LXIX. THE EFFECT OF DESICCATION UPON THE NUTRITIVE PROPERTIES OF EGG-WHITE. II.

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In a previous paper [Boas, 1927] it was shown that dried egg-white, when used as the sole source of protein in a diet for young rats, produces a train of characteristic symptoms—notably dermatitis, baldness and spastic gait—terminating in death in from 4 to 6 weeks. It was shown further that this syndrome can be completely prevented if certain substances such as yeast, potato, arrowroot, commercial caseinogen, fresh egg-white and others are added to the diet, and the presence of an unknown protective dietary factor X in these substances was postulated. The symptoms were produced only when the egg-white was dried in the undenatured state, but the results were the same whether dehydration was carried out by evaporation in air at 37°, *in vacuo* in the presence of H_2SO_4 at room temperature, or by treatment with alcohol. The two following alternative hypotheses were advanced as possible explanations of the phenomena:

(a) that dried egg-white is lacking in some essential dietary factor, which is supplied by protective factor X;

(b) that a toxic substance is formed in dried egg-white as a result of desiccation, and that this is neutralised in some way by protective factor X.

The investigation has since been carried a stage further and as there is at present no prospect of continuing it, it seems best to publish the results so far obtained although the work is still incomplete. There are now other workers in the field and it is hoped that the experiments described in this paper may be of assistance to them.

Findlay and Stern [1929], using the same diet as the author, obtained similar results and confirmed the protective power of raw potato, yeast, raw egg-white, egg-yolk and cow's milk. They found raw liver to have a high potency. They suggest that the condition produced in the rats fed upon the dried egg-white diet is analogous to "pink disease" (Swift's disease) in children, the clinical picture of nervous and cutaneous symptoms being similar. An extensive histological study of the rats showed that the most important changes were in the skin, where there was active keratinisation,

and in the spinal cord, where a diffuse infiltration of small round cells into both grey and white matter was revealed. A mild case of Swift's disease examined by them showed a similar infiltration into the spinal cord. Since Findlay and Stern were able to produce the characteristic symptoms in newborn and suckling rats by feeding the mother during pregnancy and lactation on the dried egg-white diet, they favour the hypothesis that the dried egg-white is deficient in some dietary essential, but they admit that the theory that a toxic substance is involved is not excluded by this evidence.

Parsons [1931] has also produced the dried egg-white syndrome in rats, which for the most part received diets containing as much as 66 % of protein in the form of dried egg-white, raw or cooked. In her description of the symptoms Parsons mentions in addition a scaly condition of the tails which the present author has not observed. Parsons found that dried beef-liver, if constituting 20 % of the diet, protected the rats but that 10 % did not and that 20 % of dried yeast was also ineffective.

The present paper is mainly concerned with a series of tests, some of a curative and some of a preventive nature, in which the effects of using some of the different constituents of egg-white, fresh or dried, as the source of protein were investigated. Such tests were expected to produce evidence which was in favour of or could be set against one or other of the alternative hypotheses described above.

DESCRIPTION OF DIETS AND MATERIALS USED.

Table I gives full details of the constitution of the various diets used in these experiments. Each rat received in addition to the diet 3 to 5 drops of cod-liver oil daily. All the egg-white diets were dissolved in water and coagulated by heat before incorporation in the diet.

Table I. *Details of the diets used.*

	DW diet	DG diet	Diets containing separate egg-white proteins							Casein- ogen diet	Liver diet	Steak diet
			(1 a)	(1 b)	2	3	4	5	6	7		
Dried egg-white	100	100	—	—	—	—	—	—	—	50	50	50
Pure ovalbumin solution	—	—	100 (dry)	—	—	—	—	—	—	—	—	—
Dried pure ovalbumin	—	—	—	100	—	—	—	—	—	—	—	—
Dried ovoglobulin*	—	—	—	—	200	—	—	—	16	—	—	—
Dried egg-white less globulins	—	—	—	—	—	100	—	—	—	—	—	—
Dried albumins and ovomucoid	—	—	—	—	—	—	100	—	91	50	—	—
Albumins and ovomucoid from dried egg-white	—	—	—	—	—	—	—	100	—	—	—	—
Fresh raw ox-liver	—	—	—	—	—	—	—	—	—	—	250	—
Fresh raw beef steak	—	—	—	—	—	—	—	—	—	—	—	250
Purified caseinogen	—	—	—	—	—	—	—	—	—	50	—	—
Wheat starch	250	—	250	250	250	250	250	250	250	250	250	250
Glucose	—	250	—	—	—	—	250 or 250	250	—	—	—	—
Hardened cotton seed oil	75	75	75	75	75	75	75	75	75	75	75	75
Marmite	25	25	25	25	25	25	25	25	25	25	25	25
Salt mixtures†	25	25	25	25	25	25	25	25	25	25	25	25
Distilled water	300	300	—	300	300	300	300	300	300	300	150	150

* This was 50 % NaCl.

