Anatomy. — *On the occurrence of Vascularized Enamel-organs.* By J. MULLER. (Communicated by Prof. L. BOLK.)

(Communicated at the meeting of December 18, 1926).

Is the enamel-organ vascularized, or is it not? Up to this day opinions differ concerning this seemingly simple question.

In former years it was generally assumed that an enamel-organ was never vascular i.e. provided with bloodvessels. However, in later years some cases were described in which vascularization was beyond dispute. The present paper discusses a new case which Prof. BOLK submitted to me for examination. I shall preface the description of the case with a review of what has already been published on this vascularization.

When looking up the literature of the vascularization of the enamel-organ, it strikes us that this problem has but seldom received general notice, although it is of great importance for the nutrition of the ameloblasts. Most times that vascularization was treated of only incidentally; a systematic study has never been made of the occurrence of bloodvessels in the enamel-organ.

Indeed, it was generally known that in the later stages of the development, there is a closer relation between the bloodvessels and the stratum intermedium. When in the course of development the reduction of the enamel-pulp begins, the external epithelium leans up against the cells of the stratum intermedium, so that the bloodvessels are separated from the intermediate cell-layer only by this external epithelium. Nay, some researchers even hold that the external epithelium disappears altogether, and the capillaries come into direct contact with the stratum intermedium.

LEON WILLIAMS even describes, in this developmental stage of the *rat*, bloodvessels within the stratum intermedium. MUMMERY says of WILLIAMS: "he describes bloodvessels in the stratum intermedium at this stage (the later stages of enamel formation, when the stellate reticulum has disappeared), and that there are bloodvessels in this layer is fully evidenced by the photographs in illustration of his paper".

However, my object in this paper is to discuss the occurrence of vascularization in still fully developed enamel-organs, i.e. in stages in which the internal and the external epithelium are still separated by a well-developed pulp, and the latter does not show any sign of reduction. An instance of this was not described before 1889. Before entering upon my subject I will bring forward some reports regarding vascularization, that appeared before that date.

In the edition of 1870 WEDL wrote in his Manual in accordance with the then current opinion: "Das Schmelzorgan ist gefässlos."

In complete harmony with this pronouncement is LEGROS and MAGITOT's ('79) description of the enamelorgan in a fetus of a *horse* in the 3d month of the development and in a newborn *dog*, in which they say: "cet organe étant absolument dépourvu aussi bien de système vasculaire que de système nerveux."

Just as the above researchers KLEIN and NOBLE SMITH ('80) detected that in a *dog* "no vessels are present in this middle membrane".

The first positive inquiry regarding a vascularized enamel-organ was made by POULTON ('89). This author studied the development of the dentition in *Ornithorhynchus paradoxus* and found here vascularization of the enamel-organ, as is borne out by the following quotation:

"I could not detect capillaries in the stratum intermedium — although they are certainly present in the stellate reticulum and are sometimes seen very near this layer (the stratum intermedium)", and a little further he says: "It is quite certain that blood-vessels are present in this layer (i.e. the enamel-pulp) and they extend into all parts of it." He could trace out the vessels from the adjacent connective tissue through the external epithelium down to the enamel-pulp, and observed that coincidently connective tissue forced its way in. The capillaries looked like strands of spindle-shaped cells with a narrow lumen, often difficult to distinguish.

The author further remarks that HOWES has found bloodvessels in the enamel organ of the *rat* and showed him the preparations.

Strange to say, HILL and WILSON who, in connection with POULTON's study, examined more closely the dental development in *Ornithorhynchus*, do not make mention of a vascularization in their extensive description.

The next-following observation, recorded in the literature is of much later date (1911). In this long interval we only read, in a description given by ADDISON and APPLETON of a case of vascularization found by the authors themselves, that PAUL ('96) has observed the occurrence of vascularized enamel-organs in the *calf* and the *lamb*, but that he could not find in either bloodvessels in the enamel pulpa.

As stated just now, only in 1911 do we meet with the description of a second instance, and while POULTON found bloodvessels in the enamel organs of rudimentary teeth, such as are met with in Ornithorhynchus, HOPEWELL-SMITH and MARETT-TIMS now discovered bloodvessels in the enamel organ of teeth in a later developmental stage. They examined the development of the dentition in *Macropus billardieri*. According to their description the bloodvessels penetrate in several places through the external epithelium, and the section of these vessels was large enough to show blood-corpuscles. They did not find any bloodvessels in the stratum intermedium. The authors correlate this vascularization with the peculiar feature in the dental development of Macropus, that the ameloblasts begin to form enamel even prior to the formation of dentin. SMITH and TIMS write: "It is extremely probable that the unusual vascularity of the enamel-organs is correlated with the precocious development of the enamel."

