LXXXV. EFFECT OF DEFICIENCY OF VITAMIN B COMPLEX ON THE "REDOX" SYSTEM IN THE EYE-LENS.

By SURENDRA NATH RAY,[1] PAUL GYÖRGY and LESLIE JULIUS HARRIS.[2]

From the Nutritional Laboratory, Medical Research Council and University of Cambridge, and Department of Physiology, University of Cambridge.

(*Received January 31st, 1935.*)

Previous papers from the Nutritional Laboratory have demonstrated the presence of vitamin C in the lens of the eye [Birch and Dann, 1933; 1934] and this result has since been corroborated by many others [Euler and Martius, 1933; Müller, 1933]. Euler and Martius also made the further interesting observation that, in cataract in man, ascorbic acid could not be detected in the lens. At about the same time Langston and Day [1933] reported that they had been able to produce cataract in rats by diets deficient in vitamin B_2. These two findings suggested that there might be some interrelation between the vitamin B complex and ascorbic acid and other redox systems in the metabolism of the lens, and the present investigation was undertaken with the object of testing this possibility.

The procedure adopted was to keep groups of rats on diets lacking in one or more of the various vitamins of the B group. After a certain time, when the rats had developed lesions characteristic of the particular deficiency, they were killed and their lenses examined for vitamin C by titration against standard 2:6-dichlorophenolindophenol. In certain cases the lenses of animals cured by the administration of the appropriate vitamins were also examined.

Experimental.

The animals were usually killed by coal gas and their eye-balls removed without delay. The eye-balls were carefully incised with a sharp scalpel and the lenses were gently pressed out. To remove the aqueous and vitreous humours surrounding them, the lenses were rolled on a piece of filter-paper. With the application of a little pressure, the softer material could be rubbed away, leaving the harder portion behind. This was used for the estimation of vitamin C.

The small size of the eye-lens made it difficult to estimate the amount of vitamin C by direct titration. For this reason a modification of the micro-titration method of Birch *et al.* [1933] was used. The lenses from the eyes of one rat were weighed and ground up with 0·2 ml. of 20 % trichloroacetic acid and a little washed sand, and the resulting mixture was made up to 1 ml. The solution was centrifuged and an aliquot part (0·3 ml.) of the supernatant liquid was added to a known volume (0·05 ml.) of 2:6-dichlorophenolindophenol solution. The excess of the dye was then back-titrated by a solution of pure ascorbic acid (a solution of 5 mg. of the acid in 100 ml. of 10 % acetic acid solution being generally employed). The dye solution (a 0·1 % solution by weight was usually taken) alone

[1] Sir Tarak Nath Palit Research Scholar of Calcutta University.

[2] Member of Scientific Staff, Medical Research Council.

was next titrated against the ascorbic acid solution and the difference gave the amount of ascorbic acid present in the lens extract. With a little practice and when working with lenses from a single normal rat, duplicate results agreed to within 10 %.

The following two diets were generally used and will be referred to in the text as diets 1 and 4:

Diet 1		Diet 4	
Extracted caseinogen ...	18	Glaxo caseinogen A.B. ...	18
Salt mixture	4	Salt mixture	4
Cod-liver oil	1	Cod-liver oil	1
Butter-fat	9	Butter-fat	9
Rice starch	68	Cane sugar	66
		Agar	2
		Irradiated yeast 0·1 g. daily	

Diet 4 is partially deficient in vitamin B complex, and diet 1 is vitamin B-free. In the case of rats on diet 1, the B vitamins were supplied, when necessary, in the following forms: vitamin B_1 as solutions of pure crystalline B_1 (3 pigeon doses a day), vitamin B_2 complex as pure crystalline flavin *plus* "Peters's eluate" [Kinnersley *et al.*, 1933] for antipellagra factor. No supplements were generally used with diet 4, the irradiated yeast being prepared by exposure of dried yeast in thin layers to a mercury-vapour lamp for 24 hours. Fuller experimental details regarding the preparation of experimental animals are given in an accompanying paper [György, 1935, 2]. Adult rats kept on the stock basal diet used in the Nutritional Laboratory (brown bread, milk, green vegetables, with meat and liver once a week) were taken as normal controls.

Results.

