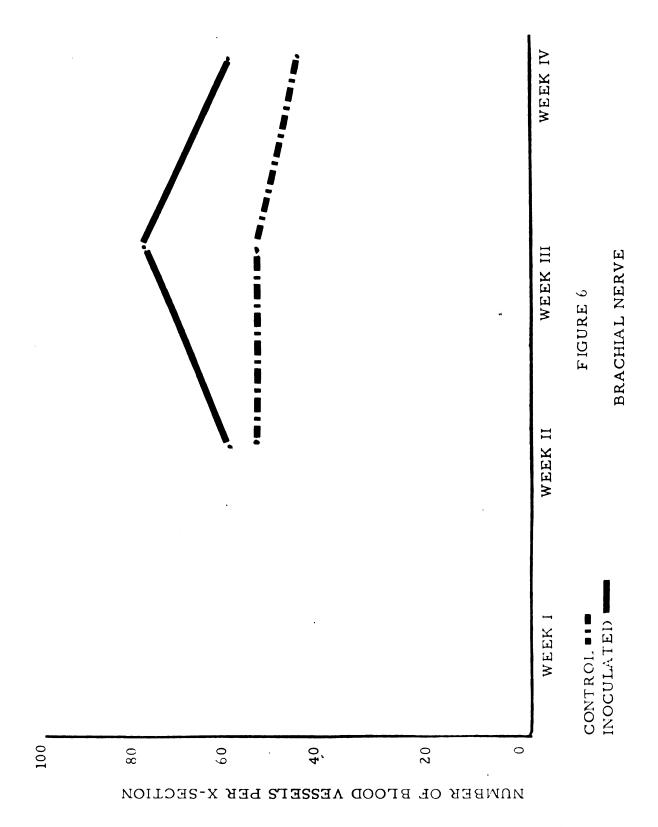
Inoculated: Demonstrated in Figure 6 is an increase in the number of blood vessels between week II (62) and week III (78), with a decrease in number in week IV (62). The value for week IV (62) was the same as that found in week II (62).

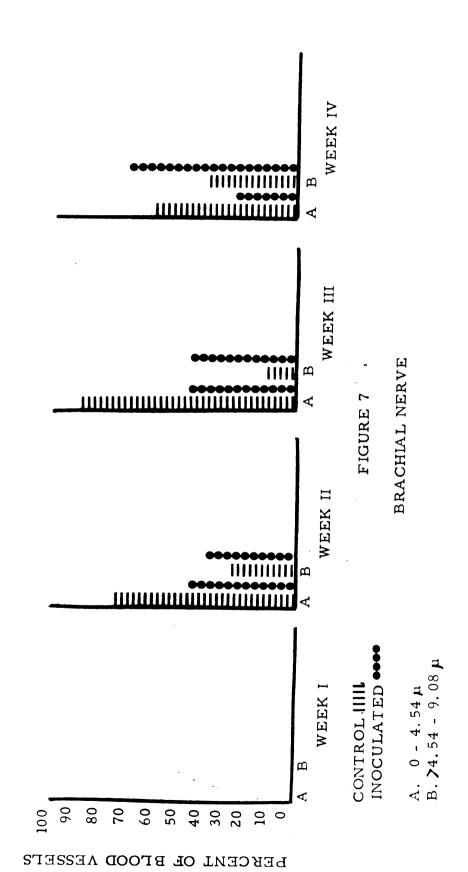
As seen in Figure 7 there is a decrease in the percentage of blood vessels having diameters of $0 - 4.54 \,\mu$, with an increase in the percentage of number having internal diameters >4.54 - 9.08 μ extending from week II to week IV. Week II had 54% in the range $0 - 4.54 \,\mu$ and 38% >4.54 - 9.08 μ , week III had 44% in the range $0 - 4.54 \,\mu$ and 42%

>4.54 - 9.08 μ , and week IV had 25% of the blood vessels with internal diameters in the range 0 - 4.54 μ and 67% >4.54 - 9.08 μ .

Control and inoculated compared: The findings for the brachial nerves correspond to both the sciatic and vagus in that the inoculated nerves contained a greater number of blood vessels for each week as shown in Figure 6. There was, in the inoculates, a difference of 10.7% in week II, a 39.2% difference in week III and a 29.1% difference in week IV.

It is shown in Figure 7 that the percentage of blood vessels having internal diameters in the range of $0 - 4.54 \,\mu$ and $74.54 - 9.08 \,\mu$ also agrees with that found in the sciatic and vagus nerves. In the control the greatest percentage was in the range $0 - 4.54 \,\mu$ for each week, while the inoculates showed an increase of those blood vessels in the range of $34.54 - 9.08 \,\mu$ during weeks III and IV, while in weeks I and II the greatest percentage was in the range $0 - 4.54 \,\mu$.





DISCUSSION AND CONCLUSIONS

When a comparison is made between the number of blood vessels in the nerves of the control and inoculated birds, it becomes clear that there is an increase in the number of blood vessels within each group from week I through week IV, and a greater number of blood vessels is found in the nerves of the inoculated birds of each week compared to the controls of the corresponding week. The greatest increase occurred during the interval between weeks II and III.

The greatest percentage of the blood vessels in all the control nerves had internal diameters in the range from $0 - 4.54 \,\mu$ for each week. The nerves of the inoculated birds had a decrease in the percentage of blood vessels with internal diameters in the range $0 - 4.54 \,\mu$, with a corresponding increase in those with internal diameter >4.54 - 9.08 μ . It is also during the interval between week II and week III when the greatest percentage of blood vessels begin to be found with internal diameters in the range >4.54 - 9.08 μ for the nerves of the inoculated birds.

