The Animal Protein Factor in the Nutrition of Chickens

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The use of the chicken for the study of the animal protein factor (APF) has brought to light several properties of vitamin B_{12} and related substances, and has contributed largely to the recognition of their significance in animal nutrition, apart from their function as anti-pernicious-anaemia factor(s). In particular, their importance in reproduction and growth has been studied with poultry.

Properties of the animal protein factor

Effect in reproduction. Many workers in the past have postulated the existence of a 'hatchability factor' essential for the successful hatching of fertile eggs from hens on all-vegetable diets. Among others, Bird, Rubin, Whitson & Haynes (1946) reported improvement in hatchability with supplements of fish meals, dried milk, fermentation products and dried cow manure; Lindstrom, Moore, Petersen, & Wiese (1949) obtained similar improvements with fish meal and a concentrate from cow manure, and Black, Getty, Coates, Harrison & Kon (1950) with a vitamin B_{12} concentrate (Examen, Glaxo Laboratories Ltd.). That the active material was in fact vitamin B_{12} was shown by Lillie, Olsen & Bird (1949), who injected solutions of the crystalline vitamin into eggs from hens on vegetable diets during incubation and obtained a marked increase in the number hatched. Carver & McGinnis (1950) confirmed their observation by injecting 2 μg vitamin B_{12} into deficient eggs before incubation with a consequent increase in hatchability from 35 to 90 %. Olcese, Couch, Quisenberry & Pearson (1950) have described signs of vitamin B_{12} deficiency in the developing embryo, including atrophy of the leg muscles, multiple haemorrhages and perosis. These authors also confirmed our own experience that embryos die most frequently about the 17th day of incubation.

As yet there is no evidence that APF deficiency has adverse effects on fertility of either male or female.

Effect on growth. It is now generally accepted that a source of the APF is essential in the diet of the growing chick. The literature on the subject is too extensive to be quoted in detail; early work has been reviewed by Schweigert (1949). It is our own experience, and that of many other workers, that hens given normal diets pass on to their progeny sufficient reserves of the APF to last through the initial growing stages. It is probably this transference of reserves from mother to offspring that has delayed for so long the discovery of the animal protein factor; its existence was only recognized when plant sources of proteins came into extensive use in American poultry rations, resulting in poorer hatchability and viability of the young chicks.

The chick, being a rapidly growing animal, very soon shows signs of a lack of essential growth factors and although, in contrast to man, no anaemia is evidenced, a severe deficiency of the APF results in very poor growth, poor feathering and usually death in 3–4 weeks. The chick appears unable to compensate to any extent for the

deficiency by the activity of its intestinal micro-organisms. These facts make the chick a very suitable subject, not only for the biological assay of the APF, but also for the fundamental study of its various components.

The chick assay of the animal protein factor

A number of workers have used chicks for the biological assay of the APF (Lillie, Denton & Bird, 1949; Nichol, Robblee, Cravens & Elvehjem, 1947; Stokstad, Jukes, Pierce, Page & Franklin, 1949; Coates, Harrison & Kon, 1950, 1951), all based on the use of chicks from hens themselves depleted of the APF. We have found it helpful to include a small amount of vitamin B_{12} as Examen (Glaxo Laboratories Ltd.), equivalent to about 0·25 μg vitamin B_{12}/100 g, in the diet of the hens in order to maintain the hatchability of their eggs at a reasonable level, while only negligible reserves are passed on to the chicks hatched from them. Our method consists essentially of maintaining groups of about ten depleted chicks on three to four dose levels of test material (incorporated into the basal vegetable-protein diet) and comparing their weight responses with those of similar groups given a standard preparation. Robblee, Nichol, Cravens, Elvehjem & Halpin (1948) recommend the inclusion of thyroprotein in the chicks' diet, thereby increasing their requirement for the APF and extending the effective range of the dose-response curve. We have examined the accuracy of our method and found that variance between birds on the same doses was very high, possibly as a result of difference in depletion rates of their dams. The variance can, to some extent, be overcome (Lillie, Denton & Bird, 1949), by keeping the chicks for up to 14 days on the basal diet and rejecting those falling outside defined weight limits at the end of the depletion period.

