The Vitamin B_2 Complex and Allied Factors : Avian Factors.

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THERE is little need to emphasise the importance of studies in nutrition to the development of the poultry industry. Research of recent years has yielded results clearly indicating the disturbances in the well-being of birds concomitant with inadequacies of supplies of essential factors in the diet. This is particularly true of those factors which may be termed water-soluble and may be classified under the heading of the vitamin B complex. In a brief review of this nature only the most salient features of recent progress can be described. Any attempt to correlate the different factors required by birds and mammals such as the rat, dog, monkey or man is at present rendered difficult from the insufficiency of data.

The several factors postulated as necessary for avian nutrition may be summarised :

Factors other than Vitamin B_1 Required by Birds.

Chick : Flavine
Anti-pellagra or filtrate factor
Vitamin B_4

Pigeon : Flavine.
Vitamin B_3
Vitamin B_5

Turkey : Flavine.

As well as these evidence for at least three other factors of fat-soluble nature has been presented : (1) Vitamin K, the anti-hæmorrhagic factor, (2) Anti-encephalomalacia factor of Goetsch and Pappenheimer and (3) a factor curative of erosions of the gizzard. At present there is very little evidence to show that these factors are needed by any other bird than the chick.

Vitamin G (Flavine) and the Filtrate Factor.

On synthetic diets and diets submitted to special treatment chicks develop a form of dermatitis described in detail by Ringrose, Norris and Heuser (1921)[1,2] as scaly incrustations of the mouth, eyes, legs and feet accompanied by a retardation in feather growth. Similar symptoms were observed by Kline, Keenan, Elvehjem and Hart[3] in chicks reared upon a diet of normal food-stuffs previously heated at 100° C. for 144 hours, a treatment which destroyed the preventive factor. The deficiency of the anti-dermatitis factor can be remedied by incorporating in the diet autoclaved yeast, skimmed milk, or liver extract. The latter is particularly efficacious in curing dermatitis and promoting growth in the chick and has served as a source of the factor in attempts on its isolation by Elvehjem and Koehn (1935).[4] A partial separation was effected by treatment of the liver extract with fuller's earth which, when removed by filtration, leaves the potent factor in the filtrate. These results have been confirmed by Lepkovsky and Jukes[5] who have designated the anti-dermatitis factor of the chick the 'filtrate factor'. For normal growth, however, another factor is required namely flavine which is removed from the liver extract by adsorption upon fuller's earth. Two factors at least are needed, therefore, in addition to vitamin B_1 by the chick (1) flavine also termed vitamin G and (2) the filtrate factor also called vitamin B_2. It should be noted that no definite nomenclature has yet been adopted for these factors ; different groups of workers employ various terms for the same factors.

Examination of the filtrate factor[6] has shown it to be a relatively inert substance untouched by oxidising and reducing agents but partially destroyed by warming with alkali. It is readily extracted from foodstuffs by hot or cold acidified water, not precipitated by phosphotungstic acid, untouched by benzoyl chloride, and not readily taken up by adsorbents. Its solubility in butyl and *iso*-amyl alcohol has provided a means for its purification.

There has been gradually accumulating evidence that concentrates of the filtrate factor, so potent in curing chick dermatitis, are also active in alleviating pellagra-like symptoms in other animals. Blacktongue in dogs[7,8,9] and pellagra in humans[10] respond to treatment with extracts of the filtrate factor from different sources such as rice, bran and liver. But it is still undecided whether the curative action of the extracts is to be attributed to the presence of one or more factors. There is the possibility that more than one factor is involved for Jukes[6]

has found that the distribution of the P–P factor and that of the filtrate factor in different food-stuffs do not run parallel.

