EXPERIMENTAL CONTRIBUTION TO THE STUDY OF THE RELATION BETWEEN NIGHT BLINDNESS AND MALNUTRITION

Influence of Deficiency of Fat-Soluble A-Vitamin in the Diet on the Visual Purple in the Eyes of Rats

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Received for publication November 28, 1924

I. Historical introduction. On the basis of clinical observation many investigators have suggested a relation between xerosis conjunctivae and certain kinds of night blindness in human beings. These two ailments are often found jointly in the same patients and under the same circumstances. Their clinical relation was recognized by Bitot in 1863 (3) who first described xerosis conjunctivae. The title of his paper is "Mémoire sur une lésion conjunctivale non encore décrite, coincidant avec l'héméralopie;" finding all his 29 patients with xerosis suffering from night blindness he thought the xerotic coverings of the eye to be the cause of the night blindness as they cut off a certain amount of the light. A. Netter (33) pointed out in the same year (1863) that this view could not be correct because several patients suffering from night blindness did not suffer from xerosis. In the course of time ophthalmologists reached the view that the three ailments, night blindness, xerosis conjunctivae and keratomalacia, were different degrees of the same disease, and this is still the point of view generally accepted.

The relation of the three ailments and especially of night blindness to malnutrition was early suggested. Night blindness often appeared under conditions of bad nourishment, as among prisoners (8), (10) or negro slaves in Brazil (13), or in long sea-voyages (15). Several observations of night blindness among soldiers and prisoners during the war 1914–18 exist (43), (37), (26), (17), (30). The night blindness among orthodox Russians during the Lenten fasts has often been described (5), (22). But several kinds of night blindness exist, and probably but one of these is related to malnutrition, viz., the kind preceding or accompanying xerophthalmia. Quite different in etiology is the congenital night blindess, the night blindness in retinitis pigmentosa, in detachment of the retina, etc. Even the cases among soldiers in the last war have apparently not all had the

same etiology (see C. Augstein (1), F. Best (2), K. K. K. Lundsgaard (26)).

When the relation between xerophthalmia and a deficiency of fat-soluble A-vitamin in the diet had been established by experiments (34), (28) and by clinical observations (32), (6), a similar relation between the deficiency of A-vitamin and the night blindness accompanying xerophthalmia became possible.

In the papers of Mori and of Bloch night blindness of some of the xerophthalmia-patients is mentioned. McCollum (27) and later the authors of the second edition of the Medical Research Council Report on vitamins (29) have compiled observations suggesting the association of night blindness in human beings with starvation for A-vitamin and have mentioned the use of liver and of cod-liver oil as remedies for night blindness, these foods containing much A-vitamin. This treatment of night blindness is mentioned by many authors (1), (10), (13), (17), (21),(39), (40), (43). O. Blegvad found night blindness in 37 out of 66 Danish xerophthalmia patients more than 3 years old and has given a summary of the question (4).

But unanimity about the importance of malnutrition in the etiology of night blindness does not exist. Some authors think alcoholism rather than malnutrition is the main cause (42), (24), (25). More weighty are the arguments in favor of the view that night blindness depends on exposure to the bright sunlight. As early as 1859 Alfr. Graefe (14) observed prisoners suffering from night blindness but recovering through a stay in darkness and relapsing by exposure to light. Several authors agree with Graefe on the basis of similar observations and consider such exposure as the main or the only cause of this kind of night blindness (10), (2), (33), (36). This view is corroborated by the occurrence of most cases of night blindness in the early spring.

Clinical observation can not settle the question of the etiology of night blindness preceding or accompanying xerophthalmia and experimental research work must be done. Experiments concerning the etiology of night blindness do not exist as far as we know.

II. Night blindness and the visual purple. The most obvious experimental treatment of the question as to the etiology of night blindness would be to try if night blindness occurs in animals on a diet deficient in A-vitamin.

It is difficult to demonstrate night blindness in animals, although it is not impossible. Night blindness consists in a difficulty or an impossibility of adapting the faculty of vision to very faint illumination. Carl Hess (16) succeeded in experimenting on this adaptation of the vision in birds. Experiments on the possible relation of night blindness to starvation for A-vitamin have to be conducted on animals whose dietary require-

ments, especially concerning the A-vitamin, are well known. In consequence we had to use rats.