The indophenol-reducing capacities (expressed in terms of concentration of ascorbic acid) of the lenses of rats on normal diet, on diet 4 and on diet 1 (supplemented by certain vitamins of the B group) are given in Table I.

Table I.

No. of rat	Wt. of eye-lens (g.)	Conc. of ascorbic acid (mg./g.)	Diet used	Nature of known deficiency
1	0·047	0·53	Normal stock diet	—
2	0·059	0·51	,, ,,	—
3	0·036	0·41	,, ,,	—
4	0·044	0·37	,, ,,	—
5	0·042	0·38	,, ,,	—
6	0·046	0·40	,, ,,	—
7	0·041	0·10	Diet 1 + vit. B_1 + 8γ of flavin + 2 g. of salmon per day	—
8	0·038	0·09	,, + vit. B_1 + 8γ of flavin + 2 g. of haddock per day	—
9	0·029	0·00	,, + vit. B_1 + 8γ of flavin	P-P
10	0·014	0·00	,, + ,, + antipellagra factor	Flavin
11	0·036	0·13	,, + ,, + ,, + ,,	Flavin
12	0·045	0·08	,, + ,, + ,, + ,,	Flavin
13	0·027	0·06	Diet 4	Partial deficiency of vit. B_1 and B_2 complex
14	0·041	0·06	,,	Partial deficiency of vit. B_1 and B_2 complex
15	0·036	0·14	,,	Partial deficiency of vit. B_1 and B_2 complex
16	0·047	0·07	,,	Partial deficiency of vit. B_1 and B_2 complex
17	0·041	0·05	,,	Partial deficiency of vit. B_1 and B_2 complex
18	0·041	0·13	,, + flavin + antipellagra factor	—
19	0·038	0·00	,, + 40 mg. of acid clay daily for the last 12 days	Partial deficiency of vit. B_2 complex

It will thus be seen that although there is a large quantity of reducing agent (vitamin C) in the lens of the normal rat, the amount present in the lens of a rat kept either on diet 1 or 4 is very low. But in addition it was found in the case of some of the rats kept on diet 4, that when the extract of the lens was added to a measured volume of 2:6-dichlorophenolindophenol, the resulting solution required a larger volume of ascorbic acid solution for complete decoloration than that necessary in control tests for the decoloration of the dye solution alone. This points to the presence of some oxidising substance in these lens. A few such results are given in Table II.

Table II.

No. of rat	Wt. of eye-lens (g.)	Amount of ascorbic acid in mg. oxidised by 1 g. of eye-lens
20	0·037	0·40
21	0·030	0·17
22	0·033	0·25

Significance of the diminished reduction titre.

This diminution of the indophenol-reducing agency almost to vanishing point in the lens of the rat may occasion surprise, since previous experience has shown conclusively that the rat is able to synthesise its own vitamin C [Parsons, 1920; Harris and Ray, 1933]. On the other hand other tissues, such as liver or kidney, from these same animals were found on examination to contain normal amounts of vitamin C. This might suggest either that the lens has a mechanism for synthesising vitamin C different from that present in liver or kidney, or that the vitamin C is not synthesised in the eye-lens but is supplied from elsewhere in the body and that the mechanism of this supply may break down in animals restricted to these special diets. An objection to the last theory may however be raised in the fact that when high doses of vitamin C (50 mg.) were injected into a rat kept on diet 4 its eye-lens showed a slight increase in concentration (about 0·15–0·20 mg./g.) of ascorbic acid. A third and more plausible theory is that the ascorbic acid in the lens forms, with some oxidising agent, part of a complex oxidation-reduction system. By the titration method we are examining only the amount of ascorbic acid in the reduced condition. It may be supposed that when animals are subjected to these special diets, the amount of oxidising substances in the eye-lens increases, thereby converting an equivalent amount of ascorbic acid into some oxidised product. Thus although the amount of total ascorbic acid (reduced + oxidised) may remain the same, it is evident that the titre would fall, thereby giving a false impression of a diminution in the amount of vitamin C. It must be pointed out however that under normal conditions the reducing titre for the lens (ox) is in fact equal to the vitamin C content as determined biologically [Birch and Dann, 1934]. Further work to elucidate these points is now being carried out.

Influence of individual dietetic factors.

