Towards the Elimination of the Fowl Leukosis Complex

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The losses among commercial flocks due to the fowl leukosis complex are enormous and fall as heavily upon the progressive man as well as the less competent. They have been put at $60,000,000 annually in the U.S.A.[2] and are proportionally as great in many other countries. It is the purpose of this account to suggest means whereby this can be reduced, and represents a brief summary of ten year's work upon the tumour viruses of fowls. Though a detailed analysis of the whole problem is not claimed, the findings seem sufficiently advanced for practical measures to be based upon them.

A particular advantage in much of this work was the fact that the Brown Leghorn flock of A. W. GREENWOOD at the Poultry Research Centre was available for the supply of experimental chicks. Since the spontaneous incidence of the condition is extremely low in this flock[3], experiments could be interpreted with some confidence. The fact that most workers had the handicap of extremely high incidences in their test animals is partly responsible for the slow progress and confusing results so typical of this field.

Under the heading of the fowl leukosis complex poultry pathologists include a series of diseases caused by a group of viruses belonging to the neoplasia-inducing series. It is usually taken to include the virus-induced sarcomas (such as the famous Rous I), myeloid and lymphoid leukaemias, erythroleukaemia, neurolymphomatosis (or fowl paralysis) including the ocular form, and osteopetrosis. Not all workers are agreed upon the exact details of the composition of the group, and the disagreement upon this and upon the pathological details can well be measured by the fact that HUNGERFORD in a recent Textbook of Poultry Diseases[4] lists 69 synonyms for the complex in the English language alone. The complex usually shows almost its complete range in each infected flock, but one or more forms may be absent and one or more especially predominate. Such viruses as have been examined experimentally are usually found to be serologically related (e.g. ANDREWES[5]). In addition, DURAN-REYNALS has shown that the classical Rous I[6] can be modified by suitable techniques to give many variants, some of which resemble forms encountered in the field, such as bone tumours and neurotropic forms, while GREENWOOD and CARR[1] produced evidence suggesting frequent mutation of Rous I to a neurolymphomatosis form. The viruses of the leukosis group are therefore either closely related or variants of a single virus, and facts obtained for one form may be extrapolated with some confidence.

Any treatment by radiation, chemotherapy, or surgery is of purely academic importance, for a sick bird is not producing eggs and in most systems of management the expense of individual caging, maintenance and treatment would never be compensated for by the value of the bird, should it be eventually restored to health and laying condition. The only feasible approach to the problem is therefore concerned with elimination of the viruses.

Immunisation against them is unsafe and uncertain. This is perhaps partly due to the fact that the whole life of the virus after infection is intracellular[2]; stages of release from the cell and reinfection do not occur. A single infected cell, which is of negligible importance in most virus diseases, will in this group be sufficient to lead to a progressive and fatal cancer. Since protection against one virus may not guarantee protection against all others[3], a very polyvalent vaccine would in any event be needed, and this might well be more in mere bulk than a newly-hatched chick could possibly tolerate.

Similar reasons may be advanced for the comparative failure of methods depending upon breeding for resistance. Lengthy and exhaustive studies upon this aspect were carried out by several workers in the U.S.A.[4]. Though the disease was reduced by this approach, it was never abolished or even reduced to negligible levels; the improvement would have been worth while commercially, but was only obtained and maintained by careful and expert attention. If more than one virus is concerned, and the cross-resistance is only partial (as with the sarcoma viruses[3]) the problem

[1] Poultry Research Centre, Edinburgh.

[2] U. S. Department of Agriculture (Regional Poultry Research Laboratory, East Lansing, Michigan), 4th ann. Rep. *1943*, 3.

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[4] T. HUNGERFORD, *Diseases of Poultry* (Wilkinshaw Co. Sydney,, 1951).

[5] C. H. ANDREWES, J. Path. Bact. *34*, 91 (1931); *35*, 243 (1932); *37*, 17 (1933); *37*, 27 (1933).

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becomes very difficult for the geneticist, while if mutation is occurring in resistant hosts, as the work of DURAN-REYNALS[1] and GREENWOOD and CARR[2] suggests, it becomes almost impossible.