It is more difficult to experiment on rats than on birds in order to examine the adaptation of the vision to faint illumination. Later one of us succeeded in doing so (published in another paper), but in the series of experiments described in this paper we have followed another and more indirect course.

According to a view accepted by many ophthalmologists, night blindness depends on an abnormality in the rod cells of the retina. This anomaly is considered to be connected with the visual purple, situated only in the rod cells. The view is often called the duplex theory of the faculty of vision. According to this theory, originated by Max Schultze in 1866 (38) before the discovery of the visual purple, the rods of the retina have another function than the cones. The ordinary faculty of vision in daylight and the perception of colors should be the function of the cones but the colorless vision at faint illumination ("twilight-vision") should be the function of the rods. After the visual purple in the rods had been discovered, Parinaud (36) supplemented the duplex theory with the assumption of the twilight-vision being dependent on the visual purple. The color of the visual purple is bleached in the light but regenerated in darkness if the retina is not removed from its situation in the eye.

The duplex theory is not accepted by all ophthalmologists. M. Tscherning (41) has opposed it on the basis of his investigations (a summary of the question is given in (18)). In spite of this we think it for the present legitimate to apply the duplex theory as a provisional starting point of our experiments in presuming the faculty of adapting the eye to vision by faint illumination to be dependent in some way or other on the visual purple in the rod cells of the retina. As a consequence of this theory night blindness is presumed to be correlated with an abnormal function of the visual purple, probably a reduced or failing function (36), (18).

III. Preliminary experimental methods. Our experiments purpose to compare the visual purple in the retina of control rats, receiving an adequate diet, with the visual purple of rats on a diet devoid of A-vitamin but adequate in all other respects. Xerophthalmia will develop in rats subjected to such an experimental diet as described by many investigators (first by Osborne and Mendel (34) and by Freise, Goldschmidt and Frank (11)) and the symptoms are identical with the symptoms of xerophthalmia in human beings (20).

When the visual purple is to be examined in the eyes of the experimental rats, the examination must take place before the development of pronounced xerophthalmia, especially earlier than the occurrence of opacity of the cornea (the reason of this necessity will be explained later). This is why a constant occurrence of xerophthalmia in all the rats on the

diet devoid of A-vitamin is a condition of the experiments. Most investigators have failed to produce xerophthalmia in all their experimental rats on such a diet but only in a certain percentage of them (Osborne and Mendel (35) in 50 per cent), only Agnes F. Morgan (31) got xerophthalmia in all. In our stock of rats we have been lucky enough to realize a constant occurrence of xerophthalmia in all rats on the diet devoid of A-vitamin used by us, when young rats were used for the experiments. The initial symptoms of xerophthalmia developed after four to seven weeks of A-vitamin starvation.

The basal food mixture of the diet had the following composition: 200 grams caseinogen, 30 grams agar, 50 grams autolysed and dried yeast, 470 grams rice starch and 50 grams salt mixture. The caseinogen was purified by repeated washing with hot alcohol and with ether, then heated in thin layers to 105° for 24 hours (as recommended by Drummond and Coward (9)). The agar was powdered and purified by being boiled in 96 per cent alcohol. The autolysed yeast was dried at a temperature lower than 40°C. The rice starch was boiled twice with 96 per cent alcohol. The salt mixture was the mixture recommended in the report of the Medical Research Council (29, p. 14).

To the basal food-mixture was added purified butter fat in the diets of control rats and linseed oil or lard in the experimental diets. The lard and the linseed oil were melted, filtered and heated in thin layers to 105°C. for 24 hours. During the experiments control rats as well as experimental rats were kept in a dark thermostat room without windows at 22°C. (except when purposely exposed to the light). The room was dimly lighted by a "glimm-lamp." Every rat was confined to its special cage, made of iron-wire net. Urine and feces fell down through the meshes of the floor. On one side of each cage was an eating-chamber with a food glass. This construction of the cages prevents the animals from eating the feces and from soiling the food.

The first experiments purposed to compare the amount of visual purple in the retinae of experimental rats on a diet devoid of A-vitamin (basal food mixture + 12 per cent linseed oil) with the amount of visual purple in the retinae of control rats (basal food mixture + 12 per cent butter fat).

