

Contribution to the Ocular Manifestation of Riboflavin Deficiency

By

Kimiho Irinoda and Seizo Sato

(入野田 公穂) (佐藤 誠三)

*From the Ophthalmological Clinic of Prof. K. Irinoda,
Faculty of Medicine, Hirosaki University, Hirosaki*

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The investigation of the pathological changes, particularly, in the visual organ, due to ariboflavinosis (vitamin B₂-deficiency) has been rare and incomplete. Though it is unusual that the deficiency of vitamin B₂ in simple form occurs, the ariboflavinosis seems to happen, not exceptionally, in complication with other various vitamins and nutrient agents, namely in the form of partial phenomenon of polyavitaminosis or malnutrition. For instance the so-called "Shibi-Gattchaki" disease, which is widespread at present in the Tsugaru-area of Aomori Prefecture, Japan, is a kind of nutritive trouble, in which ariboflavinosis and pellagra predominates, as Masuda *et al*¹⁾ have described. It is a subclinical syndrome, consisting of angular stomatitis, glossitis and proctitis. There is also roughness, fissure, edema, erosion of skin and mucous membrane, especially on a boundary part such as the outer pubic region. If the "Shibi-Gattchaki" disease becomes serious then it provokes a psychoneurogenic sign.

Clinical Observation

Thereupon we have carried on research since 1949 on the ocular manifestation in regard to a large number of patients who had complained of foggy vision, twilight blindness i.e. visual disturbance in low illumination. We found some of the following symptoms (by Irinoda²⁾) ;

- i) angular blepharoconjunctivitis
- ii) hyperemia, vascularization, pigmentation at the corneal limbus
- iii) superficial diffuse keratitis
- iv) nodular formation at the iris
- v) temporal pallor, redness, opacity of the optic nerve
- vi) retinal changes, such as silver-grayish patches, brownish pigmentation etc.

In order to ascertain the relationship between these ocular signs and riboflavin-deficiency, we have determined the quantity of riboflavin in blood by the fluorometric method, and further measured their saturation into body by a load test. Thereafter we observed the influence upon their recovery by the supply of riboflavin in the body (by Irinoda *et al*³⁾). In the first place we recognized that in school-children who seemed to have relatively little nutritive deficiency, the quantity of riboflavin in the blood and urine showed, as a rule, a low rate. In one group of out-

and hospitalized patients, who manifested not only general symptoms but ocular signs and seemed to be somewhat serious, we found a lower rate. Secondly, according to the loading examination, the saturation of riboflavin in body was markedly scanty, when a case of a neuro-retinitis with macular lesion was contrasted with a healthy adult, the curve of the total volume of riboflavin-excretion went down considerably, i.e. volume of excretion into urine was very scarce and the excretion-index very small. After the load-test we measured the quantity of riboflavin in the blood of 5 typical cases before, during and after treatment and compared it with the progress of the ocular signs. The results are significant because the low quantity of riboflavin in the blood before treatment, rapidly increased with a large dose of riboflavin, and the ocular signs therewith improved markedly.

From these examinations we deduced that the ocular manifestations of the so-called "Shib-Gattchaki" disease were for the most part due to ariboflavinosis, and that they contained the symptoms that had close connection with metabolic disturbance of nicotinic acid, thiamine (B_1), adermin (B_6) and other various vitamins. We have investigated the pathohistological findings of these changes.

Pathohistological Findings

Case 1. T. S. 26 year old male. Diagnosis: pannus corneae ariboflavinosis o. utr. (dermatological diagnosis; acne rosacea of 2. grade.)

At both cornea all over the surface, partly interstitially, opaque accompanied with vascularization. Vitamin B_2 quantity in blood 5.2 γ%, N'-methyl nicotinamide in urine 0.30mg%. Histological changes: epithelial cells of the conjunctiva and cornea were multiplied and thickened, besides between epithelial cells considerable vacuole-formation showed. At tunica propria repletion and new-formation of blood-vessels were marked, and at corneal limbus infiltration of histiogenic cells (histiocyte, monocyte, plasmacyte etc.) extended (Fig. 1, Fig. 2).

