CCXVII. THE OCCURRENCE OF CATARACT IN RATS FED ON DIETS DEFICIENT IN VITAMIN B₂.

By MARGHERITA COTONIO BOURNE AND MAGNUS ALFRED PYKE.

*From the Department of Physiology and Biochemistry, University College,
London.*

(Received June 15th, 1935.)

DAY *et al.* [1931] in America first reported that they had obtained cataract in albino rats by feeding a diet deficient in vitamin B₂. The ration used was that of Sherman and Spohn [1923] which has been widely employed in work with this vitamin. Cataracts were produced in 92% of rats after an average period of 72 days.

The work was developed by Langston *et al.* [1933] and by Langston and Day [1933] who observed an equally high incidence of cataract in mice and in young wild gray rats deprived of vitamin B₂. By feeding diets deficient in vitamin B₂ Day, Langston and Cosgrove [1934] obtained cataract in rats and chickens and Day [1934] reported cataract in rats, mice, chickens and monkeys. The importance of cataract as a symptom of vitamin B₂ deficiency was stressed by these authors.

Day and Langston [1934] published further evidence that in nearly all the cases examined rats receiving a diet free from vitamin B₂ developed cataract. They found that the appearance of cataract was delayed where small but insufficient amounts of vitamin B₂ were given; and the delayed appearance of cataract was correlated with the growth-rate data. It was concluded that cataract was probably a better criterion of vitamin B₂ deficiency than was dermatitis.

These observations are of great interest since they are related to the problem of the aetiology of cataract as well as to that of vitamin deficiency. It is important that it should be established beyond doubt whether or not degenerative changes in the eye-lens can be produced by the omission of a specific substance, such as a vitamin, from the diet. It is equally important from the point of view of vitamin studies to know if cataract, which can readily be observed, is in fact a consistent symptom of a vitamin deficiency. In addition a technique for producing cataract regularly in a laboratory animal by dietary control would prove most useful in further studies in the aetiology of cataract. For these reasons it was considered advisable to attempt to repeat the work of Day and his colleagues. Experiments were therefore undertaken in which diets deficient in vitamin B₂, including the Sherman and Spohn [1923] diet as used by the American workers, were fed to rats and the eyes examined with an ophthalmoscope at weekly intervals. The results of these experiments form the subject of the present communication.

EXPERIMENTAL.

Thirty-six young black and white rats of about 70 g. weight were obtained from a commercial source and kept in single cages with wire screen bottoms. The Sherman-Spohn [1923] diet was fed. This consists of alcohol-extracted caseinogen

18%, Osborne and Mendel salt mixture 4%, butter fat 8%, cod-liver oil 2% and maize starch 68%. Vitamin B₁ is supplied in the form of an alcoholic extract of rice polishings which is evaporated on a portion of the starch and mixed with the remainder of the constituents.

In addition to the thirty-six test animals twelve control rats were given the deficient diet with a supplement of 1 ml. per day of a standard 50% solution of alkaline-autoclaved marmite as a source of vitamin B₂. The animals were weighed and their pupils dilated with atropine and the eyes examined with the ophthalmoscope at weekly intervals.

Nature of the ocular symptoms.

The ocular changes occurring in this type of cataract have been fully described by Day *et al.* [1931] and by Langston, Day and Cosgrove [1933]. The lesions observed by us conform to the description of these authors, but in our experiments the incidence of lens changes was much lower than that found by Day and his collaborators. The highest percentage of cataract obtained by us in any series of rats was 31, whereas the American workers repeatedly observed an incidence of above 90.

In those cases in which cataract did occur the progress of the lens changes, as observed with the ophthalmoscope, followed the course described by Langston, Day and Cosgrove [1933]. The first change noted was an irregularity of refraction manifested by the appearance of shadows in the clear substance of the lens. In a few cases striae were seen extending from the periphery to the nucleus of the lens. The lens gradually became more opaque and the red reflex from the retina could no longer be obtained; in the final stage development of the cataract progressed to maturity when the whole lens appeared milky white.

The most consistent ocular symptom was that of a superficial keratitis; inflammatory changes in the cornea were observed in 92–100% of rats on the Sherman and Spohn diet. In nearly all cases this inflammatory condition cleared up as the experiment progressed leaving the cornea clear; in a few cases it reappeared for a second time. Vascularisation and residual scarring of the cornea were observed in a few cases.

Skin symptoms.