In order to estimate the amount of visual purple in the retina of a rat we first tried extraction with a solution of bile salts as indicated by W. Kühne (23). The extraction took place in red light. The method had to be abandoned because only a fraction of the visual purple was extracted in this way. Afterwards we adopted a colorimetric method. The principle of this method is indicated by S. Garten (12). Our way of proceeding was the following: the rat was carried in a dark box from the thermostat room to a photographic red lighted dark-room and narcotized by chloroform (in the first few experiments) or by subcutaneous injection of ure-

thane-solution (in most experiments). The eye-ball was enucleated and opened by an equatorial cut. The retina was dissected and the isolated retina, after washing in water, spread on a white porcelain plate. Then the plate with the retina was taken to a room with subdued daylight and quickly compared with a color scale. In the first experiments the color scale reproduced in the paper of Garten (12) was used. It is possible to complete the colorimetric comparison before the bleaching of the visual purple of the retina begins.

In preliminary experiments (expts. 1 and 2) the amount of visual purple in the eyes of four pied rats, taken directly from the dark thermostat room, was estimated. Two of the rats had for 5 and 6 weeks received a diet, devoid of A-vitamin (basal food mixture + 12 per cent linseed oil). The two other rats were control animals on adequate diet (basal food mixture + 12 per cent butter fat). No distinct difference between the amounts of visual purple in the eyes of experimental rats and of control rats was observed.

We did not think it profitable to continue the experiments in this way. The result of these few experiments showed that a constant and conspicuous difference between the amount of visual purple in the eyes of experimental rats and control rats, all kept in darkness, was not to be found. For this reason the experimental method was altered.

IV. Regeneration of the visual purple and night blindness. Possibly other abnormalities connected with the visual purple than a diminution of its amount existed in rats starved for A-vitamin. If such abnormalities had relations to night blindness in the animals the clinical experience about night blindness in human beings could probably point out the way to be followed in further experiments. As mentioned above, some observations suggest a bearing of the action of bright light on the etiology of night blindness in human beings. Several investigators consider the action of light to be the main cause of night blindness. This view may contain a part of the truth.

The principal symptom of night blindness is the difficulty in adapting the faculty of vision to faint illumination. According to existing observations this difficulty is most pronounced when the eyes of the patients have been exposed to intense light, but after a period of rest in darkness this difficulty becomes much less. Parinaud (36), who originated the theory of the relation between night blindness and abnormalities in the visual purple, pointed out that the difficulty of the patients in adapting their vision to faint light augmented toward evening and was aggravated by exposure to intense light. H. de Gouvea (13) observed night blindness in badly nourished slaves in Brazil and describes how the slaves were unable to see, when returning from their work after sunset, but had no difficulty in seeing when starting in the morning before sunrise, although

it was much darker in the morning than in the evening. Patients suffering from night blindness have told one of us about their more pronounced difficulty in seeing in the evening than in the morning.

Since night blindness is more pronounced after exposure of the eyes to light, the abnormality in the function of the eye causing the night blindness is perhaps more difficult to ascertain in eyes not having been exposed to light. In the introductory experiments the rats had been kept in darkness. The color of the visual purple is bleached in light and regenerated in darkness. If the difficulty in adapting the night blind eye to faint illumination has relation to a defect in the function of the visual purple, this defect might possibly be a difficulty in regenerating the color of the visual purple when the color had previously been bleached by exposure of the eye to light. Although present a defect of this kind might not be found if the animal was examined after a stay in darkness.

On the basis of this reasoning we have examined the regeneration of the visual purple after bleaching in the light in rats starved for A-vitamin and compared the results with the regeneration of the visual purple in control rats on adequate diet.

V. EXPERIMENTS ON THE REGENERATION OF THE BLEACHED VISUAL PURPLE IN PIED RATS AFTER A-VITAMIN STARVATION. The procedure adopted in these experiments was the following: the rat was taken from the dark thermostat room and placed in a big box with white floor, roof and walls. The box was illuminated by a 50-light Nernst-lamp, placed under the roof of the box. In the first experiments (no. 3 to 15) control rats and experimental rats were not exposed to light at the same time. In the later experiments (16 to 27) an experimental rat, kept on a diet devoid of A-vitamin, and the corresponding control rat, kept on an adequate diet, was at the same time placed in the lighted box. After a certain time the animals were narcotized and one eye in each of the rats enucleated. The eyes were opened in a dark-room in red light and the color of the retinae was observed. When the retinae were white without any reddish tint the bleaching of the visual purple had been complete. If so the next stage of the experiment was to examine the regeneration of the visual purple in the remaining eye of each of the two rats. The animals were placed in darkness, and after the lapse of a certain time, the other eye was enucleated in both the rats. After opening of the eyes and dissection of the retinae, it was examined by colorimetry as already described to see to what stage of color the regeneration of the visual purple in the retinae had proceeded during the stay of the animals in darkness, and a comparison was made between the amounts of visual purple regenerated in the retinae of the experimental and of the control rats.

