XCV. THE EFFECT OF DESICCATION UPON THE NUTRITIVE PROPERTIES OF EGG-WHITE.

By MARGARET AVERIL BOAS (*Beit Memorial Research Fellow*).

From the Department of Experimental Pathology, Lister Institute, London.

(*Received May 3rd, 1927.*)

In 1922 I was carrying out some experiments upon the calcium and phosphorus retention of rats, for which it was desirable to use a basal diet containing only small amounts of calcium and phosphorus. Egg-white was suggested as a suitable source of protein. Osborne and Mendel [1913, 1, 2; 1915] had shown that young rats could satisfy their nitrogen requirements from a diet in which ovalbumin formed 12 % of the dry weight of the diet and was the sole source of protein, and Bond [1922] had demonstrated satisfactory growth in young rats fed upon a diet containing dried egg-white at a 20 % level as the only protein. For reasons of economy it was decided to use the same source of egg-white as that employed by Bond; this was a dried undenatured preparation of Chinese origin. It was dissolved in water and coagulated by heat before being incorporated in the rest of the diet, for both Bond and Luce had found that the ingestion of raw egg-white by rats was invariably followed by diarrhoea.

The diet, which will be referred to as the DW diet, had the following composition:

Dried egg-white	100 g.	Lemon juice	25 cc.
Wheaten starch	250 g.	Marmite	25 g.
Normal salt mixture[1]	25 g.	Distilled water	300 cc.
Hardened cotton seed oil	75 g.		

Each rat received in addition 3 to 5 drops of cod-liver oil daily.

It was found, however, that when 3 weeks old rats were fed on this diet they invariably began to lose weight after a period of about 21 days, and developed a particular pathological condition which is described in detail below, and of which the most striking symptoms were dermatitis, baldness and spastic gait leading on to death.

The symptoms. The rats are as a rule from 21–24 days old when the experiment is started. They grow well and are usually in good health for from 2 to 3 weeks. Then red scaly patches appear at the corners of the mouth, the coat becomes rough and sticky and the long hairs fall out. The fur on the abdomen shows at first a characteristic ribbed appearance, followed by the development of bald areas. Meanwhile the red patches spread to other parts

[1] McCollum, Simmonds and Pitz [1917].

of the body and the picture is one of an eczematous dermatitis. There are even skin haemorrhages in severe cases. The region round the mouth is always the most severely affected, though there is often such marked blepharitis that the eyes are closed. The loss of hair is often extensive. In a few cases oedema of the feet has been seen but this does not usually occur. These rats always have a distinctive somewhat musty smell, probably due to some constitutent of the urine. The body weight remains stationary for a week or two, but falls slowly during the second stage of the disease. This is reached about 2 to 3 weeks after the development of the first signs of deficiency. To the dermatitis, symptoms of nervous upset are now added. There is pronounced spasticity of the limbs, particularly of the hind legs, and the back is arched. The rat assumes in many cases a kangaroo-like posture. The accompanying plate shows the condition at this advanced stage. Some of the rats do not show marked spasticity but assume a crouching attitude and display a curious swimming movement with the front paws. Death, which occurs in the final phase, is preceded by a rapid loss of weight, and the animal shows signs of extreme cyanosis. *Rigor mortis* sets in rapidly. *Post mortem* there is an almost complete absence of fat and the skin is infiltrated and vascularised, but these are the only apparent abnormalities, the organs seeming perfectly normal. No extensive histological study has yet been carried out but sections of liver, kidney, thyroid, spleen and adrenals failed to show any marked changes.

These results were so striking that it appeared that egg-white could not contain proteins satisfactory for complete nutrition and a short note was published [Boas, 1924, 1] to this effect. After this paper appeared, Prof. Mendel wrote suggesting that there must be some important difference between the methods I used and those employed by him, since he had repeatedly found egg-white to be an adequate source of protein for young rats. Shortly after this Dr Helen Mitchell told me that she had used the dried white of hard-boiled eggs with complete success as the sole source of protein for rats [Mitchell, 1925].

