CCLXXVIII. THE RELATIONSHIP OF DERMATITIS IN CHICKS TO LACK OF VITAMIN B₂ AND TO DIETARY EGG-WHITE¹.

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PELLAGRA-LIKE symptoms in the rat have been observed to result from well-supplemented rations containing coagulated Chinese dried egg-white [Boas, 1924, 1] or from raw or slightly cooked fresh whole egg [Friedberger, 1926]. Scheunert and Wagner [1927], Stenqvist [1928] and others² found that whole eggs cooked sufficiently, *i.e.* at 100° for from 3 to 30 minutes, lost the capacity to produce the characteristic symptoms in rats when fed as the sole diet. Parsons [1932] and Parsons and Kelly [1933] showed that Chinese fermented dried egg-white requires a distinctly longer interval of moist heat treatment than does fresh egg-white in order to lose its harmful properties (18 times as long at 80°), thus explaining the observation of Boas [1924, 2], that whereas well-supplemented rations containing mildly heated egg-white produce pellagra-like symptoms when Chinese fermented egg-white is used, they do not if this is replaced by fresh egg-white.

Norris and Ringrose [1930] and Ringrose *et al.* [1931] reported a "pellagra-like" syndrome occurring in chicks which were fed rations containing purified or commercial caseinogen or dried egg-"albumin." The condition obtained on the caseinogen rations was prevented by the addition of 5 or 10 % of autoclaved yeast or 10 % of "milk vitamin concentrate." Kline *et al.* [1932] described a similar condition obtained in chicks on feeding a natural grain ration heated at 100° for 144 hours, attributable to a destruction of vitamin B₂ by the heating process. This condition was characterised by scabby incrustations at the corners of the mouth and under the mandible, ruffled feathers and involvements of the eyes and feet. These symptoms were prevented by the addition of skim-milk powder, liver extract (Lilly, No. 343) or autoclaved yeast.

Several facts pointed to the probability that Norris and his co-workers were dealing with two separate conditions rather than a single vitamin B₂ deficiency. Egg-white has been shown to be rich in vitamin B₂ [Aykroyd and Roscoe, 1929; Chick *et al.*, 1930]. Kuhn *et al.* [1933] prepared ovoflavin from egg-white, which they believed to be vitamin B₂ or closely related to it. Boas [1927; Boas-Fixsen, 1931] stated that the "protective factor X," *i.e.* the factor protective against egg-white injury, is not vitamin B₂. Although the distribution of the two was

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² The literature has been reviewed by Friedberger and Seidenberg [1927], Stenqvist [1928], and Parsons and Kelly [1933].

somewhat similar, she found certain differences in occurrence and extractability. Boas-Fixsen [1931] also showed that whereas Factor X was present in whole yeast little could be detected in an extract made by boiling yeast with dilute acetic acid, the larger proportion remaining with the extracted residue. Factor X was absent also from marmite (an extract of autolysed yeast), *ca.* 5 % of which was included in the basal diet used [Boas, 1927]. This circumstance explains the failure of Bell [1933] to reproduce the effects described by Boas-Fixsen, inasmuch as whole yeast was included in the basal diet in place of marmite.

Similar differences between the two factors were also noted by other workers [Salmon and Goodman, 1934; Parsons, Lease and Kelly, 1933; Lease and Parsons, 1934; Parsons and Lease, 1934]. The latter found that liver extract (Lilly, No. 343), although high in vitamin B₂, did not cure the dermatitis produced in rats by egg-white diets, nor were cures effected on rations very rich in egg-white by amounts of milk or yeast which would be adequate to cure the pellagra-like symptoms if due to the absence of vitamin B₂.

In spite of the above evidence, the hypothesis was frequently put forward that the essential factor in egg-white injury was lack of vitamin B₂. It was desired to make the test more nearly quantitative than it had previously been, with a suitable animal. In the rat the symptoms of vitamin B₂ deficiency are very slow in developing and, as Harris states [1934], "The conditions which determine whether rats deprived of vitamin B₂ will develop the characteristic skin lesions or remain free from them are not yet properly understood." Inasmuch as the pellagra-like condition was shown to be relatively easily and speedily produced in chicks by lack of vitamin B₂ and was characterised by definite symptoms similar to those in the rat, this animal was chosen.

The plan of the experiment was to produce the pellagra-like symptoms in chicks by feeding rations low in vitamin B₂ and rich in egg-white respectively testing, with each, the effect both of a liver extract (Lilly, No. 343), known to be rich in vitamin B₂, and of the liver residue remaining after the above extract is removed, which is rich in the new protective factor. If the liver extract, rich in vitamin B₂, cured the symptoms on the heated ration, but several times the amount of extract did not cure those due to egg-white, the syndrome would certainly not be due to a simple lack of vitamin B₂. The feeding of equivalent amounts of the liver residue to both groups would be an additional check.

