CXCVI. EGG-WHITE AS A SOURCE OF THE ANTIDERMATITIS VITAMIN B₂.

BY HARRIETTE CHICK, ALICE MARY COPPING
AND MARGARET HONORA ROSCOE.

From the Department of Experimental Pathology, Lister Institute.

(Received October 28th, 1930.)

It has already been recorded that the fresh egg-white of the hen's egg contains vitamin B₂ unaccompanied by the antineuritic vitamin B₁ [Chick and Roscoe, 1929, 1]. In this property egg-white stands alone amongst the foodstuffs hitherto examined, for in all others in which vitamin B₂ has been found, vitamin B₁ has also been present, although the relative amounts of the two have shown a wide variation [Aykroyd and Roscoe, 1929; Roscoe, 1930].

This unusual property makes egg-white a valuable material for use in the investigation of the various members of the B group of vitamins. It has, therefore, seemed worth while to publish a short account of the evidence upon which the above statement is based.

Vitamin B₁ deficiency of egg-white.

If young rats immediately after weaning receive an artificial diet deprived of vitamin B₁, but otherwise complete and containing egg-white as source of protein and of vitamin B₂ [see Chick and Copping, 1930, 3, p. 1765, Diet II], growth takes place for 2-3 weeks, after which appetite fails and a decline in weight sets in. If this is not checked death will often follow and will be preceded by the paralysis characteristic of vitamin B₁ deficiency, which is promptly cured if vitamin B₁ is given. If, at the point where weight has become stationary or has begun to decline, vitamin B₁ is given as a daily dose of some foodstuff containing it, or as Peters's antineuritic concentrate, growth is immediately restored and the gain in weight will be in relation to the amount of vitamin B₁ administered. These facts have been applied in a method for estimation of vitamin B₁ in foodstuffs [Chick and Roscoe, 1929, 1].

Vitamin B₂ content of egg-white.

The content of vitamin B₂ can be estimated by measuring the minimal daily dose required to restore growth which has ceased in young rats after short periods, 1-2 weeks, on a synthetic diet containing vitamin B₁ and lacking only vitamin B₂ [Chick and Roscoe, 1928]. It has been found that a daily dose of 2.5-5.0 g. of fresh, cooked, egg-white (dry weight 0.3-0.6 g.) or of an

extract (see below) equivalent to 5–10 g. will restore “normal” growth (average weekly increase in weight of 10–12 g.). This result places egg-white among the foodstuffs richer in vitamin B₂, being of about equal value with milk and meat (reckoned on the dry weight), though much inferior to liver and yeast [Aykroyd and Roscoe, 1929].

Additional, more conclusive, evidence that vitamin B₂ is abundantly present in egg-white has been obtained from the cures which take place when the fresh material, or concentrates prepared therefrom, are administered to rats suffering from the characteristic skin lesions which develop in young rats after several weeks of vitamin B₂ deprivation.

A full description of these lesions is contained in a recent paper [Chick and Copping, 1930, 1; see also Chick and Roscoe, 1928]. The more “florid” dermatitis of ears and paws (see rats 513 and 529, Table I) has been rarely seen since the caseinogen in the basal diet has been carefully purified and it seems possible that its development is encouraged if the diet is not entirely free from traces of vitamin B₂ [see also Leader, 1930].

The details of some typical cures are set out in Table I. For these tests vitamin B₂ concentrates were prepared from egg-white after removal of the coagulable proteins as follows.

Fresh egg-white is diluted with 3·5 times its weight of distilled water and sulphuric acid added (ca. 4 cc. to 1000 g. egg-white) till the p_H is 4·2 (bromocresol green). The mixture is raised to the boil and boiled for about 5 minutes, care being taken to keep the mixture well stirred owing to its tendency to froth. The coagulum is filtered with slight suction and pressed in a hand-press, the press liquor being added to the filtrate. The press cake is weighed, and the quantity of adherent filtrate calculated on the assumption (which has been verified) that egg-white contains 10 % coagulable protein. Allowance for this loss is made in reckoning the amount of egg-white corresponding to the collected filtrate. This is evaporated in flat dishes under a fan in a hot room at 37° and afterwards on a water-bath at 100° till 1 cc. contains the equivalent of about 4–5 g. egg-white. The sulphates present are removed by addition of barium chloride solution, after making a preliminary trial with an aliquot portion to indicate the required amount. The removal is made slightly incomplete to avoid any risk of administering traces of barium to the experimental animals. The concentration of the filtrate is then continued until 1 cc. contains the equivalent of 5 g. egg-white and the p_H is adjusted to about 3·0 for storage.