The use of fresh egg-white. The experiments were therefore repeated using the fresh white of English or imported eggs instead of the Chinese preparation, and the results showed that I had been too hasty in concluding that egg-white was unsatisfactory as a source of protein. The rats fed upon the fresh egg-white diet grew well and remained in good condition (see Fig. 1). A second note was published [Boas, 1924, 2] withdrawing my former conclusions, and attributing the results obtained to some unknown factor peculiar to the Chinese egg-white.

The question of preservatives. It seemed probable that this unknown factor in the Chinese egg-white might be some toxic preservative. A very thorough investigation carried out in the Government Laboratory by the courtesy of Sir Robert Robertson, however, failed to reveal the presence of any known preservative.

The influence of the nature of the starch. I also communicated with Miss

Bond and learnt that although she had long used the Chinese dried egg-white, yet she had never observed in her rats the symptoms I described. The only qualitative difference between the two diets lay in the nature of the starch, as Bond used potato-starch and I used wheat-starch. This difference did not seem likely to be significant but upon investigation it proved to be of the utmost importance. Rats were fed upon the DW diet until the symptoms were developed to such a degree that death would have been expected in a week or less. They then received a diet similar in all respects to the DW diet except for the presence of potato-starch instead of wheat-starch. There was an immediate increase in weight, new hair appeared and a rapid and lasting cure resulted. Furthermore rats fed on the diet prepared with potato starch from the time of weaning grew well and showed none of the symptoms (Fig. 1). That the wheat-starch was not in itself responsible was clear, for when caseinogen was the protein used the nature of the starch was immaterial.

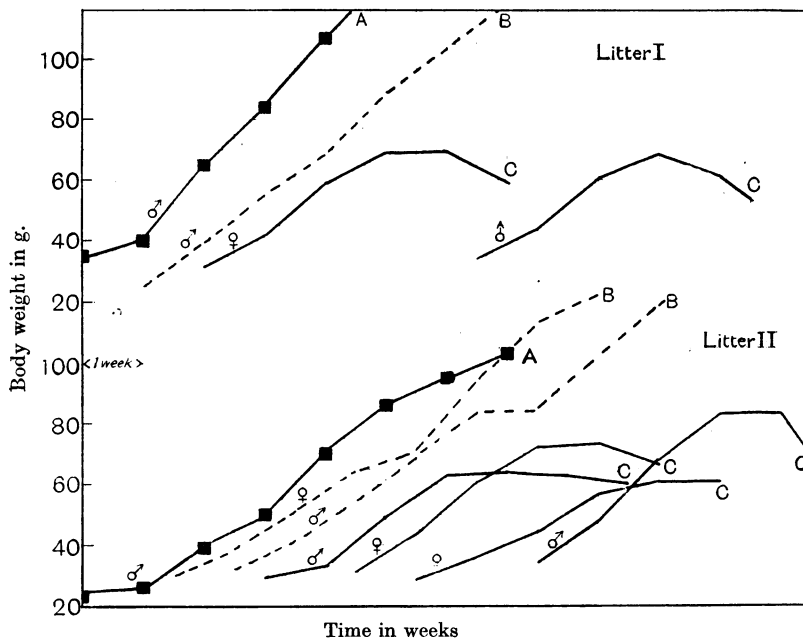

Fig. 1. Curves showing change in body weight of rats receiving diets containing the following different combinations of protein and carbohydrate. A, fresh egg-white and wheat-starch; B, dried egg-white and potato-starch; C, dried egg-white and wheat-starch.

The results of using egg-white dried in the laboratory. The next step was to prepare a sample of dried egg-white in the laboratory. Fresh egg-white was placed in thin layers in flat porcelain dishes in a hot room maintained at 37° and a stream of air blown over it by means of an electric fan for from 12 to 24 hours. In this way a dry flaky undenatured substance was obtained. When this was dissolved in water and used as the protein in the DW diet, exactly the same symptoms resulted as when the Chinese preparation was

used. Thus it was demonstrated that when dried by evaporation at 37°, egg-white suffers some radical change in its nutritive properties which renders it unsuitable for use as the sole protein of the diet of young rats unless potato starch (or some other food substances) are ingested at the same time. Two alternative hypotheses seemed feasible to account for this fact.

(1) The dried egg-white contained some toxic substance which was neutralised by the potato starch.