The Garten color scale used in the first experiments proved to be impractical because it contained too many colors. This prolonged the colori-

metric estimation. Quickness was essential because the estimation as mentioned had to go on in subdued daylight. We then prepared a very simple scale, containing only five colors, viz.: 1, deepest red to be found in the retina; 2, tile-red; 3, light red; 4, faint light red, and 5, with a reddish tint. This scale made a very quick estimation possible, the difference between its stages being pronounced. Rats, which had not been in the light but were taken directly from the dark cellar, showed a color of their retina corresponding to no. 1 or no. 2 of the scale.

In introductory experiments the conditions of the total bleaching in the light of the visual purple of the rat had to be determined. It proved to be difficult to bleach the visual purple in the eyes of a living rat, unless the animal were an albino. Pied rats contract the pigmented iris and all rats wink when exposed to intense light. On this account it proved necessary to instill atropin in the eyes of pied rats (1 drop of a 1 per cent solution of sulphate of atropin) and to narcotize them during their exposure to light, in order to prevent the animals from shutting the eyes. Urethane narcosis proved best. For deep narcosis of a 100 gram young rat the subcutaneous injection of 25 to 30 centigrams of ethyl urethane is required. In the experiments 10 to 20 centigrams, giving a light nacrosis, sufficed. During the enucleation of the eye the light narcosis was supplemented by ether inhalation.

In the atropinized and urethanized pied rats the visual purple was totally colorless after two exposures of 15 minutes each in the light box. Between the two exposures there was a pause of 15 minutes. This proved to be better than continual exposure to light for 30 minutes in order to avoid the production of opacities in the cornea and of "visual yellow" in the retina by the action of the light (further particulars especially concerning the production of visual yellow are published in (19)).

In albino rats the atropin treatment was superfluous, because the iris did not contain any pigment, and the bleaching out of the visual purple was completed after 15 minutes' exposure to light in the box.

In most experiments the completeness of the bleaching out of the visual purple was controlled, as mentioned, by enucleating and examining one eye after exposure of the rat to the light. Sufficient experience having been gained, it was in some of the later experiments deemed superfluous to continue this control.

The experimental rats had to be used in the experiments dealing with the regeneration of the visual purple before pronounced symptoms of xerophthalmia appeared, because these symptoms very soon are followed by keratitis; and opacities of the cornea impede the effect of the light on the visual purple. The experimental rats were examined when their weight commenced to decrease and enophthalmus and conjunctival secretion began (about the initial symptoms in xerophthalmia in rats, see (20)).

Eighteen experiments were made on pied rats (expts. 3 to 21). In reality these experiments turned out to be introductory or rather of guidance for the final experiments, because some unexpected difficulties appeared.

In table 1 the results of the first 9 experiments on regeneration of the visual purple are represented as described under the table. Five of these experiments (3 to 7) were made on control rats and 4 on experimental rats (8 to 11), having been starved for A-vitamin 4 to 7 weeks. In the controls the color of the visual purple was completely regenerated after 2 hours' stay in darkness. The regeneration seemed to be a little slower in the

TABLE 1

Results of experiments 3 to 11

COLOR SCALE	1 HOUR	1½ HOURS	1½ HOURS	2 HOURS
No. 1				
No. 1–2				**6** **7**
No. 2				*5* *8*
No. 2–3				
No. 3	**3**	**4**		
No. 3–4				
No. 4	*9*		*10* *11*	
No. 4–5				
No. 5				
No regeneration				

Each horizontal line corresponds to a number of the colorimetric scale. No. 1 is the most intense red color, no. 5 the faintest.

Each column represents a stay in darkness (after the total bleaching of the visual purple in the light) of the stated duration (1 hour, 1¼ hour, etc.). Consequently the table shows to what extent (to what number of the colorimetric scale) the completely bleached visual purple had regenerated after stay of the rats in darkness for periods of different duration.