A similar observation has been made by MUMMERY in an allied species, viz. in *Macropus rufus*. In his "The microscopical and general anatomy of the teeth" he says on this point: "bloodvessels are seen crossing the external epithelium and also lying within the stellate reticulum, an appearance seen in many sections."

An extensive description of a vascularized enamel-organ in another marsupial — viz. *Phascolarctos cinereus* — is given by BOLK ('15). He lays stress on the fact that a network of bloodvessels forms in the surrounding connective tissue at the transition from the enamel-septum to the external epithelium. From this network vascula force themselves into the enamel septum and thence into the pulpa, but also in other places bloodvessels grow through the external epithelium. Two layers are distinctly noticeable at the vascular reticulum in the pulpa the one more or less superficial, consisting of the larger vessels, and the other, situated deeper down, contiguous to the ameloblasts, and consisting of narrower vessels. The author adds that between the enamel pulp and the amelogenetic cells there lies only a thin layer of less differentiated cells, which should not be considered as a true stratum intermedium.

Also in the premolars of the *calf* vascularized enamel-organs were found by BROOMELL and FISCHELIS ('17), as is briefly indicated in a communication by ADDISON and APPLETON. A peculiar feature of this case is that the vessels could be traced as far as the stratum intermedium.

The observations of HOPEWELL-SMITH, MARETT TIMS and MUMMERY on the vascularization of the enamel-organs in the genus Macropus have been corroborated by CARTER. He says: "HOPEWELL-SMITH and MARETT TIMS referring to the vascularity of the enamel-organ in Macropus billardieri, state that they were unable to trace the capillaries beyond a point midway between the outer and inner enamel epithelium, but in my preparation vessels of a lumen from 20 μ to 30 μ in diameter are frequently seen lying in contact with the cells of the stratum intermedium."

CARTER found these relations in *Macropus ruficollis*.

ED. PASCALIS ('18) and SKILLEN ('21) worked with a technique quite new for this inquiry. They injected their preparations. PASCALIS studied the vascularization of the teeth during the development, and to this end he examined a *cat's*-embryo of 10 cm in length — a newborn *cat* — one of 1 week, one of 2, 3, 4, 5 weeks and a full-grown *cat*; also a newborn *dog* and a *human fetus*.

With all of them he finds: "les vaisseaux adamantins forment, *à la surface* de l'organe de l'émail un réseau comparable à celui de la pulpe, mais à mailles plus serrées et formées de capillaires de plus gros calibre."

SKILLEN also worked with much material and examined the development of the dentition in the *pig* — the *dog* — the *sheep*, the *rabbit*, the *cavia* and some earlier stages in *man*. He used injected and non-injected preparations, but could not detect any bloodvessels in the enamel-organ.

JORDAN's researches date from the same year. He studied an "anlage" of

a tooth from the lower jaw of a *cat* and felt convinced that vascularization of the enamel-organ does not occur here.

In a second publication he makes mention of his observations of an incisor of the *white rat* in a period from 1 to 7 days after birth and of the tooth-germ from the lower jaw of a newborn *cat*, and of cats, 1—2 and 3 weeks old. He finds that the incisor of the white rat is not furnished with bloodvessels, while in the cat the bloodvessels push the outer epithelium slightly forward. Hereby vascular papillae are generated, which were first described by WILLIAMS, and are called after him WILLIAMS's papillary layer.

In 1922 ADDISON and APPLETON record the occurrence of vascularization of the enamel-organ of the first molar in the upper-, and the lower-jaw. Their material comprised different stages in the development of the *white rat*. On the 21st day of the intrauterine life vessels have really penetrated into the pulpa. In the following stages the vessels have grown deeper into the pulpa, and approach the stratum intermedium. In the three-day-old rat the vessels are contiguous to the stratum intermedium. It is interesting that ....."at the time of the entrance of the first bloodvessels into the enamel-organ (21 days' fetus) no enamel has yet been formed. As the number of entering bloodvessels increase, and with their deeper penetration into the stellate reticulum, amelogenesis begins. Not until the bloodvessels have penetrated to the vicinity of the stratum intermedium is there a well-defined layer of enamel to be seen."

The occurrence of perivascular lymph-spaces, first described by BOLK ('15) could be confirmed by them.

Although many of the above descriptions are conclusive for the occurrence of vascularized enamel-organs, still it is discredited by some observers.

JORDAN says that he is very sceptical as to the cases of vascularization described. He does not consider them conclusive.

