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The Pathogenesis of Congenital Hereditary Lymphedema in the Pig

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Summary

The morphogenesis of congenital hereditary lymphedema was studied in pigs. The disorder, which is essentially a general underdevelopment or even total non-development of the lymphatic system proved to be present during the whole period of lymphatic development. It is suggested that a retardation in the differentiation of the lymphatic primordia from the primitive veins is the early event regulated by a chromosomal aberration. The longer this delay the more serious the lymphatic malformations.

Introduction

In a preceding article the histopathological findings in newborn pigs with congenital hereditary lymphedema were reported (8). It was found that the underlying lymphatic malformations were not confined to the edematous areas but were generalized in character. They varied from minor hypoplastic changes to a complete agenesis of the whole system.

As in other kinds of congenital and/or hereditary lymphedema it is generally believed now that the basic disorder is an underdevelopment of the lymphatic system in one or more parts of the body. The more serious the lymphatic malformation the more severe the clinical manifestations and the earlier their onset (3). The precise mode of morphogenesis of this rare deformity has never been studied. Only a recent suggestion of *Luginbühl* et al. (4) pointed to the possibility that the basic disorder is a failure of the peripheral lymphatics to make adequate connections with the central trunks. Still such an investigation might help to understand better the variations met in this disease and to contribute to knowledge about principles involved in the normal development of the lymphatic system.

Material and Methods

The morphogenesis of the congenital hereditary lymphedema was studied in 98 embryos and fetuses, which were obtained by operation. 79 embryos and fetuses were born from matings between two animals affected with congenital hereditary lymphedema. They varied in age from 24 to 70 days and in C.R. length from 10 to 190 mm. They were distributed as follows: 24 days (8), 26 days (2 normal – 13 hypoplasia – 0 agenesis), 29 days (1–4–1), 33 days (0–9–1), 40 days (0–6–3), 45 days (4–3–1), 55 days (2–5–2), 60 days (1–5–2) and 70 days (1–5–0). Five 90 days fetuses were from a mating between an affected boar and an unrelated normal sow (2–3–0). In addition 15 normal control fetuses were got from 7 matings between unrelated normal pigs. These were distributed equally over the following ages: 27, 29, 31, 38, 42, 60 and 70 days.

For pedigree purposes all embryos and fetuses were studied histologically. Embryos with a gestational age of less than 60 days were totally fixed in Bouin's fixative. Of the elder fetuses sometimes small pieces were fixed in Bouin's fixative, but mostly a 4 percent's neutral formalin solution was employed. The specimens were embedded in paraplast and for the whole or in part serially sectionned at 6 microns. From the larger fetuses blocks were taken systematically according to the method applied in the newborn pigs (8).

Observations

Macroscopical findings

In the early stages of development practically no differences were observed between normal and abnormal embryos. At the age of 29 days however the first rather discrete changes became visible (Fig. 1). These consisted of a

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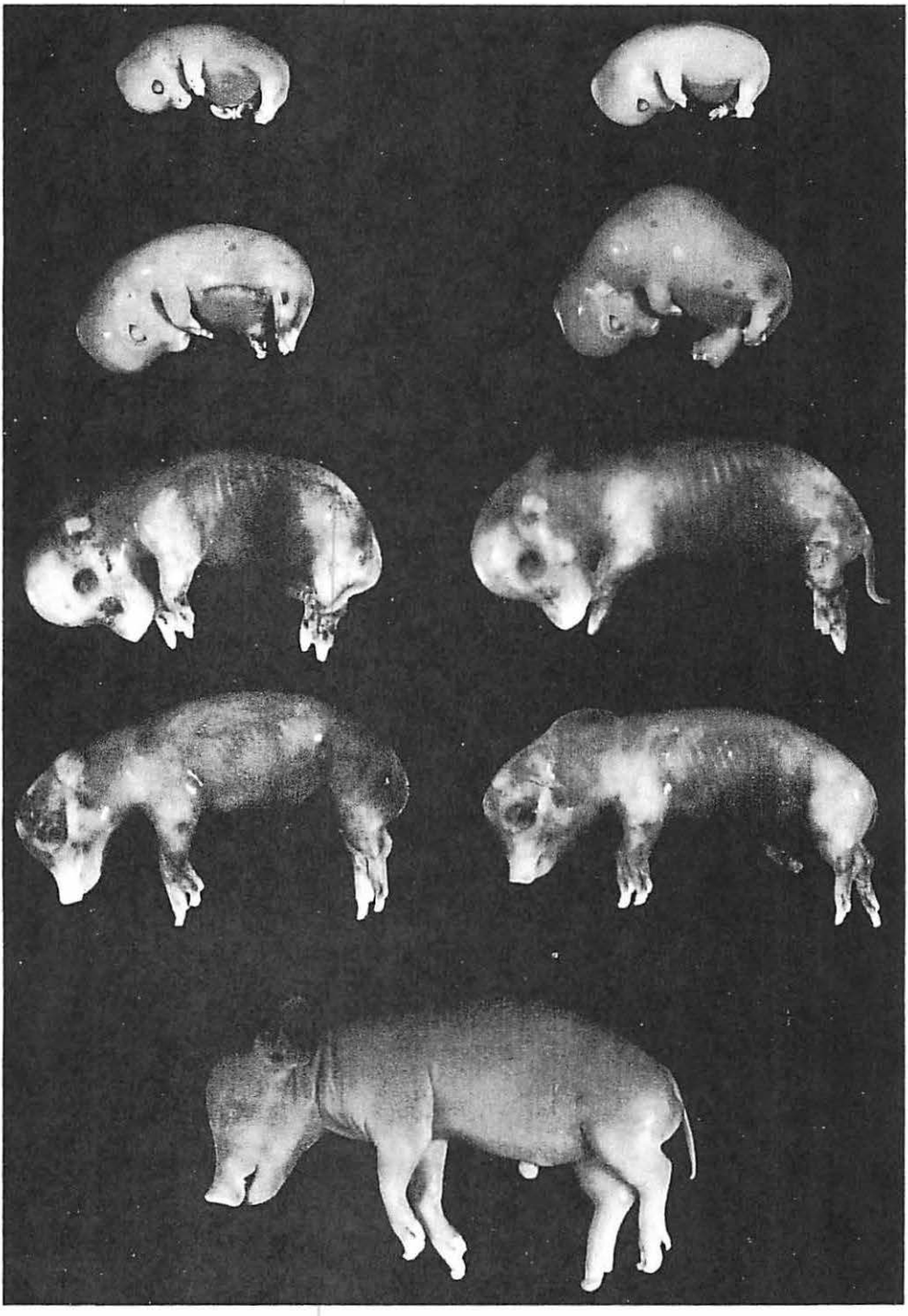