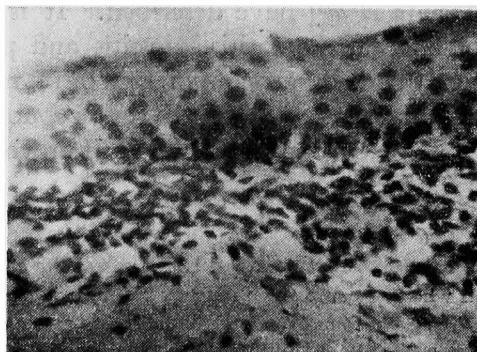


Fig. 1. New-formation of blood-vessel and cellular infiltration at anterior portion of the cornea of man (Case 1).

Case 2. T. N. 2 year old male. Diagnosis; pannus corneae ariboflavinosis o. utr.

At the limbus corneae violent circumcorneal injection showed. Vitamin B_2 quantity in blood 3.0 γ%, N'-methyl nicotinamide in urine 0.21 mg%. Histological changes; epithelial cells of the conjunctiva were markedly multiplied and thickened, besides at their superficial, upper parts keratinization happened, between epithelial cells numerous vacuoles-formation showed. At tunica propria blood-vessels developed, and in its surroundings infiltration of histiogenic cells (histiocyte, monocyte, plasmacyte, fibroblasts etc.) extended (Fig. 3).

Case 3. K. T. 17 year old female. Diagnosis; conjunctivitis catarrhalis acuta cum pig-



Fig. 2. Vacuole-formation and multiplication at the conjunctival epithelium and histiogenic cellular infiltration (histiocyte, monocyte, plasmacyte etc.) at tunica propria of the corneal limbus of man (Case 1).

Fig. 3

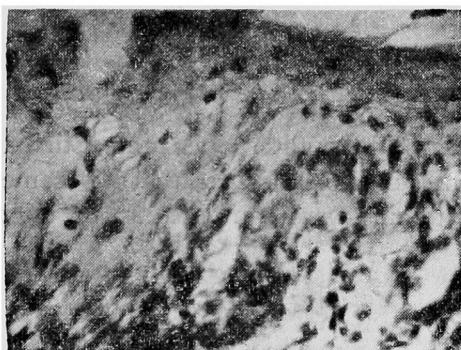


Fig. 3. Remarkable vacuole-formation, multiplication and hyperkeratinization at the epithelial layer of conjunctiva of man (Case 2).

Fig. 4

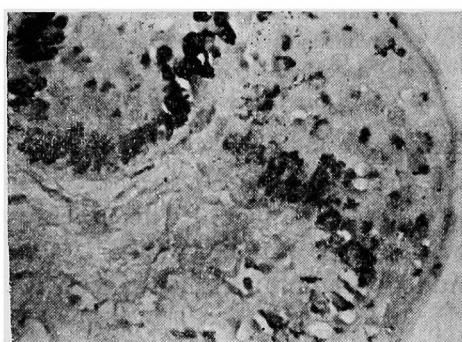


Fig. 4. Vacuolation of the protoplasmas, increase of the keratin and increased melanin-pigment at basal layer of the conjunctival epithelium of man (Case 3).

mentatio limbi cornea o. utr.

Mostly at the lidslitzone and lower part of the corneal limbus, dense deposition of brownish pigment-patch showed. Vitamin B₂ quantity in blood 4.5 γ%, N'-methyl nicotinamide in urine 0.18 mg%. Histological changes; under the basal cell layer of the conjunctiva obvious increase of melanin-pigment and destruction of the epithelial cell, namely bad staining of the nucleus, vacuolation of the protoplasms, and increase of the keratin. That is to say vacuole-formation and keratinization of the epithelial cell layer in two cases, however in the latter, increase of the melanin-pigment, such as at the skin of beginning pellagra (Fig. 4). In sum we saw that the pathohistological view showed the vacuole-formation of protoplasm, the multiplication and keratinization at epithelial cells of the conjunctiva and cornea, i.e. the repletion and new-formation of blood-vessel, chronic inflammatory histiogenic infiltration at tunica propria, and sometimes increase of the melanin-pigment at the basal cell layer.

Experimental Study

On the other hand, we have succeeded in an animal experiment that

a rabbit fed on riboflavin-deficient diet manifested various ocular changes.

Method: as experimental animals young rabbits (500–2,000 g. weighed, 1–3 months after birth) were selected. They were kept in an air-conditioned room at 20–25°C in individual metal cages (47×40×37 cm. sized) on wire screen floors preventing them access to the refuse tray and excretions. The food cups were designed to allow feeding without opening the cage. (The care and feeding of the animals have been described by Sato⁴.) 58 animals (containing paired control 12) were involved in three experiments during a two-year period.

