LIX. THE INTERRELATIONSHIP BETWEEN DIETARY EGG WHITE AND THE REQUIREMENT FOR A PROTECTIVE FACTOR IN THE CURE OF THE NUTRITIVE DISORDER DUE TO EGG WHITE¹

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THAT the pellagra-like syndrome due to the ingestion of egg white was prevented or cured by certain foods, the potency of which apparently was not attributable to their content of vitamin B₁ or B₂ but to an unknown factor (factor X), was early noted by Boas [1927]. The concentration of the active factor in an extract,² suitable for injection, has been accomplished [Lease & Parsons, 1934, 1; Lease, 1936]. This furnished a valuable approach to the study of the fundamental nature of the injury due to egg white. Early work had shown that the incidence and severity of the characteristic dermatitis were graded directly in respect to the concentration of egg white in the diet on which it was produced [Parsons, 1931]. From time to time the observation was made in this laboratory that the higher concentrations of dietary egg white required larger doses of curative foods than the lower concentrations, in harmony with the observation of Boas [1927]. It was therefore deemed of importance to determine with greater precision to what extent and under what conditions an interrelationship exists between the amount of the factor necessary for cure and the amount of egg white present in the diet.

EXPERIMENTAL

The plan of the experiment was to produce a standard grade of the characteristic disorder in rats and chicks on rations containing fermented dried egg white and well supplemented with the commonly accepted dietary essentials, the details of which have been described [Parsons & Kelly, 1933]; the potent factor was then to be administered in graded portions either by intraperitoneal injections of Extract A or by combining this extract or potent food with the ration. The injections were also graded in respect to time intervals, i.e. monthly, fortnightly, weekly and semi-weekly. Direct oral feeding of the extract was attempted for a few of the rats and chicks. In the experiments both with injecting and feeding the factor, the concentration of egg white of the ration was likewise graded during the test period so that the relationship of the potent factor to the egg white received by the animals was varied in many ways.

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² This extract is designated as Extract A [Lease, 1936].

The standard degree of severity of the nutritive disorder at the beginning of the tests was such that subsequent stages of either recovery or increasing involvement could be accurately gauged.

RESULTS

Parenteral and oral administration of the potent factor in the rat

In Fig. 1, the results of feeding graded concentrations of both egg white and the potent factor in the form of pork kidney after the establishment of a standard degree of dermatitis are given. Averages of the results from several

Fig. 1. Interrelationship between concentration of egg white and of the curative factor fed as pork kidney.

The horizontal base line in each square indicates both the body weight and the degree of intensity of the symptoms (Grade C) at the time when the curative supplement was introduced. Above this base line, narrow lines ——— indicate a change to less severe symptoms than this (Grades C to B); circles o-o-o indicate approximate or complete cure (Grades B to A). Heavy lines below the base line indicate an increase in severity over the initial symptoms (Grades C to D). The symptoms corresponding to Grades A, B, C and D have been described by Parsons & Kelly [1933]. Blocks above the base line indicate gain in body weight; the intervals marked represent 50 g.

animals have been used inasmuch as the results were quite uniform in this experiment, in which the potent factor was evenly distributed by incorporating it in the ration. It will be seen that the severity of the symptoms, represented in the figure by lines, and the changes in body weight, represented by blocks, are graded, the greater the amount of egg white in the diet or the lower the amount of the protective factor included, the more severe the symptoms and the less the growth increments. With each step-wise increase in concentration of egg white as represented in the graph, a shift toward the left to higher concentrations of the protective factor is necessary in order to find similar rates of growth and relief of symptoms. Roughly, a 230 % increase in the egg white of the ration (from 20 to 66 %) required about a 250 % increase in the amount of protective factor necessary in the diet for a similar degree of growth and recovery. The same general interrelationship was found in a similar series which is not presented here in detail, wherein the factor was fed as beef liver added to the ration.

That this interrelationship does not depend on a destruction of the potent factor by the egg white within the ration itself has been shown previously [Parsons & Lease, 1934]. That the phenomenon is not due to a lack of protein for

Fig. 2. Interrelationship between concentration of egg white in the ration and quantity of potent factor (in Extract A) necessary for cure when injected into the rat at various intervals.