In spite of the high inherent error, we have found the results of chick assays surprisingly reproducible (Coates *et al.* 1950). However, they rarely agree with those found by microbiological techniques. Broadly speaking, chick assays give much higher results for animal-protein products such as fish and whale solubles, and markedly lower values for rumen and faecal materials (Coates, Ford, Harrison, Kon, Porter, Cuthbertson & Pegler, 1951) and these discrepancies add to the already considerable evidence accumulating to show that the APF is not indeed a single entity but a multiplicity of factors with different activities for different organisms.

Evidence, with chicks, for the complex nature of the APF

When vitamin B_{12} was first obtained in pure form it was considered by some (Ott, Rickes & Wood, 1948; Nichol, Dietrich, Cravens & Elvehjem, 1949; Lillie *et al.* 1948) to be itself the APF, as its addition to the diet of chicks on all-vegetable rations resulted in considerable gains in weight. Its maximal effect was produced with doses of 3 μg/100 g diet (Ott *et al.* 1948). More recently, however, many reports indicated that chick growth can be further improved in the presence of optimal amounts of vitamin B_{12} by supplements of crude materials such as fish solubles or certain samples of dried brewer's yeast (Carlson, Miller, Peeler, Norris & Heuser, 1949; Arscott & Combs, 1950), distiller's dried solubles (Novak, Hauge & Carrick, 1947) and crude liver extracts or dried whey (Menge, Combs & Schorb, 1949).

Although it is possible that the discrepancies reported above between chick and microbiological tests of such materials are due to incomplete extraction in the microbiological assay, it is more likely that the high values found by the chick method reflect the presence in the test materials of unidentified nutrients essential for, or utilized by, the chick but not the micro-organisms employed in the assay.

In the course of our chick assays we have obtained two further indications of the multiple nature of the APF (Coates *et al.* 1950). Firstly, we have had little success in obtaining linear dose-response curves to crystalline vitamin B_{12}. A highly refined liver extract such as Examen (Glaxo Laboratories Ltd.) gives more satisfactory responses, but much better linearity is obtained with crude material such as fish solubles. It is, therefore, probable that the crude materials contain a factor necessary for the proper utilization of vitamin B_{12} by higher animals. This possibility has been strengthened with the isolation by Ford & Porter (1952) of a vitamin B_{12}-like substance from calf faeces that, although itself devoid of APF activity for chicks, considerably enhances their response to pure vitamin B_{12} (Coates, Ford, Harrison, Kon & Porter, 1952). We have also found that certain preparations from preserved herrings possess the same property. The substance is not identical with the 'intrinsic factor' of human pernicious anaemia because we have found a sample of freeze-dried normal human gastric juice ineffective in enhancing the chick's response to added vitamin B_{12}.

Secondly, we have found that the APF activity of whale and fish solubles, as measured by chicks, increased with the degree of depletion of their dams. Assays of a sample of whale solubles done simultaneously with chicks from hens freshly depleted and those from hens that had received a vegetable diet for at least a year gave values of 0·14 and 0·70 μg vitamin B_{12} activity/g respectively (Coates *et al.* 1950). Similarly, the apparent vitamin B_{12} activity of a sample of fish solubles was 1·3 μg/g with one hatch of chicks and well over 3·0 μg/g with a hatch from the same hens 6 months later. From this evidence we believe that hens given all-vegetable diets become depleted first of vitamin B_{12} and later of another member (or members) of the APF complex, and that these successive depletions are reflected in the response of the offspring to sources of the APF.

The spate of reports that so-called 'APF concentrates' prepared as by-products in the manufacture of antibiotics have growth-promoting activity for chicks beyond that of their vitamin B_{12} content culminated in the finding (Stokstad & Jukes, 1950) that this activity was due to the presence of traces of antibiotic remaining in the concentrates. Coates, Dickinson, Harrison, Kon, Porter, Cummins & Cuthbertson (1952) discuss the question whether the growth stimulation is a direct effect of the antibiotic itself or occurs indirectly as the result of increased synthesis of components of the APF in the gut of the chick, but the position is too confused at present to contribute much towards elucidating the identity of the APF.

In spite of the considerable weight of evidence for the existence of unidentified essential nutrients in the APF complex, there are still some who maintain that maximal growth in chicks can be obtained when crystalline vitamin B_{12} is the sole APF supplement. Thus Ott (1951), using a vegetable diet containing 2 μg vitamin B_{12}/100 g, was unable to improve chick growth by addition of whey, fish solubles or a crude liver

fraction. The basal diet used in Ott's experiments contained very large supplements of glycine, arginine, methionine, cystine and choline and if, as seems probable, the APF is concerned in the metabolism of protein, it is likely that large supplements of pure amino-acids might reduce the animal's need for at least some of the APF components.