Fig. 1 Embryos and fetuses with lymphatic hypoplasia (left row and bottom most specimen) and aplasia (right row) of 29, 33, 40, 60 and 90 days (C.R. lengths: 22, 26, 50, 110 and 220 mm).

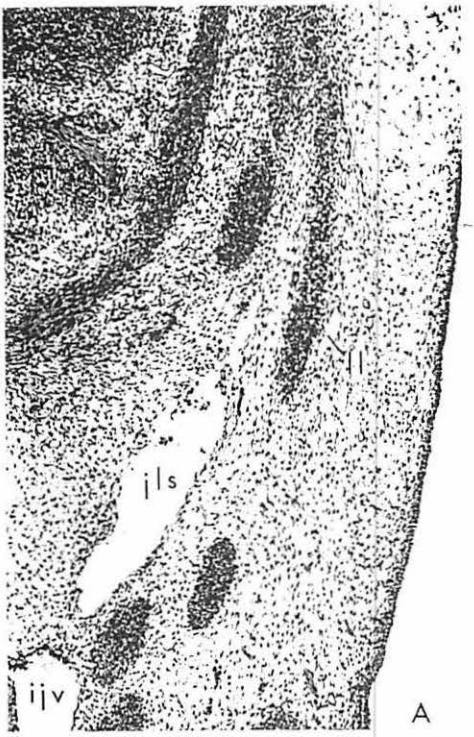


Fig. 2a Cross-section through the lateral jugular area of 26-day old embryos.
Normal configuration with lymph vessels (11) extending from the jugular lymph sac (jls) into the sub-epidermal connective tissue of the lateral and dorsolateral skin. ijv: internal jugular vein.

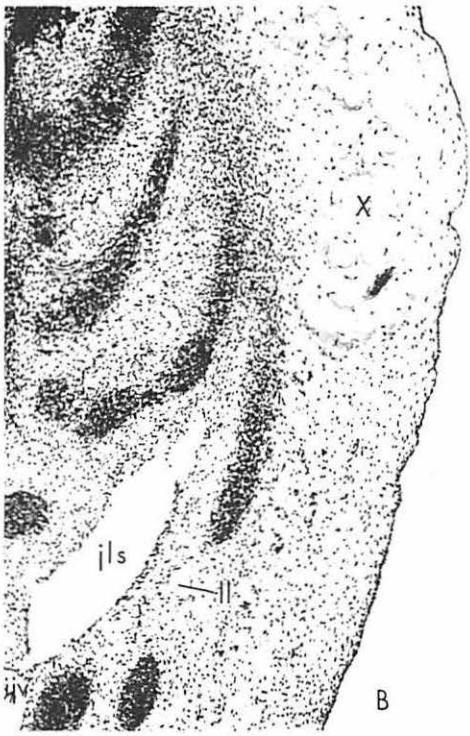


Fig. 2b Less advanced outgrowth of lymph vessels (11) form the jugular lymph sac (jls), associated with a generalised edema. This edema is most pronounced in the subepidermal connective tissue of the dorsolateral skin (X). HE. X. 50.

very slight swelling of the dorsal neck and shoulder region in most embryos and a rather marked swelling in this area in one embryo. In the older animals this swelling gradually increased and spread over the rest of the body until it was generalized in those of 40 days old. But there was always the clear distinction between moderately and severely edematous animals. In the moderately affected animals the swelling seemed to decrease anteriorly after 55 days of age, while still increasing slightly in the posterior parts. At 90 days these fetuses showed minor changes in the anterior parts of the body, moderate changes in the posterior part of the trunk and adjacent proximal parts of the hind legs, but a very conspicious swelling of their distal hind legs. In the most severely affected embryos a steady increase of the edema all over the body became associated with the formation of a series of cystic cavities in the dorsal skin of the neck and shoulders.