The symptoms at the beginning of each curve were of the intensity denoted by Grade C (see Fig. 1); lines — and o-o-o represent increasing stages of recovery; thin broken line - - - - represents symptoms more severe than the initial stage.

which a protease inhibitor in the egg white could be held responsible has also been demonstrated [Parsons, 1936]. Recent results from the laboratory of Gorter [1936] are not out of harmony with this view. Moreover, a possible destructive action of the egg white on the potent factor in the digestive tract after one or both had undergone chemical changes during some stage of digestion was made very improbable as an explanation of the injury from egg white by experiments performed in this laboratory. In these, the given portion of potent food was fed as one single dose separated from consumption of the egg white ration by a 12–24-hour fast before and after the dosage; the results of this series gave no support to the hypothesis under consideration. However, the possibility that the egg white causes changes in the membranes of the digestive tract whereby they become less capable of absorbing the potent factor suggested itself. To secure evidence on this point it was necessary to administer the potent factor parenterally to insure that any effect that the level of egg white might have on the requirement for the factor under these conditions could have only a very indirect relationship, if any, to the digestive tract. Experiments next performed showed that there is in fact a measurable influence of the level of dietary egg white in increasing the amount of potent factor which it is necessary to inject in order to cure the characteristic lesions, as was true when the factor was fed (see Fig. 2). When the ration contained 20% of egg white, a unit injection of 1 ml. of the extract administered monthly led to satisfactory recovery, whereas several times this amount was evidently necessary for the same effect when the ration contained 66% of egg white, inasmuch as 3 ml. per month, given as three 1 ml. injections on consecutive days, were not fully curative. Larger unit injections of this particular lot of Extract A could not be successfully administered, hence the series was completed in this experiment by the use of a more potent preparation¹ of Extract A; the results are given separately (Fig. 3)

Fig. 3. Interrelationship between dietary egg white and injected curative factor using a more potent extract.

to avoid confusion. A dosage of 2 ml. of this extract per month, given as 1 ml. injections fortnightly, resulted in prompt and sustained cure over 24 weeks

¹ The same method was used as for the previous sample but it was prepared from a certain lot of liver residue which was found, by feeding tests, to contain a higher concentration of the potent factor than other lots.

on rations containing either 20 or 66 % of egg white; half of this dosage permitted pronounced improvement on 20 % of egg white but resulted in an absence of a sustained cure on 66 % of egg white although life was prolonged for weeks.

It is to be noted in Fig. 2 that the extract was more effective injected as one massive dose monthly than when divided into two portions and the half doses given fortnightly. As the results of the injections given weekly and semi-weekly at the levels of 1 and 0.5 ml. total extract per month were in harmony with the others, the records have been omitted to save space.

The character of the individual response is of interest; with the injections that were spaced most widely as to time there was typically a sharp improvement after the injection, followed by a decline before the next dosage; the effects of additional injections were often cumulative. Because of these fluctuations it was considered advisable to prolong the test period far beyond that employed when the extract or potent foodstuff was more evenly distributed by incorporating it in the ration itself.

In order to decrease to a minimum any possible dietary source of the potent factor in the series in Fig. 2, the corn-starch was baked at 100° for 6 days, a period of time which had been thoroughly destructive of the factor in previous experiments [Parsons & Lease, 1934], and the B-vitamins were included in the ration in the form of alcoholic extracts¹ in which none of the potent factor had been detected by our previous experiments. It is believed, therefore, that with this precaution, the differences found in the rats' requirements for the injected factor with 20 and 66 % of egg white, respectively, were influenced negligibly if at all by any direct action of egg white on the factor in the digestive tract.

Food intake records showed that somewhat more of the 20 % egg white ration was eaten than of the 66 %; however, in no case was the consumption of the 20 % egg white ration great enough to make the intake of egg white at all commensurate with that on the 66 % ration.

Parenteral and oral administration of the potent factor in the chick

As in the case of the rat cited above, so, to a somewhat less extent with the chick, the onset of severe dermatitis was observed to be correlated with the concentration of egg white in the rations on which it was produced. Although mild symptoms were often noted in a given group of chicks on 5 % of egg white as soon as in groups on 20 %, nevertheless a much longer time elapsed as a rule before these symptoms were severe enough for use in standard tests, and a certain proportion of the chicks failed to show any very definite symptoms within the time of observation. The character of the symptoms themselves differed also somewhat from those obtained on a 20 % egg white ration, the dermatitis tending to become severe primarily on the feet and then on the neck and under the mandible with the 5 % concentration rather than at the corners of the beak.