† McCollum, Simmonds and Pitz [1917].

The materials used were prepared as follows.

Dried egg-white. See Boas [1927].

Egg-white fractions. (1 *a*) *Pure ovalbumin.* Ovalbumin crystals were prepared from fresh egg-white by the method of Hopkins and Pinkus [1898], dissolved in water and dialysed against tap-water until free from sulphate, a little toluene being added to prevent decomposition.

(1 *b*) *Pure ovalbumin, dried.* Fraction (1 *a*) was dried at 37°.

(2) *Ovoglobulin, dried.* To raw fresh egg-white an equal quantity of a saturated solution of ammonium sulphate was added and the precipitate filtered off. This was treated with a dilute solution of sodium chloride in order to dissolve the ovoglobulin. Most of the ovomucin remains undissolved [Hektoen and Cole, 1928] and can be filtered off. The ovoglobulin was then treated again with a saturated solution of ammonium sulphate and the precipitate further purified by redissolving it in water and precipitating a third time. It was then dissolved, dialysed as above, taken up with a dilute solution of sodium chloride and dried as usual. This preparation contains a little ovalbumin as well as the ovoglobulin.

(3) *Filtrate after removal of the globulins, dried.* Method (a). Raw fresh egg-white from which the globulins had been removed by half-saturation with ammonium sulphate was dialysed as above and dried in the usual way.

Method (b). To raw fresh egg-white four volumes of water were added, the whole well mixed and made neutral to litmus with dilute acetic acid. The precipitate of globulins was filtered off and the filtrate dried.

(4) *Albumin and ovomucoid fraction from fresh egg-white, dried.* Fresh raw egg-white, from which the globulins had been removed by half-saturation with ammonium sulphate, was treated with excess of solid ammonium sulphate until no further precipitate could be produced and the filtrate was protein-free. The precipitate was filtered off, dissolved in water, reprecipitated and again filtered off. After dissolving in water it was dialysed and dried.

(5) *Albumin and ovomucoid fraction from dried egg-white, dried.* 200 g. of dried egg-white were dissolved in 1600 cc. of water. From this solution the albumin and ovomucoid fraction was prepared as under (4).

Purified caseinogen. This was prepared by the method described by Chick and Roscoe [1928], to whom I am indebted for the sample used here.

Yeast extract. Pressed washed yeast was heated for 5 hours at 120° and then stirred into a large volume of boiling dilute acetic acid (0.01 %) and boiled for 5 minutes. It was then filtered and the filtrate concentrated so that 1 cc. was equivalent to 0.7 g. of the original dry yeast.

Yeast residue. The residue from the yeast extract was subjected to two further similar extractions and then dried at 37°. 1 g. of the residue was equivalent to 2.2 g. of the original dry yeast.

EXPERIMENTS ON THE PURIFIED PROTEINS OF EGG-WHITE.

Crystalline ovalbumin.

Assuming that crude raw egg-white contains some essential accessory substance X which is destroyed during drying (hypothesis (a) above) one might expect that a pure crystalline constituent protein such as ovalbumin would be freed from this factor. The use of pure ovalbumin as the sole protein of the diet would in that case be followed by the development of the same symptoms as are produced by the use of dried egg-white. Crystalline ovalbumin, prepared as described above, was used as the sole source of protein in the diet of two rats (see Table II), which had developed the usual symptoms as a result of receiving the dried egg-white diet (DW) for 8 weeks. Their condition immediately improved and rapid growth was resumed. It was, however, possible that the essential factor might still remain adherent to the albumin even after crystallisation. The solution of pure ovalbumin was therefore dried at 37°. Curative experiments on 3 rats (see Table II) proved it to have suffered no change in nutritive property.