The skin symptoms observed were not strikingly specific in nature but appeared as a general unhealthy condition. Although dermatitis was occasionally symmetrical in distribution, this was by no means the rule. Soreness, stickiness and swelling of the eyelids were common and in many cases there was alopecia around the eyes. These symptoms appeared in an average length of time of 93 days. Alopecia and soreness on the abdomen were noted. Here the sores were usually along the mid-line and were possibly aggravated by the rat pressing on the rim of the food pot. Sores at the base of the tail, on the paws and on the throat or at the back of the neck were also noted. In the case of affected paws, the back of the digits was frequently red and inflamed. Generally, the most extensive sores appeared on the neck. On the ventral side moist, red inflammation was sometimes seen and on the dorsal side of the neck some animals developed large scabs which would later be cast off leaving a bald patch. After prolonged periods on the deficient diet the rats had a characteristically dried up, dusty appearance. The fur looked grayish; quite distinct from the sleek black and white of the control animals.

Incidence of symptoms

The incidence of eye symptoms and skin symptoms in the rats in this experiment is shown in Table I. It can be seen that whilst the incidence of cataract was 31 %, different forms of dermatitis appeared in 92 % of the animals.

Table I. *Incidence of ocular changes and dermatitis in rats on the vitamin B₂-deficient diet of Sherman and Spohn.*

Symptom	Number of rats affected	Incidence %	Av. time of appearance (days)
1. Cataract	11	31	79
2. Keratitis	33	92	70
3. General dermatitis	33	92	92
(i) Affection of eyelids (bald, sore, sticky, swollen)	25	69	93
(ii) Abdomen sore or bald	13	36	79
(iii) Soreness or baldness at base of tail	11	31	115
(iv) Sore paws	9	25	85
(v) Sores on neck	5	14	77

The mortality of this batch of rats was exceedingly low. All the rats that failed to develop cataract lived for more than 88 days. The average survival of the rats that died without lens changes appearing was 135 days, while two animals were killed after 158 days and four after 179 days. Whilst the average time taken for the development of cataract, as shown in the table, was 79 days, the earliest case occurred in 56 days and the last in 123 days. No pathological symptoms occurred in the twelve control rats receiving autoclaved marmite.

Growth.

Although the appearance of dermatitis has generally been noticed in vitamin B₂ deficiency, the principal criterion of the absence of the vitamin is lack of growth. In the present case the typical flat growth curves associated with vitamin B₂ deficiency were obtained.

An analysis of the growth of thirty-four test rats and twelve controls is given in Table II.

Table II. *Maximum increase in weight of rats on the Sherman-Spohn vitamin B₂-deficient diet.*

	Av. maximum growth g.	Av. time days	Av. growth per week to maximum weight g.
12 control rats receiving 1 ml. alk.-autoclaved marmite per day	146	135	6.5
11 rats developing cataract	38	73	3.6
23 rats not developing cataract	27	88	2.1

It can be seen that whilst the growth of all test rats on the diet was very slow, the animals developing cataract actually made better growth than the rats whose lenses showed no changes. In the case of 29 of the 35 test rats listed in the table the final weight was considerably lower than the maximum weight. Also, during the first three weeks, while the animals still had stored reserves of vitamin to draw upon, the rate of growth was greatest. If the growth during this preliminary period is subtracted from the total, the average maximum growth of all the test rats is 15.2 g. and the average maximum growth of the rats developing cataract is 17.5 g.

EFFECT OF THE ADDITION OF CYSTINE ON THE INCIDENCE OF CATARACT.

The SH-containing compounds, such as glutathione and cysteine, are thought to be a part of the specialised respiration mechanism of the lens and recent work by Bourne and Young [1934] suggests that naphthalene cataract in rabbits may be due to the withdrawal of cysteine. Caseinogen which is the source of protein in the present diet contains only a small proportion of cystine and Day *et al.* [1934] stated that when egg white, which is a notoriously rich source, is substituted for caseinogen no cataract occurs. The following experiment was carried out in order to test whether the addition of cystine to the vitamin B₂-deficient diet would prevent the appearance of cataract.

Twelve rats were given the Sherman-Spohn diet as previously described but with the addition of 1 % of cystine. Two control rats were also given 1 ml. per day of alkaline-autoclaved marmite. A second batch of twenty-three rats was given the deficient diet as before with three rats receiving marmite as controls.

