VASCULARIZATION OF THE CORNEA OF THE RAT IN RIBOFLAVIN DEFICIENCY, WITH A NOTE ON CORNEAL VASCULARIZATION IN VITAMIN A DEFICIENCY

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Plates 1 to 4

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Vascularization of the cornea is an early and constant phenomenon in albino rats in riboflavin deficiency. It precedes all other demonstrable lesions of the deficiency.

The vascularization was first observed by us in the routine histological examination of rats deficient in vitamin G (the heat-stable fraction of the vitamin B complex) (1). Subsequent experimentation showed that riboflavin alone was concerned. Control rats subject to the following conditions did not develop corneal vascularization: B1 deficiency, B6 deficiency, fasting, old age, maintenance on the experimental diet plus riboflavin.

Cataract has been associated, chiefly by Day and his fellow workers, with vitamin B2 or G deficiency (the heat-stable vitamin B complex)1 in rats (2, 3) and in other animals; mice, chicken and monkeys (4). They obtained an incidence of cataract in rats of nearly 100 per cent and regarded this lesion as a better criterion of vitamin G deficiency than dermatitis (3). Recently Day, Darby and Cosgrove (5, 6) showed that cataract in their rats was due solely to riboflavin deficiency.

Cataract was of rare occurrence in our experimental rats. Animals from three different colonies were used as controls against stock variability. The few occasions of its occurrence indicated a litter susceptibility. It also appeared more often in the few animals on which we used grain extracts instead of yeast extract as a source of other essential dietary factors. However, we have made no attempt to duplicate exactly the conditions of the experiments by Day and his associates.

1 In this paper, vitamin B2, vitamin B2 complex, vitamin G and vitamin G complex as used are all equivalent to the heat-stable vitamin B complex fraction.
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Other workers also have not been so successful in producing cataract in rats by means of vitamin B2(G)-deficient diets. Bourne and Pyke (7) obtained only an incidence of 31 per cent. György (8) did not obtain cataract in a large series of rats. Richardson and Hogan (9) rarely saw cataract in their vitamin G complex-deficient rats.

While keratitis is frequently noted in descriptions of vitamin B2 or G deficiency in rats, there are only meager accounts of the histology of the cornea and only two references to the presence of blood vessels. Pappenheimer describes a section from one of Sherman and Sandel's (10) vitamin G-deficient rats as showing in the cornea “a very slight keratitis, with corneal corpuscles, a few polymorphonuclears, and some new-formed blood channels.”

Day, Langston, and O'Brien (2) gave, as their sole histological description of the cornea in vitamin G deficiency, the following: “Microscopic examination of the cornea revealed an inflammatory process in the anterior stroma. The epithelium was normal but small lymphocytic and leucocytic infiltrates accompanied by new blood vessel formation were found directly under the epithelium.”

Cataract as a consequence of riboflavin deficiency is probably dependent upon strain susceptibility and certain experimental conditions employed by Day. On the other hand, corneal vascularization invariably occurs when riboflavin is absent from the diet. Corneal changes, observable in gross, indicating the presence of vascularization, have occurred in over 300 rats in a period of more than 2 years. Microscopic examination or India ink injections have never failed to show it after the 4th week of riboflavin deficiency.

Material and Methods

The Rats.—Rats from three different colonies, including Wistar Institute rats, have been used. The employment of three types of breeding rations—(a) whole wheat and whole milk powder (Sherman diet 13); (b) dog chow and (c) scratch feed—has served as a control on the influence of the pre-experimental diet upon the deficiency. In all cases, with the exception of slight time variations, the pathology has been the same. The rats were placed on the experimental riboflavin deficient diet when 21 to 28 days old at 40 to 50 gm. weights.

The Diet.—In our earlier vitamin G-deficient experiments before the multiple nature of the heat-stable vitamin B complex was established (5), the Sherman-Bourquin (10) diet was used. We now know that this diet was primarily deficient in riboflavin (11) but also often contained only small amounts of vitamin B6 and other essential factors.

The riboflavin-deficient diet which we now employ has the following composition.
Casein\(^2\) ............................................................ 18
Osborne and Mendel salt mixture ....................................... 4
Cod liver oil .......................................................... 2
Sugar .............................................................. 20
Cornstarch\(^3\) ........................................................ 48
Peanut oil .......................................................... 8

40 \(\gamma\) of pure vitamin B\(_1\) (thiamin) were given on alternate days.