Microscopical findings

24 days: In these embryos the existence of lymphatic primordia could as yet not be ascertained. According to certain configurations it seemed quite possible that they were hidden in some vascular plexuses that had just sprouted from the anterior cardinal veins.

26 days: In the normal embryos at both sides of the neck a large jugular lymph sac had developed lateral to the internal jugular veins. There was a complicated plural

lymphatico-venous anastomosis at the confluence of the internal and the external jugular veins. From its most cranial part sprouts had started to grow out but they formed still a simple network in the subepidermal connective tissue of the posterior triangle of the neck (Fig. 2a). In the abdomen a small mesenterial lymph plexus had developed ventral to the intersubcardial anatomosis and the aorta. Other primordia could not yet separated from primitive venous tributaries.

Most embryos differed from these normal embryos in that the outgrowth of lymph vessels from the jugular lymph sacs seemed to be defective (Fig. 2b). Most remarkably, in the area thus devoid of lymph vessels i.e. the dorsolateral part of the neck, a distinct edema had appeared. The mesenteric lymph plexus too was smaller than normal. The other organ systems did not show abnormalities.

29 days: In the normal embryos in addition to the jugular lymph sacs and mesenteric lymph plexus which had further enlarged, a bilateral system of slender thoracic ducts have developed along the thoracic aorta. In the abdomen lumbar lymph plexuses had originated dorsolateral to the aorta, and iliac lymph plexuses near the confluence of the internal and external iliac veins. Most primordia had fused, but no continuity had as yet been established between the abdominal and thoracic systems. A relatively dense network was covering now the whole lateral and dorsolateral area of the neck. Extensions of the mesenteric lymph plexus had spread in the root of the mesentery.

In most of the abnormal embryos all lymphatic primordia were now present. But, except for the jugular lymph sacs, they were small and often discrete, and they had not yet fused. The peripheral extension into the posterior triangle of the neck had continued, but this network was less extensive than in the normal and showed too coarse a pattern consisting of vessels which were too wide. Sprouts of the mesenteric lymph plexus had not reached the root of the mesentery. The edema in the neck region had increased both in degree and extension.

It was less severe however than in a most seriously affected embryo, in which some cystic cavities had formed in widely distended tissue spaces of this area. A slight edema was also noticed in the praevertebral area and the skin of the rump. In this embryo not a single lymphatic primordia was found.

33 days: In the normal embryos the primary lymphatic system (that means: the part that originated directly from the primordia) had widened up a little and had gained complexity. Most of the neck was provided now with lymphatic networks. Extensions from the thoracic ducts had reached the hilar region of the lungs. Mesenteric lymph vessels surrounded the head of the pancreas and had grown out along the main parts of the mesenteric blood vessels.

In most of the abnormal embryos no marked differences with the normal existed as to the primary lymphatic system. But there were conspicuous differences in their peripheral extensions. Although varying considerably in the various embryos, growth had apparently further stayed behind as compared with the normal. The edema had progressed towards the caudal part involving most of the rump.

In the most abnormal looking embryo edema was generalized now. The cystic cavities in the neck and the shoulder region had further enlarged and were partially lined by flat cells. Still no lymph vessels were present.

40 days: In the normal embryos the lymphatic primordia were disappearing into masses of very dense networks of lymph vessels that had differentiated at their outer margins. Condensations in these networks indicated the first phase of lymph node development. Except for the most distal parts of the posterior extremities, networks of small lymphatics were found everywhere in the skin. The whole of the lungs were transversed by lymph vessels and extensive plexuses had been laid down subserously in the mesentery up to the muscular coat of the small intestine.

