Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 21, *Number* 1, *March,* 1946.

Printed in the Union of South Africa by the Government Printer, Pretoria.

The Familial Incidence of Spontaneous *Osteopetrosis gallinarum.*

By J. D. W. A. COLES and J. J. BRONKHORST†, Section of Poultry, Onderstepoort.

OSTEOPETROSIS is a rare disease of the domestic fowl and we agree with the statement of Jungherr and Landauer that one case can be expected in about every two thousand birds sent for examination by farmers. This is all the more remarkable when it is realised how comparatively simple it is to detect the abnormality, for the long bones show the diaphyses increased in their overall diameter and the osseous tissue is very much like marble. The shanks are so often involved that the diagnosis becomes even easier. Some cases are revealed only when the hardness of the bones is noticed at table.

In 1932 Hutt found two of nine fowls from the same dam affected, and very properly considered that the condition might be a genetic recessive character. During the same year, Jungherr and Landauer found a case in the flocks kept at the Storrs Agricultural Experiment Station, and in 1933 there were 30 cases, and in 1934 the number rose to 40. From 1935 onwards the cases rapidly became fewer. One might have expected support to have been forthcoming for the belief of Hutt, but Jungherr and Landauer stated, " A careful analysis of the breeding records did not suggest either a familial or hereditary tendency in the occurrence of the condition ".

In 1938 the first case of osteopetrosis was diagnosed in the pedigreed flock of Single Comb White Leghorns at Onderstepoort. It was decided to pay particular attention to the disease and see if it would not tend to stigmatize certain families more than others. Of the fowls hatched in 1937, two developed osteopetrosis; the figure for 1938 was one, for 1939 five, for 1940 twenty-three, for 1941 six, and for 1942 nought. A peak was thus reached in 1940 resembling that observed by Jungherr and Landauer in 1934.

The most striking fact about the disease at Onderstepoort has been its frequent development in the members of certain families, and the main purpose of this article is to present evidence in support of Hutt's suggestion. It is believed that at least the *susceptibility* of the fowl to osteopetrosis is a recessive character, possibly a simple recessive character. In addition to this, it was concluded that the incidence of neurolymphomatosis, carcinosis and leucosis in birds with osteopetrosis is not significantly higher than in the flocks as a whole.

Review of the Veterinary Literature.

In 1924 Ball and Auger described the disease in a hen in France. The periosteum was firmly attached to the affected bones. The viscera were normal, except for a tubercle in the liver. From this article it would appear that Besnoit and Robin encountered the disease in 1922 in a cock, that was also tubercular.

Pugh studied what was apparently the same disease in England and submitted his findings in 1927. A poultry farmer examined 30,000 fowls of both sexes over a period of years and found 44 cases, all in cockerels. Cross-bred and pure-bred birds were equally susceptible. The fowls were raised on open range, and symptoms were usually first observed at the age of six to seven months. The long bones of the legs and wings often become enormously increased in diameter, without any definite signs of ill health appearing. Lameness or a stilted gait followed when the metatarsal bones became so enlarged that the flexor tendons could not function freely. In very severe cases, the cockerel could not put its digits down flat when standing on a smooth surface. The joints were neither enlarged nor tender, and could be flexed easily without pain. The lesions were usually, but not always, developed to the same degree on both sides of the body. The tibiae and metatarsi were generally involved, the bones being dense and heavy. The periosteum over the affected bone was invariably thickened and sometimes difficult to remove. The humerus, and then the ulna were the wingbones chiefly affected. The space between the radius and the ulna was sometimes obliterated. The scapulae and coracoids were frequently thickened and shortened. The clavicles were almost invariably smaller than normal. The cranial portion of the sternal crest might be thickened; the same was true of the ilium anterior to the acetabulum. The head, beak, vertebrae and digits were unchanged. The thyroids were occasionally enlarged. The testes were reduced in size by three-quarters. The joints and articular cartilages were never affected. Growth and sexual development were retarded. The disease sometimes became arrested spontaneously after several months and, after " recovery ", the cockerel could mature sexually and become fertile. Our hen H. 8330 is an excellent example of sexual maturity being deferred until the age of over eighteen months. Finally, Pugh found no evidence of leucaemia in seven cases that were very thoroughly examined. The most striking feature of Pugh's observations was the fact that only male birds were affected. It is now conceded that both sexes are equally liable to show symptoms.

In 1929 Veenendaal referred very briefly to the disease, but added nothing to the existing knowledge.

Carpentier in 1931 described the affliction in a cockerel of three months. The diagnosis was made after the bird had been cooked.

Bayon mentioned, in 1934, that he had encountered two unrelated cases as a result of performing 1,000 autopsies. One was a cross-bred cockerel. Both branches of the wish bone or furcula were very thickened in the one bird.

In 1935 Patay saw a few cases on a large poultry farm in France, and the usual bones were involved and general development was impaired. He asserted that the parathyroids were hypertrophied.

Brochet published two short articles in 1935 on the disease in a hen, and one was illustrated by good photographs. The tibia was affected mostly,

and the diaphysis was extremely thickened. The clavicle was smaller and thinner than usual. The head, beak, vertebrae, pelvis and nails were normal.

Osteopetrosis, complicated with spirochaetosis, was diagnosed in a White Leghorn hen and cock by Venkataraman in India in 1936. The two birds belonged to the same owner.