2 gm. (yeast equivalent) of riboflavin-free yeast extract\(^4\) were given daily, after the first 10 days, as a source of vitamin B\(_2\) and other B factors.

Rats upon this diet cease to grow after 3 weeks but will often live 12 to 15 weeks, occasionally to 18 weeks. The completeness of the diet was proved by the fact that the addition of synthetic riboflavin yielded sustained satisfactory growth without demonstrable pathology in control rats.

The vitamin B\(_6\)-deficient diet was the same as the above except that 20 \(\gamma\) of riboflavin was added daily in place of the yeast extract.

**Clinical Course**

The rats cease to grow after 3 weeks. In 5 to 7 weeks the palpebral fissures become noticeably smaller, the eyeballs are less prominent and appear sunken in the orbits. The lids are slightly swollen and appear to be edematous. The tail becomes dry and scaly. The hair has lost its luster. Soon after there may be small amounts of serous blood-tinged exudate in the conjunctival sac and the lids may become stuck together. The corneas become slightly dull as if finely sanded; after 7 to 10 weeks, one or both corneas may become turbid and white. At about this time, denuded patches begin to appear about the eyes, head, shoulders and back. The skin of the paws and legs becomes dry and scaly. Later, some of the denuded areas become red, moist and even ulcerated, particularly on the paws and upon

\(^2\) Extracted twice by shaking with 60 per cent alcohol and twice by refluxing with 95 per cent alcohol.

\(^3\) Extracted twice by refluxing with 95 per cent alcohol.

\(^4\) 200 gm. of dry brewers' yeast and 1 liter of water were brought, while stirring, to boiling; cooled; 200 cc. methyl alcohol added; supernatant liquid decanted or centrifuged; the alcohol distilled off; HCl added until acid to Congo red; treated twice while stirring with small portions of fuller's earth (tonsil, L. A. Solomon Brothers, New York) until free of riboflavin as tested by absence of fluorescence to ultraviolet light. Distilled at 60°C. or evaporated until 1 cc. equalled 2 gm. yeast.
the head, neck, and shoulders and behind the ears. The distribution
and severity of these skin lesions are influenced by scratching and
gnawing by the animal. The urine becomes concentrated and highly
colored. The animal is dehydrated as indicated by the rapid increase
of weight due to water intake when riboflavin is given to a rat still
capable of recovery.

The pathology of the skin and other organs of riboflavin-deficient
rats will be described in another report. In this we shall describe
only the eye in relation to vascularization of the cornea.

**The Vascularization of the Cornea**

By the end of the 4th week of the riboflavin deficiency, there is
a marked radial ingrowth of capillaries into the cornea from the vessels
of the limbus. This happens before any change in the cornea visible
in the gross has taken place. The transparency is undiminished.
While these vessels may be seen by slit-lamp illumination, no turbidity
of the cornea or change in the corneal epithelium is revealed by this
method or by histological study. By the end of the 10th or 11th
week, the blood vessels extend inwards for more than one-third of
the diameter of the cornea and some may reach nearly to the center.
The time of onset and abundance of the vessels varies, and these differ-
ces occurred even among litter mates. Rats which had a pre-experi-
mental diet high in riboflavin developed the corneal vascularization
slightly later than those which had a low riboflavin diet (scratch feed).
We have followed the initiation, progress and repair of this process
by histological sections, injected specimens and by slit-lamp observa-
tions, through the cooperation of Dr. T. Gundersen of the Department
of Ophthalmology, Harvard Medical School.

Injected specimens gave the most information about the manner of growth of
the vessels, their source, and the circulation in the corneal limbus of the normal
eye. They were prepared by injecting 25 per cent aqueous India ink, at a pressure
of about 75 mm. Hg into the aorta, through the left ventricle of the living anesthe-
tized animal. The whole eye, with the lids and orbital glands, was fixed in 10 per
cent formalin, dehydrated in alcohol, and cleared in oil of cedarwood. In some
instances, one eye was fixed in Zenker's fluid for comparison by histological study.
The cleared injected eye was first studied as a whole. For high magnification and
for photographs, the anterior part of the eye was removed by a circumferential cut
posterior to the plane of the attachment of the ciliary body. Four radial incisions
were made in order to flatten the curved disc. The iris was next removed. The first preparations made were damaged in the removal of the lids from the eyeball by forcibly tearing away the conjunctiva at its attachment to the globe. This line of attachment extends forward above the limbus of the cornea and when pulled away often carries with it the blood vessels of the limbus. The specimens thus prepared were mounted in compression cells for immediate study. After a few days under pressure they remain flat enough to be used between slide and cover slip.

