RIBOFLAVIN AND THE CORNEA[1]

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IN 1932 Warburg and Christian (1) obtained a substance from the aqueous extract of bottom yeasts which they called "Yellow Enzyme." In 1933, three independent research groups reported the relationship between vitamin B_2 (a factor in the vitamin B complex) and the water soluble yellow green fluorescent pigments found in animals and plants. Later research proved that all these pigments were identical. Subsequent study brought to light its structure and a short time later its synthesis was accomplished. The Council on Pharmacy and Chemistry of the American Medical Association (2) in 1937 decided to call this substance Riboflavin and abandon terms vitamin G and B_2.

Experiments on animals with diets deficient in riboflavin were started even before the basic work of its chemistry and physical structure was completed. As early as 1931, Day, Langston and O'Brien (3) mentioned the development of keratitis and corneal vessels in riboflavin deficient rats. In 1936, Bessey and Wolbach (4) made a detailed study of the rat cornea in riboflavin deficiency. The experiments were carefully done and a well known ophthalmologist confirmed their findings. They found that by the fourth week of deficiency, before any other changes occurred, an ingrowth of capillaries from the limbus into the cornea took place.

These animal results called attention to the fact that similar changes could occur in humans who were riboflavin deficient. Consequently, observations were begun and reports began to be published.

Spies, Vilter and Ashe (5) published a report in 1939 entitled "Pellagra, Beriberi and Riboflavin Deficiency in Humans." Their conclusions were drawn from observations on 800 nutrition clinic patients. They noted that many patients had conjunctivitis, lacrimation, burning, and failing vision. These symptoms were relieved in some instances by riboflavin and in others by carotene. These findings were recorded and no conclusions drawn.

Another paper, published in 1939 by Sydenstricker, Geeslin and Templeton (6), was entitled "Riboflavin Deficiency in Human Subjects." Six cases were reported. They were said to exhibit symptoms of pellagra or riboflavin deficiency. Two of these patients had conjunctivitis. Since riboflavin administration caused the conjunctivitis to subside, the conjunctivitis was attributed to lack of this vitamin.

In the following year, 1940, Kruse, Sydenstricker, Sebrell and Cleckly (7) reported, under the title "Ocular Manifestations of Ariboflavinosis," their findings on 9 patients. These patients were admittedly multiple deficiencies. Several were syphilitic. The procedure was to give the patient a high caloric diet, abundant in vitamins with the exception of riboflavin, and to observe the results. Two of these cases were reported in detail.

In the first case, conjunctival injection, interstitial corneal opacities and a plexus of empty capillaries were noted. A diet without riboflavin caused the capillaries to fill with blood in 16 days. Administration of riboflavin caused the capillaries to empty in 5 days and become occluded by the twelfth day.

The second case had two previous admittances to the hospital with iritis and keratitis of undetermined origin. The corneae were so opaque that the irides could not be seen. For 11 days no change occurred. Then riboflavin was given. In 10 days the fundi were visible. When riboflavin was stopped the opacities recurred and vessels reopened. Riboflavin again caused clearing. This cycle of opacification and clearing was again repeated with riboflavin.

In this same paper was a report on 4 cases of interstitial keratitis. Two were

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treated with riboflavin and improved markedly in 10 weeks. The 2 untreated cases showed no improvement in 10 weeks. There were no pictures, drawings or precise descriptions of the vessels or opacities. An ophthalmologist is said to have observed these changes. His name was not given.

Several months later in the same year, 1940, another paper appeared by Sydenstricker, Sebrell, Cleckly and Kruse (8) entitled "Ocular Manifestations of Ariboflavinosis." Here 47 cases were presented, among which were included the 9 reported in the paper just mentioned.

In this paper the manner in which riboflavin deficiency manifests itself in the cornea is given. The very earliest sign is the obliteration of the so-called avascular zone between the limbal plexus and the corneoscleral junction. This zone became increasingly congested and soon vessels were invading the cornea in loops and twigs. All cases in this paper were relieved of subjective symptoms by riboflavin. Congested limbal regions and corneal blood vessels were also obliterated by this vitamin. The dosage had to be adjusted at times to the severity of the condition. These cases were both severe nutritional problems and people whose diets had been low in riboflavin only. The data was obtained by giving and then withholding riboflavin from the diet.

In 1941 the authors of the preceding two papers became quite conclusive in their statements and spoke in the Southern Medical Journal (9) of the normal avascular limbal zone. Limbal arcade vessels were not considered normal. The Public Health Reports of 1942 contained an article which advised slit lamp examination of the limbus to gauge riboflavin deficiency (17).

Based on the ocular signs of riboflavin deficiency set up by Sydenstricker and his co-workers, other observers began to report.

Tesdall, McCreary and Pearce (10), in 1943, reported a beneficial effect on eye fatigue and corneolimbal vascularization from riboflavin in R.C.A.F. personnel. Interestingly enough, the same authors (11) published another paper in 1944 based on a larger group. In addition, they made slit lamp and photographic studies. They also used irritants in the conjunctival sac and noted the effects on limbal vessels. Their final conclusion was that limbal vascularization could not be used to judge riboflavin deficiency.