The exact dietetic origin of this fall in the total reducing capacity of the eye-lens remains obscure. That inanition is not the cause is shown in Table III. The animals in these experiments were kept on a diet deficient in "vitamin H" [György, 1931; 1935, 1]. Besides loss of weight, they had all developed lesions characteristic of this deficiency. Yet it will be seen that the vitamin C content of the lens is quite normal.

Table III.

No. of rat	Initial wt. (g.)	Final wt. (g.)	Wt. of eye-lens (g.)	Concentration of ascorbic acid in mg./g.
23	76	69	0·040	0·53
24	73	48	0·040	0·45
25	69	57	0·015	0·44
26	73	58	0·026	0·48
27	89	77	0·022	0·49
28	71	55	0·025	0·48

These results demonstrate at the same time that "vitamin H" deficiency is not the cause of this diminution in reducing capacity. Nor does a deficiency of either flavin or the antipellagra factor appear to be responsible; for, as shown in Table I, even when these vitamins are supplied in the diet (Rats 7–12, 18), there is no improvement in the amount of vitamin C. The results with rats 7–12 and 19 also seem to rule out the possibility of vitamin B_1 deficiency being the cause. This conclusion is further brought out in Table IV where the experimental rats were kept on a diet deficient in vitamin B_1 only (alkaline autoclaved marmite being used to provide vitamin B_2 complex). All the rats exhibited advanced polyneuritic symptoms.

Table IV.

No. of rat	Wt. of eye-lens (g.)	Concentration of ascorbic acid in mg./g.
29	0·017	0·31
30	0·018	0·30
31	0·021	0·32
32	0·020	0·20
33	0·017	0·29*
34	0·020	0·27*

* Cured by administration of 1·5 pigeon doses of crystalline vitamin B_1 solution.

It will be noticed that the concentration of the reducing agent in the lens is fairly high and further that no change results when a cure is effected by an injection of vitamin B_1.

A few lenses from vitamin A-deficient rats, kindly provided by Dr W. J. Dann, were also examined, and they were also found to contain normal amounts of reducing factor.

The results seem to show that this peculiar metabolic disorder in the lens of rats subjected to certain diets is probably due to deficiency of some factor which is not identical either with vitamins B_1, B_2 (flavin or antipellagra factor), "H" or A. We have no evidence as yet as to the probable nature of this factor, but while testing several natural foodstuffs, we found raw egg-white to be a potent source of it. Liver extracts as well as fresh liver were also tested but were found to give rather irregular results. The reason for this is not known. The results are shown in Table V.

Table V shows that raw egg-white, administered in daily doses of 5 ml. possesses strong prophylactic as well as curative action. Smaller doses, 3 and 2 ml. per day, were also tried but the results then were not so clear-cut and gave rather variable values. It may also be pointed out that rats nos. 35–38 were suffering from acute dermatitis owing to deprivation of the antipellagra factor, yet the high reducing capacity of the lens emphasises the the non-identity of the antipellagra and eye factors.

Table V.

No. of rat	Wt. of eye-lens (g.)	Conc. of ascorbic acid in mg./g.	Diet used
35	0·030	0·39	Diet 1 + 5 ml. egg-white daily + vit. B_1
36	0·031	0·32	,, + ,, ,, + ,,
37	0·038	0·28	,, + ,, ,, + ,,
38	0·029	0·23	,, + ,, ,, + ,,
39	0·034	0·37	,, + ,, ,, + ,, + haddock (antipellagra factor)
40	0·028	0·43	,, + 5 ml. egg-white daily + vit. B_1 + Peters's eluate
41	0·034	0·22	Diet 4 + 5 ml. egg-white daily for the last 15 days
42	0·036	0·30	,, + ,, ,, ,,
43	0·021	0·25	Diet 1 + 2 g. liver daily
44	0·041	0·00	,, + ,, ,,
45	0·035	0·37	,, + B_1 + flavin + 2 ml. liver extract daily
46	0·047	0·11	,, + ,, + flavin + 2 ml. liver extract daily + Peters's eluate
47	0·047	0·20	,, + ,, + flavin + 2 ml. liver extract daily + Peters's eluate

It is as yet too early to suggest whether this diminution of the reducing power of the lens is due to some new vitamin. The nature of the factor present in raw egg-white is being investigated. We also hope to publish at a later date an account of the histological changes, if any, in the lens of the eye due to deprivation of this factor.