(2) The dried egg-white had been deprived of some essential dietetic factor which was supplied by the potato-starch and not by the wheat-starch. This could hardly be an amino-acid since the potato-starch was not supplying more than 2 mg. of nitrogen per day (in later experiments protection was obtained when only 0·5 mg. of nitrogen was added daily to the diet by the potato starch).

Whichever hypothesis should prove to be correct the presence of a protective factor in potato-starch must be postulated. This will be referred to as protective factor X.

Experimental details.

The problem seeming one of fundamental interest, a lengthy investigation was undertaken. All the experiments were carried out on litters of rats at the age of 3 weeks, and in all cases control rats from the same litter were included. The date on which the first signs of soreness appeared at the corners of the mouth was noted, and this fact, the weight curve and the general condition of the rats were adopted as criteria of the experiments. In many experiments male rats only were used as they were found to give a quicker and more regular response than the females. Owing to the fact that potato-starch is extremely indigestible to rats, it was found necessary to steam all diets containing more than 20 % of the carbohydrate in this form.

The general results of the experiments are given below. Since experiments involving over 600 rats were carried out, space does not permit them to be described in detail, but curves from representative cases are given (Figs. 1, 2, 3 and 4).

The result of variations in the relative amount of dried egg-white in the diet.

In order to test the first of the two above hypotheses a series of experiments was planned in which diets containing varying concentrations of dried egg-white were used. If a toxic substance were the cause of the symptoms, a decrease in their severity should result from the ingestion of smaller quantities of dried egg-white, while on the other hand an increase in the relative consumption of the egg-white should be followed by the development of a more acute form of the disease. Diets containing approximately 10, 20 and 40 % of their dry weight as dried egg-white were used. The results obtained did not offer support to this theory, for whichever diet was used the condition

was equally severe and appeared as soon. This would however be in accord with the hypothesis that the rats were suffering from a more or less complete deficiency of some essential factor.

The influence of variations in the method of desiccation.

(1) *Reaction.* It was found that the reaction of the egg-white had no influence on the change which took place during desiccation, for if sufficient acetic acid were added to raw egg-white to render it acid to litmus, it still underwent the same change during subsequent dehydration.

(2) *Heat coagulation.* The experiments of Mitchell, referred to above, suggested that if the egg-white were first coagulated by heat, the drying process would be harmless. This proved to be correct, and suggested that the change taking place when egg-white is dried at 37° might be the work of a thermolabile enzyme acting at blood-heat. This idea was however disposed of by the discovery that the egg-white could be exposed in thin layers to the temperature of 37° without undergoing any change in its nutritive properties, provided that desiccation was prevented by the use of wet cloths stretched over the dishes.

(3) *Desiccation* in vacuo. When egg-white is dried *in vacuo* over H_2SO_4 at room temperature the change still takes place, indicating that it is not an oxidation nor the result of exposure to 37°.

(4) *Dehydration by alcohol.* Some egg-white was dehydrated by treatment with successively greater concentrations of alcohol, and it was found that in this case also the egg-white had been damaged. Egg-white dried in this way was denatured during the process.

The protective factor X in potato and other starches and in whole potato.

The protective factor was found present in arrowroot as well as in potato-starch, while the cereal starches—rice and maize—were found to resemble wheat in being devoid of it. Potato-starch seemed to be rich in the factor, for a daily ration of 0·2 g. of raw starch was sufficient to protect rats fed on the DW diet from all the usual symptoms. Raw potato (Fig. 2) proved also a rich source of the factor, protection being given by 1·0 to 1·5 g. equal to 0·2 to 0·3 g. potato-starch per day. Fresh potato juice however was entirely inactive even when large amounts were ingested. The protective factor X therefore is probably so strongly adsorbed as to appear to be insoluble in water. This agrees with the fact mentioned above that raw potato does not contain more of the protective factor than the corresponding amount of potato-starch.

Attempts to deactivate potato-starch by extraction with alcohol and ether were unsuccessful.

The effect of heat and desiccation upon the protective factor X.

Some, though not all, of the protective power of potato is lost when potato is cooked by boiling or steaming. There is also evidence that destruction occurs when potato-starch is mixed with water and steamed. It was mentioned earlier in this communication that the diets containing large amounts of potato-starch were steamed before consumption in order to render them less indigestible. As these diets supplied amounts of the potato-starch far above the necessary minimum, the deleterious effect of heat upon the factor X was not apparent in these cases.

