XXXIX. EGG-WHITE AS SOLE SOURCE OF PROTEIN AND VITAMIN B₂ FOR YOUNG RATS.

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WHEN we consider the literature concerning egg-white in nutrition we are struck by the fact that some authors have found toxic properties in dried or fresh egg-white [*e.g.* Boas-Fixsen, 1931; Parsons and Kelly, 1933] and that others have used cooked egg-white as source of protein and vitamin B₂ [*e.g.* Chick, Copping and Roscoe, 1930; György, 1934]. The toxic effect, according to Boas [1927] may be counteracted by a "factor X", occurring in many foodstuffs including dried yeast, and according to Parsons [1930], by liver and yeast. Moreover the damaging property of the egg-white is counteracted by certain treatments, as, for example, thorough coagulation or denaturation by heat [Parsons and Kelly, 1933]. A very slowly acting poison which is counteracted by a normally non-stimulating foodstuff (liver, yeast) may not be considered toxic in the strict sense of the word. Many foodstuffs may become toxic, if fed in too great quantities and if certain counteracting foodstuffs are omitted or reduced. On the other hand, specific deficiency diseases are accompanied, if not caused, by an intoxication with metabolic products. The question whether egg-white, in the dried, fresh, raw or cooked state, is deleterious in the presence of all known food-substances and vitamins, and to what extent the damage may be counteracted by other foodstuffs, containing perhaps a hitherto unrecognised dietary principle, appeared to deserve further investigation. With this aim I undertook the experiments, the results of which are summarised briefly in this paper.

EXPERIMENTAL.

Section I.

Experiments were first made with egg-white subjected to various preparatory treatments, analogous to those already employed by other workers. Albino rats were used and kept in iron cages with mesh floors. The breeding diet contained raw meat, raw vegetables, wheaten bread, wheat, raw milk and a commercial chicken-food, composed of cereal, milk, peanut, meat and fish products and calcium phosphate. This food was administered until the litters were 3 weeks old. The young rats then received, with their mother's milk, an artificial diet containing: 20 % previously coagulated egg-white; 60 % potato-starch; 16 % hardened arachis fat; 4 % salt-mixture [see Stammers, 1909] with traces of CuSO₄, 5H₂O. The ration was thoroughly mixed and cooked with 4 parts of tap-water and administered in paste form, supplemented by 2 % cod-liver oil ("jeco-vitol"), to provide vitamins A and D, and by 0.5 % activated acid clay, made from rice-bran according to the method of Jansen and Donath [1927], as source of vitamin B₁. The young rats were weaned when 4 weeks old and the above

diet was continued for a further week. When 5 weeks of age, the litters were distributed evenly over three groups, in which the 20 % protein was varied thus:

A, 20 % egg-white; B, 20 % purified caseinogen; C, 10 % egg-white+10 % caseinogen.

In the A diets the treatment of the egg-white was varied as follows:

Diet A1: 20 % raw, dried, powdered Chinese egg-white mixed in the raw state through the cooled food paste.

Diet A2: 20 % (by dry weight) raw, fresh, undried egg-white mixed in the ration.

Diet A3: as A1, but the Chinese egg-white cooked 5 minutes with the ration.

Diet A4: as A2, but the fresh egg-white cooked 5 minutes with the ration.

Diet A5: as A1, but the Chinese egg-white dissolved in water (1½ hours at 30°), then thoroughly coagulated by heating the solution for 3 hours at 80°.

Diet A6: as A5, but the solution denatured by treatment with HCl at p_H 2.4 for 3 days at 37° [Parsons and Kelly, 1933], the reaction being adjusted to p_H 5 by addition of $NaHCO_3$ before mixing with the ration.

In Diets C1 and C2 the 10 % egg-white was respectively dried Chinese and fresh, treated as in Diets A1 and A2.

Diets D3–D6 and E3–E6, contained 20 % egg-white, treated exactly as in Diets A3–A6, but in the D diets 2 % dried brewer's yeast was added and in the E diets 2 % raw, dried horse-liver.

The results of these trials are summarised in Table I. 23 out of 24 young rats on Diets A1 and A2, containing 20 % untreated raw egg-white, dried or fresh, as sole source of protein and vitamin B₂, survived for 42–96 days; cooking the egg-white for a short time (A3 and A4) did not improve matters, as Parsons had

Table I.