The above articles served to draw attention to this typical, if rare, disease of fowls, but threw no light on its causation. It was left to Jungherr and Landauer to publish the first experimental work. In 1935 Jungherr described the endemic at Storrs Agricultural Experiment Station. Various breeds and both sexes were involved, especially males of the Frizzle variety. The disease often manifested itself at the age of six weeks, and was chronic in nature and seldom fatal. It was characterised by the development of large irregular swellings of the leg and wing bones, which were hard. At autopsy, the internal organs were apparently normal and the osseous changes were limited to the long bones. Jungherr also noted what we have repeatedly observed—in the acute or florid stage, the temperature over the surface of the affected bone is perceptibly increased.

In transmission experiments with bacteriologically sterile blood or bone marrow from these florid cases, Jungherr produced four typical examples of osteopetrosis in twenty-two inoculated chicks. Eleven of the remaining eighteen chicks subsequently died or were killed in a debilitated condition and, at autopsy, two showed anaemia, two haemocytoblastomatosis, one lymphoid leucosis and four had gross lesions of neurolymphomatosis. These fiindings prompted Jungherr to regard osteopetrosis as another mainfestation of the leucosis complex.

In 1938, Jungherr and Landauer reported at length on their investigations and suggested the name, *Osteopetrosis gallinarum*, owing to the fact that the disease so closely resembled the condition in man. In 1933 they raised 2,496 fowls and nineteen Creeper, seven Rumpless and four Frizzle subjects were affected. In 1934 they raised 2,035 birds and one Creeper, eighteen Rumpless, eighteen Frizzle and three Cornish fowls showed symptoms. The poultry were fed a proper commercial ration and were kept under semi-range conditions in almost complete quarantine. Both males and females were affected, and although the earliest cases were recognizable at six weeks, some diseased birds lived up to the age of two to three years.

Jungherr and Landauer also diagnosed osteopetrosis in one White Leghorn, two Barred Rocks and two Rhode Island Reds sent for examination by farmers, thus helping to emphasize that all breeds are apparently susceptible.

The following abstracts are taken from the descriptions of the gross pathology of the disease given by Jungherr and Landauer:—

> " As the metatarsus, the unfeathered part of the leg, is almost invariably affected the disease is often recognised on clinical examination. In the beginning stages the metatarsus may show a definite convexity of the anterior surface, irregular lumps in the proximal metaphyseal region or thickening of the diaphysis; the affected areas exhibit an increased surface temperature, and are firm and insensitive to the touch. In distinction from perosis, the axis of the extremities remains unaffected. Cases of long standing fail to show the local hyperpyrexia, but are characterised by enormous deformities.

" The gross pathological alterations of the skeleton are fairly consistent in the parts affected, but vary in appearance according to the duration of the condition. The metatarsus, tibia, fibula, femur, humerus, ulna, radius, metacarpus, coracoid, clavicle and sternum may be involved, in a falling order of frequency. The ischium has shown suggestive changes in one instance, while the vertebrae, phalanges and skull bones failed to exhibit either gross or microscopic lesions. The affection is ordinarily bilateral. The integument of the affected bones appears normal; the periosteal cover is usually somewhat more easily detached than in healthy bone. The articular contours of the bones appear to be unaffected. The diaphysis and metaphysis show thickening in various degrees and either a smooth tapering surface or irregular rough excrescences with porous alterations. Even bones in the early stages of the disease offer more resistance to fracture than normal ones, although the hypertrophied areas can be incised to some degree.

" Differences between the florid and arrested stages of the disease are especially pronounced in cross sections. Young lesions are characterised by increased density and enormous hypertrophy of the spongiosa, at the expense of both the marrow cavity and the external outline. Old arrested lesions are extremely hard, and consist of the spongiosa and compacta and an eccentric narrowed medullary cavity. Transitional stages between these extremes are seen. Severe encroachment upon the marrow cavity is pronounced, especially in the diaphyseal region, and seems to lead to complete obliteration in certain areas; the remaining bone marrow of the long bones has often a currant-jelly-like hyperplastic appearance. Variations in the pathologic expression of the disease, progressing from the spongy state toward extensive petrification, can be seen in the same bird. Old lesions tend to occur in the distal, young lesions in the proximal leg bones, or in the wings, and thereby suggest, in line with the clinical course of the disease, the possibility of an ascending development of the affection. A secondary widening of the marrow cavity in the old solid bones may represent an attempt at re-establishing the physiologic balance of the haematopoietic tissues.

" Gross changes in other than the skeletal tissues may be entirely lacking; this holds true especially for birds sacrificed for examination during the initial stages of the disease, or for cases which are discovered on dressing for food purposes. Cases of fairly long standing and especially those which succumb to the malady are apt to show greyish enlargement of the parenchymatous organs, particularly the spleen. In the majority of cases the parathyroids appeared normal."