Table II. *Results obtained when rats which had developed the symptoms as a result of receiving the dried egg-white (DW) diet were given instead diets containing different egg-white fractions as the source of protein.*

Litter No.	Rat No.	Length of time on DW previously (days)	Condition of rat when diet changed		New diet No.*	Result of change of diet	
			Symptoms	Weight		Symptoms	Weight g.
78	657 ♀	58	Severe	Falling	(1 a)	Healing	Gain of 27 in 16 days
78	658 ♂	58	"	"	(1 a)	"	" 24 " 16 "
81	686 ♂	51	Marked	"	(1 b)	"	" 32 " 11 "
		DW 8					
82	697 ♂	21	Appearing	Rising	(1 b)	"	67 " 18 "
82	699 ♀	21	"	"	No change	Worse	" 9 " 18 "
84	715 ♂	33	Severe	Falling	(1 b)	Healing	" 37 " 18 "
84	716 ♂	42	"	"	4	"	" 18 " 14 "
84	717 ♂	42	"	"	4	"	" 22 " 18 "
84	718 ♀	42	Marked	"	4	"	" 29 " 18 "
84	714 ♂	42	Severe	Stationary	No change	Worse	Loss of 6 " 9 "
84	719 ♂	42	"	"	"	"	" 4 " 12 "
84	720 ♀	42	"	"	"	"	" 7 " 12 "
86	731 ♀	36	Marked	"	6	Healing	Gain of 26 " 12 "
86	735 ♂	36	Severe	"	6	"	" 12 " 12 "
86	732 ♀	36	Marked	Falling	6	"	" 31 " 12 "
86	734 ♂	36	Severe	"	6	"	" 28 " 12 "
86	736 ♂	36	Marked	"	No change	Worse	Loss of 8 " 12 "
86	737 ♂	36	Severe	Stationary	3	Died in 7 days	
86	739 ♂	36	"	Falling	3	Worse	Loss of 5 in 14 days
86	738 ♂	36	"	Stationary	4	Healing	Gain of 29 " 16 "
89	761 ♀	35	Marked	"	2	"	" 48 " 21 "

* See Table I.

These results are opposed to the theory that the symptoms caused by the ingestion of dried egg-white as the sole protein are caused by a deficiency of some dietary essential. One is, therefore, forced to conclude that when egg-white is dried some toxic substance is formed (hypothesis (b)).

The source of the toxic substance.

In the hope of determining from which of the constituents of egg-white the toxic substance is derived some of the other proteins contained in egg-white were examined separately. According to Hektoen and Cole [1928] there are five different proteins in egg-white, ovoglobulin and ovomucin together constituting the fraction precipitable by half-saturation with ammonium sulphate, ovalbumin and conalbumin both precipitated by saturation with ammonium sulphate, the first crystallisable and the second non-crystallisable and ovomucoid. The albumins constitute about 80 % of the total protein, the globulins 7 % and the ovomucoid 10 %.

Use was made of this classification of Hektoen and Cole in preparing the egg-white fractions described above. From these egg-white diets (2), (3), (4) and (6) in Table I were made up and given to rats which had developed the usual symptoms as the result of receiving the DW diet for from 21 to 42 days.

The results of the change of protein are shown in Table II. It will be seen that diets (2), (4) and (6) were harmless, their replacement of the DW diet being followed by healing and resumption of growth. Diet (3), however, containing the whole egg-white dried after removal of the globulin fraction, appeared to be toxic. To confirm this result, 3 rats of one litter were fed from weaning on diet (3), 5 rats from the same litter receiving the DW or DG diets. The remaining 3 rats in the litter received diet (5), containing as the sole protein the albumins and ovomucoid fraction prepared from dried egg-white. At the end of 6 weeks it was found that all the rats with the exception of those fed on diet (5) had developed the usual symptoms, thus confirming the toxic nature of egg-white dried after removal of the globulin fraction. This experiment demonstrated also the important fact that although dried egg-white is toxic, it is possible to prepare from it a non-toxic fraction. This provides further convincing proof that dried egg-white is not lacking in some unknown essential. The growth curves of the rats in this experiment are shown in Fig. 1.

These results show that neither ovoglobulin, conalbumin, ovalbumin nor ovomucoid produces the toxic substance when it is dried. Since the toxic substance is produced when the albumins, the ovomucoid and the protein-free mother-liquor are dried together, ovomucin can also be exculpated. It follows, therefore, that the toxic substance is derived, either from some constituent of the mother-liquor or alternatively from one of the albumins or the ovomucoid when these are dried in the presence of the mother-liquor. Against the latter hypothesis must be set the harmless nature of the albumins and ovomucoid fraction when separated from the rest of the toxic dried egg-white.

One is forced to conclude, therefore, that during desiccation of egg-white a toxic substance is formed from some non-protein constituent and that this substance remains present in the mother-liquor after removal of the proteins by precipitation with ammonium sulphate. This hypothesis cannot be regarded as fully established until it has been shown that the addition of the

dried mother-liquor to an otherwise harmless diet results in the production of the characteristic syndrome, an experiment which has not as yet been carried out.