Thus it seems reasonable to assume that during the interval between week II and week III the nerves of the diseased birds are undergoing the greatest pathological change. The nerves are not only enlarging in size, but also the vasa nervorum is increasing in number and the internal diameters are larger.

The results of this experiment disagree with those of Oakberg (1950). He reported that the origin of new areas of lymphoid tissue are primarily associated with small blood vessels. As the destruction of the endothelium occurs the proliferating cells migrate into the blood stream or surrounding tissue and the continued growth of the loci leads to destruction of the circulatory supply of the nerve fibers and ganglion cells of the splanchnic nerves. This would obviously cause a decrease in the number of blood vessels which would be present.

Weiss (1943) performed experiments on rats and chickens in which he produced endoneurial edemas in the nerves by using the sleevesplicing method of severing nerves and reuniting them by means of segments of arteries. He found that both proximally and distally to the arterial sleeve appeared a bulbous edema, with the chicken showing the greater accumulation of edematous fluid. When the intraneural vascularization of the rat was studied, it was found that there was a definite decrease in the number as well as the diameter of the recognizable intraneural vessels beneath the sleeve. In the edematous regions, both proximal and distal to the sleeve, there was a slight increase in the number of blood vessels and also a dilation of the blood vessels. Weiss described this as possibly being a result of the degenerative phenomena occurring in the edematous regions. He discounted the possibility of a vascular origin of this edema by showing the vascularity beyond the edema was as abundant as within the edematous zone. Weiss felt that the edema was a result of the normal fluid content of the

endoneural spaces which had been dammed up by an obstruction.

It is possible that we could use Weiss' theory to apply to a nerve which has been affected by Marek's disease. If we assume that the numerous loci of lymphatic cells act as many small obstructions, or represent injury, throughout the length of the nerve, this would result in an enlargement of the nerve by edema by interferring with the normal flow of the endoneural fluid. The increase in vascularity that is seen in nerves affected by Marek's disease would then be a result of the degenerative processes which were occurring, not a cause of the edema.

Wight (1964) found that although the nerves infected with Marek's disease are enlarged, there is a decrease in the number of axons, probably from the degeneration occurring, and also most of the axons present were of a smaller caliber than the controls; thus probably requiring less vascularity to maintain the nerve fibers.

Wight (1965) showed that nerves which had been affected with Marek's disease possessed the capacity for regeneration. He described it as a classical type of regeneration in which there was little morphological difference between the regeneration of diseased or control nerves. The results of Wight's experiments prompted him to suggest that if the disease could be terminated, spontaneously or by therapeutic measures, then the regenerative capacity displayed by these nerves may be sufficient to restore them to function. The results of my study indicate that if regeneration is to occur, then the disease must be terminated sometime

during the interval between the second and third week of age in birds diagnosed with Marek's disease. The increase in vascularity which was due to the degenerative processes occurring would then be useful in maintaining the integrity of the regenerating nerve.

The change in vascularity of the nerve after obstruction or damage has been noted by Adams (1942b), Bacsich and Wyburn (1945a, 1945b) and Richards (1951) in which it was shown that the vascular supply has the ability to establish a collateral circulation through the nerve within two weeks. This injury to a nerve which would result in degeneration of nerve fibers may initiate not only Schwann cell activity, but also an increase in vascularity so that the integrity of the nerve is maintained. The increased vascular supply would then be explained as being necessary to maintain the new nerve fibers forming during regeneration. This theory is supported by work done in 1943 by Essex and deRezende in which they observed the repair of a nerve in vivo. The first occurrence was the development of a complex network of blood vessels which formed in the injured region. They proposed that the results of their work presented strong emphasis for the importance of the circulation in the regeneration of nerves and suggested that an adequate blood supply should be provided to injured nerves in order that their repair may be facilitated.

SUMMARY

The angioarchitecture of the sciatic, vagus, and brachial nerves of 24 normal White Leghorn chickens and 24 chickens infected with Marek's disease was studied by stereoscopic observation, after India ink injections, and by using histological sections of the nerves.

The vasa nervorum of the above named nerves from normal birds was arranged in longitudinal vascular channels with many anastomoses present. This architecture agrees with that found for the mammals by Adams (1942a) and Marcarian and Jenkins (1967a).

Comparison of infected birds with the controls revealed increase in the number of vessels in the vasa nervorum during each week of age also increased internal diameters of the blood vessels. This increase in vascularity was proposed to be a result of the degenerative processes occurring, not a cause of the large edema which is found in this disease. The increase in vascularity was considered a necessity for the possible regeneration which can occur in the nerves affected with this disease.

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FIGURE 8

A surface view of a sciatic nerve taken from a three-week-old control chicken showing the longitudinal arrangement of the blood vessels.

Cleared in methyl benzoate. 64 X.

FIGURE 9

A surface view of a sciatic nerve taken from a three-week-old Marek's affected chicken showing the longitudinal arrangement of the blood vessels. Note the increase in the size of the nerve and its vascularity.

Cleared in methyl benzoate. 64 X.

FIGURE 8

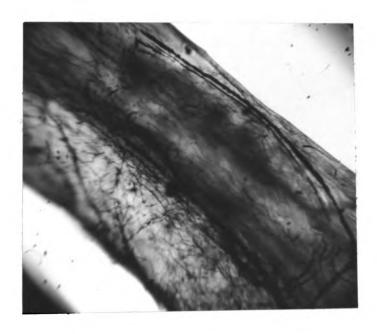


FIGURE 9