It was not until the injection of blood or bone marrow from clinically florid cases (with hyperpyretic shanks) was resorted to, that Jungherr and Landauer obtained evidence of transmissibility. The inoculum consisted of fresh, whole heparinized blood or a 10 per cent. suspension of bone marrow in Locke's solution. For preservation, these materials were desiccated over non-fuming concentrated sulphuric acid in a high vacuum, stored in the refrigerator and again suspended in Locke's solution before use. The inoculum never exceeded 0·5 c.c. For the experiments, they injected White Leghorn chicks, less than a week old, that were obtained from a commercial hatchery. Uninoculated controls of the same age were kept in separate batteries and none developed the condition. Transmission of osteopetrosis

was apparently successful with material from two donors from the endemic outbreak, and one strain was carried through four passages. The osseous changes were usually seen after an incubation period of three to five months. The worst bone lesions produced experimentally were never as severe as many found occurring naturally. Microscopically, the experimental cases exhibited well developed lesions, transitional between the florid and arrested stages and resembling in all particulars the pathognomonic alterations of spontaneous osteopetrosis.

The disease was produced when the inoculum was given intracardially, intraperitoneally, intravenously, intracranially and intramuscularly. The evidence pointed to an ultramicroscopic transmissible agent as the aetiologic factor, which could withstand desiccation for periods of up to 105 days. The agent appeared to be present in the blood, bone marrow and lymphomata found in some of the cases, but was absent from the diseased bone tissue itself. The aetiologic factor could be demonstrated only in active cases, which were characterised by the increased surface temperature already described.

Actually, Jungherr and Landauer injected 61 baby chicks in the course of four passages, and produced six gross lesion cases of osteopetrosis and six gross lesion cases associated with lymphomatosis and twenty-three cases of lymphomatosis. Thus, about 20 per cent. of the experimental chicks showed osseous changes, about 400 times the normal figure, and the results must accordingly be regarded as highly significant. It is interesting to note that no cases developed in the third passage, where the agent presumably remained dormant.

Jungherr and Landauer were impressed by the frequency with which lymphomatosis and neurolymphomatosis occurred in their injected chicks, and this matter will be returned to in due course.

Besides describing the symptoms, gross pathology and histopathology of osteopetrosis, Jungherr and Landauer made an outstanding contribution to the true appreciation of the nature of the disease, by demonstrating the significance of a transmissible agent. The fact that less than 20 per cent. of the injected chicks developed symptoms only tends to show that fowls generally possess a marked natural resistance to the disease. We believe that this natural resistance to the spontaneous, if not to the experimental disease, is an expression of an inherited character.

The Onderstepoort Flock of White Leghorns.

This flock is maintained with one aim in view; we desire to breed a strain of fowls highly resistant to neurolymphomatosis and the various neoplasms to which they are liable. Table 1 shows how the mortality from these causes has been reduced by five-sixths, but whether this achievement has been due entirely to a rigid selection of the breeding birds is at present impossible to say. We do not breed from females in their pullet year, and more and more do we insist on the males being in their second season. There are two reasons for this. Firstly, experience has shown that most birds die of malignant conditions and neurolymphomatosis before the age of twenty-one months, the time when they enter the breeding pens. Secondly, little can be known about the egg production of a hen and her sisters, if they are mated when about ten months old.

To gain a place in a breeding pen, a Leghorn hen must lay about 225 eggs in her pullet year, and the eggs must average 2 oz. and be of good shape and have firm shells. The hen must conform to the accepted standard of perfection for the breed, and side sprigs and stubs and crooked keels and spurs must be absent. Her body weight must be about four pounds. The eyes must be a rich bay colour. Broodiness is a disqualification. Early maturing birds, that lay throughout the winter and well into the following autumn, are particularly desired. Potential breeders must come from families where deaths are rare, especially from neoplastic diseases. Preference is given to moderately heavy layers from large families of all-round excellence, rather than to outstanding survivors of families where most have died of lymphoid leucosis, for instance. Cocks are chosen if their sisters have done well, and if they themselves are reasonably fine specimens of the breed. These exacting qualifications are indispensable, if the standard of the poultry is to be raised.

In assembling the breeding pens, it has been our custom to put closely related hens together.

These facts are mentioned for two reasons. Of the 643 hens and 55 cocks used in the breeding pens from 1937 to 1941 only one, a cock, had a brother or a sister that suffered from osteopetrosis, and this cock incidentally sired no cases. The brothers and sisters of cases simply failed to meet the requirements of breeding stock. Then all the sisters, except two, of males and females producing cases were themselves mated to cocks that sired cases. The significance of these two points, fortunate if unpremeditated, will be appreciated later, for we rely heavily on them in concluding that the susceptibility to osteopetrosis may possibly be inherited as a simple recessive.

The Onderstepoort Cases.

We have already described and indicated in Tables 2 and 3 how affected birds were found among those hatched from 1937 to 1941, with 1940 easily the peak years.

Table 4 gives full details of the diseased fowls, and various deductions may be made. Osteopetrosis seems to occur as readily in one sex as in the other, bearing in mind that fewer males than females are retained. Egg production, if any are laid at all, is very poor; but H.8327 was an exception and almost the same could be said of J.8877. G.741 laid during her pullet year but not during the second season, at which time the disease began to develop. H.8328 and H.8330 laid only during their second season; presumably the active stage of the disease had passed by then.

Visibly diseased hens, mated to a normal looking cock, can produce affected chicks, as well as others showing nothing unusual—refer to Table 5.

On one occasion we mated a cock and a hen, both of which were diseased. From the union we obtained six fertile eggs, but none hatched. Of course, we can deduce nothing from the fact that all the germs died.