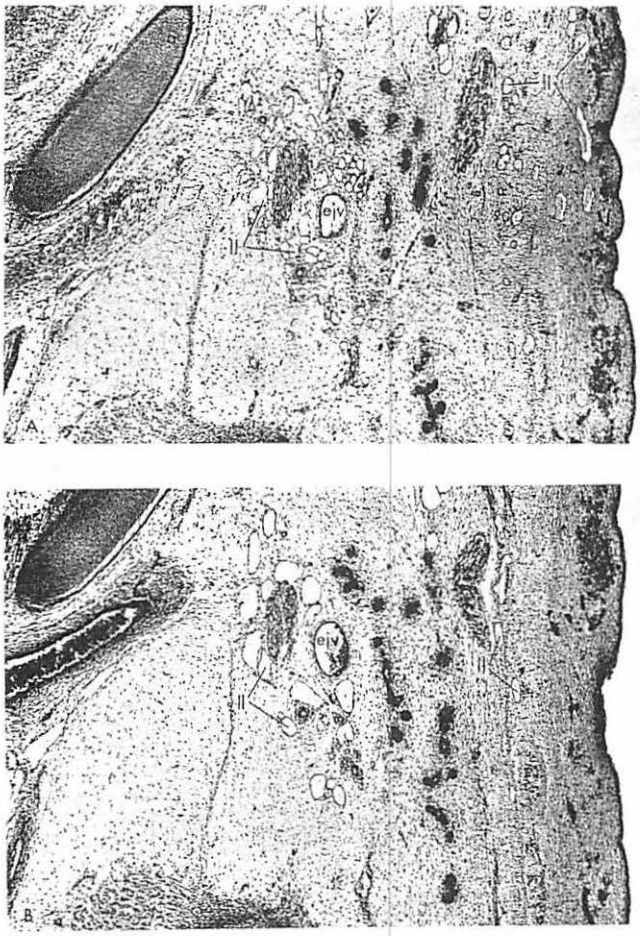


Fig. 3 Cross-section through the upper jugular area of 45-day old fetuses showing a normal (a) and hypoplastic (b) configuration of the lymph vessels (11). In the latter the plexuses of lymphatics are far less intricate and consist of much wider vessels. ejv: external jugular vein. HE. X. 32.

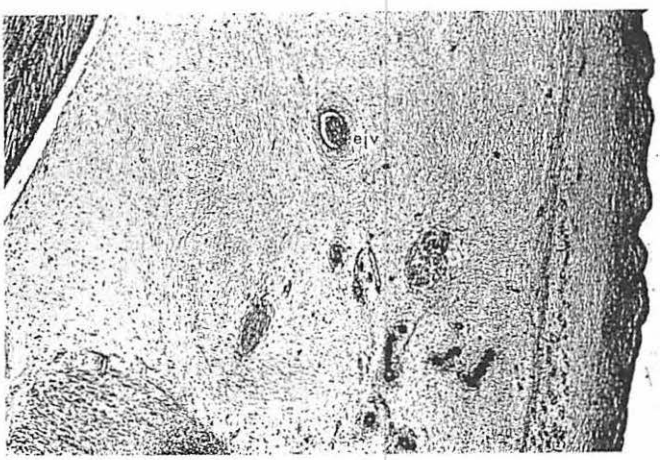
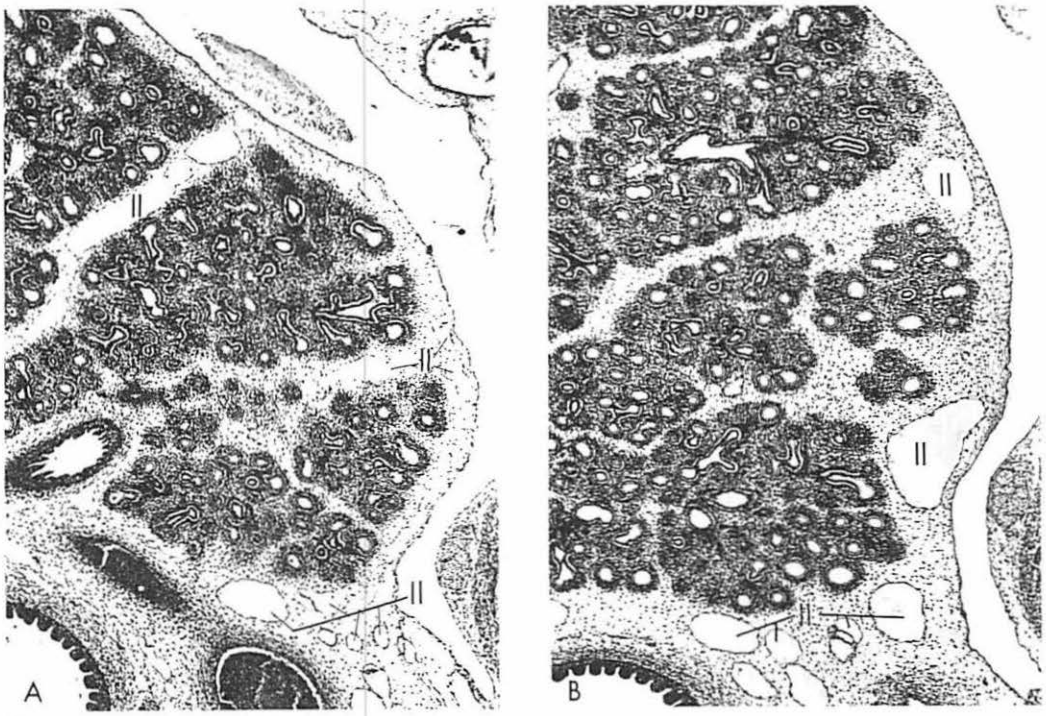


Fig. 4 Cross-section through the same area as shown in fig. 3 of a 45-day old fetus with complete agenesis of the lymphatic system. ejv: external jugular vein. HE. X. 32.



a) b)