Dietary procedures: new animals were tuberculin-tested with old tuberculin intracutaneously and quarantined for 5 days. Food was withheld for 24 hours and they were then offered a diet of following composition :

Daily basal diet :

refined rice corn	40 g.
refined casein (by Hammarsten)	10 g.
Macculum's combined mineral (No. 185)	3.5 g.
soya bean or sesame oil	6.0 g.

these were together with ca 100 cc. water and few filterpaper lumps stirred up, and then boiled in sterilizer 20 minutes in 130–150°C, so that it became soft bread-like food. The necessary vitamins were combined in the diet when it was supplied daily.

Added vitamins : in first experiment

Thiamine	1.25 mg.
Ascorbic acid	20.0 mg.
Pyridoxine	1.0 mg.
Niacin	2.0 mg.
Folic acid	1.0 mg.
Cod liver oil	0.5 cc.

in second experiment besides this

Choline chloride	10.0 mg.
Ca pantothenate	5.0 mg.

in third experiment moreover

Biotin	0.3 mg.
Inositol	0.1 mg.

Further in the diet of the control animal, riboflavin 1 mg. was added daily.

Examination: the animals were weighed daily in the morning, observed on general state and examined weekly with slitlamp, oblique illumination, retionscopy, and as occasion demanded, corneal test by fluorescein-instillation. As the riboflavin measurement by fluorometric method

was used, the catheterized urine and autopsied tissue, and load test of riboflavin, the recovery test by riboflavin-supply was carried out, too. Before the animals died, they were butchered and autopsied to prevent post-mortem change. Various organs were measured for riboflavin-quantification. Tissue fragments (visual organ, liver, brain) were fixed in 10% formalin, and then embedded in paraffin, celloidin, freezing and stained by H. E., V. G., Sudan III, mucicarmin, thionin, Kultschitzky's myelin-staining technique, Suzuki's axon cylinder-staining technique, Marchi's technique (by Osmium acid-fixation).

Results : From those experiments we obtained the following results :—

1) All rabbits suffered from such general symptom as loss of hair, brown stains of fur, slow rate of growth, diarrhea and anorexia, after 15–40 days (23 days on an average).

2) The rabbits deficient in riboflavin made a much smaller urinal excretion of riboflavin than the control group, and they showed a remarkably lower rate of urinal excretion in the riboflavin load test, indicating a smaller degree of riboflavin-saturation in the body.

3) In the viscera (liver, kidney, lung, heart) the quantity of riboflavin was smaller than in those of the control animals whether the former were loaded with riboflavin or not.

4) It was observed, both clinically and pathohistologically, that the symptoms of deficiency were well cured by injection of riboflavin.

5) In cases of only deficiency in riboflavin, 5 of 26 cases showed diffuse superficial opacity of the cornea, and 3 showed coneal vascularization.

6) Out of 20 cases of deficiency in several vitamins of VB₂-complex, 3 showed diffuse superficial corneal opacity, 1 erosio corneae, 4 corneal vascularization and 1 edematous opacity of the retina.

7) Pathohistological findings from these clinical signs were as follows ;

a) The edema and the vacuoles of the corneal epithelial layer, vascularization in the stroma below the epithelium, and subepithelial cellular infiltrations (histiocyte, monocyte, plasmacyte, etc.) that indicated chronic inflammation at the corneal limbus were found. (Fig. 5, Fig. 6, Fig. 7) b) In one case of the erosio corneae we found necrosis and falling of the corneal epitheliums and polynuclear leucocyte-infiltration between the upper lamellæ of the corneal stroma. c) In the case of the retinal edematous opacity we found the degeneration-substance like mucin (positive by the mucicarmin-staining) at the retinal nerve-fiber-layer.

d) In most of the cases signs indicating the process of degeneration of the retinal ganglion cells, namely chromatolysis and chromatophilia of the Nissle's body, and alveolar vacuole-formation of the protoplasma were

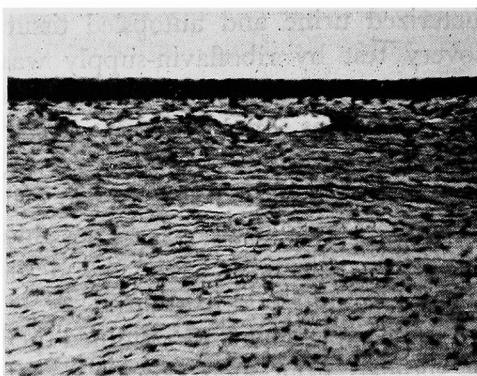


Fig. 5. New-formation of blood-vessel at anterior portion of the cornea of rabbit.