It may be argued that osteopetrosis is due to an infection picked up after birth. It is our practice never to brood chicks together, that have been hatched on different dates, and if we look at the dates of birth of the affected progeny of cock 2899 and hen E.153, for instance, it is apparent that all chicks from all hens must be equally exposed to any infection, unless of course the infective agent is present in the egg. It is possible that the

transmissible agent discovered by Jungherr and Landauer is present in the chick at birth, but the evidence about to be presented shows that a susceptibility to the disease probably has to be inherited before symptoms can develop.

To the characteristics of osteopetrosis observed by others, we would add that the plumage is inclined to look rough, instead of lying smoothly on the body, and the sexual development is noticeably retarded. In common with others, we have found that the affected bones do not fracture easily. Indeed they are very hard, like ivory. This contrasts with the frequency with which fractures characterise the disease in man.

The Familial Incidence of the Disease.

In Tables 2 and 3, which should be studied along with Tables 4 and 5, we show how strikingly this very rare disease can affect individual families. No cognisance is taken in Table 2 of the results of the special matings made in 1941, because the affected progeny on that particular occasion were begotten of hens showing the disease. This special breeding pen will be discussed later. All the parent cocks and hens listed in Table 2 looked perfectly normal.

If susceptibility to osteopetrosis is a manifestation of a simple recessive rather than just a recessive character, and if the transmissible agent of Jungherr and Landauer was always present to take advantage of its opportunities, we would expect the cases enumerated in Table 2 to have numbered 43, i.e. 25 per cent. of 173. It is interesting to note that during the peak year of 1940 there were 23 cases out of 85 chicks retained, which is a remarkably good fit. This evidence is highly suggestive, but we are fortunately able indirectly to corroborate it. Cases could have resulted only from mating carriers, as birds with the disease were never put in our ordinary breeding pens. Carriers can result only from mating cases with normal birds or carriers (and this, of course, was never done) or from mating carriers with normals. Only one fowl out of 698 cocks and hens in the breeding pens had a brother or sister that showed the disease. So when numbers of breeding birds were selected at random from normal-looking fowls which were, with a single insignificant exception, not the brothers and sisters of cases and which presumably arose from the union of carriers and true normals, the breeding pens could be expected to house both carriers and normals representing the tainted families, in equal numbers; and this is what we found, for 16 hens mated to certain cocks gave rise to cases, while 17 sisters of these tainted or carrier cocks and hens, when mated to the same carrier cocks, produced only normal looking progeny. We have not taken into consideration the two sisters, which were mated to their brother, and produced only one chick each.

We realise that definitely to prove susceptibility to osteopetrosis to be a unifactorial recessive, it would be necessary to get only osteopetrotic chicks from the mating of two affected birds, and carriers and visibly diseased chicks in equal numbers from the union of a heterozygous normal with a clinical case. As mentioned elsewhere, we attempted the first type of mating but secured no progeny, for a reason so far undetermined. The second type of mating was also essayed (Table 5), but too few visibly diseased chicks were hatched. The second failure may have been due to insufficient exposure of inherently susceptible birds to the causal agent described by Jungherr and Landauer.

The above facts, supporting the idea of an inherited susceptibility to the disease determined by the genes, remain to challenge any contention that all our cases of this rare misfortune were due simply to an infection acquired in the egg from the mother, or picked up after hatching, such as in the case of bacillary white diarrhoea.

It is thus only reasonable to suppose that susceptibility is due to a recessive, if not a unifactorial recessive.

Direct Attempts to Prove Susceptibility a Simple Recessive Character.

In order to put our suspicions, that susceptibility might be a simple recessive character, to a final test, we assembled the breeding pen represented in Table 5. Cock 2899 was used because he had already sired 17 cases. Let us analyse the results. There was never any proof at any time that G.625, H.744, G.793 and H.8305 were not completely normal in their genetic make-up, and so their failure to produce cases was not surprising. E.903 was a known carrier, but left us with only two chicks, too small a number to allow inferences to be drawn; the same thoughts were entertained about H.8328 and H.8330.

So we are left with H.8327. This mating of a clinical case with a carrier should have given equal numbers of carriers and clinical cases. In other words, seven of the fifteen chicks might reasonably have had osteopetrosis, and yet we found only 2. But just as it is possible to find thirteen or fourteen boys in a family of fifteen, so it is possible to find less than the expected number of fowls with the genetic constitution of a flagrant case. Another feasible explanation, of course, is that the susceptible birds existed, but that the transmissible agent reached only 2.

This experiment solved nothing and the fact than 8 other hens mated to 2899 yielded no progeny only served to emphasize the difficulties attendant on work of this nature.

The second abortive attempt involving the mating of a diseased cock with an affected hen has already been mentioned.

Osteopetrosis in Man.

Jungherr and Landauer are the only investigators who have made a thorough study of the histopathology of *osteopetrosis gallinarum*, and they have claimed that the picture in the fowl is essentially that of the disease in man. So it will be profitable to discuss the condition in the human being, where it also passes under such names as marble bones, *osteosclerosis fragilis generalisata*, congenital osteosclerosis and Albers-Schönberg's disease.

The most conspicuous thing about osteopetrosis in man is its rarity. Not more than 150 cases have so far been recorded. Both sexes are equally prone to it. The disease may begin in early intra-uterine life and be fully developed at the time of birth. Pirie actually diagnosed the condition in a foetus by X-raying the mother. Osteopetrosis may be found at all ages; even a woman of seventy-two revealed the abnormality.