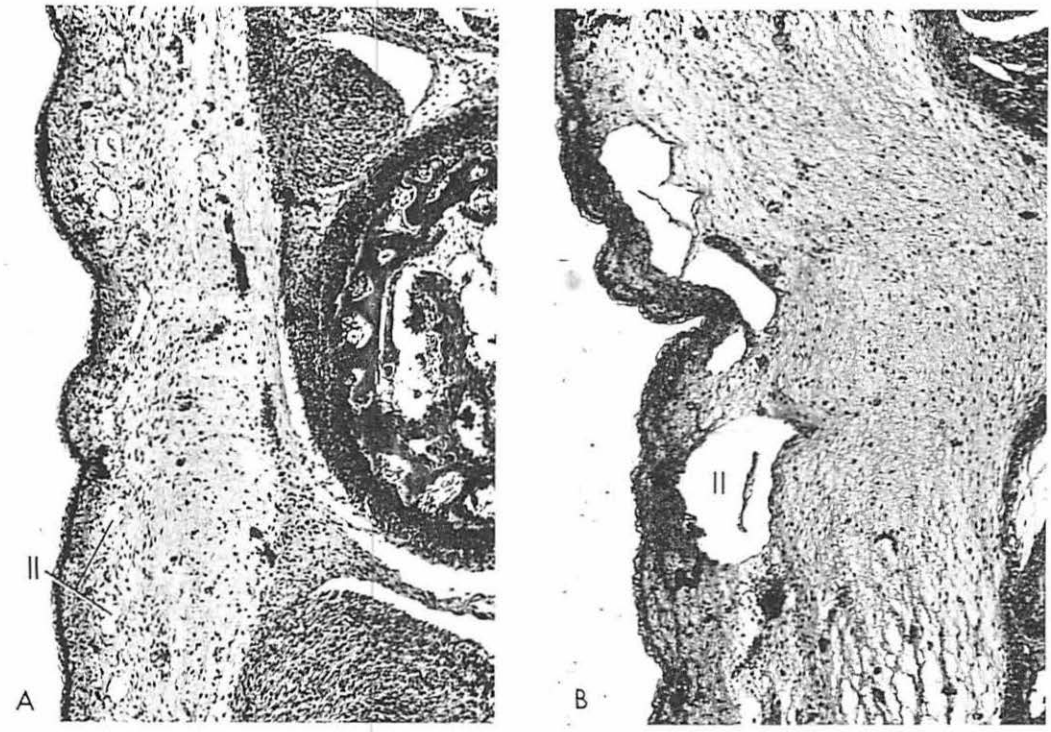
Fig. 5 Cross-section through the lungs of 45-day old fetuses showing a normal (a) and hypoplastic (b) configuration of the lymphatic system. In the last lymphatics (11) are fewer in number, wider and situated in the distinctly edematous and rather dense connective tissue. HE. X. 40.

In most of the abnormal embryos the primary lymphatic system had a more simple structure since it was not envelopped by such dense networks as that in the normal. In these networks only a few condensations had appeared. Peripheral outgrowth did not extend the lateral, dorsolateral and ventral area of the neck proceeding from here into the anterior extremities up to the humero-radial joint. Extensions of the thoracic ducts had only reached the hilar region of the lung, while no mesenteric lymph vessels still extended up to the intestine. Sprouts from the iliac lymph plexuses had just formed minor networks in the gluteal and inguinal regions. The edema was more marked now in the skin of the posterior half of the trunk being only slightly less pronounced than in the most severely affected animals. In the anterior and more central parts of the body it was rather inconspicious and evidenced mostly by a concomitant increase of connective tissue elements.

In the most severely affected animals the generalized character of the edema was preserved showing an uniform distribution. The cysts on the back had further enlarged. No lymph-vascular structures were observed.

45 days (Fig. 3, 4, 5, 6): In the normal embryos the whole skin was covered by finely meshed lymphatic networks. From the mesenteric networks sprouts had invaded the submucosal layer of the intestine. In some of the larger vessels valve-like structures had developed, while in the lymphnodes the first lymphoid cells had appeared.

In most of the abnormal embryos little progression had been made. In the anterior extremities sprouts had reached the radio-carpal joint, but even at this time no



a) b)

Fig. 6 Cross-section through the skin of the inner side of the most proximal part of the forelegs of 45-day old fetuses. a) Normal configuration of the lymphatics (11); b) hypoplastic configuration demonstrating edematous stroma with only a few very wide lymph vessels (11). HE. X. 63.

lymphatics had invaded the posterior extremities. In the lungs a few lymph vessels had reached the pleural lining. In the mesentery however still no vessels had invaded the wall of the small intestine.