EXPERIMENTAL.

Day-old white Leghorn chicks were obtained from the University of Wisconsin Poultry Department and from commercial hatcheries; they were started on the experimental rations as soon as they were received, having consumed no food until this time. The chicks were housed in two groups on raised wire screens until the supplement was started, at which time they were placed in individual wire cages. A favourable temperature was provided by a system of electric light bulbs.

The criterion for starting the supplement was as severe a crusting of the mouth as was compatible with a minimum loss of chicks. In the early experiments many deaths occurred, especially on the heated ration, before the effect of the supplement could be ascertained. In the chicks on the egg-white ration, moderate crusting and redness of the corners of the mouth with a check in growth or drop in weight were taken as criteria for starting the supplement (the 21st to 30th experimental day). At this time the feet were scaly and rough; the eyelids were occasionally swollen and slightly encrusted (Plate XII, fig. 2).

If the condition of the chicks on the heated ration low in vitamin B₂ was allowed to become as severe as that of the chicks on egg-white, death occurred before the supplement was eaten. Thus the supplement was started when a check in gain or a drop in weight occurred (the 21st to 24th experimental day); some chicks showed scabbiness of the mouth at this time (Plate XII, fig. 1). Chicks serving as a normal control experiment received the heated ration *plus* 5 % of yeast. Some chicks were allowed to continue on the egg-white and heated rations without supplements as a negative control experiment. The test period for the supplements was 30 days.

Egg-white Basal Ration I contained 30 % of fermented dried egg-white, 50 of yellow corn-meal (maize-meal), 10 of wheat embryo, 5 of yeast¹, 2 of cod-liver oil, 1 of NaCl and 2 of CaCO₃. In a preliminary experiment 10 % of yeast was included in Basal Ration I; the chicks behaved no differently from those on the regular 5 % ration so it was not continued. The heated Basal Ration II², low in vitamin B₂, was composed of 58 % of ground yellow corn (maize), 25 of wheat middlings, 12 of commercial caseinogen, 2 of cod-liver oil, 1 of NaCl and 2 of CaCO₃. With the exception of the cod-liver oil and salts, which were added later, this mixture was heated for 144 hours at 100° in a thermostatically controlled oven.

The liver extract³ (Eli Lilly and Co., No. 343) was the powdered preparation. That fraction remaining after its removal from liver is the liver residue³, herein designated as Residue A. The Residue A given to those chicks on the heated ration and certain chicks in a control experiment on the egg-white ration was leached with running water for 5 days, then extracted with 50 % ethyl alcohol for two 24-hour periods and dried at room temperature before an electric fan, thus ensuring a low vitamin B₂ content. 4 % of the liver extract (*i.e.* ten times the amount which Kline *et al.* [1932] state will furnish sufficient vitamin B₂ to meet the requirement of the chick) was fed with egg-white Ration I; 0.8 %, with the vitamin B₂-low Ration II; 16 % (*i.e.* an equivalent amount) of liver Residue A was fed with the egg-white Ration I; and 3.2 % with the heated ration. The concentrations of the liver residue were based on the fact that from the same amount of liver there is obtained 4–6 times as much liver residue as liver extract⁴. Because of the difficulty in the case of the chick in feeding the supplements quantitatively as separate doses, these were fed in most instances as a part of the basal rations, replacing an equivalent amount of corn-meal in the egg-white ration and being added to the heated ration. Individual food intake records were kept and the amounts of the supplements consumed were calculated. As a further check, in two cases the extract was fed by pipette to correspond to the amount of liver residue consumed *ad lib.* in the heated ration during a previous period.

The effect of the supplements.

There was a sharp difference in the appearance of the two groups of chicks fed the same basal ration but different supplements. At the end of the test period, those egg-white chicks which received liver Residue A were strong and healthy; those which received the liver extract exhibited severe dermatitis. Those chicks on the heated ration given the liver extract were healthy and lively; those given the liver residue showed very marked dermatitis. The pellagra-like syndrome of the latter group was strikingly similar to that of the egg-white group; on casual examination no difference was detectable. The egg-white chicks exhibited this severe dermatitis on the liver extract even though on the basis of actual food intake they received 4.4 times as much liver extract as the chicks on the heated ration.

¹ From the Northwestern Yeast Company, Chicago.

² We wish to thank Prof. Hart and his associates for an initial supply of the heated ration and for suggestions for housing the chicks.

³ Kindly furnished by Eli Lilly and Co., Indianapolis.

⁴ Personal communication from Eli Lilly and Co.