Since in the first experiment it had only been possible to obtain a 31 % incidence of cataract this second experiment could, at best, only give the most tentative results owing to the comparatively small number of rats. In fact, of the twelve rats receiving the diet *plus* cystine none developed cataract. The best survival was 94 days and the average 59 days. Of the twenty-three rats on the Sherman-Spohn diet, two developed cataract.

A summary of the experiment is given in Table III.

Table III. *Effect of the addition of cystine to the Sherman-Spohn diet.*

Symptom	Av. time of first appearance (days)		Number of rats surviving		Proportion of survivors affected	
	Diet	Diet <i>plus</i> cystine	Diet	Diet <i>plus</i> cystine	Diet %	Diet <i>plus</i> cystine %
Cataract	72	—	9	6*	22	0
Keratitis	29	31	22	12	100	100
Affected eyelids	54	50	13	11	85	82
Skin sores or baldness	66	48	11	11	73	82
Dropped penis	33	28	22	12	50	58

* Survived 72 days.

The mortality of the rats in this experiment was generally higher than before. The second section of Table III therefore gives the number of rats surviving after the average time of first appearance of each individual symptom, and the third section shows the incidence of each particular symptom among these surviving rats. Although 22 % of the rats fed on the Sherman-Spohn diet and living for 72 days developed cataract, this proportion represents only two rats.

A summary of the growth data from this experiment is given in Table IV.

Table IV. *Growth of rats on the Sherman-Spohn diet with and without the addition of 1 % of cystine.*

	Av. maximum growth g.	Av. time days	Av. growth per week to maximum weight g.
Controls	120	112	7.5
Diet: (i) cataract	2	32	0.4
(ii) others	2	27	0.5
Diet <i>plus</i> cystine	7	31	1.6

It can be seen that the growth of the test animals in this experiment was even less than before. This could be taken to indicate that their state of vitamin B₂ deficiency was more complete. Even so the incidence of cataract has dropped from 31 to 22 % on the unaltered Sherman-Spohn diet.

EFFECT OF MAIZE STARCH ON THE PRODUCTION OF CATARACT.

Previous to the present experiments a large number of rats had been kept on a diet deficient in vitamin B₂ without the development of cataract; the diet was that commonly employed in the laboratory at University College. It consisted of Glaxo "casein" 20 %, McCollum salt mixture 5 % and rice starch 75 %. To this ration daily additions of cod-liver oil and Peters's extract of yeast, to supply vitamins A, D and B₁, were made.

Workers on vitamin B₂ deficiency have in the past suspected that maize contains a toxin which might play a part in the production of the deficiency symptoms. In order to test whether the difference in behaviour of rats on the University College diet and on that used by the American workers was due to the use of rice starch in the former diet in place of maize starch the following experiment was carried out.

Eleven rats were started on the Sherman-Spohn diet as before. Fourteen rats were given the University College diet made with rice starch in the usual way and eleven animals were given the University College diet in which maize starch had been substituted for rice starch. Each batch of rats was controlled with two animals to which autoclaved marmite was given.

The growth data for this experiment are shown in Table V.

Table V. *Growth of rats fed on three diets deficient in vitamin B₂.*

	Av. maximum growth g.	Av. time days	Av. growth per week to maxi- mum weight g.
Controls	101	126	5.6
Sherman-Spohn Diet: (i) cataract	31	56	3.9
(ii) others	9	37	1.8
U.C.L. Diet: (i) cataract	14	63	1.6
(ii) others	22	70	2.2
Maize starch diet	21	61	2.4

From this table it can be seen that the average maximum growth on all three diets was negligible.

In this case, again, although the experiment was continued for 133 days, the incidence of cataract was very low. Of the total number of 36 animals on the three diets only two individuals developed cataract. One of these animals was receiving the Sherman-Spohn diet and one the University College diet. No cataract appeared in the rats receiving the maize starch diet. Results are set out in Table VI.

From this evidence, therefore, it appears that the maize starch plays no part in the production of cataract. On the contrary, those rats receiving the maize starch diet survived in better condition than did the animals on either of the other rations.