	All diets contained 60 % potato-starch.						20 % purified caseinogen	10 % egg-white + 10 % purified caseinogen	
Protein in diet	20 % egg-white								
	A1	A2	A3	A4	A5	A6	B	C1	C2
No. of rats	15	9	8	12	8	8	13	3	3
Duration of exp. (days)	96	42	62	22	42	20	53	20	12
Average weekly in- crease in wt. (g.)	4.5	2.5	8	3.5	8	9	4	15	11
Affections and symptoms	Bald- ness	Eyes Ears Mouth	Pare- sis	Eyes Ears	Pare- sis	None	Bald- ness (2 rats)	None	Eyes (1 rat)
No. of rats dying	0	1	2	4	4	0	4	0	0
Protein in diet	20 % egg-white + 2 % dried brewer's yeast						20 % egg-white + 2 % dried horse-liver		
	D3	D4	D5	D6		E3	E5	E6	
No. of rats	8	54	8	8		4	3	2	
Duration of exp. (days)	62	22	42	20		33	13	20	
Average weekly in- crease in wt. (g.)	11.5	10.5	9	7.5		7.5	14	15	
Affections and symptoms	Eyes Ears Mouth	Eyes Ears	Bald- ness (1 rat)	None		Bald- ness	None	None	
No. of rats dying	2	12	0	0		0	1	0	

already found. Diet A5, in which the egg-white was heated for 3 hours, showed an unexpectedly high rate of mortality (4 out of 8 rats). This I believe to be due to the fact that the egg-white used was not milled as finely as that of the other rations, at least during the first 3 weeks of the experiment; at the end of those 3 weeks half of the rats died. It would seem that the coagulation of a certain part of the egg-white had not been complete. Diet A6, in which the Chinese egg-white was denaturated with HCl, gave the best results.

Diet B (20 % caseinogen) was unsatisfactory in consequence of the deficiency in vitamin B₂.

Diets C1 and C2 (10 % caseinogen with 10 % Chinese dried, or 10 % fresh, egg-white) gave unexpectedly good results during the short time of the trial (12–20 days).

Diets D1–D5, in which the 20 % egg-white was supplemented with 2 % dried brewer's yeast, were not wholly harmless; diets E1–E5, with 2 % dried liver, were rather better during the relatively short time of observation.

The dried egg-white did not generally prove more injurious than the fresh material, but while the latter caused no paresis or baldness, affections of eyes, ears and mouth were often present and the fur was always dirty.

Refecation was never seen in these experiments and coprophagy was hindered by the use of wire cage floors of 1 cm. mesh. But, in order to exclude further the chance of refecation, most of the above experiments were repeated, using rice-starch and sucrose instead of potato-starch, which according to Boas [1927] has a counteracting effect on the damage caused by egg-white. These results are collected in Table II.

Diets a, b, c, *etc.* correspond to diets A, B, C, described above, with 60 % rice-starch in place of 60 % potato-starch: diets a', b', c', *etc.* contained 20 % of rice starch and 40 % sucrose.

Diets f contained 3 % (by dry weight) of egg-yolk and diets g 20 % of egg-yolk.

Of the diets marked with Greek letters, α contained 40 % egg-white and 40 % rice-starch; α' 40 % egg-white, no rice-starch and 40 % sucrose; β 40 % purified caseinogen and 40 % rice-starch; γ 20 % egg-white, 20 % purified caseinogen and 40 % rice-starch. Diet γ' contained 20 % egg-white, 20 % purified caseinogen, no rice-starch and 40 % sucrose; diet δ' contained 40 % egg-white, 40 % sucrose and 2 % dried brewer's yeast.

In the different experiments summarised in Table II the results regarding growth are not altogether comparable with one another, for the trials of longer duration naturally show lower average growth-rates, owing to the fact that the rats are becoming more nearly adult (see *e.g.* diets α 3 and γ 3). From these experiments it may, however, be concluded that egg-white in the raw, dried state, is not so very detrimental as source of protein in a diet if all known vitamins are present, including the factors supposed to occur in yeast, egg-yolk and liver, *e.g.* the "factor Y", of Chick and Copping [1930] or "factor X" of Boas [1927; Fixsen, 1930]. If these factors are omitted, damage occurs in some animals, but not in all, unless the egg-white is treated with HCl according to Parsons and Kelly [1933]. The process of drying in itself did not prove to result in damage to the rats, as was thought by Boas [1927].