It is generally agreed that most infections take place in the first eight weeks of the chick's life[3]. There is a curious discrepancy at this point. The laboratory worker finds that injection with cell injury is necessary to produce the disease and that the sarcoma group at any rate do not produce immediate effects if they enter with minimal damage, as was first recognized by ROUS and co-workers[4]. It was also first shown by ROUS and colleagues that infected and normal fowls can safely run together, and even be fed upon tumour without infection; a fact that our own practice at Edinburgh fully confirms, for no precautions in isolating the experimental birds are taken. Yet it is also quite clear that infection in the field is easy. When birds of the Centre flock, for example, were sent to an area where the disease was extant, and were kept under normal field conditions, a very heavy loss due to the disease was experienced[5]. Clearly, some factor is operating in commercial conditions that is absent in laboratories.

The contention in the present account is that this factor is the parasites of fowls, especially the blood-sucking ectoparasites. The blood of leukaemic fowls may be infective at a dilution of one part in a million, and all subsequent workers agree with ROUS and colleagues[4] that the blood of sarcoma-bearing birds contains infective virus. It is therefore to be expected that blood-sucking parasites may transmit the condition, as such parasites must usually precede their meal by injecting a clot-preventing secretion into the host. In 1939, an infection of red mite at the Lister Institute of Preventive Medicine, London, spread ROUS sarcomas from the tumour-bearing birds to all normals in a few weeks. The new tumours mostly appeared on the inner aspect of the wing, and were all found to resemble Rous I and to contain a virus of this type. When the mites were cleared away, such unwished transmissions of the tumour also ceased, and were not encountered again. It was not thought necessary to publish these findings since JOHNSON[6] had reported the transmission of leukosis by red mite two years previously. Little attention has been paid subsequently to this work, however. Perhaps the finding that the closely-related virus condition, rabbit myxomatosis, is mainly spread in epizootic form as a mosquito-borne infection may serve to focus attention of the possibilities of parasitic transmission. Consideration of the field experiments also suggests that some such mechanism is essential to explain the manner in which the disease can spread to flocks with strict contact isolation. Only a flying or bird-carried vector could be responsible. In this connexion it is also interesting to note that the majority of "spontaneous" sarcomas appear to arise in the external musculature (4 out of the original 5 of ROUS, for example) and rather often on the wing like the mite-transmitted ROUS tumours. The blood cysts, which BLAKEMORE and INNES[1] regard as a related condition are also external rather than internal.

It might seem at first sight that this would not agree with the field reports that infection is mainly restricted to the first eight weeks of the chick's life (e.g. HUTT *et al.*[2]). This is, in fact, a consequence that can be predicted, for it is known that the Rous I virus in a 6-week old bird will induce sufficient antibody to cause neutralization when extracted from the infected cells into the tissue fluids in just over 40 days[3]. If this holds true for the other viruses, and making a slight extension to allow for the lesser antibody production of very young animals, the answer comes out about right.

Since modern insecticides give effective means for combatting the parasites, this mode of transmission should be controlled readily. Since the first eight weeks are the most critical, and in many parts of the world the chicks must be in closed rooms supplied with artificial heat for this period, the control need only be applied to the brooder rooms, making prevention both easier and cheaper.

This, however, was never regarded as sufficient to explain all the incidence and spread of the disease. The second mode of transmission of the complex is regarded as through the egg. This mode is recognized in the laboratory work[4] but has been denied as an effective contribution in the field. The evidence for and against this was summarized by COLE[5]. This valuable account at once shows that the apparent discrepancy does not exist. The evidence usually relates to the offspring of birds which died of leukosis (usually up to 500 days) versus those of the birds which survived up to this age. While the former birds were obviously carriers, it would not be correct to conclude that all survivors are not carriers. Those which would only die after a longer period are ignored. Furthermore, it is known that those fowls which recover from Rous I tumours remain carriers of this virus for life[6], the latent virus being liable to a

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sudden and fatal recrudescence of activity[1]. The blood of fowls bred for resistance to erythroleukosis is also infectious after the bird has resisted an inoculation (CARR, unpublished). Non-appearance of a fatal disease during a short period cannot therefore be regarded as proof that the bird does not carry the virus, especially if there has been some selection for resistance to the virus. Such resistant carriers may be a worse risk than the bird which dies of the infection, and thereby terminates its reproductive career.