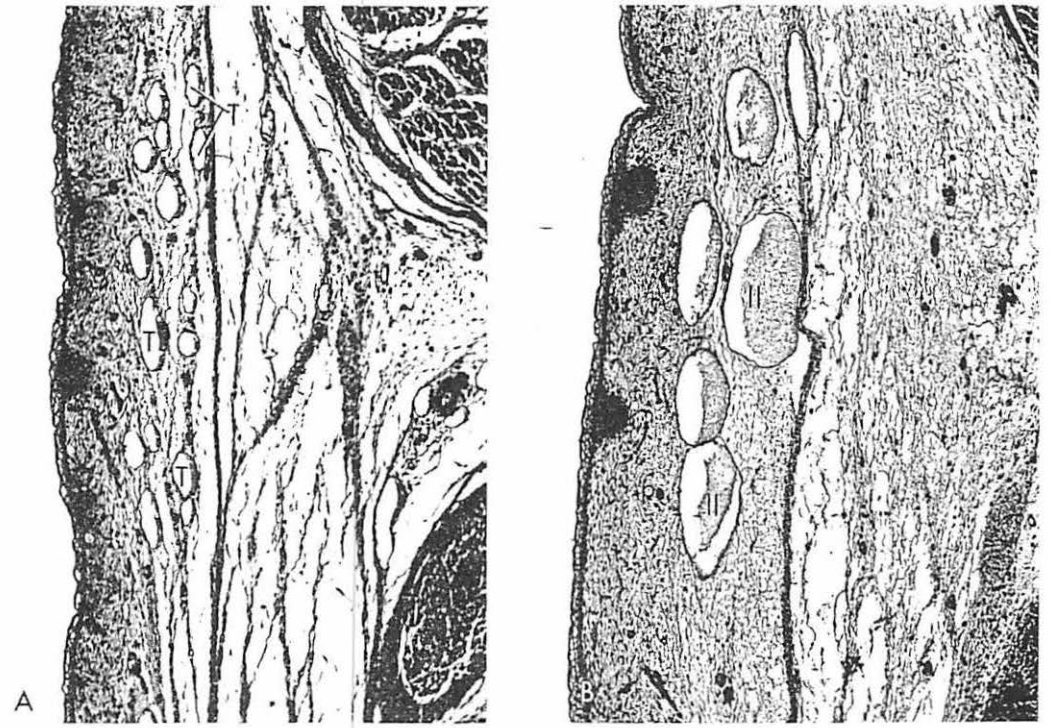
The most severely affected embryos did not differ much from those in the preceding group.

55 and 60 days: In the oldest part of the dermal lymph plexuses of the normal fetuses a differentiation had taken place into a superficial dermal network of small lymphvessels and larger more longitudinal running trunks situated subcutaneously (Fig. 7a, 8a). The lymph nodes had become more complex and new ones had developed in the mesentery, and in the axillary and inguinal regions.

In most of the abnormal fetuses peripheral outgrowth had advanced now to the distal metacarpal region and some differentiation had taken place into dermal networks and subcutaneous trunks (Fig. 7b). But the trunks were too few in number and, as everywhere, the networks were still too coarse and consisted of too wide and irregular vessels.

Only in a few of the 55-day-old fetuses lymphatics had reached the most proximal parts of both hind legs. They extended into the deepest layers of the subcutis of the medial side of the legs and consisted of very irregular and often cystic cavities (Fig. 9a).

In all of the moderately edematous 60-day-old fetuses except one, lymph vessels had grown out into the proximal parts of the hind legs. They formed few irregularly distributed more or less longitudinally running channels situated superficially in the



a) b)

Fig. 7 Cross-section through the same area as shown in fig. 6 in 55-day old fetuses. a) Normal configuration of the lymph vessels with a superficial plexus (hardly visible) and deep longitudinal trunks (T). b) Lymphatic hypoplasia showing no differentiation. The few very wide lymph vessels (11) are situated in edematous finely fibrillar connective tissue. HE. X. 50.

subcutis of the medial side of the legs (Fig. 9b). No lymphvessels had developed as yet in the deeper tissues and the remaining parts of the skin.

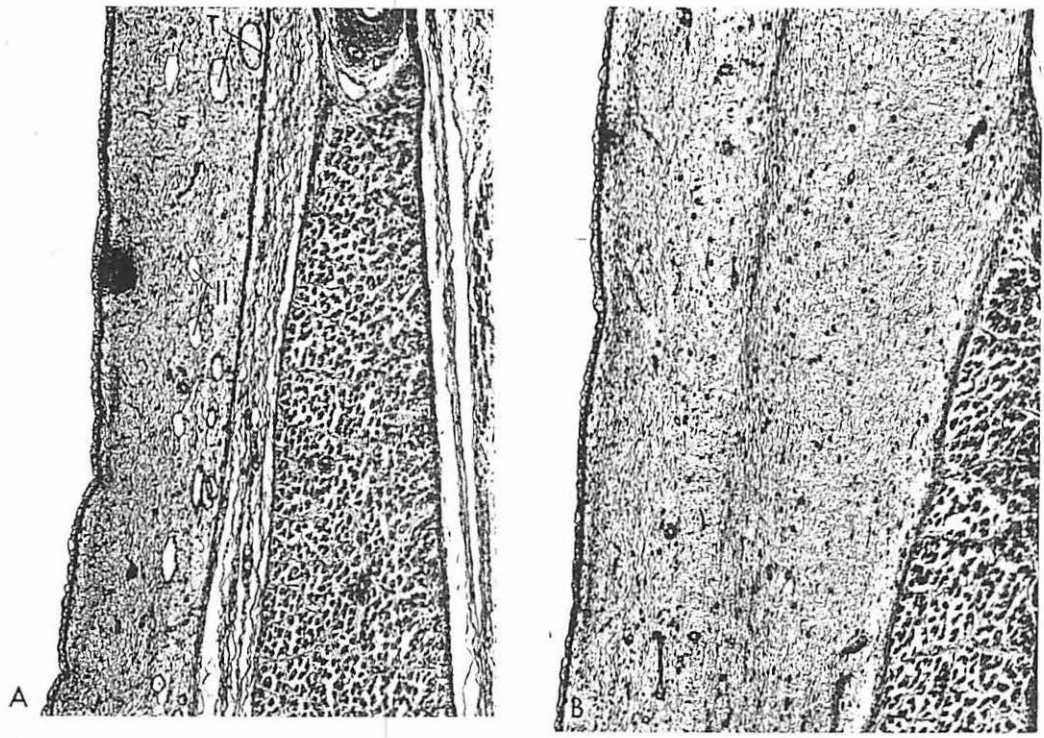
In most fetuses lymphatics had started to grow out into the skin and deeper parts of the lateral thoracic wall. In the lungs coarse networks of often almost cystic dilated lymph vessels were found, while in the mesentery lymph plexuses were unevenly distributed and did not extend into the wall of the intestine. In these fetuses lymph nodes were only present in the prevertebral area and mesentery. Their number and proportion were smaller than usual. The edema had the same distribution as in the 45 days embryos.