When the diets contained 60 % cooked potato-starch (Table I) growth was promoted and symptoms prevented rather more effectively than when rice-starch and sucrose (Table II) were used as source of carbohydrates; compare *e.g.* diets A3, A5 with a3, a5 and diet C1 with c1. When, in addition, yeast or liver was given, there was no difference; compare *e.g.* diets D3, D5, E3, E5 with diets

Table II.

Carbohydrate in all diets given as rice-starch or sucrose.

Protein in diet	20 % egg-white						20 % purified caseinogen			
	a1	a'1	a3	a4	a5	a6	b	b'		
No. of rats	16	12	13	8	10	6	47	20		
Duration of exp. (days)	50	50	62	31	42	40	70	50		
Average weekly increase in wt. (g.)	4	5.5	5.5	2	5	4.5	2.5	1.5		
Affections and symptoms	Baldness, paresis	Baldness, shaggy coat	Baldness, paresis (2 rats)	Baldness	Baldness, paresis	None	Baldness	Baldness, shaggy coat		
No. of rats dying	9	4	7	0	6	2	12	3		
Protein in diet	10 % egg-white + 10 % purified caseinogen		20 % egg-white + 2 % dried brewer's yeast					20 % egg-white + 2 % dried horse-liver		
	c1	c6	d1	d3	d4	d5	d6	e3	e5	e6
No. of rats	12	4	12	8	7	8	8	4	2	2
Duration of exp. (days)	40	40	57	62	35	42	20	33	20	20
Average weekly increase in wt. (g.)	6	10.5	12	12.5	9.5	11	10	8	17	14
Affections and symptoms	Baldness, paresis	Baldness	None	Eyes Ears (2 rats)	Slight baldness	Baldness	None	Baldness (1 rat)	None	None
No. of rats dying	4	1	1	2	0	0	0	0	1	0
Protein in diet	20 % egg-white + 3 % and 20 % egg-yolk		40 % egg-white				40 % purified caseinogen			
	f4	g4	α1	α'1	α3	α4	β			
No. of rats	8	8	4	3	8	4	10			
Duration of exp. (days)	31	31	40	33	76	20	28			
Average weekly increase in wt. (g.)	7	15	6	4	(2)	10	8			
Affections and symptoms	None	None	Baldness (1 rat)	Shaggy coat	Shaggy coat	Shaggy coat	Shaggy coat			
No. of rats dying	0	0	2	0	0	0	0			
Protein in diet	20 % egg-white + 20 % purified caseinogen					40 % egg-white + 2 % dried brewer's yeast				
	γ1	γ'1	γ2	γ3	δ'1 δ'4					
No. of rats	4	6	6	10	1 8					
Duration of exp. (days)	24	28	8	76	19 20					
Average weekly increase in wt. (g.)	9	11	12	(5.5)	10 14.5					
Affections and symptoms	Baldness	Shaggy coat	None	Shaggy coat	None Shaggy coat					
No. of rats dying	0	0	0	0	0 0					

d3, d5, e3, e5. These results confirm the conclusion of Boas [1927] that a "factor X", occurring in potato-starch, as well as in yeast, commercial caseinogen and several other foodstuffs, was able to counteract the toxic properties of egg-white in the diet.

During the above experiments (section I) my attention was attracted to the extraordinarily good growth on diets C, c and γ , which contained both purified caseinogen and egg-white as sources of protein. Boas-Fixsen found that factor X occurred to a relatively small extent in commercial caseinogen, but although a special growth-promoting property has been ascribed to commercial "light white casein" (Coward) no such property has been shown to exist in "purified" caseinogen. Further evidence on this subject is described in the following section.

Section II.

In order to accustom 124 young rats 3 weeks old to egg-white, 5 % (dry weight) of fresh rapidly coagulated egg-white was added to a b-ration, which already contained 20 % purified caseinogen, which had been purified by washing the caseinogen in a mixture of 10 l. "Monster" tap-water, 5 l. 96 % alcohol and 28 ml. *N* HCl (p_H 4.1 measured by glass-electrode) during 1 week, subsequently washing in acid 85–90 % alcohol (p_H 5.1) during 1 week and finally extracting with ether in a Soxhlet-apparatus for 2 weeks. After 2 weeks, when 5 weeks old, the rats were distributed over 2 groups, one of which received 20 % purified caseinogen as sole source of protein (39 rats), the other 20 % (dry weight) of fresh, rapidly coagulated egg-white. For both groups the increase in weight during the following 6 weeks (18–20 g.) was about equal to that of the comparable groups in section I above (a4, b see Fig. 1 and Table II) and in both the greater part of

Fig. 1.