Heavy type represents control rats on adequate diet.

Italic numerals represents experimental rats starved for A-vitamin.

The numbers refer to particular experiments.

experimental rats than in the controls but the difference was very trivial and in consequence doubtful.

In the next series of experiments the rats were exposed daily to the light in the period previous to the examination of the regeneration of the visual purple. This was done on the basis of the following supposition: if the experimental rats regenerated the visual purple more slowly than the control rats, the difference could be expected to be more pronounced when the function of regeneration had been "fatigued" by daily bleaching of the purple.

The daily exposure to light was accomplished either by placing the

animals every day for two hours before a window facing the south (expts. 12, 14 and 16) or in most experiments (expts. 13, 15 and 17 to 21) by placing the rats several times daily in the light box. The rats had always atropinized eyes when exposed to the light. The experimental rat and his corresponding control rat were in every case treated exactly in the same manner and exposed simultaneously to the same amount of light in the same way. Previous to the examination the experimental rats had received the basal food mixture + 15 per cent or 12 per cent of lard and the control rats the basal food mixture + 15 per cent or 12 per cent butter fat.

Twenty-four examinations of the regeneration of the visual purple in experimental rats and corresponding control rats were made in this way (expts. 12 to 21). Experiments 12 to 15 comprise each a single rat. In nos. 16 to 21 each number comprises the experimental rat and the cor-

TABLE 2

Representing experiments 12 to 21

COLOR SCALE	1 HOUR	1½ HOURS	2½ HOURS	3 HOURS
No. 1				**18** *19* *19*
No. 1–2			**17**	
No. 2		**16**	*17*	*21* **21**
No. 2–3	*20*			
No. 3	**20**	*12* *14* *16*		
No. 3–4	**17** **18** **19**			*18*
No. 4	*18* *19* *21* **21**			
No. 4–5		*15*		
No. 5				
No regeneration	*13* *17*			

The explanation is given under table 1.

responding control rat. In nos. 17 to 19 and 21 the regeneration was examined in both eyes of each rat.

In spite of all precautions disturbing factors were present in these experiments. In nos. 13, 14 and 15 the experimental rats suffered from keratitis. In experiments 17 to 21 a new disturbing factor appeared. It was observed that during the process of regeneration of the visual purple an adhesion of the pigment from the pigment cell layer to the dissected retina in most cases occurred. This adhesion of the pigment did not take place as was to be expected from statements in the literature either in the bleached retina or in the retina of rats taken directly from the dark cellar. The adhesion only occurred during the regeneration of the visual purple after complete bleaching of its color in the light. The phenomenon occurred in experimental rats as well as in control rats. The adhesion of the pigment imparts to the retina a brownish color and often makes the

colorimetric estimation of the amount of visual purple very difficult and in consequence uncertain.

In spite of these defects connected with experiments 12 to 21, the results are represented in table 2. On the whole the regeneration of the visual purple is slower and—during the time of observation—more imperfect in the retinae of the experimental rats than in the retinae of the control rats. In the control rats the regeneration was complete after $1\frac{1}{2}$ to $2\frac{1}{2}$ hours' stay in darkness.

To get a better survey over all the 33 regeneration examinations in the 18 experiments, 3 to 21, the average results have been computed in table 3. The way of computing was the following: the amount of visual purple regenerated after each given period of stay in darkness was estimated in several experiments; in each estimation the amount of regeneration was expressed by the number of the color scale, matching the color of the

TABLE 3

Thirty-three estimations in pied rats of the regeneration of the bleached visual purple (from expts. 3 to 21).

Average numbers from the color scale after regeneration in periods of different duration. The number of rats from the observation of which the numbers are taken is given.