The most severely affected animals were essentially similar to those aged 45 days.

70 and 90 days: Although slight differences were found between the fetuses of these two ages, those 70 days old did not differ very much from the 60-day-old fetuses while those 90 days old were very much alike the newborn pigs (8).

In the normal fetuses lymphatic development had proceeded by a further differentiation of large and small trunks from capillary networks which themselves became increasingly more complicated. Valves and lymph nodes were more numerous.

In most of the abnormal fetuses no more than a very modest progression as to lymphatic outgrowth and differentiation was made. In the 90 days fetuses the median strip of the skin of the lower back and the posterior extremities distal to the tibio-tarsal



a) b)

Fig. 8 Cross-section through the skin of the inner side of the most proximal part of the hind legs of 55-day-old fetuses. a) Normal configuration with a superficial plexus of lymph vessels (11) and deeper longitudinal lymph trunks (T). b) In hypoplasia no lymph vessels have grown in yet. The subepidermal connective tissue has thickened considerably by edema and fibrosis. M. skin muscle. HE. X.s 50

joint were still lacking lymph vessels, although the tips of the anterior extremities were reached and the mesenteric lymphatics had locally spread into the submucosal layer of the intestine. For the rest all peripheral lymph plexuses had remained distinctly abnormal and inconsistent in form, pattern and extension.

One of these fetuses was very interesting as it showed a lymph-vascular configuration that came rather near the normal. For although definite abnormalities did exist as to the form and pattern of the lymph vessels they were less pronounced than in the others and the vessels had reached all parts of the body. The edema was correspondingly less severe. In the others a very severe edema was seen in the lower back region and the posterior extremities, while elswhere but a slight to moderate increase of finely fibrillar connective tissue was found.

No specimens with lymphatic agenesis were obtained.

Comment

The main characteristics of this disorder are generalized lymphvascular abnormalities and edema. Between both a strong relationship exists as may be concluded from their simultaneous occurence and the strong positive correlation between the severity of the edema and the grade of lymphatic malformation. From the similarity in edema between the fetuses with hypoplasia and those with complete agenesis of the lymphatic system and from the concurrent fibrosis in elder fetuses it seems justified to postulate that the edema actually is a lymphedema caused by lymphvascular abnormalities.

The maldevelopment of the lymphatic system comprises two variants: a complete agenesis

a) b)

Fig. 9 Cross-section through the same area as shown in fig. 8, of a 55-day-old fetus (A) and a 60-day-old fetus (B) to show two different types of vascular malformation in lymphatic hypoplasia.
a) Large cystic cavity (C) under the skin muscle (M) in a cutis otherwise devoid of lymph vessels.
b) Superficial plexus of more or less evenly distributed wide lymph vessels. HE. X. 40.

and a hypoplasia of the whole lymphatic system. The nature of the underdevelopment is best understood by comparing lymphatic outgrowth between the anterior and posterior extremities in normal and abnormal fetuses. The conclusions drawn however may be applied equally to all anterior and central parts on the one side and posterior and more distal parts on the other.

As has been demonstrated lymphatic outgrowth into the fore legs took place between the 33rd and 40th day with those of the hind legs laying a few days behind (Fig. 10). By contrast in the fetuses exhibiting hypoplastic changes of the lymphvascular system lymphatic outgrowth into the anterior extremities did not start before the 40st day and into the posterior extremities not even before the 55th day of gestation. In both there was also an impairment of the further extension which became increasingly more serious with the length of the gestation. Thus the tips of the fore legs were not reached before the fetuses were 70 days old. In the hind legs the inhibition of growth was disproportionately more serious and large distal parts would even never receive a lymphatic contributuary. From this it is clear that in this abnormal developmental process delayed outgrowth of peripheral lymphatics and subsequently an everincreasing inhibition of this growth are most important elements.