PRENANT ('24) sides with JORDAN and holds that the would-be capillaries apparently found in the pulpa are nothing else but severed vascular papillae of WILLIAMS, so that the vessels are still enclosed by outer epithelium.

I do not agree with these authors, and feel convinced that bloodvessels may indeed occur in the enamel pulp, but that this depends on the species and the age of the animal. Vascularized enamel-organs are by no means present in every animal species, and they are found only in a definite developmental stage.

When summarizing the various publications on the vascularization of the enamel-organ it appears, that most authors deny its occurrence, but that the experience of some workers is that an entrance of bloodvessels into the enamel-organ can unmistakably be witnessed. What strikes us most is that the same positive results were obtained with various species of Marsupialia, viz. with Phascolarctos cinereus, Macropus rufus, Macropus

ruficollis and Macropus billardieri, in all of which bloodvessels were found in the enamel-pulpa. This indicates that with the species of Marsupialia the occurrence of this vascularization may reasonably be expected to be much more general than has hitherto been found.

Secondly the conviction grows upon us, that there is some connection between this supply of bloodvessels and amelogenesis.

HOPEWELL-SMITH and MARETT TIMS already pointed to this, as they found that the vascularization was attended with a precocious development of the enamel. ADDISON and APPLETON also came to the conclusion that: "The vascularization of the enamel-organ is closely connected chronologically with the beginning of amelogenesis.

Let us state in the third place that BOLK established the absence of a stratum intermedium in Phascolarctos cinereus, and correlated this with the precocious vascularization.

CARTER arrives at the same conclusion in his examination of Macropus ruficollis, when he says: "when growth is very rapid the stellate reticulum may be seen in contact with the ameloblasts without the intervention of the stratum intermedium."

That this is not always the case, is shown by the inquiry of ADDISON and APPLETON, in which the stratum intermedium was present in all the stages they studied.

In the fourth place we may ask why the vascular system comes so soon into contact with the stratum intermedium and the ameloblasts.

When there is no vascularized enamel-organ, we see that, after the reduction of the pulpa, the bloodvessels come into close contact with the ameloblasts. The pulpa provided nourishment for the ameloblasts, and after the reduction of the pulpa this function is assumed by the bloodvessels. What, however, should we think of those cases in which the enamel-organ is still fully developed and the bloodvessels penetrate into the pulpa and are contiguous to the stratum intermedium? At the conclusion of this paper I hope to recur to this point.

My own observation of vascularization of the enamel-organ was made on an embryo of Dactilomys.

Frontal sections of 20 μ of the preparation were stained with hematoxylin. Three tooth-germs were present in the upper- and the lower-jaw, on the left as well as on the right.

Dactilomys belongs to the Hystricomorpha, a subdivision of the Rodentia, whose complete dental formula is

$$I\,\tfrac{1}{1}\,P\,\tfrac{4}{4}\,M\,\tfrac{1\,2\,3}{1\,2\,3}.$$

There is no succession, so that the germs of I—P and M_1 are present.

Amelogenesis has not yet begun, but dentinification has. In the incisors the dentin already consists of a thick layer, as may be seen in fig. 1 representing a section through the "anlage" of an incisor from the

upper jaw. The enamel-organ has slightly receded from the adjacent connective tissue, also a fissure has arisen on either side of the dentin through the retraction of the dental papilla and the ameloblasts. A stratum intermedium is well-developed. The section has cut the tooth-band only a little near the mouth-epithelium.

However, what attracts our attention most is the occurrence of blood-vessels in the enamel-pulp. Vascularization can be seen in all the sections of this tooth. The bloodvessels may distinctly be traced from the adjacent connective tissue into the pulpa. The vascula that appear as strands of

Fig. 1.

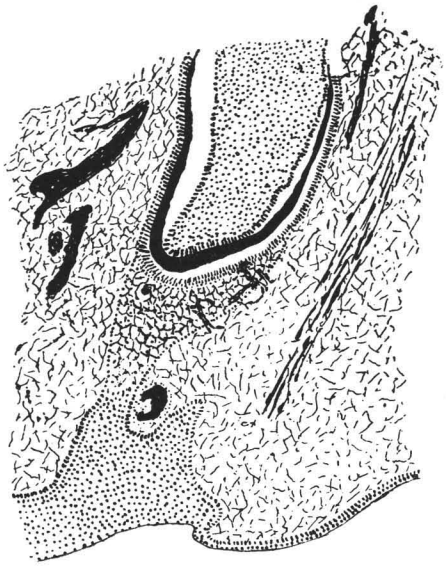

Fig. 2.

spindle-shaped cells, pass through the enamel-organ in all directions, but never enter the stratum intermedium. They can only be observed as far as this layer.

