Congenital Hereditary Lymphoedema in the Dog*

Part I. Clinical and Genetic Studies

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Congenital hereditary lymphoedema has been reported in man (Milroy, 1892; Esterly, 1965), cattle (Donald, Deas, and Wilson, 1952; Morris, Blood, Sidman, Steel, and Whittem, 1954), and swine (Wiesner, 1960). In each species, oedema of variable degree involves primarily the extremities and may have little effect upon general health. The underlying cause of the oedema is poorly understood.

This report deals with the clinical and genetic features of congenital hereditary lymphoedema in the descendents of one affected dog. Evidence from genetic studies and lymphangiograms in these animals indicates that the oedema results from a dominantly inherited developmental abnormality of the peripheral lymphatic system. In the rear limbs there is lymphatic obstruction at the level of the peripheral regional lymph nodes, and distal lymph channels are very dilated. The results of pathological studies are described in Part II of this report (Luginbühl, Chacko, Patterson, and Medway, 1967).

Materials and Methods

Genetic Studies.

Lymphoedema Dogs. The proposita and one of her sons were used in test matings. Both had pitting oedema of the rear limbs, below the femoro-tibial joint; the affected male had pitting oedema of the forepaws as well. Lymphangiography in both dogs demonstrated extensive dilatation of lymphatic channels distal to the femoro-tibial joint. Popliteal lymph nodes could not be palpated and were not demonstrated by lymphangiography.

Unaffected Dogs. Two males and three females used in breeding experiments were judged to be free of limb oedema by clinical examinations, and had easily palpable popliteal lymph nodes. One male and two of the females were not of the same breed as the proposita, and thus were not closely related.

Lymphangiography. Lymphangiography of the rear limbs was carried out by direct cannulation of peripheral lymphatic vessels as described in the dog by Skelley and Prier (Prier, Schaffer, and Skelley, 1962; Skelley, Prier, and Koehler, 1964). After making an incision through the skin of the dorsal metatarsal region, sterile Evan's blue dye in 0-5% solution was injected into the interdigital space. Within 10 seconds, the dye appeared in the local lymphatic vessels. In the dorsal metatarsal region, two main lymphatic vessels accompany the dorsal metatarsal vein. One of these was cannulated with a 26-gauge needle attached to a length of polyethylene tubing. Ethiodol* was infused at a rate of 0-25 ml./minute with a Harvard infusion pump.† Radiographs of the limb were taken at intervals after the start of injection.

The lymphatic channels of the rear limb of a normal dog, outlined by this method, are shown in Fig. 1. One or two main lymphatic channels extend from the dorsal metatarsal region upward to the region posterior to the femoro-tibial joint, where the popliteal lymph node is seen. From the popliteal node, two or three larger lymph vessels extend to the pelvic region where they enter the pelvic and iliac lymph nodes. In normal dogs, injection of Ethiodol at the rate of 0-25 ml./minute opacifies the main lymphatic channels of the leg and popliteal node within 5 minutes after the start of injection. The segmented appearance of the vessels results from the presence of lymphatic valves. Collateral branches of the lymphatic vessels proximal or distal to the site of the injection are normally not opacified.

Lymphatic vessels of the forelimb proved more difficult to cannulate, and satisfactory lymphangiograms were not consistently made.

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† Harvard Apparatus Company, Dover, Massachusetts, U.S.A.
Clinical Laboratory Studies. Routine red blood cell and white blood cell counts (total and differential), haematocrit, haemoglobin and total plasma protein determinations, and electrophoretic analysis of plasma proteins were carried out on all dogs which survived to weaning, and in a few pups which died in the neonatal period.

Cytogenetic Studies. Cytogenetic studies of peripheral blood lymphocytes in one affected dog were performed, using the method described by Moorhead, Nowell, Mellman, Battips, and Hungerford (1960).*

Results

The Proposita. A 10-week-old female mixed poodle pup was presented to the University of Pennsylvania Veterinary Clinic because of swelling of the rear limbs, first noticed at about 4 weeks of age. She was one of eight pups and, according to the owner, the only one affected. Information about the condition of the parents was not available. Non-painful pitting oedema of both rear limbs was pronounced below the tibio-tarsal joint. The popliteal lymph nodes were not palpable. Other physical findings were within normal limits. Cardiovascular and renal function tests and examination of the serum proteins failed to reveal the cause of the oedema. The dog was donated for further study.