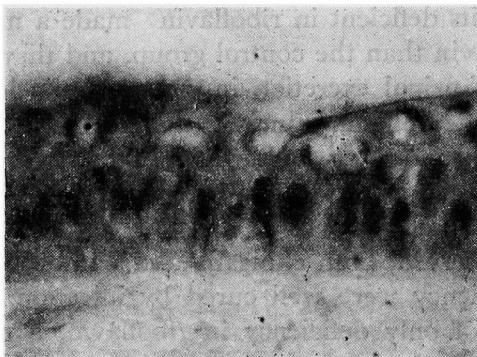


Fig. 6. Remarkable vacuole-formation at epithelial layer of the cornea of rabbit.



Fig. 7. Histiogenic cellular infiltration (histiocyte, monocyte, plasmacyte etc.) at tunica propria of the corneal limbus of rabbit.

found. Such changes were found at the ganglion cell in the cortex of brain, too. (Fig. 9, Fig. 10, Fig. 11, Fig. 12, Fig. 13) e) In the liver central fatty degeneration was marked. (Fig. 14).

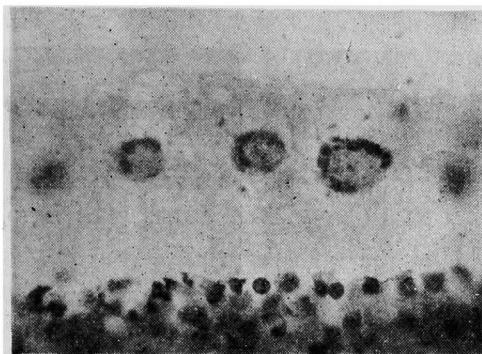


Fig. 8. Nissle's body at the retinal ganglion cell of contrast-animal (by thionin-staining).

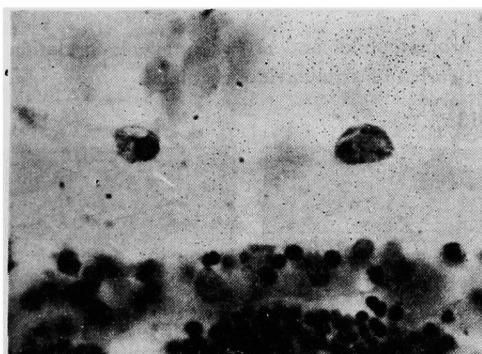


Fig. 9. Chromatophilia at the retinal ganglion cell of a riboflavinotic animal.

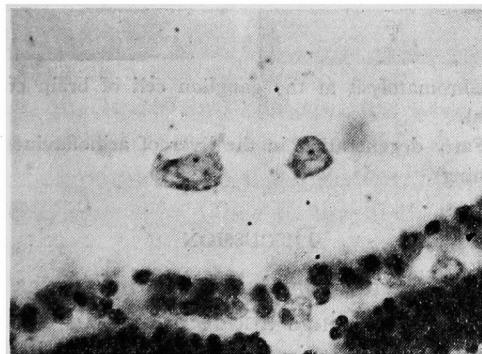


Fig. 10. Chromatolysis and vacuole-formation at the retinal ganglion cell of a riboflavinotic animal.

Thus we recognized that with a lack of riboflavin there occurs pathological changes, chiefly at the cornea and the retina, and then a decrease of visual acuity. We believe, therefore, that we have sufficient data to explain the clinical symptoms of riboflavin-deficiency in man.

