Epizootic Reticulum Cell Sarcoma in a Sequestered Colony of Japanese Quails

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SUMMARY

A sudden appearance of a malignant lymphoid tumor, which we characterized as reticulum cell sarcoma, was observed in a sequestered colony of Auburn strain 571 Japanese quails on the campus of the University of Hawaii, Honolulu. Tumor infiltrations were present in thymuses, spleens, livers, and, less commonly, in the caeca and bursa of Fabricius. In the advanced stages, other organs, such as kidneys, lungs, and gonads, were similarly involved.

Electron microscopy revealed virion-like, double-walled, spherical bodies measuring approximately 1200 Å in the nucleoplasm of tumor and hepatic cells. The significance of these bodies remains speculative.

INTRODUCTION

Spontaneous avian leukosis and other related hemopoietic tumors, although not uncommon in domestic fowls, are infrequently encountered in the Japanese quail, Coturnix coturnix japónica. In 1963, Wight (8) reported an outbreak of 5 cases of spontaneous avian leukemia in his C. coturnix japónica flock maintained at the Poultry Research Centre in Edinburgh. Lesions conforming closely to Wight's original description suddenly appeared in quails in Honolulu in the early winter of 1967. Tumors developed in a sequestered colony of Japanese quails, Auburn strain 571 (4), which was housed on the Manoa Campus of the University of Hawaii, Honolulu. The 1st tumor-bearing quail in this group exhibited several nodular masses in the neck. In subsequent months, over 60% of the 47 quails in the closed colony developed the tumor (Table 1, Chart 1). This was in striking contrast to the stock colony of several hundred Auburn strain 571 quails maintained earlier on the University's poultry farm, located 40 miles northwest of the city. The farm quails remained free of avian leukemia during this year and during prior years of observation.

A paucity of information exists in the literature with reference to spontaneous leukemia involving this species. The etiological agent responsible for this specific quail tumor has not been identified; nevertheless, some evidence of transmissibility of an oncogenic agent from Auburn strain 571 to another designated Auburn strain 661 was recently observed in our laboratory and will be the subject of a separate report. The purpose of this communication, therefore, is to describe the epizootic nature and the pathological features of the tumor.

MATERIALS AND METHODS

The Japanese Quail. The genus Coturnix is the Old World quail whose prevalence has been recorded in Europe, Asia, Africa, and Australia. Of this genus, 14 races have been recognized by Peters (5), and the quails used in this investigation fall into 1 of the 2 major races, viz., Coturnix coturnix coturnix (the European) and Coturnix coturnix japónica (the Japanese). Auburn strain 571 of the Japanese quail was developed in 1957 by Dr. Robert Howes of the University of Auburn and was introduced to the University of Hawaii Poultry Farm in 1963. Auburn strain 661 was developed in 1966 again by Dr. Howes, and the quails from this strain were brought to Hawaii late in 1967 to replace the earlier stock of Auburn 571. This change was made for reasons of "greater genetic uniformity" in strain 661, but no striking difference was apparent in the morphological characteristics of the 2 strains. The replacement with Auburn 661 took place shortly after a small colony of strain 571 was separated, as reported in the present study, and no additional birds of the latter strain have been maintained in Hawaii since that time.

After hatching on the University's poultry farm late in March 1967, a colony of 47 Auburn strain 571 quail of both sexes was randomly segregated and transferred for brooding on the same day to a temporary wooden bungalow of the Poultry Science Division on the Manoa Campus. After 5 weeks, the quails were taken to the main animal quarters of the campus, where they were separated by sex into groups of 6 to 10 birds, living in close proximity to each other in metal cages. Other fowls (chickens and pheasants), as well as some mice, were housed in the same area. The quails were allowed free access to water and fed Trip-L-Duty bird feed (Alber's Milling Co., Los Angeles, Calif.). Overhead fluorescent lights were left on only during regular working hours, and no special lighting arrangements were provided.
Unfortunately, strain 571 quails were no longer available at the University farm by the time the investigation of tumor-bearing birds was begun on the Manoa Campus. Since the possibility existed that the newly acquired strain 661 quails may have been exposed to identical conditions of breeding and care on the farm, the untreated controls of 91 adult stock quails of this new strain were randomized and sacrificed for pathological examination.

Pathological Examination. Autopsies were performed on all control and experimental animals that died spontaneously, as well as on those sacrificed. Tissues were fixed in Carnoy's or Zenker's fixative and processed for routine histological examination. Hematoxylin and eosin stains were used throughout. Gridley stain (2) was used for the demonstration of reticulum fibers.

Fixation of Tissues for Electron Microscopy. Minute samples of tissue from the liver, spleen, and thymus were fixed in 1% osmium tetroxide and embedded in Epon 812. Sections were prepared with an MT-2 Porter Blum ultramicrotome for survey of the ultrastructural features of the involved reticular and hepatic cells. Electron microscopic examination was carried out on a Philips EM 300.