Since the blood of carriers may be infective, it seems logical that any blood-spots produced after the yolk detaches from the ovary and which pass into the egg are an obvious risk. Though the larger spots are detected by candling, smaller ones of merely a few thousand cells will be missed. Some confirmation that these suggestions have validity comes from an extension of the work on the egg-transmitted neurolymphomatosis from ROUS carriers by GREENWOOD and CARR already described[2].

The Rous-resistant line had the highest frequency of blood-spots of any of the 8 lines comprising the Edinburgh flock. That blood-spot frequency is inherited was shown by SHARMA[3] and confirmed by LERNER *et al*[4]. When this resistant line was outcrossed and reselected for resistance, the frequency of blood-spots and of neurolymphomatosis in the offspring both decreased. Though elimination of blood-spots is very desirable in itself, there is, actually, a more effective control. It was recently shown by CARR[5] that the infectivity of ROUS tumours falls off with age of the host, and DURAN-REYNALS[6] indicated that after about 2 years of age the "spontaneous" sarcomas of fowls are less likely to contain infective material. Therefore, any egg-transmitted infections of these viruses should be primarily a disease of pullet-hatched birds rather than hen-hatched birds. This was at once found to be the case with the Centre flock. The pullet-egg breeding over the last 10 years was only about 10% of the total hatching, but was found responsible for 50% of the cases of fowl paralysis (in the restricted sense). A similar but less marked trend was found for the other forms of the leukosis complex. Owing to the very low spontaneous incidence, and the fact that many birds are kept to an advanced age, it was expected that some of the cases classified under these headings would be non-virus forms resembling leukosis, which are a rarity in commercial flocks. Fowl paralysis is probably almost always a virus-induced condition, so was chosen for analysis. As there is no reason to suppose that the non-virus neoplasms are related to age of parent, their inclusion will reduce the actual age effect. There was no correlation of incidence of leukosis with age of the *male* parent. The low spontaneous incidence and complex breeding programme of the Centre flock make an exact incidence of the relative risk of using pullets versus hens for breeding not easy to deduce, but it is certainly ten times greater, and may well be more.

Some confirmation of this can be seen in going through the literature, for many workers give figures indicating that the birds hatched from hen eggs are less affected than those from the same parents as pullets (e.g. JOHNSTON and WILSON[1]), but most of these experiments are complicated by lack of control of possible parasitic spread.

The investigations are obviously not complete as yet. It is possible that the mechanisms outlined for the transmission of the disease are not the only ones concerned in its spread. Since, however, the problems that this condition raises demand urgent attention, and the measures described here imply little more than good and careful poultry husbandry involving little outlay and no risk, it seemed desirable to publish the results for consideration by other workers in the subject.

All expenses in connexion with this work were borne by the British Empire Cancer Campaign.

Résumé

L'auteur propose des méthodes simples pour lutter contre les pertes énormes dues aux leucoses qui sévissent dans les élevages commerciaux de nombreux pays. Ces leucoses comprennent des leucémies, des sarcomes, des neurolymphomatoses et des ostéopétroses. Elles sont provoquées par un groupe de virus étroitement apparentés.

L'auteur étudie deux modes de propagation:

Le premier, intéressant surtout les poussins, s'effectuerait par l'intermédiaire d'arthropodes parasites. De bonnes conditions d'élevage et l'utilisation des insecticides modernes permettrait d'y remédier.

Le deuxième s'effectue par transmission du virus des poules infectées à leur descendance par l'intermédiaire de l'œuf. Comme le pouvoir infectueux du virus diminue avec l'âge de l'hôte, cette transmission se produit surtout lorsqu'on utilise pour les faire couver des œufs du premier cycle annuel de ponte et est très réduite si les œufs utilisés dans ce but proviennent de cycles plus tardifs.

Etant donné qu'entre les virus l'immunité croisée est incomplète et que le virus reste intracellulaire pendant l'évolution de la maladie, il y a peu de chances que la vaccination soit efficace. La sélection de souches résistantes ne peut pas non plus être complètement efficace, car la résistance croisée est seulement partielle, et aussi parce que les virus semblent être assez facilement capables de produire des mutations chez les hôtes résistants.

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