Source of the Corneal Vessels.—The corneal vessels arise from a rich arteriovenous plexus which encircles the cornea in the limbus and which corresponds to the superficial marginal plexus of the cornea of the human eye.

The arterial supply of this plexus is an encircling artery formed by the anastomosis of the branches of two short trunks which usually arise, one each from the lateral and mesial long posterior ciliary arteries just before these divide to form the greater arterial circle of the iris. Each short arterial trunk divides once into superior and inferior branches which join their fellows of the opposite side. This circular artery of the limbus may have slightly different origins. The main trunks may arise from either branch of the long posterior ciliary artery or each branch of the latter may give rise to an artery. There may be combinations of these methods of origin in the same eye (Fig. 1).

There is great variation in the veins which accompany the circular artery. In general there are two or more roughly parallel freely anastomosing veins, one or more on each side of the artery. In general, the veins lie deep to the artery. The veins empty into several large trunks and communicate as well with the venous plexus of the sclera.

The plexus of the limbus consists of a series of loops, freely inosculating with one another and forming a band 0.2 mm. to 0.3 mm. in width internal to the arterial circle. We have made no attempt at a complete study of this plexus. It is more complex in the young rat than in the old. Fig. 2 is from a normal weight control rat and therefore 3 to 4 weeks old. Fig. 3 is from a normal age control rat and therefore 13 to 14 weeks old. These are representative of a few examples at each age period. The drawings are presented in lieu of an attempt at a more detailed description.

The circular artery of the limbus also gives rise to branches which go to the palpebral conjunctiva, to the nictitating membrane and to the sclera. We could find no branches to the ciliary body and iris.

Manner of Growth of the Corneal Vessels.—The early pattern made by the advancing capillaries, 4th to 7th week of the deficiency, is more complex because of the great abundance of anastomoses (Figs. 4 and 5). The pattern is lace-like and on the advancing border fringed with glomerulus-like loops and arrow-head-like pointed sprouts. Venous connections are even more abundant than arterial and it is impossible to avoid concluding that simultaneous growth from arterial and venous sources takes place. Except for very short pointed sprouts usually situated near the middle of loops, the advancing zone of capillaries is composed
of anastomosing loops. The appearance suggests that the pointed sprouts split behind as they grow, which would be one way of maintaining a pressure gradient. Another possibility is that pool-like expansions of loops advance toward the center of the cornea while contracting on the peripheral side. Lateral bands from arms of loops and from short pointed sprouts give abundant evidence of the establishment of anastomoses by coalescence.

At later periods, 7 weeks or more, (Figs. 6, 7, 8, 9) we find conspicuous radially directed capillaries, arterial and venous in relation to their sources, which are connected by very small calibered vessels, often barely brought out by the India ink mass. This appearance we interpret as due to the closure of many of the early loops. Serial observations on the living animal will be required to determine the exact manner of growth of the capillaries into the cornea. The many publications of the Clarks (12, 13) and their associates and Sandison (14) upon the growth of blood vessels as observed through transparent chambers in the rabbit's ear may well serve as a model for such studies.

The invading capillaries at first lie just beneath the corneal epithelium, but soon others come to lie deep in the tunica propria. By the end of survival time in attempted absolute riboflavin deficiency, 12 to 18 weeks, the blood vessels extend across the cornea nearly to its center.

Behavior of the Blood Vessels in Recovery from the Deficiency

Slit-lamp observation reveals the capillaries in the 4th to 6th week of the deficiency. Turbidity of the cornea does not appear until many days and often several weeks after the vessels are conspicuous under the slit-lamp. Moderate degrees of turbidity of the cornea disappear as soon as 12 hours after giving 60 $\gamma$ of riboflavin by mouth. Unless severely damaged, the cornea is clear within 48 hours. After 2 weeks' treatment with 20 $\gamma$ of riboflavin daily, the blood vessels can no longer be seen by an experienced observer with the slit-lamp. They may, however, be demonstrated in sections and by India ink injection. We do not know how long they persist, but they are present in abundance in perfectly clear apparently normal corneas as late as 58 days under adequate riboflavin treatment. They persist long after leucocytic infiltration has disappeared and the tunica propria is restored to normal in all other respects.