A lymphangiogram of the left rear limb of the proposita was made at 2 years of age (Fig. 2). Incision of the skin over the dorsum of the metatarsal region resulted in a profuse outpouring of oedema fluid. On injection of Evan's blue dye into the interdigital space, an extensive network of enlarged lymph vessels was outlined in the subcutis and dye soon coloured the fluid oozing from the incision. The two main lymph channels lateral to the dorsal metatarsal vein were greatly dilated. One was cannulated for injection of contrast medium. The lymphangiogram in Fig. 2a was made 12 minutes after the start of the infusion. In this film the collateral lymphatic channels below the femoro-tibial joint were seen to be tortuous and greatly increased in number and size. The main vessels were dilated and appeared to end blindly in the region normally occupied by the popliteal lymph node, which was not seen. At 36 minutes, after injection of a total of 9 ml. Ethiodol (Fig. 2b), a few fine

Fig. 1. Normal lymphangiogram of the left rear limb of a 2-year-old female mixed dog. Both exposures were made approximately four minutes after beginning infusion of Ethiodol into a large lymphatic channel in the dorsal metatarsal region (infusion rate, 0.25 ml./minute).

a. Distal portion of limb, showing one major lymphatic channel extending upward from the metatarsal region (m) to the popliteal lymph node (p). The needle used for injection is seen distally.

b. Proximal portion of the limb showing two afferent lymph channels entering the popliteal lymph node and several larger lymphatic vessels extending from the node to the pelvic region. The segmented appearance of the more central vessels results from the presence of lymphatic valves.
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Fig. 2. Lymphangiogram of the proposita. (a) Exposure made 12 minutes after the start of injection of contrast medium into a large lymphatic vessel in the dorsal metatarsal region. A total of 3 ml. contrast medium were infused. The main lymphatic channels end blindly in the popliteal fossa (f). The popliteal lymph node is not seen. Distally, the lymphatic vessels are increased in number, size, and tortuosity. (b) Exposure made 36 minutes after the onset of infusion. A total of 9 ml. contrast medium were infused. A few fine lymphatic vessels extend upward in the thigh region, but the popliteal lymph node and major channels to the trunk are not seen. An extensive plexus of fine lymphatic vessels is seen in the metatarsal and digital areas.

lymphatic channels were seen in the region, but neither the popliteal lymph node nor the main lymphatic channels which normally lead from the node to the pelvic region were outlined. As in the previous film, a rich system of dilated, collateral lymphatic channels was seen in the lower portion of the leg, above and below the site of injection.

The proposita was retained for breeding studies and at this writing is 5 years of age. She has borne 17 pups in three litters. Pitting oedema of the rear limbs is still present, but she is otherwise in good health.

Genetic Studies. A pedigree chart showing six test matings is presented in Fig. 3. The proposita was mated to an unaffected male poodle (P), an unaffected German shorthair pointer (G), and one of her affected sons (No. 1). The affected son (No. 1) was also mated to his normal litter sister (No. 3), an unaffected collie (C), and an unaffected keeshond (K).

The results of these matings are summarized in Table I. In five matings between affected and unaffected dogs, 20 of 40 pups had obvious oedema when examined within one week after birth. Twelve of 24 females and 8 of 15 males were affected. The sex was not recorded in one unaffected dog (No. 2). One mating between two affected dogs produced four pups, all of which were affected. The proportion of affected pups in each of the 3 mating types closely approximates that expected under the hypothesis of autosomal dominant inheritance.