Desiccation at 37° does not appear to damage the protective factor in raw potato to any appreciable extent.

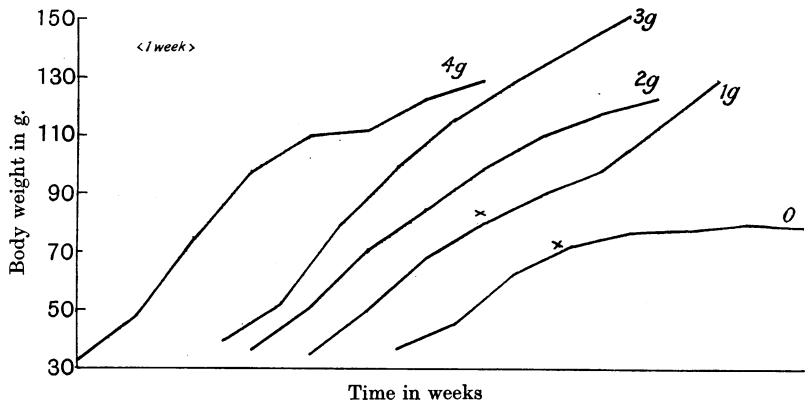

Fig. 2. Curves showing the change in body weight of male rats receiving the DW diet and a daily ration of raw potato. Each curve represents average figures for two or three rats. At × the symptoms of skin lesion appeared.

The question of roughage.

It was noticeable that when rats received potato-starch or raw potato in the diet they produced faeces many times more bulky than those of rats fed upon the egg-white diet prepared with wheat-starch. This bulkiness was due to the presence of undigested starch. If the dried egg-white were supposed to contain some toxic substance, the protective action of the starch might be due simply to mechanical removal of this substance from the intestine before complete absorption could take place. To test this theory agar-agar equal to 10 % of the dry weight of the diet was added to the DW diet. Although by this means bulky faeces were produced, the appearance and course of the usual symptoms were unaffected.

The effect of desiccation upon other proteins.

It was of interest to ascertain whether proteins other than those contained in egg-white suffered a like change when dried. It is quite evident that this is not the case of the vast majority of proteins, seeing that these are commonly dried and preserved in the dry condition before being incorporated in the diet, and can then be used as the sole source of protein

with complete success. In blood-serum the proteins are present in colloidal solution as they are in egg-white, and it therefore seemed possible that if horse-serum were dried in the same way as egg-white it might suffer a similar change in its nutritive properties. This did not prove to be the case. Moreover, the dried serum was found to contain the protective factor.

The effect of supplementing with other proteins.

At this stage in the investigation an incident occurred which, although unfortunate at the time, yet gave information of interest. I was unable to obtain any more Chinese egg-white from the firm which had up to then supplied it. A sample from a different source was tested and found to give the usual results. A larger supply was then obtained from this source and used for a series of experiments, but the results were irregular and unsatisfactory. Although some symptoms of the disease were obtained, in many cases they were only transitory, and followed by spontaneous cures. Only in few cases was the disease at all severe. This egg-white, unlike the first sample, was not entirely soluble in water, as (owing presumably to having been dried at too high a temperature) some of it had become denatured during the drying process. It had already been found that egg-white previously denatured by heat was not damaged by desiccation (p. 716) and the irregular results obtained with this partially denatured specimen suggested that denatured dried egg-white (and presumably fresh egg-white) was able to counteract the deleterious effects of the dried egg-white. If this were also true of fresh egg-white, the facts could be explained by the hypothesis that fresh egg-white contains a dietetic factor not present in the rest of the diet, this factor being destroyed during desiccation, but preserved by heat denaturation.

To test this theory the following experiment was carried out. Six male rats of the same litter were divided into three groups at the age of 21 days. Group A received the ordinary DW diet in which the dried egg-white formed 20 % of the dry weight, and the other groups received the same diet modified in two different ways. In group B the dried egg-white was reduced to 10 % of the dry weight without any addition. In group C the protein of the diet consisted of 10 % dried egg-white and 10 % fresh egg-white.