	EXPERIMENTAL RATS	CONTROL RATS
1 hour regeneration	4.6 (7 rats)	3.4 (5 rats)
$1\frac{1}{4}$ hours' regeneration		3.0 (1 rat)
$1\frac{1}{2}$ hours' regeneration	3.6 (6 rats)	2.0 (1 rat)
2 hours' regeneration	2.0 (2 rats)	1.3 (3 rats)
$2\frac{1}{2}$ hours' regeneration	2.0 (1 rat)	1.5 (1 rat)
3 hours' regeneration	2.2 (3 rats)	1.3 (3 rats)

retina (the simplified color scale containing five colors) after, say, one hour's stay in darkness, seven experimental rats were examined and seven values for the amount of regeneration of the visual purple in a period of this duration was accordingly ascertained, these values being represented by numbers; the average of these 7 numbers is 4.6 and this value of 4.6 is taken as representing the average amount of regeneration of the visual purple after one hour's stay in darkness after the complete bleaching of the purple. In the same way the number 3.4 is found to represent the average amount of visual purple regeneration in 5 control rats after 1 hour's stay in darkness, and so on. It is to be remembered that no. 1 means the deepest red color and no. 5 the faintest. Number 6 is taken as representing no regeneration at all.

These average scale numbers for the regeneration of the purple after periods of varying duration are put down in table 3 and represented as a

diagram in figure 1, the ordinates corresponding to the average numbers of purple regeneration, the abscissae to the corresponding duration of the stay in darkness.

Table 3 as well as figure 1 shows how the regeneration of the visual purple on an average proceeds slower in the experimental rats than in the control rats. Want of A-vitamin in the diet seems to make the regeneration of the visual purple slower.

But the results are not decisive, partly because the "average error" of the results is very great, partly because disturbing factors had been present, especially the fastening of pigment to the retina.