Cytogenetic studies in one male with lymphoedema (No. 12) revealed a normal modal chromosome number of 78 with a normal male karyotype (Moore and Lambert, 1963; Gustavsson, 1964).
Sex not known, unaffected
Female, unaffected
Male, unaffected
Lymphoedema
Transient lymphoedema
Died (not sacrificed)
Necropsy

Fig. 3. Pedigree of test matings. The proposita (arrow) was mated to a normal poodle (P), a normal German shorthaired pointer (G), and her affected son (No. 1). The affected son (No. 1) was mated to a normal litter sister (No. 3), a normal keeshond (K), and a normal collie (C). The other numbered dogs are referred to in the text.

Clinical Features. Affected dogs had swollen extremities which pitted on pressure (Fig. 4). The swellings were apparently not painful, and the skin of the swollen parts was not excessively warm or cool to touch. Although oedema was usually bilateral, the degree of swelling often was greater on one side than the other.

The distribution of oedema varied. Three types of distribution were recognized in the 23 affected offspring: (1) oedema of the rear limbs only; (2) oedema of the rear and forelimbs; and (3) oedema of all four limbs, trunk, and tail.

The distribution of oedema in the offspring of the three types of test matings is shown in Table II. The distribution varied within litters in matings between affected and unaffected dogs. In the one

<table>
<thead>
<tr>
<th>TABLE I</th>
</tr>
</thead>
<tbody>
<tr>
<td>LYMPHOEDEMA—DISTRIBUTION OF AFFECTED OFFSPRING IN VARIOUS TYPES OF MATINGS</td>
</tr>
<tr>
<td>Matings</td>
</tr>
<tr>
<td>Unaffected male x affected female</td>
</tr>
<tr>
<td>Affected male x unaffected female</td>
</tr>
<tr>
<td>Affected male x affected female</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

* Expected numbers based on the hypothesis of autosomal dominant inheritance.

\[ \chi^2 = 0.85, \text{ df } = 2, p \approx 0.60. \]
mating between two affected dogs, all four pups had extensive oedema of the trunk, tail, and limbs.

The amount of oedema usually decreased with age in those pups that survived the neonatal period. In the litter that resulted from mating the affected son of the proposita to a normal collie (C), four of the pups had oedema of the rear limbs only. The oedema in the four pups was no longer clinically detectable at 3 months of age. A lymphangiogram from one (No. 17) is shown in Fig. 5. There is a slight increase in the number, tortuosity, and size of the smaller lymphatic vessels below the femoro-tibial joint. The popliteal lymph node is not seen in the lymphangiogram, and could not be found on necropsy.

Pups with oedema of the trunk and limbs appeared weak and assumed a spread-eagled position, with limbs abducted. The ventral thoracic wall was flattened. All but one of these died within the first three weeks after birth, probably because they were unable to crawl and nurse normally. One pup with generalized oedema (No. 12) was fed by hand and is now 2 years of age. Moderate oedema of the fore and rear limbs is still present, but oedema is not clinically detectable in the trunk or tail.

Lymphangiograms of the rear limbs in surviving offspring which remained clinically affected closely resembled those of the proposita, showing a marked increase in the number, size, and tortuosity of lymphatic channels in the distal portion of the rear limb, with main vessels ending blindly in the region normally occupied by the popliteal lymph node (Fig. 6). Subsequent radiographs of the rear limb up to five days after lymphangiography showed little decrease in the amount of contrast medium in the lymphatic channels below the femoro-tibial joint.

**TABLE II**

<table>
<thead>
<tr>
<th>Mating</th>
<th>Rear Limbs</th>
<th>All 4 Limbs</th>
<th>Limbs and Trunk</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unaffected male × affected female</td>
<td>0</td>
<td>2</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Affected male × unaffected female</td>
<td>9*</td>
<td>3</td>
<td>3</td>
<td>15</td>
</tr>
<tr>
<td>Affected male × affected female</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>5</td>
<td>10</td>
<td>24</td>
</tr>
</tbody>
</table>

* In four of these, oedema was no longer present at 3 months of age.
Fig. 5. Lymphangiogram of a 3-month-old female dog (No. 17) following recovery from transient congenital lymphoedema of the rear limbs. This lymphangiogram was made 3 minutes after the start of infusion of contrast medium into a lymphatic vessel in the dorsal metatarsal region of the left rear limb (infusion rate, 0.25 ml./minute). An unusually large number of fine branches of the main lymphatic channels is seen in the mid-tibial region (t), and the main channels appear irregular in shape. The popliteal lymph node is not seen. A sublumbar lymph node (1) is visible at the upper centre of the picture, medial to the wing of the left ilium.