5 $\gamma$ daily is an adequate protective dose against vascularization.
The earliest recovery period studied by the injection method was 7 days. At this time, evidences of growth activity were almost absent. The glomerulus-like structure, the pools and pointed sprouts, had largely disappeared. The inner border of the vascular zone presented only loops and rare V-shaped loops with pointed extremities. There was marked diminution of caliber of many vessels, particularly of lateral anastomotic branches. A few vessels showed numerous constrictions. In preparations after 12 to 16 days of recovery, the simplification of the vascular pattern was considerable (Fig. 10). All evidences of growth were absent. The constrictions of the radially directed vessels were much more pronounced, producing an effect of beading. By the 25th day of recovery, the pattern was greatly simplified (Fig. 12). The beading effect was generally present. Many long radially directed capillaries now appeared as flattened spirally twisted bands (Fig. 11). The rate of change from the 25th to the 58th day was not so marked and may be summarized as a steadily increasing diminution in number and caliber of the capillaries and a retreat of the vessels from the center of the cornea toward the periphery (Fig. 13).

**Correlation of the Injected Specimens with the Histological Changes in the Cornea**

1. **The Vascularization.**—The first capillaries to be seen lie just beneath the corneal epithelium, close to the limbus. Later they appear deep in the tunica propria. They may extend far toward the center of the cornea before leucocytes appear outside of the vessels (Fig. 14). The corneal epithelium remains unchanged until late in the deficiency and then undergoes degenerative changes which we regard as secondary to the lesions in the tunica propria.

Capillaries in cross section have sharply defined circular outlines, and the collagen lamellae are bowed around them. In longitudinal sections, the capillaries are seen to lie between lamellae. The more advanced the deficiency, the more prominent are the endothelial cells and the more numerous are the mitotic figures in the capillary walls. The capillaries can be traced obliquely downward into the depths of the tunica propria; rarely they descend almost perpendicularly to the surface. Only rarely and in very advanced stages of the deficiency have we found the blood vessels deeper than the junction of the middle and lower (deep) third of the tunica propria. While the blood vessels reach a considerable size, they remain capillaries in structure. We could find no indication of the formation of a muscular coat even in the experiments of longest duration, 16 to 18 weeks.

Leucocytes are found in small numbers within a week or two after the vessels have penetrated the cornea. They become progressively more numerous and consist chiefly of polymorphonuclear leucocytes. A few lymphoid cells can be recognized and rarely a mononuclear wandering cell corresponding to the monocyte or mononuclear phagocytic cell. The leucocytes, for the most part, are strung out between the lamellae. They may accumulate in great numbers beneath the
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corneal epithelium, especially near the limbus. In advanced stages of the deficiency, great numbers of leucocytes collect in the central non-vascularized portion of the cornea and the epithelium becomes invaded by them (Fig. 15). The collagen of the central portion becomes markedly changed; the collagen fibrils are replaced by a lightly staining non-fibrillary material, as if the fibrils had swollen and fused. When blood vessels have entered such regions, we find an increase of fibroblasts. The newly formed fibroblasts lie adjacent to the blood vessels; there may be numerous ones in sections. There is no special arrangement of these fibroblasts and they may have their long diameter obliquely or perpendicularly aligned with regard to the normal plane of the lamellae of the corneal connective tissue. Rarely we have found areas of densely stained hyaline collagen without fibrillary structure which we have interpreted as evidence of necrosis.

The corneal epithelium over the regions with the most advanced vascularization and infiltration is often markedly changed. The deep layer of cells remains surprisingly persistent. The superficial cells become separated and vesicles may form between the superficial and deep layers of the epithelium. Necrosis and ulceration are very late consequences. The split-lamp, after vascularization is well established but before the cornea becomes turbid, shows at most an occasional point of light reflection, probably indicative of separated superficial cells of the epithelium. Histologically, desquamating cells can be found but not in greater numbers than in the various types of control eyes we have used. Even should this inconstant slit-lamp observation be indicative of an epithelial lesion, it is not conceivable that a lesion of such insignificance and elusiveness should be responsible, in any familiar sense, for the vascularization and subsequent degeneration of the tunica propria.