The disease is characterised essentially by increased thickness and density of the cortical and spongy portions of the entire osseous system. The base of the skull, the bodies of the vertebrae and the long bones are those

generally most affected. The dense compact bone encroaches on the medullary cavity, which sooner or later becomes almost entirely obliterated. These areas of sclerosis sometimes appear to be of a uniform density throughout, or they may show transverse lines of rarefaction. One suspects that a case examined very fully by Pirie was so characterised by rarefaction that he found the bones to be of the nature of chalk, and he suggested that the bones should be called chalky bones instead of marble bones. However, all other investigators have testified to the excessive weight, hardness and inelasticity of the bones.

Due to the frequency with which rarefaction occurs, even in very limited areas of the bone, the patients are particularly liable to fracture their limbs and hips as a result of slight injuries. Sometimes a fracture is unaccompanied by pain, and no one is aware of it until the bone is X-rayed. Most cases of osteopetrosis would never be suspected, especially in adults, were the fractured limbs not examined roentgenographically. As it is now customary to X-ray the relatives of cases, more and more instances of the disease are being brought to light.

Roentgenographically, the affected bones appear diffusely opaque and heavy and the finer markings are lacking. The shadow of the marrow cavity tends to disappear.

The disease is never confined to a single bone or to an isolated section of the body. It is widespread in its distribution, but often some bones such as the skull escape.

While the diaphysis of a long bone of the fowl is the region showing the severest changes, the site in man is by no means so fixed. A few illustrations may be given to make this clear. In McPeak's cases there was a definite increase in diameter of the femur, beginning at the juncture of the middle and lower thirds and extending to the supracondylar region, at which point the bone returned to its normal diameter. An increase in diameter was also noted in the lower end of the tibia.

Ghormley examined an eight years old boy and found the cortex of the ribs and the narrow cavity in places apparently obliterated. There was definite thickening of the cortex of all the long bones, more marked in the femora and humeri, and the thickening was greater in the proximal portion of each bone, than in the distal.

Ellis contends that the contour of the bone is not altered by the sclerosis, although clubbing of the posterior clinoid process and of the ends of the long bones, occurs sufficiently often to be regarded as the rule rather than the exception. Ellis thinks the clubbing is not directly due to the sclerosis, for in his two cases the main area of sclerosis occurred in the middle third of the shaft of the long bones and completely outside the area of clubbing. He believes that the site of sclerosis within the bone varies considerably in different cases, and that multiple cases in any one family tend to show a uniform set of lesions. He admits that several authors have insisted that the areas of sclerosis appear at the ends of the long bones. The radiological report on Ellis' two cases stated that the ends of the diaphyses of the long bones were greatly expanded with cortical thickening.

Many cases show a slight anaemia, usually of the hypochromic type. There is no conclusive evidence that the chemical compositon of affected bones deviates from the normal.

The picture presented so far applies mainly to what may be called a benign type of the disease, which is seen in most adults and many children. The people are to all intents and purposes normal, except for a tendency to break bones easily. In infants a far more dangerous and lethal form of the disease is often encountered. All the lesions occur that have so far been enumerated. In addition, there may be some retardation in the longitudinal growth of bone, and the general build may be stocky. The child may have a pigeon breast and a square forehead. Dentition is often arrested and the teeth tend to decay. Owing to the contour of the face, there is nearly always a purulent rhinitis. Sexual development is delayed. Severe damage to the base of the skull often leads to partial occlusion of the foramina through which the optic and other nerves emerge. Thus we find blindness due to optic atrophy, and nystagmus and deafness and facial palsies and sometimes hydrocephalus and not infrequently defective mentality. The prognosis in these severe cases is virtually hopeless.

Some investigators have found leucaemia, carcinosis and sarcomatosis associated with human osteopetrosis, but there is no suggestion that the disease is necessarily connected with these malignant conditions.

The cause of the disease in man is unknown, but the familial incidence of the condition has often been noted and a few examples will be given. Ghormley found lesions in a father and his son; the boy's mother was normal. Pirie's cases included a mother and her son and two daughters, and it was this boy who was examined roentgenographically while still in utero. McPeak found three generations involved—a grandmother, her two daughters and five children (2 boys and 3 girls) of one of these daughters. A sister of the five affected children was normal. A daughter and a son of the other affected daughter of the grandmother were also normal.

Clifton, Frank and Freeman's child was born of relatives who had married. They state that Harnapp found the disease in a father and five of his seven children.

Ellis' two cases were brothers and their parents were second cousins.

Higinbotham and Alexander found an adult family of two brothers and two sisters all affected, and they also showed prognathism and syndactylism.

Other workers' observations have been reviewed by McCune and Bradley. In Kudrjawtzewa's series of cases, both parents had been married before, the man having three normal children and the woman one. By their consanguineous marriage, these people had five children, two of whom died in infancy and three of whom had the disease. D'Istria had a similar experience. The man had three normal children by the first marriage. Then he married a cousin and three children died in infancy and one more was mentally defective and a fifth had osteopetrosis. Lorey and Reye diagnosed the disease in three sisters, and a cousin of these girls was found to be affected by Sick. Lauterburg saw the condition in two brothers. Cohn and Salinger found three children diseased in one family; Frank had the same experience with another.