The biochemical significance of the APF in chick nutrition

The biochemical functions of the APF are still obscure. That it is involved in the metabolism of proteins is apparent, as an increase in the protein content of chick diets lacking APF accentuates the signs of its deficiency in the chicks (Rubin & Bird, 1947). McGinnis, Hsu & Graham (1948) found higher than normal levels of non-protein-nitrogen in the blood of chicks deprived of APF and suggested that the APF was concerned in the utilization of amino-acids. Their suggestion received further support from Charkey, Wilgus, Patton & Gassner (1950), who measured microbiologically arginine, lysine, methionine, tryptophan, histidine, threonine and valine in chick blood and found higher values for all seven amino-acids in the blood from depleted chicks as compared with controls given vitamin B_{12}.

There are numerous indications from work with chicks that the APF is concerned in transmethylation processes. The suggestion was first made by Gillis & Norris (1949*a*) who showed that in supplying the chick's need of APF in the form of a liver paste they reduced its requirement for choline and betaine. Later the same authors (Gillis & Norris, 1949*b*) found that crystalline vitamin B_{12} had the same sparing action on methylating compounds. Similarly, Schaefer, Salmon & Strength (1949) demonstrated that the choline requirement of APF-deficient chicks was reduced six-fold when vitamin B_{12} was added to their diet. Later, the same authors (Schaefer, Salmon, & Strength, 1951) showed that certain precursors of choline, in particular dimethylaminoethanol, could be utilized by chicks in place of choline itself, but only if vitamin B_{12} was present in the diet. Briggs, Hill & Giles (1950) also studied the relationship between choline and vitamin B_{12} in chicks, and although they found that choline exerted a sparing action on the birds' requirement for vitamin B_{12}, DL-methionine was far more efficient in this respect and they concluded that, under certain conditions at least, chicks may do without extraneous vitamin B_{12} if sufficient methionine is present in the diet. Patrick (1950) arrived at similar conclusions, but Jukes & Stokstad (1951), though they confirmed the sparing action on vitamin B_{12} of both methionine and choline, were unable to produce optimal growth in chicks without both methionine and vitamin B_{12}. The differences in results obtained by different workers can undoubtedly be explained by slight variations in experimental conditions.

The relationship between vitamin B_{12} and other vitamins has been investigated in experiments with chicks by a number of workers. Schaefer, Salmon, Strength & Copeland (1950) postulated the interrelationship of folic acid, vitamin B_{12} and choline. Dietrich, Monson & Elvehjem (1951) presented data demonstrating that vitamin B_{12} is concerned in the conversion of folic to folinic acid in the chick, but we ourselves have been unable to diminish signs of vitamin B_{12} deficiency in chicks by injections of folinic acid. Others (Yacowitz, Norris & Heuser, 1951) have reported a mutual sparing action between pantothenic acid and vitamin B_{12}. The interrelation-

ship of other vitamins or amino-acids and vitamin B_{12} may account for some of the evidence for the complexity of the APF. No doubt, with the identification of the components of the APF its biochemical significance will be explained, and it is clear that work with chicks can throw considerable light on the problem of the nature and functions of the APF.