Fig. 1. Growth curves of rats fed from weaning on diets containing as the source of protein:

- A. Albumin and ovomucoid fraction from dried egg-white.
- B. Egg-white dried after removal of the globulin fraction.
- C. Dried egg-white.

In A, B and C the carbohydrate was glucose, in D wheat starch.
All rats were from the same litter.

THE PROTECTIVE FACTOR X.

Solubility. It was shown previously [Boas, 1927] that the protective factor X appeared to be insoluble in water or alcohol. Further evidence on this point has been obtained.

(1) The factor is not removed from caseinogen by repeated washing with dilute acetic acid followed by extraction with hot alcohol. Rats reared from weaning on the caseinogen diet described in Table I, in which the protein consisted of dried egg-white and purified caseinogen in equal quantities, grew well and appeared in perfect health. (It was shown before [Boas, 1927] that a reduction of the dried egg-white in the diet to one-half the usual amount without the addition of any other substance did not decrease the severity of the symptoms produced.)

(2) Only a small amount of the factor is extracted from fresh washed yeast by boiling in dilute acetic acid (0.01 %). A daily dose of yeast extract equivalent to 1.4 g. of dried yeast did little to prevent the syndrome in rats fed upon the DW diet, whereas a dose of the yeast residue equal to 1.3 g. of dried yeast gave good protection.

(3) Fresh egg-white possesses protective power. Complete protection resulted when fresh egg-white was ingested in a proportion to dried egg-white of one to one, but when the ratio was one to three, only partial protection was afforded. It has now been found that when a precipitate of albumins and ovomucoid is formed by saturation with ammonium sulphate of fresh egg-white, from which the globulins have been removed, the protective substances are also carried down. This fraction can neutralise the toxic material in dried egg-white if it is given to rats in the proportion of one part of the fraction to one part of dried egg-white.

The experiments briefly described above are shown in full detail in Table III.

Table III. *Details of experiments showing the presence of protective factor X in certain substances.*

Litter No.	Rat No.	Diet*	Length of exp. (days)	Gain in body-weight g.	Condition of rat at close of exp.
83	705 ♀	DW	41	63	Marked symptoms
83	712 ♂		41	79	"
83	707 ♀		41	67	Good condition
83	708 ♀	Caseinogen	41	79	"
83	709 ♂		41	111	"
83	710 ♂		41	97	"
82	695 ♂	DW	35	—	Dead
82	699 ♀		42	48	Marked symptoms
82	700 ♀		42	42	"
82	702 ♀	DW + daily dose of 2.0 cc. yeast extract (last 3 weeks)	42	66	Moderate symptoms
82	703 ♀		42	69	"
82	696 ♂		42	42	"
82	694 ♂	DW + daily dose of 0.6 g. yeast residue (last 3 weeks)	42	85	Good condition
82	704 ♀		42	72	"
90	772 ♀	DW	47	44	Moderate symptoms
90	773 ♀	Egg-white fraction, diet 7	47	88	Good condition
90	771 ♂		47	85	"
81	683 ♂	DW	42	59	Severe symptoms
81	684 ♂		42	27	"
81	690 ♀		42	25	Marked symptoms
81	685 ♂	Steak diet	42	113	"
81	686 ♂		42	91	"
81	687 ♂		42	89	"
81	691 ♀		42	77	"
81	688 ♂		23	107	Excellent condition
81	689 ♂	Liver diet	23	64	"
81	692 ♀		23	62	"
81	693 ♀		23	71	"

* See Table I.

Distribution in nature. The paucity of the protective factor X in lean meat has been confirmed. Rats reared on a diet (see Table I) in which the protein consisted of 50 g. of dried egg-white and 250 g. of raw lean beef steak (68 g. dry weight) showed a condition little superior to that of the controls receiving DW diet. On the other hand, raw liver is a rich source, for 250 g. (83 g. dry weight) of raw ox-liver proved amply sufficient to counteract the effects of 50 g. of dried egg-white. Parsons [1931] found that the amount of dried beef-

liver needed to neutralise 66 g. of dried egg-white was between 10 and 20 g. and that 20 g. of dried yeast were inadequate. On the other hand, I have found that a rat ingesting from 1 to 2 g. of dried egg-white daily was kept in good health by a daily dose of 0.4 g. of dried yeast. Findlay and Stern [1929] have also confirmed the high protective potency of dried yeast.

REFECTION.