All these observations are very impressive but may, nevertheless, have their value enhanced if we allude to the studies of Herzenberg and Lewit, who uncovered two pedigrees to indicate that marble bones are recessive in man. Some workers have suggested that the susceptibility to this very rare disease of human beings is a simple recessive character. This may or may

not be so. If it is so, we must assume that the grandmother and one of her daughters mentioned by McPeak, must both have married men, who were at least carriers. Such a coincidence is not impossible, even if it is improbable. Only extensive studies in the future can settle the point.

Discussion.

In this article we have discussed the spontaneous occurrence of *osteopetrosis gallinarum* in the Onderstepoort flock of White Leghorn fowls. Our motive in maintaining this flock is to attempt to breed a strain of fowls highly resistant to neoplastic conditions which, as we know, are responsible for such appalling economic losses in the poultry industry. We have described the criteria for the selection of breeding birds, and we have indicated the considerable degree of success already achieved.

Except for assembling the one breeding pen in 1941, we made no effort to increase the incidence of osteopetrosis. Rather did we prefer to see just what the ravages of the natural disease would be. We also made no attempt to search for the transmissible agent found by Jungherr and Landauer. It remains for future workers to correlate their findings with ours.

We think that we have unearthed very telling evidence in support of our belief that the susceptibility to *osteopetrosis gallinarum* is a recessive character, maybe a simple recessive one. No cases appeared in the birds hatched in 1942, but of those brought out in 1943, two, a brother and a sister, have already developed lesions, and this is additional support for our contention.

It is by no means improbable that the appearance of symptoms depends on an inherited susceptibility, together with the presence of a transmissible agent. Whether this agent is of exogenous or endogenous origin, matters little at this stage. Obviously the next step in the investigation of the disease is to see whether the dual cause hypothesis is correct. That Jungherr and Landauer could not produce lesions in more than 20 per cent. of their experimental chicks rather indicates that susceptibility is very much conditioned by one or more factors, possibly of a genetic nature.

Even if we concede that the histopathological picture is the same as that in osteopetrosis of man, we cannot be sure that the two diseases are identical in all essential respects. Both conditions are very rare. Both tend to affect a number of subjects in each family concerned. In the two diseases both males and females are susceptible and an affected parent may give rise to diseased progeny. Both are characterised by retarded sexual development. The affected bones are dense, heavy and hard, and lack elasticity and the marrow cavity is inclined to disappear. Diseased subjects of both species sometimes show anaemia.

A few differences are also well worth noting. The skull, vertebrae, and phalanges of the fowl do not seem to suffer, as they almost invariably do in man. No one has observed the increase in temperature over an affected human bone, such as Jungherr, Landauer and we have repeatedly witnessed in the case of the fowl. It is the diaphysis of the long bone of the hen that is so badly involved. From the descriptions of bones at our disposal, it is obvious that such a definite site for the development of the abnormality does not exist in the human being.

In man the contour of the diseased bone remains more or less unaltered, whereas in birds there are hard, rough, osseous excrescences above the

surface and very often the overall diameter of the bone is two to four times the normal. Fowls show no clubbing at the ends of the long bones.

Nobody has seen a fractured bone in an affected fowl, but this may be due to the fact that birds do not fall as heavily as people.

On the whole, the points in common seem to outweigh those that are dissimilar and, for the time being at least, we should join with Jungherr and Landauer in thinking that osteopetrosis in the fowl is probably the same as marble bones in man.

Summary.

Thirty-nine spontaneous cases of osteopetrosis have been studied in the Onderstepoort experimental flock of fowls, and the disease has shown a striking tendency to affect some families more than others. Evidence has been advanced indicating that a susceptibility to the disease depends on a recessive character. There is also some indirect evidence that susceptibility depends on a unifactorial recessive.

Malignant conditions, such as leucosis and carcinosis, were not found more frequently in fowls with osteopetrosis than in the flock as a whole.

Families stigmatized with osteopetrosis almost never provide birds worthy of inclusion in a high class breeding pen. We have stated the grounds on which a hen should be admitted to a good breeding pen, and we have indicated what success has crowned our efforts to evolve a strain of fowls resistant to neoplastic conditions, while being also highly desirable in all other respects.

The literature dealing with osteopetrosis in fowls and man has been reviewed and the points of similarity and dissimilarity have been discussed.

Acknowledgments.

We wish to thank Mr. T. Meyer for preparing the photographs. We are also indebted to Mr. C. H. Driessen and Miss M. Bellingan for caring for the fowls and keeping the records, respectively, and for their sustained interest in this work.

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Fig. 1.—White Leghorn cockerel with osteopetrosis.

Fig 2.

Fig 3.

Fig. 2.—Leg of fowl showing how the bones are thickened.
Fig. 3.—White Leghorn pullet with osteopetrosis.

TABLE 1.

Summary of Cases of Osteopetrosis, Neurolymphomatosis, Leucosis, Carcinosis and other Malignant Disease Diagnosed in the Onderstepoort Flock of Pedigreed White Leghorns from 1937 to 1942.