A similar concentrate can be prepared with acetic in place of sulphuric acid. If the egg-white is diluted 4–5 times and acetic acid added to a concentration of 0·3 %, the p_H will be found optimal for precipitation of the protein. Separation and filtration of the coagulum is usually easier than in the sulphuric acid preparation, but the large amount of acetate in the final product is a drawback, rendering it unpalatable and likely to cause diarrhoea. Preparations made with sulphuric acid, on the other hand, are usually well taken and, if not entirely acceptable, can be made so by adding a few crystals of sugar to the daily dose.

Rats 400, 403 and 405 (Table I) had not suffered vitamin B₂ deficiency long enough for the development of skin lesions but show the effect of cooked fresh egg-white and of the purified concentrate in restoring growth after 7 weeks' deprivation. The restoration of growth, however, was not permanent and the rate fell off after a few weeks, irrespective of the size of the daily ration. This failure, which seems to vary in degree according to the duration of the previous period of deprivation, we believe is not to be attributed to

Table I. *Vitamin B₂ content of egg-white products.*

Restoration of growth and cure of dermatitis in young rats previously maintained on a diet (P2L) deprived of vitamin B₂; vitamin B₁ provided as a daily dose 0.1 cc. $\equiv$ 0.6 g. yeast of Peters's antineuritic concentrate [Peters, 1924; Kinnorsley and Peters, 1925, 1927].

Material	Dose, expressed as equivalent of fresh egg-white g.	Rat No. and sex	Time previously on "B ₂ " diet Weeks	Symptoms and general condition at beginning of cure	Duration of treatment Weeks	Body weight			Result of treatment
						At beginning of cure g.	Total increase during treatment g.	Average weekly increase g.	
Fresh egg-white	2.5	403 ♀	7	No skin symptoms. Body weight stationary	8	51	50	6.5	Growth good at first, but rate decreasing towards end of the period
"	5.0	400 ♂	7	"	10	59	73	7	"
Egg-white 3 concentrate (acetic acid)	5	405 ♀	7	"	8	46	66	8.5	Growth good at first, but weight stationary during last 2 weeks
"	5	387 ♀	16	Skin symptoms moderate. Eyes sunken "spectacled." Fur very poor, stringy in places, epidermis peeling off nose and mouth, abdomen stained	4	38	30	7.5	Good cure. Marked improvement in 7 days, eyes better, skin healthy and new fur beginning to grow. In 3 weeks coat good and eyes nearly normal. Increase in weight substantial and not improved by doubling the dose
Egg-white 3 concentrate (acetic acid)	5	392 ♂	13	No definite dermatitis. Both eyes closed, eyelids stuck together, general condition miserable	5	47	49	10	Cure. After 7 days epidermis on face desquamating; after 3 weeks skin and fur normal, eyes nearly normal
Egg-white 11 concentrate (acetic acid)	5 later 10	513 ♀	22	Skin symptoms severe. Eyes sunken "spectacled." Skin of ears "pawery." Fur "moist," "stringy," raw place under chin. Abdomen stained. General condition miserable	2) 3)	52	20 33	10.5	Rapid cure. Marked improvement in 5 days, eyes less sunken, skin beginning to look healthy, raw patch healed, appetite improved. In 4 weeks skin normal

Egg-white 11 concentrate (acetic acid)	2.5 later 10	529 ♀	21	<i>Skin symptoms severe.</i> Eyes sunken, "spectacled," right almost closed. Fur poor, raw patches of skin on fore limbs and on right chest, marked, "florid" dermatitis on fore paws	2) 3)	47	13) 37)	10	<i>Slow cure.</i> Definite improvement in 7 days, slower than with rat 513. Raw patches not healed in 12 days. Skin healed and coat normal after 3 weeks on increased dose
Egg-white 18 concentrate (sulphuric acid)	10	663 ♂	30	<i>Skin symptoms severe.</i> Eyes sunken and secreting. Coat very bad, moist and stringy; dry scurfy patches on haunches. Raw place slowly de- veloped during 6 weeks on right fore limb, extending from neck to paw. General condition bad, dropped penis	3	99	29	10	<i>Slow cure.</i> General condition deterio- rated during first few days with de- velopment of helplessness in hind limbs and spasticity of muscles; marked improvement in this and skin condition after 9 days. After 2 weeks raw place on right fore limb almost completely healed. In 3 weeks skin soft and healthy, new fur growing. Penis still dropped
Egg-white 18 concentrate (sulphuric acid)	10	710 ♂	19	<i>Skin symptoms moderate.</i> Eyes sunk- en and secreting. Nose and paws blood stained. Fur poor, nearly bald on head and neck, dry scurfy patches on haunches. General condition poor, dropped penis	3	56	39	13	<i>Rapid cure.</i> After 9 days skin soft and velvety, no blood stains on wrists. After 2 weeks scurfy patches much improved, though persisting. After 3 weeks skin healthy and clean, new fur growing. Penis still dropped