The last column of Table VI is of some interest. It was shown in Table V that no appreciable growth occurred on any of the diets. Thus, from the point of view of growth alone, each was equally deficient in vitamin B₂. But as regards

Table VI. *Symptoms appearing in rats fed on three diets deficient in vitamin B₂.*

Symptom	Av. time of first appearance			Number of rats surviving			Proportion of survivors affected		
	Sherman-Spohn diet days	U.C.L. diet days	Maize starch diet days	Sherman-Spohn diet	U.C. diet	Maize starch diet	Sherman-Spohn diet %	U.C. diet %	Maize starch diet %
Cataract	79	101	—	5	7	9*	20	14	0
Keratitis	32	58	87	11	11	9	100	55	89
Affected eyelids	52	67	76	7	11	11	71	36	27
Skin sores or baldness	81	44	103	4	13	9	100	15	22

* Survived 100 days.

affected eyelids and skin sores, whilst the incidence on the practically identical U.C.L. and maize starch diets was 36, 27, 15 and 22% respectively, the percentage on the American diet was 71 and 100%. It therefore appears possible, either that the Sherman-Spohn diet is lacking in some additional factor, which may or may not be a part of the vitamin B₂ complex (since we must now accept the vitamin as such), or that the diet contains some toxic factor.

DISCUSSION.

The results obtained by us differ in two important respects from those reported by Day and his associates. First, whereas the American workers have obtained a very high incidence of cataract, 92–100% of all rats fed on the Sherman and Spohn diet, the highest proportion found by us when the identical ration was fed was 31%. Second, Day and his collaborators have found that the occurrence of cataract is a more consistent symptom of vitamin B₂ deficiency than dermatitis. This is not the case in our experiment where in rats on the Sherman and Spohn diet dermatitis appeared in from 73 to 100% of cases while cataract occurred in only 20–31%.

The consistency with which Day and his associates have been able to repeat their observations is impressive, and the evidence which they present is convincing. At the same time our own experiments and the experience of other workers show that it is possible for rats to survive for long periods on diets deficient in vitamin B₂ without developing cataract [*v. György, 1935*]. Our own experiments in which cataracts occurred irregularly and at best in only a small percentage of animals suggest that some dietary factor may be concerned, but it is at present unidentified and therefore cannot be controlled. Whether this factor is a part of the vitamin B₂ complex or whether it is some other substance cannot be decided without further investigation. The recent work on the separation of vitamin B₂ into flavin and a supplementary substance should facilitate the solution of this problem.

SUMMARY.

Experiments were undertaken in an effort to repeat the work of Day and his collaborators who found that a deficiency of vitamin B₂ regularly produced cataract in rats. The results were as follows:

(a) 31% of 36 young rats fed on the Sherman-Spohn diet deficient in vitamin B₂ developed cataract in an average period of 79 days.

(b) 22 % of 9 rats surviving on the Sherman-Spohn diet developed cataract in an average period of 72 days. No cases of cataract occurred among 6 rats surviving for this length of time on the same diet to which 1 % of cystine had been added.

(c) The incidence of cataract among 5 surviving rats on the Sherman-Spohn diet was 20 % in 79 days; the incidence among 7 rats fed on a diet composed of caseinogen, salt mixture and rice starch was 14 % in 101 days; no cases of cataract occurred among 9 rats surviving for 100 days on a diet of caseinogen, salt mixture and maize starch.

Since the incidence of cataract in our experiments is relatively low and since it was found that numbers of rats could survive for long periods on diets deficient in vitamin B₂ without developing cataract, it is concluded that the relationship of cataract to vitamin B₂ deficiency still remains obscure. The results of our experiments suggest that some dietary factor may be concerned in the production of cataract, but whether this factor is the whole of the vitamin B₂ complex or some component of it, or whether it is some new and unrecognised substance is not yet established.

We are indebted to Prof. J. C. Drummond for his advice and encouragement during the progress of this work. One of us (M. C. B.) is grateful to the Medical Research Council for a grant and the other (M. A. P.) wishes to acknowledge the generosity of Messrs Vitamins Ltd, in giving him the opportunity of taking part in this investigation. We are indebted to Mr H. T. Fawns for the preparations of large quantities of pure cystine and for his generous assistance in other respects.

REFERENCES.

- Bourne and Young (1934). *Biochem. J.* **28**, 803.
Day (1934). *Amer. J. Pub. Health*, **24**, 603.
— and Langston (1934). *J. Nutrition*, **7**, 97.
— — and Cosgrove (1934). *J. Nutrition*, **7**, 12 (Proc.).
— — and O'Brien (1931). *Amer. J. Ophthal.* **14**, 1005.
György (1935). *Biochem. J.* **29**, 741.
Langston and Day (1933). *Southern Med. J.* **26**, 128.
— — and Cosgrove (1933). *Arch. Ophthal.* **10**, 508.
Sherman and Spohn (1923). *J. Amer. Chem. Soc.* **45**, 2719.