The physical and chemical properties of flavine are too well known to necessitate recapitulation. Its exact function in chick metabolism is obscure although it is probably related to the oxidative metabolism of the tissues. No severe symptoms develop from a deficiency of flavine in the diet of the chick; growth is retarded and there is a tendency to diarrhœa which responds readily to flavine treatment.[11,12] Some claims have been made for its efficacy in curing leg weakness in the chick and in increasing the hatchability of eggs.[13,14] Its chief effect on the chick is growth promoting. A strange contrast to the chick is the case of the turkey. This bird on certain diets develops symptoms of dermatitis which can be cured by the administration of flavine.[11]

The symptoms of dermatitis developed in chicks on a heated ration of normal food-stuffs resemble closely those produced by feeding diets rich in egg-white. Lease and Parsons[15,16] have shown that thoroughly extracted liver residue alleviated the condition while liver extract rich in vitamin B_2 complex had no effect. Similar results were obtained by Tully and Franke (1931)[17] who considered that the effects of egg-white are due to a toxic agent which cannot be counteracted by vitamin B_2. It has, therefore, been concluded that dermatitis due to excessive amounts of egg-white originates from some other cause than a deficiency of the vitamin B_2 complex. This agrees with the work of György[18] who finds that the factor curative of the egg-white syndrone, termed by him vitamin H, is rendered water-soluble after enzymic hydrolysis of food-stuffs.

Other Chick Factors.

The need of the chick for flavine and the filtrate factor is definitely established. While the need of other factors is not to be doubted, further investigation is necessary to elucidate their functions and properties and to distinguish them from the known vitamins. The difficulty of rearing chicks on a synthetic diet, so frequently encountered,[19,20,21] was attributed to a deficiency of a number of factors resulting in multiple lesions. This was first indicated by the work of Hogan[22] and Boucher who found that chicks on a synthetic diet supplemented with the fat-soluble vitamins and vitamins B_1 and B_2 exhibit subnormal growth, paralysis, 'brain degeneration'; these conditions could be alleviated by the administration of hydrolysed yeast, ether extracted egg yolk, liver extracts and tiki-tiki, each of these materials presumably supplying a missing factor. The paralysis observed by Hogan and Boucher is not confined to chicks on synthetic and experimentally designed diets but is frequently seen in birds on practically normal diets (*e.g.*, range paralysis). The paralytic symptoms and the subnormal growth of such affected birds have been attributed by Elvehjem and his colleagues[23,24] to deficiencies in the diet of two factors present in liver. The anti-paralytic factor is considered to be vitamin B_4 since it was found that concentrates from hogs' liver or kentucky grass prepared similarly to the method of Kinnersley *et al.*,[25] for vitamin B_4 concentrates were active in curing paralysis and 'brain degeneration' but did not alone produce a maximum response in growth. Normal growth was attained when the vitamin B_4 concentrates and the liver residue were administered simultaneously. In more recent investigations, Kline *et al.*,[26] have discovered that the factor present in the liver residue is arginine, a fact of some interest since the basal diet appeared to contain adequate amounts of this amino-acid.

Microscopical examination of the brains of chicks suffering from paralysis revealed lesions, designated by Elvehjem and colleagues as 'brain degeneration'. Localised in the cerebrum and sometimes in the cerebellum, the affected areas were white and caseous and the cells œdematous. The similarity of this condition to that observed by Goetsch and Pappenheimer[27,28] in paralytic chicks led them to consider that they were dealing with a deficiency of the same factor, the encephalomalacia factor. The factor of Goetsch and Pappenheimer is, however, fat-soluble; it is present in vegetable oils, such as those of cotton seed and soya-bean. Saponification of the soya-bean oil with the exculsion of air leaves the factor in the non-saponifiable fraction. The fact that 'vitamin B_4' may be present in oils and the close resemblance of the symptoms observed by the two groups of workers suggests that only one factor may be involved. Elvehjem and his colleagues[29] claim to have differentiated the symptoms due to deficiencies of the two factors.

The use of different basal diets, generally designed to produce a deficiency of one particular factor and particularly of synthetic diets has yielded results suggesting that the chick requires for its normal development a number of water and fat-soluble factors. The failure to appreciate the necessity of the latter substances may have obscured the true response of the chick to the lack of the water-soluble factors. Substantial evidence has been presented for the existence of two fat-soluble factors other than vitamins A and D and the encephalomalacia factor namely. the anti-hæmorrhagic factor[30–35] or vitamin K and a factor protective against gizzard erosion.[6,36,38] A deficiency of vitamin K manifests itself by a delayed clotting time of the blood, a fact which has been utilised in devising a method for the assay of the vitamin. This factor is stated to be a complex hydrocarbon of molecular weight about 600, stable to heat and light and present in liver fat, hempseed, alfalfa grass, etc. A condition frequently developing concurrently with the symptoms of vitamin K deficiency is a disorder of the gizzard characterised by erosion and necrosis. The preventive factor is present in kale, alfalfa, wheat, bran, lung tissue, etc. Some confusion exists as to the exact nature of its properties and effects. The work of Almquist and Skotstad[37] appears to show that the factor is present in the saponifiable fraction of oils from greens ; on the other hand, Kline *et al.*[38] state that it is insoluble in water, alcohol and ether. These discrepancies await elucidation.