Some interesting light has been thrown on the problem by the discovery that rats showing characteristic symptoms when fed upon dried egg-white sometimes exhibit spontaneous cures. At the same time they produce bulky faeces containing undigested starch. These effects appear to be of a similar nature to the spontaneous cures occasionally observed in the case of rats reared upon a diet devoid of water-soluble vitamins [Fridericia *et al.* 1927; Roscoe, 1927]. In this condition, called refection by Fridericia, the rats pass bulky faeces white in colour with a large content of undigested starch and as long as such faeces are produced the rat is able to grow and enjoy perfect health in spite of the absence of water-soluble B vitamins from the diet. Refection can be transmitted by feeding the faeces of a relected rat and Fridericia *et al.* have postulated the existence of a virus of refection, cultivated in the intestine of the affected rat and passing out in the faeces.

In the spontaneous cures observed in rats receiving the dried egg-white diet the condition could also be passed from one rat to another by ingestion of the faeces. Furthermore, rats showing characteristic symptoms on the dried egg-white diet exhibited cures after receiving doses of the faeces from relected rats (see Fig. 2). It appears, therefore, that the agency in both cases of refection may be identical and this suggests two possibilities. If, as Fridericia suggests, refection is due to the growth in the gut of an organism which synthesises the members of the vitamin B complex, then this organism may also be supposed to synthesise the protective factor X. This is not improbable for yeast has been found to be a rich source of all these factors. On the other hand, the spontaneous cures of the rats fed on the dried egg-white diet may be simply due to some union of the toxic substance with the undigested starch thus preventing absorption of the former.

Fig. 2. Growth curves of rats showing:
 At A. Spontaneous cures.
 At B. Cures induced by a daily dose for 7 days of 0.7 g. of the faeces of a relected rat.
 At C. Cure induced by a daily dose for 12 days of 0.5 g. of the faeces of rat 713, showing a spontaneous cure.
 All rats were receiving the DW diet throughout.

DISCUSSION.

The weight of the evidence offered above is certainly on the side of the hypothesis that dried egg-white contains some toxic substance created during the process of drying. It is for the most part opposed to the theory that the nutritive disorder is due to a deficiency. These facts afford little support to Findlay and Stern's proposed identification of the egg-white syndrome with Swift's disease. Their case is based on pathological and clinical findings and until evidence of a therapeutic or dietetic nature is obtained must be regarded as unproved.

The protein-free mother-liquor has been suggested as the source of the toxic substance, but this deduction is based on a process of exclusion and much further work is needed before it can be regarded as established.

It has already been pointed out [Boas, 1927] that the protective factor X presents in its distribution in nature striking analogies with the water-soluble B vitamins, in particular with vitamin B₂. Its presence, however, in purified caseinogen which, so far as is at present known, contains none of the members of the vitamin B complex, and its absence from marmite clearly prove that it is not identical with any of these factors. It seems highly probable that it will ultimately prove to be a closely allied substance.

The conception of a toxic substance in the diet, neutralised by a specific accessory factor, is not a new one in dietetics. Mellanby [1926] has demonstrated a toxamin in oatmeal the action of which is antagonised by vitamin D, and there is considerable evidence that the harmful effects of an excess of certain proteins in the diet can be counteracted by a factor probably identical with one of the constituents of the water-soluble B complex [Hartwell, 1922, 1924, 1925; Reader and Drummond, 1926; Hassan and Drummond, 1927; Parsons, 1931].

SUMMARY.

1. Pure ovalbumin, prepared by crystallisation from crude egg-white, forms a satisfactory protein for young rats. The same is true of ovoglobulin and of the total albumin and ovomucoid fraction.

2. Desiccation does not alter the nutritive properties of these materials as is the case with crude egg-white.

3. It therefore appears probable that the alteration in nutritive value observed when egg-white is dried is due not to the loss of some essential factor but to the formation of a toxic substance.

4. Evidence is adduced suggesting that the toxic substance is formed from some non-protein constituent of the egg-white.

5. It is possible to prepare from dried egg-white a protein fraction uncontaminated by this toxic substance.

6. The protective factor X which is capable of neutralising the toxic substance in dried egg-white is present in raw liver, in a dried preparation of

the total albumin and ovomucoid fraction of egg-white, but not in raw beef steak.

7. It is only partially extracted from yeast by boiling dilute acetic acid.

8. It is not removed from caseinogen by washing with dilute acetic acid and extraction with alcohol.

9. Rats suffering from the effects of the dried egg-white can become spontaneously refected in a similar manner to that observed by Fridericia and his colleagues and by Roscoe in the case of rats fed upon diets devoid of water-soluble B vitamins.

10. This condition can be transmitted from one rat to another by ingestion of the faeces.

11. Refection can be induced in rats, receiving the dried egg-white diet, by feeding the bulky white faeces of refected rats on diets deprived of B vitamins. This suggests that the agency is the same in both cases.

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