Direct indication of the interrelationship between concentrations of egg white and curative factor in chicks was furnished by feeding Extract A, evaporated on the bran and middlings of the ration, in curative experiments similar to those just described for the rat in which pork kidney and liver were fed. The increases in the amount of curative factor made necessary by the increases in concentration of egg white are clearly seen in Fig. 4. It seems worthy of note, that these data do not give any evidence that the factor was more sensitive to increasingly

¹ These extracts are the 50 % ethyl alcohol-soluble portions of yeast, and of wheat embryo previously extracted with a commercial fat solvent.

large amounts of egg white in the ration when the two were combined after the factor had been extracted and concentrated than it did in the form of pork kidney used in the series with rats. This was borne out by an experiment in which Extract A was allowed to stand with various dietary ingredients, then evaporated on them, and in which it was found that this factor in its extracted

Fig. 4. The interrelationship between dietary egg white and potent factor is here shown for the chick as it was for the rat in Fig. 1.

The potent factor in this experiment is furnished by Extract A dried on a part of the ration. The symbols for changes in symptoms and body weight are identical in Figs. 1 and 4. A detailed description of the symptoms is given by Lease & Parsons [1934, 2].

Fig. 5. The same interrelationship is here demonstrated for the chick as was shown in Fig. 2 for the rat.

The symbols for changes in symptoms are the same as in Figs. 1 and 4.

form was no more sensitive to losses when dried on egg white than when dried on starch. This confirms the previous observation that the injurious effect of egg white is not due to changes which may take place in the ration mixture as such.

In the next series of experiments doses of Extract A ranging from 0.125 to 3 ml. were injected into chicks three times a week; the egg white in the ration

varied from 5 to 30 %. The exact details of the experiments with rats could not be repeated with the chick inasmuch as a level of egg white as high as 66 % was usually fatal to the chick in a short time, and the minimum curative requirement of potent factor for this level was so high that injections adequate for cure were too large to be well borne by young chicks. Haemorrhages from injections occurred more frequently in the chick than in the rat and there was much more evidence of shock in the small chicks. On the other hand, the degree of cure by injection seemed to be approximately as consistent in the chick as in the rat for the test period used. However, in only a few instances were the chick experiments continued for intervals at all comparable with those for the rat, inasmuch as the chicks grew so large after a few weeks that their housing, feeding and care involved considerable difficulty with the equipment at hand in the laboratory; therefore, in Fig. 5, experiments of 5 weeks' or less duration are presented. It is apparent, however, that the need for the potent factor at any given level of egg white is distinctly greater for the chick than for the rat, but that a similar interrelationship between egg white and factor exists in the two animals.

The question arose as to whether the extract was being less completely absorbed and utilized in the chick than in the rat, inasmuch as adhesions and thickening of the abdominal walls were occasionally noted on autopsy in the case of some chicks on long experiments, but no evidence for this hypothesis was obtained by testing the effect of carefully varying the site of injection in contrast to the constant use of the right abdominal wall. It is believed that this consideration, therefore, was of little significance in the results. In the rat the absorption of the extract from intraperitoneal injections took place so rapidly that the pigment was observed in the urine about 15 min. after the injection.

DISCUSSION

The effectiveness of the intraperitoneal injections of Extract A in proportion to the small amount of solids introduced was striking; with a basal ration containing 40 % of egg white, 4.5 ml. of a very potent lot of extract containing 0.78 g. of total solid material produced satisfactory, nearly complete, cure in 14 days; shortly thereafter the remaining slight symptoms disappeared.

While the role of linseed oil noted by Salmon & Goodman [1934] and confirmed by ourselves in delaying and slightly modifying the inevitable manifestations of injury on egg white diets is not yet understood, it seems to be entirely distinct from the clear-cut protective and curative action of the potent factor in Extract A. In our laboratory, neither linseed oil nor lard had any measurable curative effect on the syndrome, either used alone or with low levels of the potent factor; even the protective capacity of these fats was very limited. Moreover, treatment of Extract A with ether did not lower its potency measurably.

The substantial evidence which has already been presented against the possibility that the potent factor might be identical with one of the members of the vitamin B complex already known is rendered more significant by the dissimilar results obtained in these experiments with the injection of Extract A and of Eli Lilly and Company's Extract No. 343 respectively; the latter was entirely without beneficial influence on the syndrome due to egg white at a level of 3 ml. per week as was also Liver Extract No. 171 of Parke, Davis and Company.