The curves of increase in body weight of these rats are shown in Fig. 3. In the first two groups the usual symptoms developed but in group C the fresh egg-white was able to give protection. It appeared that this was the minimum amount which would give this result, for after receiving the diet for 3 weeks all the rats showed a slight soreness at the corners of the mouth which lasted for a few days and then disappeared.

The storage of the protective factor X by rats.

In the spring and summer of 1926, the rats receiving the DW diet began to show a greater variability than those of previous work. Some rats or even some whole litters exhibited an unusual resistance to the DW diet. Finally

in September three separate litters which were receiving the DW diet grew well and showed only slight signs of skin affection even after they had received the DW diet for 6 to 7 weeks. There was no variation in the sample of dried egg-white or in the wheat-starch used, and it seemed possible that the rats might in some way have themselves acquired greater reserves of the protective factor than was usual. This idea was based on the assumption that a storage of the factor could take place as has been demonstrated in the case of some of the other accessory factors.

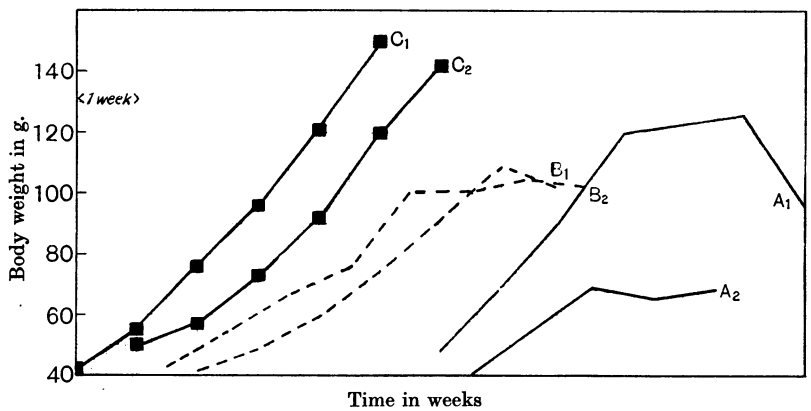

Fig. 3. Curves showing the change in body weight of 6 male rats of the same litter.
Rats A_1 and A_2 received the DW diet.
Rats B_1 and B_2 received the DW diet modified by the reduction of the protein to one-half the usual amount.
Rats C_1 and C_2 received the DW diet modified by the replacement of one-half of the protein by fresh egg-white.

All the rats used had been bred in the Institute on a diet of milk, white and brown bread, marmite, cabbage, carrot, raw meat and various grains. During the past year the amount of water-soluble vitamins in the diet had been increased, in particular by the addition of large amounts of rolled oats and of wheat embryos. It was arranged that some pregnant does should receive a restricted diet during the last week or so of pregnancy and throughout lactation. This diet consisted of white bread, milk, cabbage, carrot and meat. Some litters which did not appear to thrive received a little brown bread occasionally. Although on this diet well-grown healthy litters were obtained it proved a severe strain on the mothers. They were often in such poor condition at the end of the lactation period that they could no longer be used for breeding purposes. I also obtained some litters of rats from an outside source bred on a diet inferior to that of the Institute stock.

Young rats from these two sources were fed upon the DW diet and the results were compared with those obtained with litters the mothers of which had received the full stock diet during lactation. An analysis was made of the condition of the three groups of rats after they had received the experimental diet for 5 weeks. A note was made as to whether the body weight

was rising, falling or stationary and a number of crosses indicated the physical condition of the animal. × indicated no definite symptoms except a rough coat and soreness round the mouth. ×× meant definite dermatitis and loss of hair but unaccompanied by nervous trouble, and when ××× was given both dermatitis and nervous symptoms were severe.

When analysed in this way the results of the experiments showed that the *rapidity* with which the disease developed in young rats was controlled by two things:

(*a*) the weight of the rat at the beginning of the experiment;

(*b*) the diet received by the mother during pregnancy and lactation.

When rats of equal initial body weight were compared, those whose mothers had been fed upon the restricted diet showed a more advanced stage of the disease after they had received the DW diet for 5 weeks than did those from mothers fed upon the usual stock diet.