REFERENCES

Arscott, G. H. & Combs, G. F. (1950). *Poult. Sci.* **29**, 746.
Bird, H. R., Rubin, M., Whitson, D. & Haynes, S. K. (1946). *Poult. Sci.* **25**, 285.
Black, D. J. G., Getty, J., Coates, M. E., Harrison, G. F. & Kon, S. K. (1950). *Biochem. J.* **46**, viii.
Briggs, G. M., Hill, E. G. & Giles, M. J. (1950). *Poult. Sci.* **29**, 723.
Carlson, C. W., Miller, R. F., Peeler, H. T., Norris, L. C. & Heuser, G. F. (1949). *Poult. Sci.* **28**, 750.
Carver, J. S. & McGinnis, J. (1950). *Poult. Sci.* **29**, 752.
Charkey, L. W., Wilgus, H. S., Patton, A. R. & Gassner, F. X. (1950). *Proc. Soc. exp. Biol., N.Y.*, **73**, 21.
Coates, M. E., Dickinson, C. D., Harrison, G. F., Kon, S. K., Porter, J. W. G., Cummins, S. H. & Cuthbertson, W. F. J. (1952). *J. Sci. Food Agric.* **3**, 43.
Coates, M. E., Ford, J. E., Harrison, G. F., Kon, S. K. & Porter, J. W. G. (1952). *Biochem. J.* **51**, vi.
Coates, M. E., Ford, J. E., Harrison, G. F., Kon, S. K., Porter, J. W. G., Cuthbertson, W. F. J. & Pegler, H. F. (1951). *Biochem. J.* **49**, lxvii.
Coates, M. E., Harrison, G. F. & Kon, S. K. (1950). *Biochem. J.* **46**, vii.
Coates, M. E., Harrison, G. F. & Kon, S. K. (1951). *Analyst*, **76**, 146.
Dietrich, L. S., Monson, W. J. & Elvehjem, C. A. (1951). *Proc. Soc. exp. Biol., N.Y.*, **77**, 93.
Ford, J. E. & Porter, J. W. G. (1952). *Biochem. J.* **51**, v.
Gillis, M. B. & Norris, L. C. (1949*a*). *J. biol. Chem.* **179**, 487.
Gillis, M. B. & Norris, L. C. (1949*b*). *Poult. Sci.* **28**, 749.
Jukes, T. H. & Stokstad, E. L. R. (1951). *Fed. Proc.* **10**, Part. 1, 386.
Lillie, R. J., Denton, C. A. & Bird, H. R. (1948). *J. biol. Chem.* **176**, 1477.
Lillie, R. J., Denton, C. A. & Bird, H. R. (1949). *Poult. Sci.* **28**, 772.
Lillie, R. J., Olsen, M. W. & Bird, H. R. (1949). *Proc. Soc. exp. Biol., N.Y.*, **72**, 598.
Lindstrom, R. G., Moore, P. R., Petersen, C. F. & Wiese, A. C. (1949). *Poult. Sci.* **28**, 464.
McGinnis, J., Hsu, P. T. & Graham, W. D. (1948). *Poult. Sci.* **27**, 674.
Menge, H., Combs, G. F. & Schorb, M. S. (1949). *Poult. Sci.* **28**, 775.
Nichol, C. A., Dietrich, L. S., Cravens, W. W. & Elvehjem, C. A. (1949). *Proc. Soc. exp. Biol., N.Y.*, **70**, 40.
Nichol, C. A., Robblee, A. R., Cravens, W. W. & Elvehjem, C. A. (1947). *J. biol. Chem.* **170**, 419.
Novak, A. F., Hauge, S. M. & Carrick, C. W. (1947). *Poult. Sci.* **26**, 604.
Olcese, O., Couch, J. R., Quisenberry, J. H. & Pearson, P. B. (1950). *J. Nutrit.* **41**, 423.
Ott, W. H. (1951). *Poult. Sci.* **30**, 86.
Ott, W. H., Rickes, E. L. & Wood, T. R. (1948). *J. biol. Chem.* **174**, 1047.
Patrick, H. (1950). *Poult. Sci.* **29**, 923.
Robblee, A. R., Nichol, C. A., Cravens, W. W., Elvehjem, C. A. & Halpin, J. G. (1948). *Proc. Soc. exp. Biol., N.Y.*, **67**, 400.
Rubin, M. & Bird, H. R. (1947). *J. Nutrit.* **34**, 233.
Schaefer, A. E., Salmon, W. B. & Strength, D. R. (1949). *Proc. Soc. exp. Biol., N.Y.*, **71**, 202.
Schaefer, A. E., Salmon, W. D. & Strength, D. R. (1951). *J. Nutrit.* **44**, 305.
Schaefer, A. E., Salmon, W. D., Strength, D. R. & Copeland, D. H. (1950). *J. Nutrit.* **40**, 95.
Schweigert, B. S. (1949). *Nutr. Rev.* **7**, 225.
Stokstad, E. L. R. & Jukes, T. H. (1950). *Proc. Soc. exp. Biol., N.Y.*, **73**, 523.
Stokstad, E. L. R., Jukes, T. H., Pierce, J., Page, A. C. & Franklin, A. L. (1949). *J. biol. Chem.* **180**, 647.
Yacowitz, H., Norris, L. C. & Heuser, G. F. (1951). *J. biol. Chem.* **192**, 141.