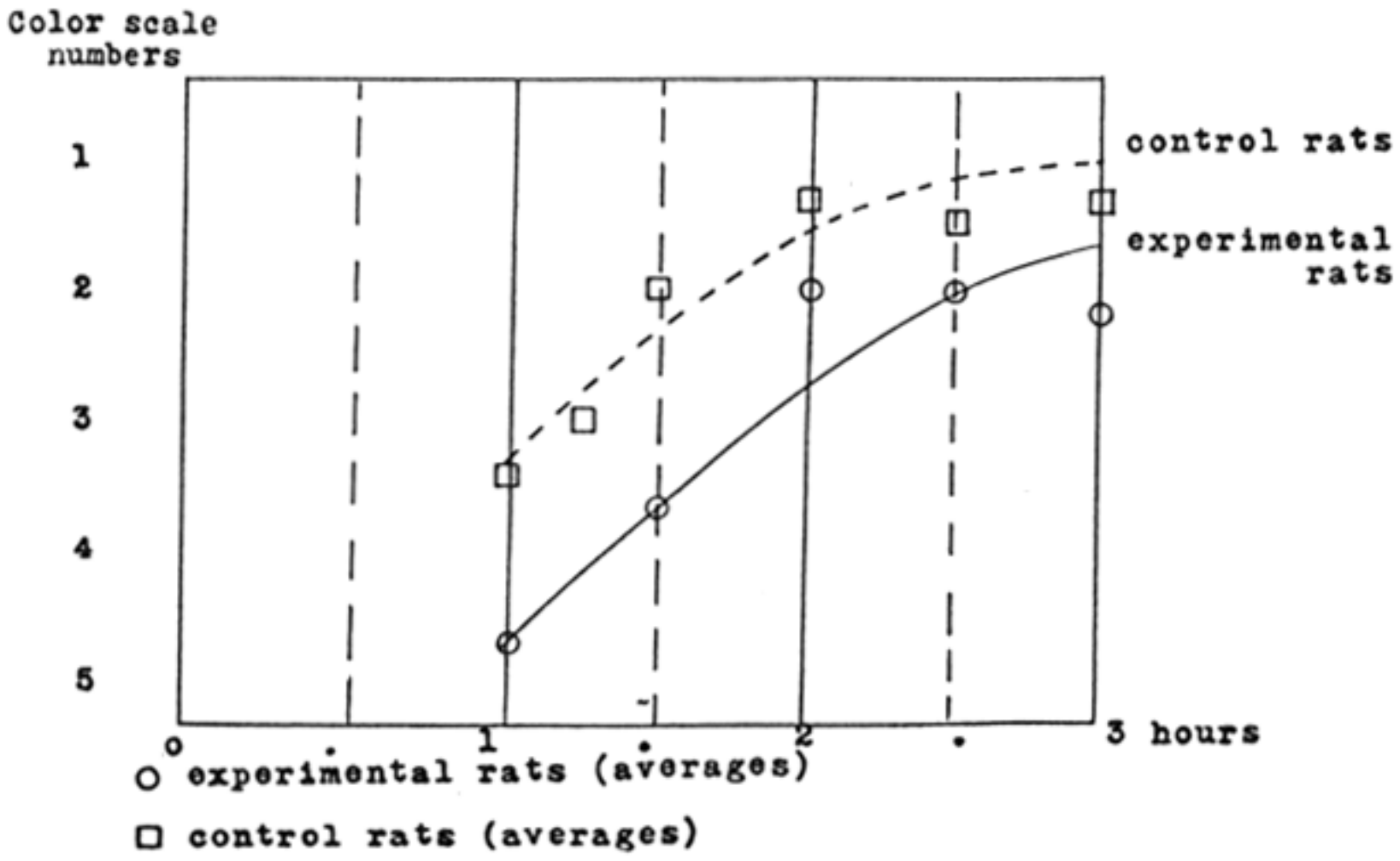

Fig. 1. Diagram of the regeneration of the visual purple in experimental and control rats.

VI. Experiments on albino-rats. The difficulty in the colorimetric estimation dependent on the adhesion of pigment from the pigment cell layer to the dissected retinae can be avoided in experiments on albino rats. No visible pigment is to be found in the pigment cell layer or in the iris of the albinos. This last makes the instillation of atropin during the exposure to light superfluous.

In the albinos 22 estimations of the regeneration of the visual purple after complete bleaching were made in 12 rats (expts. 22 to 27). In these experiments advantage could be taken of the experience regarding disturbing factors gained during the experiments on pied rats. Accordingly the main features of the experimental method were the following: *1.* The experimental rat and the corresponding control rat had the same

weight, when the feeding with the special diet commenced. The experimental rats got the basal food mixture + 15 per cent lard, the control rats the basal food mixture + 15 per cent butter fat. *2.* In the period previous to the examination of the visual purple all rats had daily been exposed to the action of light by being placed for 6 hours in a window. An experimental rat and the corresponding control rat were always placed in the same window during the same time. *3.* The regeneration of the bleached visual purple in an experimental rat was examined as soon as the rat commenced losing in weight before the occurrence of other xerophthalmic symptoms than enophthalmus and conjunctival secretion. The regeneration was in every case examined in the control rat simultaneously with the examination of the corresponding experimental rat. *4.* The bleaching of the visual purple preceding the examination of its regenera-

TABLE 4

Representing experiments 22 to 27 in albino rats

COLOR SCALE	¾ HOUR	1½ HOUR	2 HOURS	2½ HOURS	3 HOURS	4 HOURS
No. 1					**25 26**	
No. 1–2			**22 25 26**			**27**
No. 2					**27**	
No. 2–3					**24**	
No. 3	**22**			***22***		
No. 3–4			**23**			
No. 4	***22***	**23**				***27***
No. 4–5			***23***			
No. 5			***25***		***24 25 27***	
No regeneration		***23***	***26***		***26***	

The explanation is given under table 1.

tion was always complete. Twenty minutes' exposure to the 50-light Nernst-lamp in the light box sufficed in narcotized albino-rats. The narcosis was effected by the subcutaneous injection of 10 centigrams of ethyl urethane in aqueous solution, supplemented by ether inhalation during the enucleation of the eyes.

The results of the 22 examinations in albino rats are represented in table 4. From this table it is evident that the regeneration of the bleached visual purple is much slower and—within the time of observation—much more incomplete in the experimental albino rats, starved for A-vitamin, than in the control rats on an adequate diet. After 3 to 5 hours' stay in darkness the visual purple was almost completely regenerated in the retinae of the control rats, but very little color had been regenerated in the retinae of the experimental rats. In table 5 the average amounts of regenerated visual purple at different points of time have been computed on the basis

of the corresponding numbers of the color scale in the same way as in table 3. The difference between experimental rats and control rats is evident. But the main point in the results of the albino experiments is that a pronounced difference not only appears between these averages but between every experimental rat and its corresponding control rat.

From these results we think it warranted to conclude that starvation for A-vitamin in rats produces a defect in the function of the visual purple, the defect consisting in a slowness in the regeneration of the visual purple after its having been bleached through exposure to intense light.