When proceeding to the following sections we notice (Fig. 2) another peculiarity of the vascularized enamel-organ, viz. that here vessels penetrate into the enamel-organ exclusively on the buccal side. This, however, is the exception, but when comparing the various sections of the incisivi, we shall observe that most vessels make their way into the enamel organ on the buccal side of the dental ledge. This is also the case with the premolars.

Something like this was also observed by ADDISON and APPLETON in their preparations. They ascribed it to the structure and the position of the enamel-organ. They correlated this phenomenon in the first place with the difference in the length of the lingual and the buccal sides of the organ, measured from the point of attachment of the dental ridge to this organ. The authors say: "This difference in length of the lingual and buccal portions, seems to be of importance in connection with the site of entrance

of the blood-vessels into the enamel-organ." This difference in length also occurs in my preparations.

But the relations in my case are the reverse of those found by ADDISON and APPLETON. This may be seen from a comparison of fig. 2 with what they write. In the figure the buccal area is considerably longer than the lingual area. The said authors write: "The fact that the palatal surface of the external epithelium is considerably wider than the buccal or lateral surface would (other things being equal) in itself afford opportunity for the entrance of more bloodvessels through the palatal surface, than through the buccal surface."

The authors have preceded these descriptions by the statement that the dental ledge is attached medially to the enamel-organ and say that: "the lingual surface of the dental ledge is almost in a straight line with the adjoining lingual surface of the external enamel epithelium", so that I may be allowed to conclude that this reversion of the relations is the consequence of a misconception, and that, therefore, the relations in their preparations agree with those I found in Dactilomys.

Secondly ADDISON and APPLETON ascribed it to the position of the dental germ relative to the alveolus of the tooth, as is expressed in the following quotation: "This advantage is further increased by the fact that the buccal surface of the external enamel epithelium is for almost its full extent in close contact with the osteogenetic membrane or with the developing bone itself of the maxilla. This proximity would seem to interfere with the free access of blood-vessels to this side of the enamel-organ."

After applying the correction alluded to above we see, then, that the relations are the same also here.

On close inspection of fig. 2 our attention is arrested by the presence of a rudimentary tooth. Buccally to the dental ledge we see it as a conoid mass of dentine. It is contiguous to the epithelium of the mouth-cavity and I could not detect an enamel-organ, but I observed that the connective tissue has thickened round the toothlet.

Similar rudimentary toothlets were present in the upper- and in the lower-jaw. They were always disposed labially to the germ of the incisors. They have often been described in papers on rodents and have been considered as the remains of a milk dentition.

Fig. 2 shows us still more. Hitherto we have always been speaking of "dental ledge". May we do so? In the figure the ledge is obviously many cells broad and it seems to me that the enamel-organ is rather suspended directly to the mouth-epithelium, without the intervention of a dental ledge. The epithelial cells are seen to pass gradually into the pulpal cells.

The germ of the incisivi from the lower-jaw is much smaller than of those of the upper-jaw. Fig. 3, representing a cross-section through such an incisor, shows us a veritable network of vessels in the enamel-pulp. This

vascular reticulium has penetrated into all the parts of the pulpa, and is supplied by vessels that have grown through the outer epithelium in several

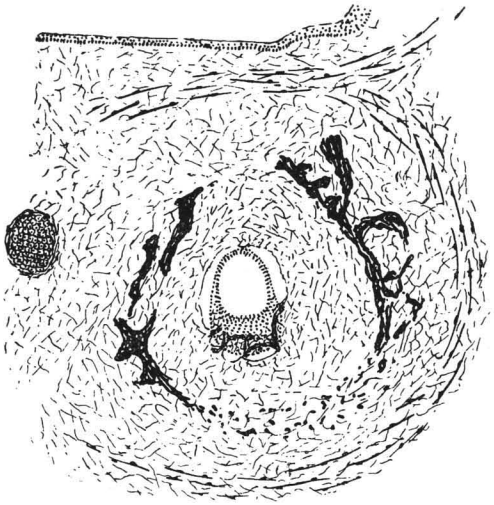

Fig. 3.

places. The stratum intermedium is very broad, the vessels do not enter here either. What we do see again, is that the vessels are seen to run up against this layer. The outer-, and the inner-epithelium are separated only on one side from the enamel-organ by pulpal tissue. On the other side they are adjoined. This is easy to understand, if we reflect that we have to do here with the rudiment of an incisor of a rodent, which displays amelogenesis only on the labial surface.