Vitamin C.

The vitamin C content of the eye-lens of guinea-pigs, both normal and scorbutic, has also been examined. As may be imagined, the vitamin C in the lens appears to depend upon the amount given in the food but curiously enough quite an appreciable amount of the vitamin (about 0·2 mg./g.) is held back tenaciously by the eye-lens, even when the animal has died from acute scurvy. The effect of chronic scurvy as well as combined deficiency of vitamins B and C is under investigation, and fuller details will be published later.

Oxidising agent present in blood.

According to van Eekelen *et al.* [1933; see also Emmerie and van Eekelen, 1934] vitamin C is present in a reversibly oxidised form in the blood. Direct titrations of trichloroacetic acid extracts of the blood of normal animals against 2:6-dichlorophenolindophenol, indeed, fail to show any reduction. With guinea-pigs or human beings given large doses of vitamin C, extracts of blood show small reducing values which however are almost negligible compared with the amount of vitamin C ingested. Nevertheless when extracts of normal blood were examined by the new back-titration method, the presence of some oxidising agent, of the same nature as that occasionally seen in the lens of rats fed on deficient diets (p. 737), was encountered. This finding suggests that there may be some agency present in blood which is capable of oxidising ascorbic acid, possibly into the reversibly oxidised form. If the amount of ascorbic acid ingested is very great, it is evident that after it has reduced the whole of the oxidising agent in the blood, some of the ascorbic acid may still remain and this surplus will give a net reducing value to the blood. Conversely, an increased concentration of the oxidising agent may be expected to be found in scorbutic animals. Only a few experiments have been performed in this direction and though the results seem to confirm this theory, much additional work is required before anything more definite can be said.

The results are summarised in Table VI.

Table VI.

No. of rat	Nature of animal	Wt. of blood taken (g.)	Amount of ascorbic acid in mg. capable of being oxidised by 1 g. of whole blood
48	Normal	8·65	0·019
49	,,	4·75	0·026
50	Diet 4	3·30	0·022
No. of guinea-pig			
G1	Normal	9·40	0·00
G2	19 days on scorbutic basal diet	8·55	0·039

SUMMARY.

1. A new technique for estimating vitamin C by a method of back-titration is described. By its means the indophenol-reducing capacity of the lens from a single normal rat can readily be estimated, the error of determination being within ± 10 %.

2. The indophenol-reducing capacity of the eye-lens as determined by this method is greatly diminished in rats which have been subjected to certain experimental diets lacking in various constituents of the vitamin B complex. In extreme cases, extracts of the lens, instead of showing a reducing capacity, actually oxidise ascorbic acid. The cause of the abnormality does not appear to be due to deficiency of vitamins B_1, B_2 (either flavin or antipellagra factor), "H" or A. It is however too early to say whether a new vitamin is involved.

3. Raw egg-white possesses marked prophylactic and curative action in this disorder.

4. The blood of normal and of deficient animals contains an oxidising agency similar to that found under certain conditions in the lens.

REFERENCES.

Birch and Dann (1933). *Nature*, **131**, 469.
—— —— (1934). *Biochem. J.* **28**, 638.
—— Harris and Ray (1933). *Biochem. J.* **27**, 590.
van Eekelen, Emmerie, Josephy and Wolff (1933). *Acta Brev. Neerl.* **3**, 168.
Emmerie and van Eekelen (1934). *Biochem. J.* **28**, 1153.
Euler and Martius (1933). *Z. physiol. Chem.* **222**, 65.
György (1931). *Z. ärztl. Fortbildung.*
—— (1935, 1). Handbuch der Kinderheilkunde by Pfaundler-Schlossmann (Berlin).
—— (1935, 2). *Biochem. J.* **29**, 741.
Harris and Ray (1933). *Biochem. J.* **27**, 2006.
Kinnersley, O'Brien, Peters and Reader (1933). *Biochem. J.* **27**, 225.
Langston and Day (1933). *Southern Med. J.* **26**, 128.
Müller (1933). *Nature*, **132**, 280.
Parsons (1920). *J. Biol. Chem.* **44**, 587.