Stannus (12), in the British Medical Journal, and Scott (13), in the Journal of the Royal Army Medical Corps, both denied any relationship between riboflavin intake and corneolimbal vessel changes.

At the Annual Congress of the Ophthalmological Society of the United Kingdom in 1946 (14) a vast work on the "ocular disturbances associated with malnutrition" was presented. The observations on over 3,000 Japanese prisoners with eye difficulties were summarized in this report. The data was that compiled by doctors who were prisoners themselves and who made examinations all during their internments, as well as doctors who treated them since their release. In their conclusions corneal vascularization was negligible.

Vail and Ascher (15) produced a detailed study of the limbus area. This proved by pictures and diagrams to workers, who were not ophthalmologists, that there is no avascular area adjacent to the cornea; furthermore, that the vitamin deficient patients they observed developed no corneal vascularization.

All this work regarding riboflavin deficiency in humans was done on clinic patients. Many of them had multiple deficiencies; previous eye pathology was admitted in some; conjunctivitis and syphilis were also factors. The diet in many of these patients, especially out-patients, is open to question.

Accordingly, a real conclusive experiment could be done if normal humans were put on a diet deficient in riboflavin only, and results observed.

Only one such experiment was attempted—by Boehrer, Stanford and Ryan (16). They put 3 college students on a diet containing 0.5 mgm. of riboflavin daily and used 3 for controls. The experiment ran 5 weeks. No corneal or limbal changes occurred in the students on the diet. However, one of the controls developed vascular corneal loops. The subjects in this experiment admitted

occasional dietary indiscretions. This experiment could not be called conclusive.

The experiment we are reporting was set up by the National Research Council for the purpose of observing the changes in human subjects on a riboflavin deficient diet. Each branch of medicine concerned is represented on this Council by one of its own qualified members. Dr. Derrick Vail represents the ophthalmologists.

Report of Experiment

This work was done at the Elgin State Hospital. Thirty male patients were selected who were normal physically and who were cooperative.

A separate locked building was used. Special nurses, who were acquainted with the experiment, were with the patients at all times. The building had its own diet kitchen where the special diet was prepared. Each meal was assayed. Twenty-four-hour urine specimens were examined weekly to make sure the intake of riboflavin was proper. A physician was employed as a full-time fellow to oversee conditions and make observations. A laboratory was available for photography and miscellaneous tests.

The experiment was set up as follows: Fifteen patients were employed as controls. Fifteen were given the deficiency diet. Both groups of patients ate the same food, only one group had sufficient riboflavin and the other had the reduced amount. The amount of riboflavin to include in the diets posed quite a question. The figure given as a minimum requirement in adults varies with each investigator. However, more than 1.0 mgm. per day is the lowest basic requirement given. The control group was given 2 mgm. per day; the deficiency group 0.6 mgm. per day. This figure of 0.6 is at most one-half of what is estimated as the barest necessity. A diet without riboflavin was not contemplated since life would then be endangered.

The experiment was conducted as follows: Both groups were given a diet with 1.0 mgm. per day for two months. This was to stabilize the experiment and to get the patients' reactions. After this two-month period, the controls were given 2.0 mgm. daily and the deficient group 0.6 mgm. daily.

The whole group was examined with the slit lamp several times at the start, then at weekly and monthly intervals. As other symptoms or signs developed, more frequent examinations were made. The limbal blood vessels in each patient were photographed at intervals with and without instillations of dionin to insure an accurate record.

The slit lamp examinations were also standardized. Each cornea was examined by quadrants. The limbal vessels were noted. The loops and twigs at the margin of clear cornea or in clear cornea were counted and recorded. The record of each patient was compared after each observation. Any change or discrepancy was investigated. It was often difficult to state whether a vessel was in clear cornea or still in the transitional zone because of observing the vessel at different times from different angles. However, whether the vessel changed in size was the important thing, not its position.

It became evident after a while that the limbal blood vessels behaved like any other capillary bed. Without apparent reason segments varying in size would dilate and fill with blood, or empty. A heavy meshwork of vessels at one observation would be a cobweb of fine threads at another—or any stage of dilatation in between. The vascular bed itself, however, did not change. The observations and the photographs revealed no change in limbal or corneal vascularization. The average time of deficiency diet and observation was one year and three months; the shortest time was nine months; the longest time was one year and six months. The length of time the patient was kept on the diet depended on the other physical changes that occurred. When skin, tongue, scrotal, or other lesion became severe the patient was reversed, since any serious threat to health was not permitted.

It seemed obvious that observations of a changing vascular bed over a short period of time could be interpreted many ways. Especially if one postulated an avascular perilimbal zone. This avascular perilimbal zone has never been reported following anatomical or adequate

slit lamp studies. It was more or less arbitrarily established by some workers.

Conclusions

Study of a group of patients on a riboflavin deficient diet for one year and three months revealed no change in the limbal blood vessels or corneal vascularity.

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