Fig. 2.

Fig. 3.

x Denotes division of the rats into 2 groups, each group receiving a different kind of protein.

this increase (ca. 12 g.) occurred in the first 2 weeks, that is in the 6th and 7th weeks of life (Fig. 1). The young rats described above in section I had never shown this peculiarity; those fed on diet a4 increased by 2 g. during the first 10 days and those on diet b by 3.5 g. during the first 2 weeks, *i.e.* by rather less than the average weekly rate of growth shown over the longer period, which was 2 and 2.5 g. respectively. It was evident that in the present instance the previous presence in the rations of egg-white and purified caseinogen exerted a beneficial effect on the growth even for 2 weeks after one of them had been omitted. If this is true, the growth should also be enhanced for a period of about 2 weeks when a change is made from 20 % egg-white to 20 % purified caseinogen or *vice versa*. Special experiments were made to test this. 47 rats 3 weeks old received diet a4 (egg-white), 65 others diet b (caseinogen). When 4 weeks old they were weaned and, when 5 weeks old, distributed over 4 groups; some which had been fed on diet a4 (egg-white) now received diet b (caseinogen) and some which had been fed on diet b, now received diet a4. The remainder in each

group were continued on the previous diet. The result was a clear-cut corroboration of my expectation (Figs. 2 and 3).

When the diet during the 4th and 5th weeks of life had contained 20 % egg-white, the average growth on the changed diet (caseinogen, 24 rats) was 9.5 g. during the first 10 days, while that on the unchanged diet (23 rats) was 0.5 g. When the diet during the 4th and 5th weeks had contained 20 % purified caseinogen, the average growth on the unchanged diet (49 rats) was 3.5 g. during the 6th and 7th weeks and on the changed diet (egg-white) 12.5 g. (16 rats). Thus, in either case when the protein of the diet was altered, the growth was at first stimulated, presumably because the changed protein provided some factor absent from the previous one. After 10–14 days, the rate diminished (1–3 g. weekly), presumably because the reserve of some factor present in the first protein, but absent from the second, was becoming exhausted. When, on the contrary, the protein was not altered, there was no improvement in either growth or condition. Change in appetite could not have played any part, for the food intake was kept the same in all groups by giving a relatively small supply every day, so that each rat could eat it all.

We may conclude, therefore, that purified caseinogen, preferably in combination with potato-starch, can partially replace the growth-promoting action of yeast, liver or egg-yolk when added to diets containing egg-white as source both of protein and vitamin B₂. Vitamins A, B₁ and D were always given in sufficient doses. In such diets both purified caseinogen and egg-white are required to maintain an appreciable though subnormal growth; the first supplies an unrecognised food principle, the second vitamin B₂.

Section III.

In the following experiments additional evidence is given that growth may be almost completely restored by incorporation of 40 % purified caseinogen (see group 3) into the ration containing egg-white as source of protein, and that the essential dietary factor, which is thus added, is not extractable by washing the caseinogen with dilute acid alcohol (27 %), acid, 85–90 % alcohol or ether.

Two litters of young rats during the 4th and 5th weeks of life received a diet containing as much as 50 % (dry weight) egg-white, this high proportion being at the expense of the potato-starch, which was reduced to 30 %. In other respects the diet resembled diet A3 of section I. No symptoms of "proteintoxico" [Glanzmann, 1934] caused by the large amount of rapidly coagulated, previously dried egg-white were to be seen during these 2 weeks. (Glanzmann often observed paresis and death within 2 days with dried buttermilk.) The litters were then distributed over three groups, 6 rats in each, maintained on diets containing different proteins, with results as follows:

Group 1: 50 % egg-white; weekly growth 10.5 g. during 3 weeks.

Group 2: 50 % purified caseinogen; weekly growth 12 g. during 3 weeks.

Group 3: 10 % egg-white and 40 % purified caseinogen; weekly growth 19.5 g. during 4 weeks.