insufficiency of vitamin B₂, but to lack of a further, at present unknown factor, which accompanies vitamin B₂ in many foodstuffs, but is absent from, or deficient in, egg-white [see Chick and Copping, 1930, 3].

The remaining observations in Table I illustrate the swift and certain action of egg-white concentrates in curing the rat dermatitis developed after long periods (13–30 weeks) of vitamin B₂ deprivation. In most cases the skin was restored to a normal state in about 3 weeks with doses equivalent to 5–10 g. of the fresh egg-white. Restoration of growth invariably accompanied healing of the skin lesions, but was usually checked after 2 or 3 weeks.

Attempts at further purification of vitamin B₂ preparations from egg-white.

Alcohol fractionation was tried [see Narayanan and Drummond, 1930; Chick and Copping, 1930, 2], but the precipitate (ovomucoid) formed in alcohol of 70 % strength (by weight) and the filtrate, both proved to be inactive. Using weaker alcohol of 50 % strength, both precipitate and filtrate were found to contain a portion of the original activity. An attempt to make an extract from egg-white by boiling in 50 % alcohol at p_H 4.0 also failed; in this case the filtrate after separation of the precipitated proteins was inactive.

Precipitation with basic lead acetate [Chick and Roscoe, 1929, 2; Narayanan and Drummond, 1930] at p_H 8.0 was also tried, but the solution obtained after decomposition of the precipitate by H₂S was inactive.

Separation of vitamin B₁ from the white in the egg.

The agency by which the vitamin B₁, abundantly present in the yolk, is prevented from diffusing into the surrounding egg-white, is unknown, since both are water-soluble and capable of free diffusion through membranes. A mixture of vitamins B₁ and B₂ contained in a watery yeast extract was found to dialyse freely through a cellophane membrane [Chick and Roscoe, 1929, 2]. It was conceivable, however, that in egg-yolk vitamin B₁ might exist in some combination which prevented free diffusion. Undiluted egg-yolk was therefore placed in a cellophane membrane surrounded by distilled water and left to stand for 4 days at about 0°, but vitamin B₁ was found present in the surrounding fluid.

It is probable that the diffusion of vitamin B₁ in the egg from the yolk to the white is prevented by some property of the living yolk membrane.

SUMMARY.

1. Egg-white of hen's eggs, while a relatively rich source of the anti-dermatitis vitamin B₂, is lacking in vitamin B₁, and in this respect is unique among the foodstuffs hitherto examined.
2. Convenient vitamin B₂ concentrates can be prepared from egg-white by removal of the heat-coagulable proteins. Growth can be restored in young rats suffering from vitamin B₂ deficiency, and the characteristic skin lesions

healed, by daily doses of this concentrate equivalent to 5–10 g. of the original egg-white.

3. Attempts to purify these concentrates further by alcohol fractionation or precipitation with lead acetate have been unsuccessful.

REFERENCES.

- Aykroyd and Roscoe (1929). *Biochem. J.* **23**, 483.
Chick and Copping (1930, 1). *Biochem. J.* **24**, 932.
—— — (1930, 2). *Biochem. J.* **24**, 1744.
—— — (1930, 3). *Biochem. J.* **24**, 1764.
—— and Roscoe (1928). *Biochem. J.* **22**, 790.
—— — (1929, 1). *Biochem. J.* **23**, 498.
—— — (1929, 2). *Biochem. J.* **23**, 504.
Kinnnersley and Peters (1925). *Biochem. J.* **19**, 820.
—— — (1927). *Biochem. J.* **21**, 777.
Leader (1930). *Biochem. J.* **24**, 1172.
Narayanan and Drummond (1930). *Biochem. J.* **24**, 19.
Peters (1924). *Biochem. J.* **18**, 858.
Roscoe (1930). *Biochem. J.* **24**, 1754.