Details of results. The chicks on egg-white Basal Ration I *plus* 4 % of liver extract became steadily worse although they gained slowly in weight. The crust on the mouth increased in amount and severity so that at the end of the test period the corners of the mouth were sore and red, with the crust often extending under the mandible (Plate XII, fig. 3). The feet became more scaly, with sore cracks between the scales in a number of cases. An occasional chick showed gangrenous toes. The eyes grew more swollen, often to closure and in many instances were encrusted. The feathers were bedraggled and dull. There was one exception to this out of a group of 12, wherein the only apparent abnormalities were rough feathers and a marked slackening of growth. This chick was one of the last to show symptoms and was still gaining in weight when given the supplement. On the other hand, those chicks on the egg-white ration *plus* liver Residue A, either leached or non-leached, gained weight rapidly. The redness and, more slowly, the crust disappeared from the mouth. At the end of the test period, the mouth was clean and pale; the eyes were wide open and not swollen (Plate XII, fig. 4). The feathers became clean, glossy and thick, in spite of a certain degree of fraying due to the rubbing of the feathers against the wire cage. The epidermis of the feet desquamated; and at the end of the experimental period there was a pronounced degree of healing, although in some cases desquamation was not complete.

The liver residue, both leached and non-leached, was fed not only at a 16 % but also at an 8 % level with the egg-white ration. 8 % was a trial level and was not used extensively; it appeared to be a minimum dose as the results were variable. One chick which was not cured on this level gained markedly in weight when changed to 16 % of liver residue; its symptoms disappeared, except that the feet showed some residual scaliness.

The chicks on the ration low in vitamin B₂ supplemented with liver Residue A soon showed pellagra-like symptoms. The mouth became crusted, the crust growing heavier and more extensive with time. The eyes became swollen. The feet grew cracked and scaly. At the end of the test period there often was a large bare area on the neck exhibiting large flakes of heavy scurf; in some chicks this scurf extended over the region of the crop and under the wings; the legs became very emaciated. There was one exception to this out of a group of 7, wherein the chick showed no symptoms beyond slight scabbiness at the corners of the mouth. Although it continued to grow, it did not attain the weight increase of the poorest of the liver extract-supplemented group (Fig. 1, V). The chicks on the heated ration *plus* 0.8 % of liver extract grew rapidly. Throughout the experimental period the mouth was clean and smooth; the eyes were bright and shiny; the feet and legs were smooth and sturdy. There were no exceptions in this group.

Two chicks having very severe involvements of the mouth and feet on the heated ration *plus* 3.2 % of liver residue were changed to 0.8 % of liver extract. The chicks gained weight markedly; the heavy crust and redness quickly disappeared from the mouth; the feet desquamated and became clean and smooth. The legs grew to be sturdy. The feathers lost their ragged, unkempt appearance and became a smooth, glossy, thick coat. At the end of the 30-day test period the average gain of the chicks on the egg-white ration with 4 % liver extract was 51 g.; with 16 % of liver Residue A, 143 g. The average gain of the chicks on the heated ration with 0.8 % of liver extract was 160 g.; with 3.2 % of liver residue, 30 g. (Fig. 1). The average food intake of the chicks for the 30-day test period on the heated ration with liver extract was 416 g., and that of chicks on the egg-white ration *plus* liver extract was 365 g. The total average intake of liver extract of the former was 3.3 g. and of the egg-white chicks 14.6 g. The largest intake of extract of any chick on the heated ration was 4.5 g., and the lowest on the egg-white ration was 11.2 g., *i.e.* 2.5 times as much.

Chicks continued on the heated ration without any supplement showed, without exception, the usual effects of lack of vitamin B₂. Those which lived long enough on the ration exhibited the typical pellagra-like symptoms. This group contained some of the sturdiest of the chicks, since the last ones to drop in weight were left on the basal ration. Several necrosed areas were found on autopsy in the gizzards of the chicks showing pellagra-like symptoms on both the egg-white and the heated diets. The intestines were often flabby. Those chicks given heated ration *plus* 5 % of yeast, serving as a positive control experiment, grew well and showed no signs of dermatitis.

Fig. 1. Weight curves of chicks on dermatitis-producing rations showing effects of supplements.

— = Basal ration.

— = Supplement.

— = Preliminary period of 8% liver residue.

— = Preliminary period of 3.2% liver residue.

+ = Died.

P = Pronounced pellagra-like symptoms.

M = Moderate symptoms.

A = Absence of symptoms.

A- = Practically all symptoms absent.

? = Varied symptoms but no dermatitis.

DISCUSSION.

Since a thoroughly extracted liver residue cured the chicks on the egg-white ration, but not those on a ration low in vitamin B₂, and an equivalent amount of a vitamin B₂-rich liver extract cured those chicks on the latter ration, but not those on the egg-white ration, the factor curative of the egg-white injury is not vitamin B₂. These results are analogous with those of Boas-Fixsen cited above in which yeast extracts proved to be unable to prevent the occurrence of the egg-white syndrome in rats, whereas the extracted yeast was protective. The results in the present work are the more remarkable since the symptoms obtained on both rations were so nearly identical in appearance. Our conclusions are therefore in accord with those of Boas [1927] and Salmon and Goodman [1934].