Factors influencing lymphatic outgrowth may be intrinsic and extrinsic. Although the operating of intrinsic factors can not be ruled out with certainty much is in favor of extrinsic factors playing a dominant role as

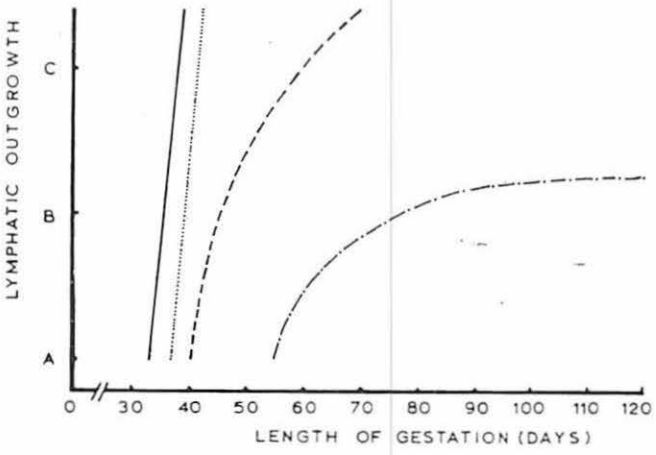


Fig. 10 Rates of extension of the lymphatics into the fore (——) and hind legs (·····) of normal fetuses and into the fore (------) and hind legs (·-·-·-·) of fetuses with lymphatic hypoplasia. The levels of extension are indicated by the position of the humeroradial/femoro-tibial (A), carpo-metacarpal/tarso-metatarsal (B) and metacarpo-phalangeal/metastarso-phalangeal (C) joints.

will be demonstrated here. From the experiments of *Clarke* and *Clarke* (1, 2) it transpired that lymphatic outgrowth is greatly influenced by increasing pressure and structural changes in the surrounding tissues. Assuming the same mechanism operating during embryonic development the forming of edema and fibrosis might thus cause an inhibition of lymphatic outgrowth, which in turn causes an aggravation of the lymphedema and fibrosis. This creates a vicious circle that in the extreme would end in a complete arrest of growth. And this is exactly what seems to happen in these abnormal pigs. It would be conceivable then that most parts of these animals, though rather late, are still reached by lymphatics before growth has stopped completely but that in some other parts, as the distal hind legs, this process will proceed to this unfortunate last phase. Slight variations in the time of onset of lymphatic ingrowth may thus lead to the variability in peripheral extension of the lymphatics observed in these affected pigs.

In addition this decreasing tendency for sprouting may well account for the forming of too coarse plexuses and, since the development of lymph nodes depends on the forming of very dense networks of lymph vessels, also for hypoplasia of the lymph nodes.

The structural abnormalities of the vessels themselves i.e. dilatation, irregularity and deficiency of valves may be due to increased lymph flow through the too few vessels available.

From the preceding it may be concluded that the inhibition of growth is secondary to a retardation in the time of onset of this outgrowth. Such a delay was traced to the very first peripheral lymphatic spread in the neck region of 26-day-old embryos. Since no well-marked separation exists between the development of the lymphatic primordia and their peripheral extension one may well reckon that these primordia are involved in the same pathological process. Although it is difficult to differentiate between a normal variability and abnormal development in the very short period involved, it was strongly suggested by the simultaneous occurrence of edema, the too short peripheral extensions of the jugular lymph sacs, and too small mesenteric primordia that a delay in the development of the primordia may well be an underlying mechanism. This brings this maldevelopment to the same point where the other variant of this disorder – a complete agenesis – may be expected to start namely to the process of differentiation of the lymphatic system from the primitive veins. This process must be a very critical one because even under normal conditions after that moment the veins apparently have lost completely their ability to form lymph vessels or to fuse with them. It is quite conceivable that too great a delay in this process of differentiation thus

results in a complete agenesis. In conclusion it is thought that the basic disorder, evoked by a chromosomal aberration, is a disturbance, or more specifically a retardation in the differentiation of the lymphatic primordia from the veins. If this retardation is too great it will lead to a complete agenesis. If less it will cause a delay in the outgrowth of the lymph sprouts into more peripheral parts which leads to lymphedema, inhibition of growth and ultimately to a general hypoplasia.

As for the normal development of the lymphatic system this investigation brought new proof of the untenability of the still common theory that lymphatics originate in the peripheral parts of the body (5, 6, 7). And by this it affects seriously the proposition on which the idea of *Luginbühl* et al. (4) about the pathogenesis of this disorder in dogs is based. Furthermore it was made clear that the lymphatic system functions, and must function from the very beginning, although it became equally clear that even the complete absence of any function as is evidently the case in those with lymphatic agenesis may be well compatible with life up till the last month of gestation (8). In addition it was a remarkable finding that even a very incomplete lymphatic system consisting only of the centrally situated primary trunks exerts its influence throughout the whole body as was indicated by the differences in grade of edema at most distant places between animals with hypoplasia and those with agenesis.

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