Fig. 11

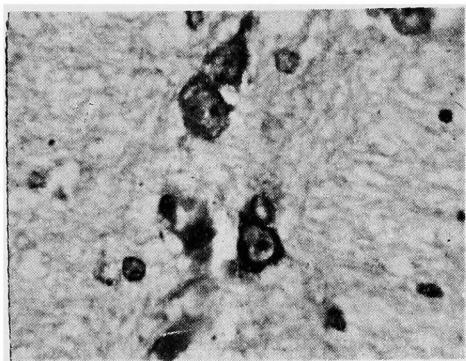


Fig. 12

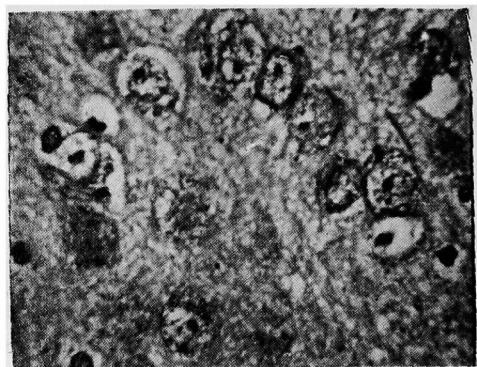


Fig. 11. Nissle's body at the ganglion cell of brain cortex of contrast-animal.

Fig. 12. Chromatophilia and alveolar vacuole-formation at the ganglion cell of brain cortex of arboflavinotic animal.

[Fig. 13]

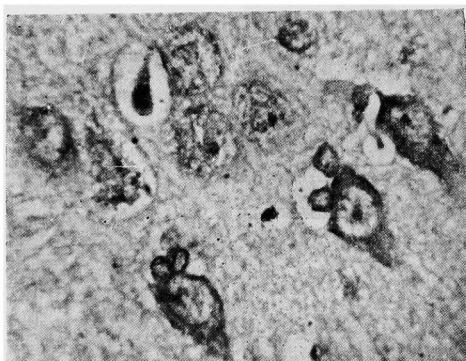


Fig. 14

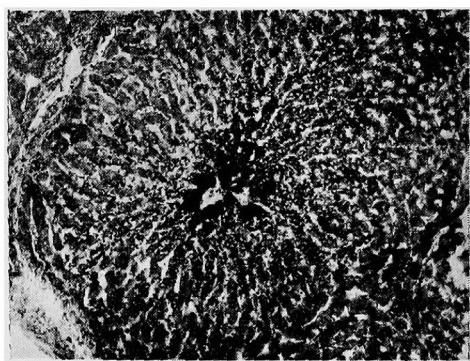


Fig. 13. Chromatolysis at the ganglion cell of brain cortex of arboflavinotic animal.

Fig. 14. Fatty degeneration in the liver of arboflavinotic animal (by Sudan III staining).

DISCUSSION

Our observation indicates that in human beings, as well as in animals, the deficiency of riboflavin brings about numerous and various signs, not only in the general body, but in the visual organ. Previously, Sydenstricker⁵⁾ and his associates reported in 47 cases of arboflavinotic patients with cheilitis, glossitis, seborrhoea ocular manifestation such as 37 conjunctivitis, 45 circumcorneal injection, 37 corneal vascularization, 18 corneal opacity, 19 pigmentation of the iris, 4 iritis, 6 cataract, and H. J. Stern, Landau,^{6),7)} J. J. Stern⁸⁾ observed that in arboflavinosis, vascularization of the cornea (so-called eczematous keratitis) often occurred, which was

reactively influenced by the administration of riboflavin. Experimentally, Day and O'Brien⁹⁾ by employing a diet lacking in the B₂ group, especially riboflavin, produced blepharitis, conjunctivitis, interstitial vascular keratitis and cataract in the rat. Although others have been unable to confirm, this observation led to corneal vascularizations in man being regarded as a riboflavin deficiency. Later, Bessey and Wolbach¹⁰⁾ brought out corneal vascularity and Suda¹¹⁾ brought out diffuse superficial keratitis in white rats bred with riboflavin-deficient diets.

Clinically, in riboflavin deficiency the following ocular symptoms were recently described; smarting, itching feeling as of the presence of a foreign body, and moderate photophobia; objectively, there are found injection of the bulbar conjunctiva, vascular keratitis, congestion of the iris and visual disturbances. The keratitis begins with engorgements of the perilimbal group of vessels, which is followed by proliferation of the capillaries in the superficial and middle corneal strata and interstitial infiltration. The vascular condition is attributed to the need of the cornea to obtain from the vessels the oxygen which it lacks, owing to the deficiency of the yellow respiratory ferment of Warburg (of which flavine is a component).