Pigeon Factors.

Of the factors of the vitamin B complex evidence has been presented indicating that the adult pigeon requires at least three others in addition to vitamin B_1. The fact that pigeons on a polished rice diet supplemented by a vitamin B_1 preparation failed to attain their maximum weight led Williams and Waterman (1928)[39] to conclude that another factor, vitamin B_3, was necessary. This has been amply confirmed.[40, 41, 42, 43] O'Brien (1934)[44] found that full weight restoration could be obtained in the pigeon by the addition of a hydrolysate of wheat germ to the above diet supplemented by Peters concentrate (containing vitamins B_1 and B_5). A peculiar property of such vitamin B_3 extracts was their extreme sensitivity to oxidation. A more detailed investigation of the vitamin requirements of pigeons by Carter and O'Brien (1934, 1935, 1936) [45, 46, 47] has revealed that the problem was complicated by the inadequacy of the amount of protein in the diet and the period of preliminary depletion to which the birds were submitted to decrease their reserves of vitamins. A partial recovery in weight occurred in pigeons on polished rice and Peters concentrate receiving extra protein in the form of casein to correct the low protein content of the rice. But the extent of the restoration in weight was dependent upon the intensity of the initial depletion. Whereas most birds of short depletion recovered their maximum weight, a large percentage of the birds of long depletion failed to do so unless supplied with an alcoholic extract of liver. Treatment of this extract with fuller's earth yielded two fractions : one of high flavine content adsorbed on the fuller's earth and a filtrate carrying the vitamin B_3 activity. When supplementing one another the two fractions produced restoration to maximum weight. The flavine fraction was further purified on the lines usually adopted for the isolation of flavine. These results suggest that the pigeon requires flavine and vitamin B_3 in addition to vitamins B_1 and B_5. Further confirmation of the need of factors other than vitamin B_1 by the pigeon is found in the work of Ammerman and Waterman (1935).[48] A failure to regain their maximum weight was observed in pigeons on a diet of autoclaved whole wheat supplemented by doses of crystalline vitamin B_1 up to 180γ/day. This result has recently been confirmed by us.[49]

In 1930 Carter, Kinnersley and Peters[50, 51] submitted evidence for the existence of a factor distinct from vitamin B_3. Their experiments indicated that certain highly purified concentrates of vitamin B_1, administered in large doses, could not maintain the weight of pigeons. This was attributed to the lack of a factor which was found to be present in Peters concentrate, an alcoholic extract of norite charcoal obtained in the isolation of vitamin B_1 from yeast. The factor was termed vitamin B_5 and is present in yeast, marmite and whole wheat.

Heart Block in Pigeons.

Pigeons on a rice or synthetic diet are, as shown by Carter,[45, 52, 53] subject to

bradycardia and heart block. The condition manifests itself earlier than polyneuritis and is abolished by vagal section and atropine. It can be cured by the administration of yeast or substitution of a whole wheat diet from which it may be concluded that it is of dietary origin. It should be noted that rats on vitamin B_1 deficient diets develop a bradycardia without bundle block. But in contrast to the pigeon they are cured by the administration of vitamin B_1 (Drury, Maudesley and Harris, 1930[54]).

A discussion of the possible relation of avian factors to those required by man and animals would be premature. Nevertheless it is of interest to note that some of the water-soluble factors are needed by more than one species. Flavine for instance promotes growth of the rat, chick and pigeon and is curative of dermatitis in the turkey. The filtrate factor concentrates have been shown to be active in curing not only dermatitis in the chick but also black-tongue in the dog and pellagra in humans. More recently Lepkovsky and his colleagues[55] have found that concentrates of the filtrate factor promote growth in rats when supplemented by anti-dermatitis factor, vitamin B_6. It should also be noted that the liver concentrate containing vitamin B_3[47] is also active in stimulating growth in the rat in conjunction with the anti-dermatitis factor.[56] Whether these effects are due to the action of one factor is, at present, undecided.

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