In group 3 growth was almost maximum, because the two factors contained in egg-white and in purified caseinogen, respectively, supplemented each other in their growth-promoting actions. Maximum growth (25 g. weekly, as was obtained with the breeding diet) was only secured by the addition of 3 % dried yeast (suggesting the further action of the factor Y of Chick and Copping [1930]).

In groups 1 and 2 the weight increase stopped after 1 month; in group 3 it diminished, but remained almost maximum during 2 months, even when no

yeast was added, showing that not only "factor Y" but also another factor may be growth-promoting when added to the egg-white rations. The rats of group 1 (50 % egg-white) when 6 weeks old, developed bald patches on head, neck and back, giving them a rather moth-eaten appearance. The rats of group 2 (50 % purified caseinogen), after recovering from a slight degree of baldness evidently due to the preceding feeding with egg-white during the 4th and 5th weeks, developed rather shaggy coats when 8 weeks old. The rats of group 3 remained in good condition, but after 2 months incipient depigmentation sometimes appeared when pied rats were used [Gorter, 1934]. One may conclude that the baldness is associated with egg-white feeding and that this can probably be prevented by a dietary factor which occurs to a relatively small extent in purified caseinogen.

DISCUSSION.

The question arises, whether 20 % egg-white is to be regarded as an incomplete, or as a toxic, source of protein and whether it contains enough vitamin B₂ for growth. Mitchell [1925] found 18 % coagulated egg-white to be a satisfactory source of protein; in this work dried whole yeast was the source of B-vitamins, but this contains protein also. Boas [1927; Boas-Fixsen, 1931] and Parsons [1930] described, however, specific ill-effects caused by 20-66 % egg-white (dry weight) in the diet; as source of B-vitamins they used marmite (Boas) and wheat embryo or an extract of wheat embryo.

According to Parsons and Kelly [1933] the toxic effect of egg-white cannot be due to an amino-acid deficiency; for when thoroughly denatured (with HCl), 20-66 % egg-white in a diet always proved harmless. The possible influence of the protein provided by the 10 % whole wheat embryo used in their most successful growth experiments should, however, be remembered, as a hint that an amino-acid deficiency of the egg-white is still possible.

In respect of the vitamin B₂ quality of egg-white, Chick, Copping and Roscoe [1930] concluded that 20 % egg-white (dry weight), as sole source of protein and of vitamin B₂, provided an ample supply of vitamin for a few weeks only.

If egg-white be regarded as adequate for provision of vitamin B₂ there must be some other nutritive fault of diets containing 20 % coagulated egg-white as sole source of protein and vitamin B₂, a defect which, however, does not influence the growth for the first 2 weeks. If purified caseinogen is also present in sufficient quantity in the diet (preferably in combination with potato-starch), the damage associated with egg-white does not appear for a period of 3 months. The hypothetical dietary factor, occurring in purified caseinogen, possibly an amino-acid, is distinguished from all known B-vitamins except B₃ [see O'Brien, 1934] by its relative insolubility in acid dilute alcohol. It may be connected with the equally insoluble factor X [Boas, 1927; Boas-Fixsen, 1931; Glanzmann, 1934; Lease and Parsons, 1934, 1] which counteracts the deleterious effect of egg-white in the dried raw state, or perhaps with the dietary factor described by Coward *et al.* [1929] in "light white casein" (see also Mapson [1933]). The latter, however, was not present in (purified) "Glaxo casein" and was soluble in 96 % alcohol.

Lease and Parsons [1934, 2] recently extracted a factor curative of the egg-white dermatitis in rats from kidney or liver, after previous digestion with papain. I have not yet tried this method with caseinogen.

SUMMARY.

1. The toxic effects of egg-white, when present as sole source of protein and vitamin B₂ in a diet, which cause the well-known skin disorder in young rats, can be counteracted by substitution of purified caseinogen for part of the egg-white.

2. The dietary factor contained in caseinogen and lacking in egg-white is present also in liver, yeast and egg-yolk; it is insoluble in dilute or strong acid alcohol or in ether.

3. While differing in solubility from the known B-vitamins and from the dietary factor in commercial caseinogen described by Coward *et al.*, it would appear to be similar in distribution and function to the "factor X" described by Boas, and partly also to the "factor Y" described by Chick *et al.* The possibility that it may be an amino-acid is not excluded.

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