These views, however, are not yet fully confirmed. Some authors have observed an improvement in the corneal lesions through riboflavin administration. Others affirm that corneal vascularization is not a sure sign of deficiency of vitamin B and deny altogether any relation between perilimbal engorgement of vessels and vitamin deficiency. If this is so, what are the reasons for the occurrence of these signs in the visual organ? When one follows a molecule of riboflavin from its entrance into the body to its final position in cellular oxidation, one finds that it has to undergo numerous changes before it is capable of entering reactive processes, i.e. phosphorylation, known to occur in the intestines and possibly also performed in the cells of the body, and combination with a protein, believed to take place within the cells, or combination with the remaining portion of the Hogness's riboflavin-adenine-dinucleotide (Warburg's yellow enzyme) complex, which may take place in the liver, with subsequent combination with a protein. It is conceivable that a defect in the chain of oxidative reactions other than in the metabolism of the riboflavin-containing enzyme may be present. This defect may conceivably be in the reducing system (coenzyme I and II) or in the oxidizing system (cytochrome C, succinate-fumarate system, hexose monophosphate, L-, D-aminoacid, hypoxanthine, xanthine, certain aldehydes). That is to say, the recent investigations on oxidative enzymes which contain riboflavin indicate that there exists, in addition to the yellow enzyme, which acts as a hydrogen acceptor in oxidation of carbohydrate, yet other riboflavin-protein complexes (amino-acids) which act as activators of the reduced coenzyme. So

the end results of riboflavin deficiency would not be affected by these newer concepts, for such a deficiency would result in a severe disturbance of the oxidative systems in the body.

From the preceding consideration, it can be seen that the function of the yellow enzyme is to aid in the transfer of hydrogen from the tissue cells to the blood stream, where free oxygen is available from the breakdown of oxyhaemoglobin to hemoglobin, and where the oxidation of the hydrogen received originally from the substrate (hemin analog chlorophyll), through the coenzyme, can be accomplished. It is believed that if hemin substances are not present in a tissue (such as the avascular cornea) the oxidation within the cell is accomplished by Warburg's yellow enzyme. Because in avascular or hypovascular tissue such as the cornea, inner layer of the retina hemin substances are not brought into close proximity with the tissue cells, so it may be assumed that the greater part of gaseous interchange in oxidation is effected through Warburg's yellow enzyme. Therefore, in the avascular or hypovascular tissue riboflavin is deficient, so local asphyxia is brought about and at first degenerative changes occur. Further, on the other side, as an attempt to overcome local asphyxia by bringing available oxygen into closer proximity with tissue cells, the proliferation of capillaries was brought out.

We are going to suggest that in the event of a deficiency in any of these elements, or in some one of the elements, namely, riboflavin, the capillary endotheliums will be one of the first, if not the first, of the tissues to suffer the effects of interference with normal respiration and metabolism. The result of the anoxia upon the capillaries is a derangement of function, which we shall refer to as "capillary dysergia" with metabolic disorder of the cells of the neighbouring tissue. So accompanied with these changes, chronic inflammation was involved as a consequence.

Hitherto nothing has been described about the retinal changes due to ariboflavinosis. In experimental study we saw a case of retinal edematous swelling with pathohistological mucous degeneration of nerve-fiber-layer due to lack of riboflavin and some of the other factors of the vitamin B₂ group. Excepting the one above, all the cases without the ophthalmoscopic finding in the ganglion cells, showed remarkable degeneration such as chromatophilia or chromatolysis of the Nissle's body and alveolar vacuole-formation of protoplasms. These changes of the retinal ganglion cell must be discerned as the beginning stage of degeneration, caused by the nutritional deficiency of the cells in riboflavin, because the same changes occurred in the ganglion cell of the brain cortex by the same cause.