Popliteal lymph nodes were not palpable in any of the dogs with clinical oedema. Prescapular and submaxillary lymph nodes could be palpated in most of the affected dogs. An abdominal radiograph of the proposita, made three months after lymphangiography, showed residual contrast medium in the sublumbar lymph nodes, which appeared to be of normal size. This feature was not investigated in other dogs.

Eleven (45%) of the 24 affected pups died before weaning. Of the 20 unaffected pups, 3 (15%) died before weaning, and one died at 3 months of age.

Clinical Laboratory Studies. Laboratory studies in surviving pups revealed mild anaemia and neutrophilic leucocytosis in a few of the dogs, but there were no consistent differences between affected and unaffected dogs, and the values were usually within the normal range. Total plasma protein values and electrophoretic patterns of plasma proteins were within normal limits, and showed no consistent differences between dogs with lymphoedema and normal relatives. Laboratory data from the proposita at 2 years of age, and from five offspring of the mating with a normal poodle (P), at 3 months of age, are given in Table III. Blood samples for these determinations were obtained on the same day from all six animals.

Discussion

Congenital hereditary lymphoedema in cattle (Donald et al., 1952) and swine (Wiesner, 1960) is reportedly inherited as an autosomal recessive trait. Inheritance of congenital lymphoedema in the dog resembles that in Milroy's disease in man; the number of affected offspring in test matings fits well the hypothesis of autosomal dominant inheritance. Expressivity is variable, as in man, but the range of...
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TABLE III
LABORATORY DETERMINATIONS ON BLOOD FROM PROPOSITA AND 5 OFFSPRING

<table>
<thead>
<tr>
<th>Pedigree No.</th>
<th>Sex</th>
<th>Age (mth.)</th>
<th>PCV (%)</th>
<th>Hb (g./100 ml.)</th>
<th>RBC (milions/mm³)</th>
<th>WBC (thousands/mm³)</th>
<th>Segmented (%)</th>
<th>Non-segmented (%)</th>
<th>Lymphocyte (%)</th>
<th>Mononuclear (%)</th>
<th>Eosinophil (%)</th>
<th>Basophil (%)</th>
<th>Total protein (g./100 ml.)</th>
<th>Albumin Globulin Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1*</td>
<td>Male</td>
<td>3</td>
<td>36</td>
<td>11.2</td>
<td>5,118</td>
<td>19,800</td>
<td>62</td>
<td>8</td>
<td>20</td>
<td>6</td>
<td>4</td>
<td>5-0</td>
<td>1-50</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Unknown</td>
<td>3</td>
<td>40</td>
<td>12.5</td>
<td>5,680</td>
<td>17,650</td>
<td>72</td>
<td>6</td>
<td>21</td>
<td>1</td>
<td>1</td>
<td>5-6</td>
<td>1-29</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Female</td>
<td>3</td>
<td>40</td>
<td>12.5</td>
<td>5,680</td>
<td>14,750</td>
<td>62</td>
<td>7</td>
<td>25</td>
<td>6</td>
<td>6</td>
<td>5-6</td>
<td>1-83</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Female</td>
<td>3</td>
<td>37</td>
<td>11.7</td>
<td>5,254</td>
<td>14,300</td>
<td>82</td>
<td>3</td>
<td>13</td>
<td>2</td>
<td>2</td>
<td>5-6</td>
<td>1-43</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Male</td>
<td>3</td>
<td>34</td>
<td>10.9</td>
<td>4,828</td>
<td>13,300</td>
<td>61</td>
<td>10</td>
<td>25</td>
<td>3</td>
<td>1</td>
<td>5-6</td>
<td>1-80</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Female</td>
<td>40</td>
<td>12.5</td>
<td>5,680</td>
<td>22,900</td>
<td>72</td>
<td>9</td>
<td>14</td>
<td>1</td>
<td>4</td>
<td>6-0</td>
<td>1-35</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Lymphoedema.
PCV = packed cell volume.