It appears therefore that rats are able to store the protective factor and that the degree of this reserve in young rats depends not only on their own body weight but also on the amount of the factor in their own previous diet and in the diet of the mother. When possessing large reserves of the factor the young rat can grow well and have an almost normal skin and coat for many weeks on the DW diet, but eventually the reserves are used up and the disease appears and takes its usual course. In an experiment with adult rats of 200–300 g. on the DW diet three months elapsed before the symptoms appeared.

The connection between the protective factor X and the water-soluble vitamins.

The above experiments upon the storage of the factor showed that when the water-soluble vitamin content of the diet was reduced there was an accompanying decrease in the amount of the unknown protective factor. It seemed possible that there might be some connection between this factor and the active principles contained in McCollum's vitamin B, more especially as they possess a similar distribution in many substances, notably egg-white and raw potato. They could not be identical, for potato-starch, one of the most potent sources of the protective factor, is devoid of vitamin B, although from Eijkman's work [1906] it appears to possess some antineuritic power. Further, marmite, which is capable of supplying the water-soluble vitamins, did not contain the protective factor. The work of Goldberger and his co-workers [Goldberger, Waring and Tanner, 1923; Goldberger and Tanner, 1924, 1925; Goldberger, Wheeler and Tanner, 1925; Goldberger, Wheeler, Lillie and Rogers, 1926; Goldberger and Lillie, 1926] was of great interest in this connection. Most of the work was done on cases of human pellagra. Goldberger demonstrated that water-soluble B consisted of two different factors, one the antineuritic or antiberiberi, and the other the pellagra-preventive. He found that the first was more easily destroyed by heat than was the second, for

yeast autoclaved at 120° for 5 to 6 hours was found to have retained its curative power towards pellagra, although the antineuritic potency was lost. Although often found together in nature, the two factors did not show an identical distribution, wheat germ for example being much richer in the antineuritic factor than in the pellagra-preventive.

The rats fed upon the DW diet showed symptoms which in many ways resembled those of pellagra, and it seemed of interest to determine the content of the protective factor X in those substances which Goldberger had found to be rich in his pellagra-preventive factor. Table I shows roughly the content of the protective factor X and of the supposed two constituents of the water-soluble vitamins in a number of foodstuffs. It will be seen that all three are present in dried yeast (Fig. 4); when the yeast is heated for a long time at a high temperature (4 to 5 hours at 120°) the antineuritic vitamin is destroyed but the other two are unaffected. Milk also contains all three, which are therefore found as impurities in commercial caseinogen. Extraction of the caseinogen with alcohol removes the B vitamins but not the protective factor X.

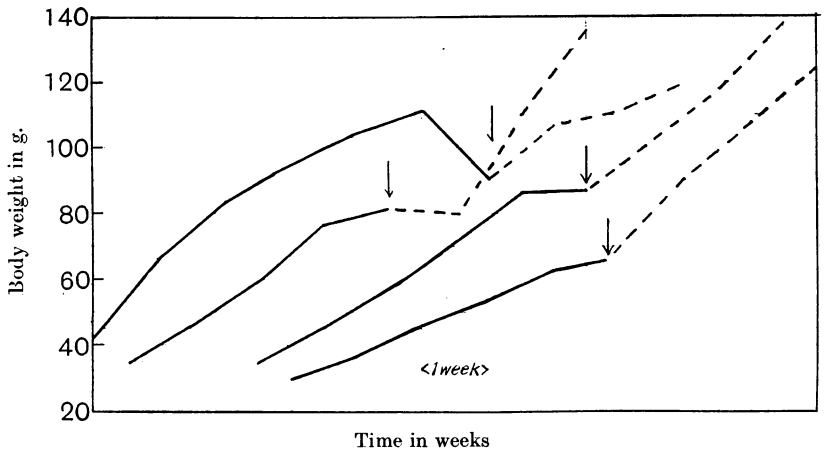

Fig. 4. Curves showing the change in body weight of rats fed upon the DW diet. At the arrows daily rations of dried yeast (0·2 to 0·4 g.) were given in addition.

Raw potato contains both the antineuritic vitamin and the protective factor (it has not yet been tested for the pellagra-preventive). The protective factor remains associated with the starch throughout its preparation, while the antineuritic is present in potato juice and is washed out of the starch.