Throughout the progress of the deficiency, Descemet's membrane and the endothelium covering it show no changes that could be demonstrated by any of a variety of staining methods.

2. Repair.—By the 7th day after restoration of riboflavin to the diet, leucocytes, with rare exceptions, have disappeared from the tunica propria and from the corneal epithelium. The capillaries by this time have become smaller in caliber. After the 12th day of repair, the vessels could not be seen by an experienced observer using the slit-lamp illumination. They can be found with ease in histological sections, though these give no indication of the number and size of the capillaries that are brought to view by the India ink injections.

In general, the study of sections gives the impression that in repair there is a progressive diminution in the number of vessels and in size. It would seem that the pressure used in making the injections opens capillaries that were collapsed. Sections of cornea injected with India ink confirm this impression in that thin lines of the pigment may be found, without accompanying blood corpuscles and with no recognizable endothelial cells, or the latter indistinguishable from the cells of the cornea. Serial sections show that the ink occupies slits of considerable width, indicating compression of the capillaries between the layers of connective tissue lamellae. Careful search is required at all periods of repair to find any evi-
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dence of degeneration of capillary endothelium or of entrapped red blood corpuscles.

The former is indicated by a rare swollen finely vacuolated endothelial cell in situ. The latter is shown by phagocytosed hemosiderin granules. In all corneas, after 2 weeks of repair, all evidences of degenerative changes of the epithelium and of the tunica propria were absent. Fibrin was never found. The appearances in sections, whether of non-injected or of ink-injected eyes, suggest that circulation in part ceases before the endothelium disappears and that the final closure is accompanied or brought about by compression between the connective tissue lamellae. The persistence of vessels for periods up to 58 days, inasmuch as some of these contain normal appearing blood corpuscles and are lined by normal looking endothelial cells, suggests that some of them may persist indefinitely.

Vascularization of the Rat's Cornea in Vitamin A Deficiency

Wolbach and Howe (15) in 1925 described vascularization of the cornea of rats in vitamin A deficiency, accompanied by leucocytic infiltration and changes which were interpreted as edema of the tunica propria. The vascularization was regarded as a phenomenon secondary to the hyperkeratinization of the corneal epithelium and it was suggested that the vascularization was a physiological response to the increased growth rate of the corneal epithelium. We have reviewed the vitamin A deficiency material and apart from the hyperkeratinization of the corneal and conjunctival epithelia, find great similarities with our findings in riboflavin deficiency. The accumulation of desquamated cornified cells in the conjunctival sac and the consequent inflammatory response might be regarded as adequate cause for ingrowth of blood vessels. However, in vitamin A deficiency, the ingrowth of capillaries takes place concurrently with the epithelial changes. Considerable vascularization may be present before there is any considerable accumulation of desquamated cells and before there are more than early histological signs of inflammatory reaction in the conjunctival limbus. We are disposed to discard an inflammatory explanation of the vascularization. Always, however, the presence of capillaries in the cornea was accompanied by a characteristic change in the corneal epithelium, indicative of the shift to keratinizing metaplasia. The speculation is warranted that an important factor in causation may be a change in permeability of the epithelium in its effect upon the respiration of the cornea as a whole. The details of repair following restoration of vitamin A to the diet
by the addition of butter fat or cod liver oil, though not followed by the injection method, in general parallel those of repair from riboflavin deficiency.

The closure of the vessels takes place in the same manner but apparently takes place more rapidly. This we infer by the ease with which entrapped and disintegrating red blood cells are found. Also swollen and vacuolated endothelial cells are found with ease and after the 2nd week hemosiderin granules are often present within endothelial cells and strung out in closed capillaries. It has been possible to follow in serial sections strings of hemosiderin granules into an endothelial lined space containing granular deeply eosinophilic red blood cells, thus confirming the riboflavin repair closure of capillaries by intermittent constriction. Our impression is that for the periods of repair studied, up to 43 days, obliteration of the vessels due to vitamin A deficiency is more complete than that due to riboflavin deficiency.

DISCUSSION

The riboflavin used in the repair experiments and in the purified diets used in control experiments was pure synthetic riboflavin made by one of us. Therefore, the effects described by us could not have been due to an impurity.