No such antagonistic effect was found between the factor potent against egg white injury and the various members of the vitamin B complex as that reported

by Kellogg & Eddy [1933] in regard to the effect of vitamin B₁ on the requirement for vitamin B₂. Whether fed or injected simultaneously with Extract A, neither the concentrated vitamin B₁ nor the Liver Extract No. 343 of Eli Lilly and Company had any effect in either hindering or aiding the curative action.

It seems improbable from these experiments that the results are explicable by the hypothesis that the effectiveness of the potent foodstuffs is only an indirect one in protecting some recognized antipellagric substance in the digestive tract and in making its absorption possible, inasmuch as in these experiments both Extract A and No. 343 were introduced into body tissues without direct connexion with the digestive tract.

White [1936] has recently demonstrated an interesting type of deficiency in the rat, involving sulphur-containing amino-acids, by administering cholic acid. Here a relative rather than an uncomplicated deficiency of these amino-acids was involved through the capacity of cholic acid to react with them in the body tissues. The relationship of egg white to the unknown potent factor, brought out in these experiments, would seem to indicate that this disorder also is metabolic in nature.

The lack of precision involved in the use of the term "toxic" for such a physiological effect as that due to egg white has not been unrecognized by us, but we have been forced to employ it in this sense for want of a word which more fully defines the syndrome produced. We have refrained, as has Boas, from assigning this factor, protective against egg white injury, even a tentative place in the vitamin series, for the reason that it has not yet been shown to be a normal dietary essential although no results so far obtained preclude the possibility that it may be so. We believe that the need for this factor in normal nutrition is not demonstrable if egg white is included in the basal ration because of the peculiar influence of egg white on the need for the factor. Although György [1935] has employed the term vitamin H for the factor which is curative of the dermatitis in his experiments (*status seborrhoicus*) and which he states to be identical with the protective factor which we have studied, the rations on which this dermatitis is produced in his published experiments uniformly contain egg white.

The assumption of Ringrose & Norris [1936], that the physiological effects of egg white in chicks are due to an uncomplicated deficiency of a protective factor which, as they believe, was formed or released in their experiments during the autoclaving of the egg white, was not subjected by them to the experimental procedure of feeding the harmless heated form of egg white in the same ration with the toxic form to test the hypothetical production of the protective factor; this test has been made, with no beneficial results, in the case of detoxicated steamed egg white [Parsons & Kelly, 1933].

SUMMARY

1. The nutritional disorder in the rat or chick, due to egg white, is not attributable to a deficiency, either in the diet or in the tissues, of any factor of the vitamin B complex so far clearly characterized.

2. There is a definite interrelationship between the egg white of the ration and a certain potent factor (factor *X* of Boas), such that the greater the concentration of egg white the greater the amount of the factor necessary to cure the nutritional disorder.

3. This interrelationship is demonstrable when the potent factor is given parenterally as well as when given orally. This fact would seem to eliminate the

hypotheses that the nutritional disorder is due to a deficiency of the potent factor created by the direct destructive effect on it of the egg white in the ration mixture or in the digestive tract, or by a change brought about in the membranes of the digestive tract whereby the absorption of the potent factor is hindered or prevented. The implication of these data is that the interrelationship is metabolic in nature. It has not yet been demonstrated that the protective factor is a vitamin or that it is required in normal nutrition.

4. The requirement for the active factor is higher in the chick than in the rat.

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EXPLANATION OF PLATE I

- Fig. 1 illustrates a rat with severe symptoms of egg white injury after the initiation of treatment but before improvement was apparent: Note the swollen bare lips, swollen and encrusted eyelids, swollen bare paws, necrosed tip of tail, fuzzy dead hair with scurf and pigmentation visible, and a nerve disorder resulting in an abnormal posture. The ears are not noticeably involved. This is a more severe stage of the injury due to egg white than Grade C at which curative tests are begun in the standard technique of this laboratory.
- Fig. 2. The same rat 10 days after initiation of injection with Extract A, given as 0.5 ml. doses twice a week. Note the following signs of improvement: the loss of dead hair, scurf and pigment which were shed at the appearance of the new epidermis, reduction of swelling of eyelids and paws, somewhat better posture and greater alertness.
- Fig. 3. The same rat 23 days after the beginning of injections. Note the further improvement in the rat's condition. 9 days before this picture was taken the extract was replaced by a similar one prepared from pork kidney rather than from beef liver and of greater potency.

Fig. 1.

Fig. 2.

Fig. 3.