Thus it appears that the protective factor X, though having in many ways a similar distribution to vitamin B, does not coincide with either of its supposed constituents. This is confirmed by the nature of the symptoms produced when the protective factor is absent from the DW diet. The nervous symptoms (spasticity of the limbs) are not the same as the paralysis induced in rats by deprivation of the antineuritic factor, nor are the skin lesions identical with

those observed in cases of experimental pellagra in rats as described by Goldberger [Goldberger and Lillie, 1926] and confirmed by Chick and Roscoe [1927]. Rats deprived of the pellagra-preventive factor by these workers developed scaly thickened ears and inflamed oedematous feet, whereas the rats in my experiments never showed the first and only in a few cases the second.

Table I.

Showing the distribution in certain substances of the two constituents of vitamin B and of the protective factor X. The figures for the antineuritic vitamin are taken from the Medical Research Council's Report on the Present State of Knowledge of Accessory Food Factors [1924] and those for the pellagra-preventive factor from Goldberger's work and from the work of Chittenden and Underhill [1917] and Underhill and Mendel [1925] on "black-tongue," a pellagrous-like condition in dogs.

Substance	Protective factor X	Water-soluble B of McCollum: Eijkman's anti-neuritic	Water-soluble B of McCollum: Goldberger's pellagra-preventive
Raw potato	+ + +	+ +	
Cooked potato	+	+	
Dried potato	+ +	+ +	
Potato-starch	+ + +	+ (Eijkman)	
Cooked potato-starch	+		
Potato-juice	0	+ +	
Arrowroot	+ + +	0	
Cereal starches	0	0	0
Dried yeast	+ + +	+ + +	+ + +
Autoclaved yeast	+ +	0	+ +
Marmite	0	+ +	+ +
Harris vitamin B concentrate	?	+	+ +
Fresh egg-white	+	0	
Dried egg-white	0	0	
Dried coagulated egg-white	+	0	
Dried egg-yolk	+ +	+	
Fresh milk	+	+	+
Commercial caseinogen	+	+ +	+
Crude lactalbumin	+	+	+
Extracted heated caseinogen	+	0	0
Dried horse-serum	+		
Wheat-germ	?	+ +	+
Wheat-bran	0	+	
Wheat-flour	0	0	0
Wheat-starch	0	0	0
Wheat-gluten	+		
Malt-extract	0	+	
Raw meat	0	+	+ +
Fresh spinach	+		
Fresh cabbage	+	+	
Banana	+	+	
Butter	0	0	+ Black tongue

Discussion.

In the earlier stages of the investigation it seemed possible that the phenomena observed might be explained by one of the following two alternative hypotheses.

(1) When fresh crude egg-white is dried an essential dietetic factor in it, the existence of which has been hitherto unrecognised, is destroyed. In the absence of this factor from a diet in which the nitrogen is entirely supplied by the egg-white and is complete in all other respects young rats develop a

characteristic universal dermatitis accompanied by nervous disorder which is finally terminated by death. This factor X is present in fresh eggs, both in the yolk and in the white, in dried yeast, raw potato, potato-starch and arrowroot, milk, blood-serum, banana and in fresh spinach and cabbage leaves.

(2) Some toxic product formed when fresh egg-white is dried is responsible for the symptoms which develop when rats are fed on a diet containing dried egg-white as the sole source of protein. The toxic substance can be neutralised by a factor X present in the foodstuffs enumerated in hypothesis (1).

The investigation has not yet reached a stage at which it is possible to decide which, if either, of the above hypotheses is correct. Neither seems entirely satisfactory. So far as at present investigated, egg-white is the only crude protein mixture which is susceptible to this deterioration of its nutritive properties on drying. Dried horse-serum, commercial preparations of caseinogen, gluten and lactalbumin, when they were the only proteins supplied, did not produce the symptoms which were shown by rats receiving a diet in which dried egg-white was the sole protein.

Many facts of the problem which appear irreconcilable may ultimately prove to be due to differences in the amount of the factor X present. Suppose egg-white to contain only just sufficient of it, then a partial destruction of the factor during desiccation would result in the appearance of the symptoms, whereas caseinogen, serum, etc., might contain so much that the loss of a part of it would still leave sufficient for the needs of the animal.

Experiments now in progress however indicate that there is a relationship between the amount of dried egg-white ingested and the amount of the protective factor X needed to give protection.