The clinical observations and histological studies rule out demonstrable injury of any nature as the inciting factor of the vascularization. The role of riboflavin as a respiratory carrier suggests that the vascularization is a response to asphyxia. Since the prevailing opinion is that the cornea respires through its external surface, the failure of the epithelium to transport oxygen seems to be an explanation of the sequence of histologic events. However, it is also reasonable to assume that the vascularization may be a response to the respiratory needs of the epithelium itself. If respiration of the cornea is dependent upon the epithelium by virtue of riboflavin activity, the vascularization in vitamin A deficiency could also be accounted for on the basis of an altered physiology accompanying the keratinizing metaplasia. We have not seen vascularization of the cornea of guinea pigs, either in riboflavin deficiency or in vitamin A deficiency. In the rat in riboflavin deficiency, there is no vascularization of cartilage.
As a means for the study in a mammal, of growth and regression of capillaries, riboflavin deficiency has obvious advantages which we have made little attempt to explore. Our only histological observation that may possibly be relevant to the problem is that in common with vitamin A deficiency, there is disappearance of the yellow material in the acini of the Harderian glands. In both deficiencies, the vascularization is present before the pigment has entirely disappeared.

SUMMARY AND CONCLUSIONS

Vascularization of the cornea of the rat in the absence of antecedent pathology is probably a specific and the most reliable criterion of riboflavin deficiency.

Its initiation and repair may be used for testing the biological activity of compounds structurally related to riboflavin.

The facts that the invading capillaries are easily visible in the living animal and that the growth and regression of the blood vessels are under dietary control and for a considerable period of time unaccompanied by other pathological reactions, make this method very suitable for the study of problems related to capillary growth.

We believe that the best hypothesis in explanation is that the vascularization is a response to asphyxia of the tunica propria.

BIBLIOGRAPHY

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EXPLANATION OF PLATES

PLATE 1

Fig. 1. Drawing of cornea of an injected rat. 77 days on riboflavin-deficient diet. Reproduced principally to show the circular artery of the limbus. Figs. 6, 7, 8, and 9 are from this animal.

Fig. 2. Normal rat, weight control. India ink injection to show the plexus of the corneal limbus. Arteries black. Veins stippled. × about 91.

Fig. 3. Normal rat. Age control. India ink injection. The limbic plexus. Arteries black, veins stippled. × about 91.
PLATE 2

Fig. 4. Early growth of blood vessels into the cornea. Rat 5 weeks on riboflavin-deficient diet. India ink injection. × about 23.

Fig. 5. Riboflavin-deficient rat. 7 weeks. India ink injection. The number of new vessels and the complexity of the pattern are greater than usual. × about 23.

Fig. 6. Same rat illustrated in Fig. 1. Photomicrograph × about 9.

Fig. 7. A detail of Fig. 6. × about 23.

Fig. 8. A detail of Fig. 6. × about 23.

Fig. 9. A detail of Fig. 6. × about 23.
(Bessey and Wolbach: Corneal vascularization in riboflavin lack)
Fig. 10. India ink-injected rat. Corneal vessels showing the circular artery and some of the veins of the plexus. Riboflavin-deficient rat 91 days, followed by 12 days with riboflavin restored, 40 \( \gamma \) daily. Shows the cessation of growth and irregular closure of vessels. \( \times \) about 32.

Fig. 11. A detail from Fig. 12. \( \times \) about 147.

Fig. 12. India ink-injected rat. Corneal vascularization in repair. This rat was kept for 56 days on riboflavin-deficient diet and then received 20 \( \gamma \) daily for 25 days. \( \times \) about 32.

Fig. 13. Corneal vascularization in repair. Rat 56 days on riboflavin-deficient diet, then 58 days with the addition of 20 \( \gamma \) daily. \( \times \) about 32.
**PLATE 4**

**FIG. 14.** Drawing of cornea of riboflavin-deficient rat 70 days. Shows vascularization with beginning leucocytic infiltration. Stained with Mallory's eosin-methylene blue, after Zenker's fixation. × 144.

**FIG. 15.** Drawings of cornea of rat kept on riboflavin deficient diet with inadequate vitamin B₆ content 120 days. Shows deep vascularization of the tunica propria, heavy leucocytic infiltration and lesions of the epithelium. Stained with Mallory's eosin-methylene blue, after Zenker's fixation. × 144.