Year Hatched.	Autopsies.	Cocks in Breeding Pens.	Hens in Breeding Pens.	Osteopetrosis.	Osteopetrosis plus Neurolymphomatosis.	Osteopetrosis plus other malignant conditions.	Neurolymphomatosis	Neurolymphomatosis plus Leucaemia.	Other malignant conditions.	Total Neurolymphomatosis plus other malignant conditions.
1937. D fowls.....	431	8	95	1	—	1	36 8·4%	19 4·4%	129 29·9%	185 42·9%
1938. E fowls.....	755	10	109	1	—	—	42 5·6%	16 2·1%	253 33·5%	311 41·2%
1939. G fowls.....	931	10	135	2	—	3	57 6·1%	22 2·4%	289 31·0%	371 39·8%
1940. H fowls.....	1,119	15	160	14	2	7	21 1·9%	6 0·5%	203 18·1%	239 21·4%
1941. J fowls.....	777	15	144	6	—	—	3 0·4%	1 0·1%	80 10·3%	84 10·8%
1942. K fowls.....	418	9	76	—	—	—	6 1·4%	—	26 6·2%	32 7·7%

Actually only 55 cocks were used from 1937 to 1941. Three cocks served for two years. During the same period 643 hens were mated.

There are still 81 J and 171 K fowls alive and, as they are all worthy of inclusion in the breeding pens, the figures of 10·8 per cent. and 7·7 per cent. given for " Total neurolymphomatosis plus other malignant conditions " will ultimately be improved upon.

On birds hatched from 1937 to 1941, 4,013 autopsies have been performed and 29·7 per cent. of these showed neurolymphomatosis and/or some other malignant condition.

Of 37 cases of osteopetrosis, 13 or 35·1 per cent. showed associated neurolymphomatosis, carcinosis, etc.

Thus, there is no significant increase of leucosis, etc. in fowls suffering from osteopetrosis.

TABLE 2.

Matings that Produced Osteopetrosis gallinarum.

Year.	Cock.	Hen.	Cases.	Chicks retained over age of two months.
1937	B. 536	A. 237	1	16
		B. 416	1	12
1938	520	B. 462	1	10
1939	42	E. 96	1	3
1939	317	E. 119	1	16
		E. 400	1	14
		E. 919	2	4
1940	2572	E. 223	3	12
1940	2788	E. 903	1	12
		E. 985	2	14
1940	2899	E. 69	4	9
		E. 153	7	18
		E. 268	4	14
		E. 406	1	4
		E. 440	1	2
1941	307	G. 959	3	13
	8	16	34	173

Cock 2788 and G. 959 were brother and sister.

Hens E. 69, E. 268 and E. 440 were full sisters.

Nineteen full sisters of hens and cocks producing the disease were mated to the above eight males without revealing cases in their progeny, but only one chick was retained from each of two of these nineteen, and so we have reliable evidence concerning the progeny of only seventeen.

TABLE 3.

Familial Incidence of Osteopetrosis in 1940, the Year of its Greatest Frequency.

Sire.	Dam.	Type of Offspring.	Progency Retained over Age of 2 Months.		
			No.	Osteopetrosis.	
				No.	Per cent.
2560 Resistant Progeny	10 Hens	Resistant	100	0	0
2581 Resistant Progeny	10 Hens	Resistant	130	0	0
2596 Resistant Progeny	7 Hens	Resistant	54	0	0
2626 Resistant Progeny	14 Hens	Resistant	98	0	0
2671 Resistant Progeny	10 Hens	Resistant	95	0	0
2683 Resistant Progeny	12 Hens	Resistant	138	0	0
2685 Resistant Progeny	10 Hens	Resistant	92	0	0
2734 Resistant Progeny	8 Hens	Resistant	60	0	0
2812 Resistant Progeny	7 Hens	Resistant	13	0	0
2829 Resistant Progeny	7 Hens	Resistant	85	0	0
3102 Resistant Progeny	9 Hens	Resistant	64	0	0
3139 Resistant Progeny	9 Hens	Resistant	69	0	0
J 5637 Resistant Progeny	15 Hens	Resistant	156	0	0
2572 Susceptible Progeny	8 Hens	Resistant	55	0	0
	E 223	Susceptible	12	3	25
2788 Susceptible Progeny	10 Hens	Resistant	61	0	0
	E 903	Susceptible	12	1	8·3
	E 985	Susceptible	14	2	14·3
2899 Susceptible Progeny	6 Hens	Resistant	72	0	0
	E 69	Susceptible	9	4	44·4
	E 153	Susceptible	18	7	38·9
	E 268	Susceptible	14	4	28·6
	E 406	Susceptible	4	1	25
	E 440	Susceptible	2	1	50

Total chicks from susceptible hens = 85.

Total cases from susceptible hens = 23.

The above table emphasizes how susceptible families are to osteopetrosis gallinarum, while the great majority of families seem to be resistant.

TABLE 4.

Detailed Summary of the Onderstepoort Cases of Osteopetrosis gallinarum.