Those changes should cause various visual disturbances such as "nutritional amblyopia" (Moore,¹²⁾ Landor and Pollister¹³⁾, Scott¹⁴⁾, Rodger¹⁵⁾ and "Twilight-blindness" (already described by Pock-Steen¹⁶⁾

and also clinically noticed by us). Besides if reconsidering the hyperkeratinization of the corneal and conjunctival epithelia, which were shown in our clinical cases, we found great similarity in nature between the ocular lesions of avitaminosis A and ariboflavinosis. The former, however, responded to vitamin A. As the processes in conjunctivitis or avitaminosis A extend through the corneal epithelium and down into the cornea, it is not surprising that they disturb the cornea with a typical, but probably secondary, response like that seen in ariboflavinosis. Since the site of the first reaction of riboflavin is just beneath the corneal epithelium, it is possible to conceive how riboflavin may thus be secondarily disturbed. As the process reaches the lamellae just under the corneal epithelium, it is possible that the keratitic manifestation represents a secondary riboflavin disturbance. In consequence, avitaminosis A concurrent with ariboflavinosis might be expected to add to the extent or intensity of the keratitis due to the ariboflavinosis. And according to Spies *et al*¹⁷⁾ the enzymatic function of riboflavin has so a close connection with other various vitamins, that nicotinic acid in the form of its amide is an important factor in forming the co-zymase (von Euler) and co-ferment (Warburg). Thiamine is also an important co-enzyme in phosphorized thiamine (cocarboxylase). So ariboflavinosis may happen as a partial phenomenon of polyavitaminosis.

Hills *et al*¹⁸⁾ have reported on the experimental deficiency of riboflavin in the human being. Under the conditions of their study the tendency of small vascular twigs in the margins of the cornea to disappear and reappear months later was frequently noted, but no proliferation of the vessels of the limbic plexus was seen. Certainly these must have been a latent ariboflavinosis, if they become manifest by the joining of other factors, such as injury and light exposure. The "yellow enzyme" is necessary in such a large number of cellular reactions that it is surprising that abnormalities beneath the dermal layer were absent or minimal. The inability to find any changes in the metabolites in the body fluids may be because the cell, once matured, requires only a minimum amount of riboflavin. However, the rate of replacement of such cells with others may be dependent on a more abundant supply of riboflavin, obtainable either from the diet or through the breakdown of less vital tissue.

SUMMARY

By clinical observation on so-called "Shibi-Gattchaki" patients and the experimental study on young riboflavin-deficient rabbits we have obtained the following results;

1. The deficiency of riboflavin mostly caused degenerative changes (vacuolar degenerations), regenerative process (compensatory vascularization) and reactive chronic inflammation in the avascular or hypovascular

organ such as the cornea, inner layer of the retina.

2. Ocular manifestation of ariboflavinosis including its latent state occurred considerably frequently in human beings as well as in animals, as compared with other signs.

3. Ocular symptom, like other various clinical signs, are pathognomically reliable.

References

- 1) Masuda, & Aoyama, Tohoku J. Exp. Med., 1951, **55**, 1.
- 2) Irinoda, Rinsho Ganka (Jap.), 1952, **6**, 167.
- 3) Irinoda, *et al*, Acta Soc. ophthalm. jap., 1952, **56**, 1902.
- 4) Sato, Acta Soc. ophthalm. jap., 1953, **57**, 1027.
- 5) Sydenstricker, *et al*, J.A.M.A., 1940, **114**, 2437.
- 6) Stern, H. J. & Landau, Am. J. Ophth., 1942, **31**, 952.
- 7) Stern, H. J., Arch. Ophth., 1949, **42**, 438.
- 8) Stern, J. J., Am. J. Ophth., 1950, **33**, 1127.
- 9) Day, Langston, & O'Brien, Amer. J. Ophth., 1931, **14**, 1005.
- 10) Bessey & Wolbach, J. Exp. Med., 1939, **59**, 1.
- 11) Suda, Acta Soc. ophthalm. jap., 1953, **57**, 1027.
- 12) Moore, West African Med. J., 1930, **4**, 46.
- 13) Landor, & Pollister, Transact. Roy. Soc. Trop. Med. Hyg., 1935, **19**, 121, cit. fr. Bicknell & Prescott; *The Vitamin in Medicine*, 1948.
- 14) Scott, Ann. Trop. Med. Parasitol., 1918, **12**, 109.
- 15) Rodger, Arch. Ophth., 1952, **47**, 571.
- 16) Pock-Steen, Geneesk. tschr. Ned.-Indie, 1939, **79**, 1986, Abstracted in J.A.M.A., 1939, **113**, 2102.
- 17) Spies, Vilter, & Ashe, J. A. M. A., 1939, **113**, 931.
- 18) Hills, Liebert, Steinberg, & Horwitt, Arch. Int. Med., 1951, **87**, 682.