Ringrose *et al.* [1931] tested no supplement with their egg-"albumin" ration. 5 or 10 % of autoclaved yeast or 10 % of "milk vitamin concentrate" when added to their purified caseinogen ration prevented the pellagra-like symptoms; 2.5 % of autoclaved yeast did not. This is in accord with the findings of Kline *et al.* [1932] that 6 % of autoclaved yeast or 8 % of skim-milk powder will prevent symptoms in the chick due to lack of vitamin B₂. With the rat, 80 % of skim-milk powder was necessary with 20 % of egg-white to cure the dermatitis due to egg-white [Parsons and Lease, 1934]. Ox-liver was about one-half to three-fourths as potent after autoclaving [Parsons, 1931]. Thus the 25 % egg-white ration of Norris and co-workers presumably would require about 25 % of untreated yeast, of the type used in this laboratory, or 30-50 % of autoclaved yeast to prevent injury. It is significant that the inclusion of 10 % of yeast with the egg-white basal ration did not prevent the occurrence of symptoms in the present experiments. The Cornell workers assumed the syndromes on the caseinogen and the egg-white rations to be identical and both due to lack of vitamin B₂; this error is attributable to their failure to test the value of supplements with the egg-white rations.

Since it had been shown that the greater the amount of egg-white present, the greater the amount of the protective factor necessary in the diet to cure or prevent dermatitis [Boas, 1927; Parsons, 1932; Parsons and Kelly, 1933], it was conceivable that the toxic effect was due to a destructive action of egg-white either on the new factor or on the vitamin B₂ content of the ration during an extended period of storage, so that the function of the new factor would then be merely to prevent the destruction of vitamin B₂. An analogous effect is the destructive action of lard on the vitamin E content of a ration mixture and the opposing action of wheat germ oil [Evans and Burr, 1927]. That this hypothesis is untenable in the case of egg-white injury was shown by the fact that there was no difference in the rate of loss of potency, during a year or more of storage, between the loss which took place in a ration mixture containing both egg-white and a minimum protective amount of dried liver or liver residue and that which occurred in the ingredients of the ration when stored separately for the same length of time and mixed at the time of feeding. In either case the loss was very slight [Parsons and Lease, 1934; and unpublished observations].

No experiments herein cited, however, furnish any evidence which bears on an alternative hypothesis, *i.e.* a failure of the vitamin B₂ of the diet to reach the blood-stream of the rat, due to some inhibiting effect of the egg-white in the digestive tract. It may be stated that this hypothesis has not been substantiated by some further experiments with rats in our laboratory (unpublished observations).

Fig. 1.

C 53. Heated ration + 0.8 % liver extract.

Fig. 2.

C 45. Egg-white ration + 16 % liver residue.

Fig. 3.

C 31. Egg-white ration + 4 % liver extract.

Fig. 4.

C 39. Egg-white ration + 16 % liver residue.

SUMMARY.

The dermatitis produced in chicks with a well-supplemented ration rich in egg-white is cured by an extracted liver residue poor in vitamin B₂. It is not cured by a liver extract (Lilly, No. 343) rich in vitamin B₂.

The dermatitis in chicks due to a lack of vitamin B₂ is cured by the liver extract, but not by the liver residue.

The syndrome produced on egg-white is therefore not due to a vitamin B₂ deficiency in the ration, although the two conditions are strikingly similar in appearance. Some hypotheses are discussed.

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DESCRIPTION OF FIGURES IN PLATE XII.

- Fig. 1. Chick on ration low in vitamin B₂ on the day the supplement was started. Note the encrusting of the corner of the mouth. This chick was given 0.8 % liver extract and the symptoms rapidly disappeared.
- Fig. 2. Chick on egg-white ration plus 16 % liver residue, at beginning (3rd day) of supplementary period. Note the dark scab at the corner of the mouth, ruffled feathers and slightly swollen eyelids. The similarity to the chick on the vitamin B₂-low ration (Fig. 1) is striking. At the end of the test period these symptoms had disappeared.
- Fig. 3. Chick on egg-white ration plus 4 % liver extract on the 27th day of the supplement, 3 days before the end of the test period. Note the bedraggled feathers, thickened, roughened toes, and crust at corner of the mouth.
- Fig. 4. Chick on egg-white ration plus 16 % liver Residue A, on 27th day, 3 days before the end of the test period. Note the clean mouth, bright eyes and thick, glossy feathers. The ordinary posture of this chick when not restricted was erect. It had been on the supplementary period as long as the chick in Fig. 3. Note the contrast.