The section has not cut the dentine. Owing to the retraction it does not lie in the plane of the figure.

I fancied I had also found in this preparation the perivascular lymphspaces described by BOLK. We see them already in fig. 2, but better still in fig. 4. The vessels are surrounded here by broad spaces. Are these the same lumina that BOLK observed? The spaces shown by the figure may be artefacts. The enamel-pulp is extremely aqueous. Fixation of course makes it shrivel, thus causing the spaces. This view is still favoured by the fact that they are connected with that fissure round the dental germ, which is no doubt the result of the shrivelling.

These fissures in the incisivi I also found in the premolars. They also have a vascularized enamel-organ.

Fig. 5 represents a premolar from the upper-jaw. Here again we observe retraction through shrivelling. The odontoblasts have scarcely started the formation of the dentine. Only an extremely thin layer lines these cells. The vessels penetrate from the enclosing connective tissue into the enamel-organ, but they never reach the stratum intermedium, as was the case with the incisivi. There is always a layer of stellate reticulum between the

capillaries and the stratum intermedium. But here also the vessels cross and recross the pulpa, being most numerous between the folds of the molars.

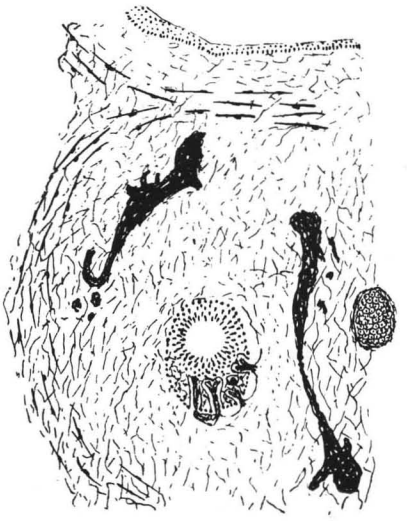

Fig. 4.

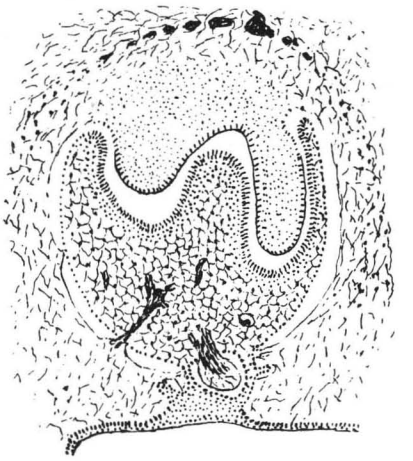

Fig. 5.

Another peculiarity is shown by this picture. There is namely an enamel-niche in the premolars of the upperjaw and in all the molars. In the false molars they have already developed into tunnels.

Whereas in the incisivi and the premolars the enamel-organ was completely developed, this appears not to be the case with the molars. The odontoblasts just begin to arrange themselves and as yet there is no question about dentinification. Neither has the enamel-pulp been differentiated, while the external epithelium is cubic. We do not meet with vascularization here either. I was going to say *as yet* for I am convinced that the bloodvessels enter into the enamel-organ only during the development. The incisivi that began to develop first, display a vascularization that is furthest advanced. The vessels close up to the cells of the stratum intermedium. In the premolars this process has not yet advanced so far. Here the vascularization is of a more recent date, whereas in the molars no blood-vessels have at all entered into the enamel-organ.

Amelogenesis begins only after the bloodvessels have penetrated far into the pulpa. Dentinification, however, begins about simultaneously with the entrance of the vessels, and has proceeded far when the vessels have reached the stratum intermedium.

But why do the bloodvessels enter into the enamel-organ at so early a stage? If what BOLK says, is true, viz. that in non-vascularized enamel-organs the enamel-pulp is intended for the nourishment of the ameloblasts, of which I am convinced, I am bound to conclude that in the case of vascularization of the enamel-organ the pulpa can no longer meet the demands of this nutrition, and that this higher function is assumed by the

bloodvessels themselves. If the enamel-organ is not vascularized, the bloodvessels come into contact with the ameloblasts only after reduction of the pulpa, but according as the demands of this nutrition increase, the bloodvessels are sure to assume sooner the task of the pulpa, i.e. they enter into the enamel-organ.

In Dactilomys we saw that amelogenesis occurs only after the bloodvessels have reached the stratum intermedium. So the bloodvessels grow into the enamel-organ at an early stage, and take over the nutrition of the ameloblasts. But I insist on it that here also the amelobasts are fed first by the enamel-pulp and only later on by the bloodvessels.

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