The findings, considered together, suggest that in the dog there is a localized or generalized disturbance in the morphogenesis of peripheral lymphatic channels, including the lymph nodes, with failure to establish normal connexions with the more central lymphatics. In a few cases, morphogenesis apparently may continue after birth, with formation of abnormal lymphatic vessels and nodes, but with functionally adequate lymphatic drainage from the distal portion of the limb. Limited evidence from films taken after lymphangiography in the proposita suggests that sublumbar, and possibly other central lymph nodes are present in affected dogs.

The underlying anatomical changes in hereditary lymphoedema of Ayrshire cattle may be similar to those in the dog. Morris et al. (1954) found abnormalities of the peripheral lymph nodes and dilatation of afferent and efferent lymphatic vessels in two carefully studied calves. Periperal lymph nodes, however, were not absent.

In congenital hereditary lymphoedema of swine (Dickbeinigkeit), oedematous thickening of the legs is said to be associated with large, irregular, lymph-filled lymphatic vessels in the subcutis (Wiesner, 1960), but the lymph nodes and other parts of the lymphatic system were not described.

The basis for the oedema of Milroy's disease in man is unknown. Milroy (1892) originally suggested that there might be some obstructive or vasomotor abnormality which interferes with the activity of blood vessels or lymphatics. Schroeder and Helweg-Larsen (1950) found groups of unusual arteriole-like structures in the deep dermis, and they suggested that an arteriolar abnormality might interfere with the balance of fluid flow between the capillary bed and the tissues, producing filtration oedema. Similar vascular structures were seen in Esterly's proposita (1965), but, as he points out, their origin and significance are obscure.

Kinmonth, Taylor, Tracy, and Marsh (1957)
failed to demonstrate lymphatic vessels by lymphangiography in two cases of familial lymphoedema in man. Wood and Esterly (1960) had a similar experience with the proposita of the family reported by Esterly (1965). More recently, Ersek, Danese, and Howard (1966) found that Evans blue dye, injected intradermally in two affected members of the same family, diffused slowly and in a random manner in the feet. Surgical exploration of the area did not reveal any lymphatic vessels. When dye was injected in the distal thigh, it moved towards the popliteal lymph nodes in a typical ‘spider-like’ fashion, outlining lymphatic vessels on the way. These findings have led to the view that in Milroy’s disease the peripheral lymphatic vessels are either absent, or too hypoplastic to be demonstrated. The state of the regional lymph nodes in Milroy’s disease has not been described.

Baikie has noted that familial lymphoedema is one of a group of inherited diseases in which affected families appear to have an increased incidence of acute leukaemia (Baikie, 1966). In three sibships with congenital lymphoedema which he studied, five deaths from acute leukaemia occurred among 11 members. Baikie suggests that the lymphoedematous limb may provide a favourable environment for neoplastic change.

Summary

The clinical and genetic features of congenital hereditary lymphoedema in the descendants of one affected dog are described. A marked range of severity was observed. In the most mildly affected dogs, only the rear limbs were oedematous, and the oedema disappeared with age. In the most severely affected dogs, oedema was generalized. The mortality rate was high in pups with generalized oedema, but one was hand fed and raised to maturity. Oedema of the trunk and tail disappeared in this dog, but persisted in all four limbs.

Evidence from lymphangiograms and breeding experiments indicates that congenital hereditary lymphoedema in the dog results from a dominantly inherited defect in the morphogenesis of the peripheral lymphatic system. In the rear limbs there is lymphatic obstruction at the level normally occupied by the popliteal lymph node. In dogs in which rear limb oedema persisted, this lymph node was not palpable, nor was it demonstrated by lymphangiography; the distal lymphatic vessels of the limb were much increased in size, number, and tortuosity. Dogs in which oedema of the rear limbs disappeared with age, had lymphangiographic evidence of minor lymph vascular changes, and absent popliteal lymph nodes.

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References