Fowl.	Sex.	Date Hatched.	Sire.	Dam.	Eggs Laid.	Age at Disposal in Days.	Remarks.
D 734	Female	7/9/37	B 536	A 237	36	327	Osteopetrosis. Lymphoid leucosis of liver, spleen and lungs.
671	Male	25/8/37	B 536	B 416	—	?	Osteopetrosis.
349	Male	25/8/38	520	B 462	—	?	Osteopetrosis.
3955	Male	22/9/39	42	E 96	—	230	Osteopetrosis. Killed.
G 746	Female	18/8/39	317	E 119	32	298	Mild osteopetrosis. Erythroleucosis.
G 741	Female	1/9/39	317	E 400	76	869	Osteopetrosis. Cystic vestigial remains of the right Mullerian duct. Laid only during pullet year. Disease first diagnosed at age of 818 days. This was the oldest case diagnosed.
2786	Female	1/9/39	317	E 919	0	140	Osteopetrosis. Lymphocytoma of the gizzard.
G 141	Female	1/9/39	317	E 919	9	302	Osteopetrosis. Lymphoid leucosis of liver. Embryonal nephroma of kidney.
92	Female	30/8/40	2572	E 223	0	369	Osteopetrosis. Lymphocytoma in abdomen on lateral surface of cloaca. Lymphocytomatosis of myocardium.
H 651	Female	13/9/40	2572	E 223	6	495	Slight osteopetrosis. Killed.
H 8327	Female	30/8/40	2572	E 223	217	575	Moderate osteopetrosis. Lymphoid leucosis of liver and spleen. Haemorrhage into an ovum. Aerocystitis of one abdominal air sac. Mother of the cases Male 3771 and Male 4118.
1668	Female	20/9/40	2788	E 903	0	263	Mild osteopetrosis. Lymphoid leucosis of liver and kidneys.
53	Male	23/8/40	2788	E 985	—	141	Osteopetrosis. Bullied to death by other fowls.
1255	Female	13/9/40	2788	E 985	0	218	Osteopetrosis.
1463	Female	20/9/40	2899	E 69	0	176	Osteopetrosis. Sexually retarded. Killed.
1464	Female	20/9/40	2899	E 69	0	187	Osteopetrosis. Carcinoma of ovary. Killed.
4842	Female	23/8/40	2899	E 69	0	204	Osteopetrosis. Sexually retarded. Killed.
4844	Male	23/8/40	2899	E 69	—	48	Osteopetrosis. Killed. This was the youngest case.
625	Female	6/9/40	2899	E 153	0	201	Osteopetrosis. Killed.

TABLE 4.—(*continued*).

Fowl.	Sex.	Date Hatched.	Sire.	Dam.	Eggs Laid.	Age at Disposal in Days.	Remarks.
1055	Female	13/9/40	2899	E 153	0	181	Osteopetrosis. Chronic cardiac dilatation. Cirrhosis and venous stasis of liver. Deposit of fibrin on surface of liver.
H 8310	Female	20/9/40	2899	E 153	0	434	Osteopetrosis.
H 8328	Female	20/9/40	2899	E 153	18	484	Osteopetrosis. Moderate anaemia. No eggs were laid in the pullet year.
H 8329	Female	16/8/40	2899	E 153	0	359	Marked osteopetrosis. Chronic salpingitis. Lymphocytomata of ovary and peritoneum and of skin at the side of the tail.
H 8330	Female	30/8/40	2899	E 153	61	496	Osteopetrosis. Myeloid leucosis. No eggs laid in pullet year. Mother of Male 3354.
4846	Female	23/8/40	2899	E 153	0	204	Osteopetrosis. Sexually retarded. Killed.
1476	Female	20/9/40	2899	E 268	0	187	Osteopetrosis. Neurolymphomatosis of the right brachial and right sciatic nerves.
H 8294	Female	6/9/40	2899	E 268	0	489	Osteopetrosis and neurolymphomatosis.
H 8297	Female	30/8/40	2899	E 268	0	281	Mild osteopetrosis. Multiple haemangiogenous endotheliomata of the skin, left kidney, lungs and liver. Chondromata of the skin and subcutis.
H 8336	Female	20/9/40	2899	E 268	0	310	Mild osteopetrosis.
646	Female	6/9/40	2899	E 406	0	225	Osteopetrosis. Killed.
1500	Female	20/9/40	2899	E 440	0	225	Osteopetrosis. Sexually retarded. Hepatic cirrhosis with cardiac dilatation, hydropericardium and pulmonary oedema and intestinal catarrh.
3354	Male	18/8/41	2899	H 8330	—	162	Very severe osteopetrosis. Mother had the disease.
3771	Male	25/8/41	2899	H 8327	—	317	Marked osteopetrosis. Killed. Mother had the disease.
4118	Male	1/9/41	2899	H 8327	—	199	Slight osteopetrosis. Disease more developed in left leg. Septic iridocyclitis of right eye. Killed. Mother had the condition.
4466	Female	8/9/41	307	G 959	0	181	Osteopetrosis. Killed.
J 8877	Female	15/9/41	307	G 959	140	449	Slight osteopetrosis.
J 9080	Female	15/9/41	307	G 959	64	449	Slight osteopetrosis. Killed.

TABLE 5.

Special 1941 *Mating—Cock* 2899.

Hen.	Remarks.	Chicks Retained.	Pullets Leg-banded.	Cases.
G 625	Sister of cock 2899	12	5	0
H 744	Sister of cases 625, 1055, H 8310, H 8328, H. 8329 H 8330 and 4846	7	3	0
G 793	Sister of cock 2899	5	4	0
E 903	Had produced a case when mated to cock 2788	2	0	0
H 8305	Sister of cases 625, 1055, H 8310, H 8328, H 8329, H 8330 and 4846	14	9	0
H 8327	Had osteopetrosis	15	9	2
H 8328	Had osteopetrosis. Daughter of cock 2899	1	0	0
H 8330	Had osteopetrosis. Daughter of cock 2899	1	0	1

Eight other hens were also mated, but either laid no eggs or gave no progeny.