It seems therefore probable that the true explanation of the problem is a more complicated one than either of the two above theories. It may have some relation to the problem studied by Hartwell [1924, 1, 2; 1925] and also recently by Reader and Drummond [1926]. They find that a dietary factor having a similar distribution to vitamin B was able to correct a diet overbalanced by excess of protein. Since marmite contains this factor, it cannot be the same as the protective factor X, but it is quite conceivable that in the problem set out in this paper a similar question of balance between two constituents of the diet is involved.

Summary.

(1) A diet of crude egg-white, boiled and supplemented with wheat-starch, cotton-seed oil, cod-liver oil, lemon juice, marmite, salts and water, supports young rats in growth and health.

(2) If the egg-white is previously dried the diet is inadequate unless the carbohydrate is supplied in the form of potato-starch or arrowroot, or unless certain substances are added in small amounts.

(3) The condition which is developed by rats fed on this unsatisfactory diet is described in detail.

(4) The change which takes place in dried egg-white is independent of the reaction of the solution during drying.

(5) It is not the work of a thermolabile enzyme.

(6) It does not appear to be a process of oxidation.

(7) Egg-white previously coagulated by boiling is not damaged by desiccation.

(8) The crude proteins of horse-serum and milk do not suffer a similar change during desiccation.

(9) The foodstuffs which possess the power of counteracting the ill-effects resulting from the ingestion of the dried egg-white are raw potato, potato-starch, arrowroot, dried yeast, fresh egg-white, egg-yolk, milk, commercial caseinogen, crude lactalbumin, spinach and cabbage leaves, banana, and dried horse-serum. The presence of a protective factor X in these substances is postulated.

(10) This factor shows a similar distribution in many ways to that of the water-soluble B vitamins. It is not however identical with either the anti-neuritic factor or Goldberger's pellagra-preventive.

(11) Its resistance towards heat and desiccation varies according to the substance in which it is found.

(12) The resistance shown by rats to the ill-effects of the dried egg-white diet depends chiefly on the body weight of the animal at the beginning of the experiment. It is also influenced by the diet of the mother during pregnancy and lactation. It seems probable therefore that rats can store reserves of the protective factor X.

I should like to record my gratitude to Sir C. J. Martin and Dr H. Chick for their unfailing help and advice throughout the course of this investigation.

REFERENCES.

Boas (1924, 1). *Biochem. J.* **18**, 422.
—— (1924, 2). *Biochem. J.* **18**, 1322.
Bond (1922). *Biochem. J.* **16**, 479.
Chick and Roscoe (1927). *Biochem. J.* **21**, 698.
Chittenden and Underhill (1917). *Amer. J. Physiol.* **44**, 13.
Eijkman (1906). *Arch. Hyg.* **58**, 49.
Goldberger and Lillie (1926). *U.S.A. Public Health Report*, **41**, 1924.
Goldberger and Tanner (1924). *U.S.A. Public Health Report*, **39**, 87.
—— —— (1925). *U.S.A. Public Health Report*, **40**, 54.
Goldberger, Waring and Tanner (1923). *U.S.A. Public Health Report*, **38**, 2361.
Goldberger, Wheeler, Lillie and Rogers (1926). *U.S.A. Public Health Report*, **41**, 297.
Goldberger, Wheeler and Tanner (1925). *U.S.A. Public Health Report*, **40**, 927.
Hartwell (1924, 1). *Lancet*, ii, 956.
—— (1924, 2). *Biochem. J.* **18**, 785.
—— (1925). *Biochem. J.* **19**, 1075.
McCollum, Simmonds and Pitz (1917). *J. Biol. Chem.* **29**, 521.
Medical Research Council (1924). *Special report series, No.* 38.
Mitchell (1925). *Amer. J. Physiol.* **64**, 359.
Osborne and Mendel (1913, 1). *J. Biol. Chem.* **15**, 311.
—— —— (1913, 2). *J. Biol. Chem.* **16**, 423.
—— —— (1915). *J. Biol. Chem.* **20**, 351.
Reader and Drummond (1926). *Biochem. J.* **20**, 1256.
Underhill and Mendel (1925). *U.S.A. Public Health Report*, **40**, 1087.