VII. EXPERIMENTS ON THE REGENERATION OF THE BLEACHED VISUAL PURPLE IN RATS STARVED FOR B-VITAMIN. It may be asked if the ascertained defect in the regeneration of the visual purple is something exclusively connected with starvation for A-vitamin, or if other dietary defects are able to produce the same result. A thorough investigation of this

TABLE 5

Twenty-two estimations in albino rats of the regeneration of the bleached visual purple (exps. 22 to 27)

Average numbers from color scale after regeneration in periods of different duration.

	EXPERIMENTAL RATS	CONTROL RATS
¾ hour's regeneration	4.0 (1 rat)	3.0 (1 rat)
1½ hours' regeneration	6.0 (1 rat)	4.0 (1 rat)
2 hours' regeneration	5.2 (3 rats)	2.0 (4 rats)
2½ hours' regeneration	3.0 (1 rat)	
3 hours' regeneration	5.2 (4 rats)	1.6 (4 rats)
5 hours' regeneration	4.0 (1 rat)	2.0 (1 rat)

question would require many experiments. We made only two experiments on rats receiving a diet adequate in respect to A-vitamin, but deficient in B-vitamin (expts. 28 and 29). In these experiments, performed in the way described, no difference was observed between experimental rats and control rats concerning the regeneration of the bleached visual purple. In all the visual purple was completely regenerated after 2 hours' stay in darkness.

DISCUSSION OF THE RESULTS. A deficiency in the diet of rats of the fat-soluble A-vitamin produces a series of well-known symptoms, the most characteristic being the effect on growth and the eye disease xerophthalmia. In addition to the known symptoms of this deficiency our experiments have pointed out another symptom, viz., a delay in the regeneration of the visual purple of the retina, succeeding the bleaching of the purple in the light. This symptom is an early one, being manifested as soon as the

growth of the young rat stops and earlier than the onset of pronounced xerophthalmic symptoms.

In human beings a relation exists between xerophthalmia and a certain kind of night blindness, the night blindness often preceding the xerophthalmia. Some authors have suggested a relation of this kind of night blindness to a deficiency of A-vitamin in the diet, but others have made observations suggesting a connection between night blindness and exposure of the eyes to intense light. If this kind of night blindness depends on a defect in the function of the visual purple, identical with that observed in the rats starved for A-vitamin, both of these views contain a part of the truth.

In rats a deficiency in the diet of A-vitamin produces a defect in the faculty of regenerating the visual purple after bleaching of the purple in the light. But this defect is only to be observed after the eyes of the rats have been exposed to the action of intense light. The night blindness behaves in the same way. The ailment is presumably caused by an identical deficiency in the diet, but the symptom, the night blindness proper, is only manifest after exposure of the patients' eyes to intense light. This analogy suggests the relation of night blindness to a defect in the function of the visual purple identical with that here reported for rats.

SUMMARY

1. Methods of estimating the amount of visual purple in the retinae of rats by colorimetry are described. The conditions of complete bleaching of the visual purple in eyes of living rats by action of the light are investigated. Methods of examining the regeneration of the bleached visual purple are described.

2. Rats starved for A-vitamin and corresponding control rats receiving an adequate diet are examined as to the amount of visual purple in their retinae and their faculty of regenerating the color of the bleached visual purple.

3. No influence of starving for A-vitamin on the amount of visual purple in the retinae of rats kept in darkness has been found.

4. When the visual purple of the retinae has been completely bleached by exposure of the rats to light, the regeneration of the purple is delayed in rats starved for A-vitamin as compared with control rats receiving an adequate diet. This abnormality is much more pronounced in albino rats than in pied rats. In pied rats a fastening of the pigment from the pigment cell layer to the dissected retina impedes the examination of the visual purple. Previous to the examination of the regeneration of the bleached visual purple, the rats have to be daily exposed to the light. This has to be done in the same way in experimental rats and control rats.

5. The abnormality in rats starved for A-vitamin as to the regeneration of the bleached visual purple occurs earlier than pronounced symptoms of xerophthalmia.

6. The abnormality in the regeneration of the bleached visual purple has not been found in rats starved for B-vitamin.

7. In human beings a relation between xerophthalmia and a kind of night blindness, often preceding the xerophthalmia, has been suggested by several authors. This kind of night blindness is by some authors thought dependent on a deficiency in the diet of A-vitamin, by others on the exposure of the eyes to intense light. If this kind of night blindness depends on a defect in the function of the visual purple, identical to that observed in rats starved for A-vitamin, both views contain part of the truth.

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