RESULTS

Epizootic Reticulum Cell Sarcoma in a Segregated Colony of C. coturnix japonica, Auburn Strain 571. The onset of tumor epizooty in 47 sequestered quails was observed early in February 1967, when 1 of the quails exhibited several large nodules in the neck (Fig. 1) at approximately 10 months of age. This bird was first bled from the jugular vein and then sacrificed for pathological examination. A few weeks later, 3 or 4 birds of this colony were found to be listless and unusually warm to touch. Several were similarly sacrificed for pathological examination during the subsequent months, as shown in Chart 1. Although spontaneous deaths were recorded, accurate mortality figures could not be ascertained from this study, since some quails were sacrificed for the purpose of studying the transmissibility and transplantability of the neoplasms from freshly sacrificed donor quails to recipients of strain 661. The details of that study will be reported elsewhere.

After 22 months, the spontaneous occurrence of tumor ended abruptly (Chart 1). Eight of the original birds are still alive and apparently healthy at the time of this writing. The incidence of the spontaneous tumor development (Table 1) for the 22-month period was 30 out of 47 or 63.8% of the total (3 birds are not included in the data shown in Chart 1, since the exact date of death was not recorded).

Pathological Observations. Gross pathological findings were similar in all animals with the tumor. In some instances, a marked thymic prominence was noted, as in the 1st quail (Figs. 1 and 2), while in others only a slight to moderate enlargement was observed. The spleen was definitely enlarged in every instance (Fig. 3), while the bursa of Fabricius was infiltrated with tumor cells in approximately two-thirds of the involved birds. The liver showed frequent evidence of small, focal, grayish-tan infiltrations, giving a mottled appearance to the normally homogenously dark red organ. The duodenum and caeca were common sites of tumor involvement, which gave a thickened and edematous appearance to their walls (Fig. 4). Tumor infiltration of the peripheral or central nervous system was not observed. Osseous tissues and eyes were not infiltrated by the tumor cells in any of the animals examined.

The histological pattern of the tumor was of monotonous and homogeneous appearance. The typical tumor cell consisted of a clearly defined cell border with prominent eosinophilic or amphophilic cytoplasm and a hyperchromatic...
nucleus. The nucleolus was centrally located and very often appeared as an enlarged eosinophilic mass which resembled an inclusion body (Fig. 5). Mitotic figures were commonly observed, but tumor giant cells were not present in any of the sections. There was no necrosis or fibrosis of the tumor and special stain revealed only slight to no apparent elaboration of reticulin fibers by the tumor cells. The reticulin fibers were derived chiefly from the vascular adventitia.

The liver was heavily infiltrated by tumor cells in the advanced stages of the disease. The hepatic lobules and the portal triads were equally involved, usually as focal islands of infiltrating tumor cells, but in rare instances the entire liver was diffusely permeated by the neoplastic reticular cells. Blood vessel lumens and sinusoidal spaces were commonly invaded by the infiltrating tumor cells (Fig. 6).

The thymus (Fig. 7), bone marrow, and spleen were usually extensively disrupted by tumor cells. The marked splenomegaly observed in affected quails was due to the uniform infiltration and displacement of splenic tissues by the neoplastic reticulum cells. The bursa of Fabricius was often infiltrated, but not infrequently it was seen to be free of tumor cells in quails which displayed the evidence of the neoplasm. The neoplastic cells were rarely evident in the lungs and kidneys.

Although Wight (8) described an abscessed tumor in 1 of the 5 quails of his series, no evidence of inflammation accompanied the tumor in any of ours.

**Pathological Observations in Untreated Auburn Strain 661 Quail (Stock Animals).** No gross or histological evidence of reticulum cell sarcoma was observed in 89 of the 91 quails of Auburn strain 661 which were maintained at the University farm. Two quails disclosed small, gray, focal infiltrations of the liver. These lesions were shown to be lymphocytic infiltrates and differed significantly from the neoplastic cells described above for strain 571 quails. The thymus, spleen, and bursa of Fabricius were not involved in these quails.

**Electron Microscopy of Tumor-bearing Liver and Splenic Tissues.** The electron micrographs of the liver, spleen, and tumor tissues of affected quails frequently demonstrated electron-dense spherical bodies which were apparent in an occasional nucleus of the tumor cells as well as in parenchymal cells of the liver. The sections of the affected livers disclosed tumor cells in the perisinusoidal spaces which impinged upon the cell membrane of hepatic cells. Intracytoplasmic organelles of hepatic cells did not appear to be significantly altered, except for the presence of infrequently dilated cisternae of endoplasmic reticulum. Regenerating activity of the liver was evidenced by double nuclei in the liver cells (Fig. 8). Intranuclear spherical bodies were observed in both hepatic and tumor cells (Figs. 8 and 9). These spherical bodies were estimated to be about 1200 Å in diameter and showed a hollow center surrounded by a fine concentric and somewhat fibrillar double wall. A small electron-dense dot was usually present, eccentrically positioned in the hollow centers of the spherules. The dots measured about 200 Å in diameter (Fig. 9). The ultrastructural appearance of the nucleolus, nuclear chromatin, and nuclear envelope did not otherwise appear unusual. No evidence of such spherical bodies was found in the cytoplasm of the tumor and liver parenchymal cells, nor were such bodies visible in the cytoplasmic membranes and the interstitial spaces.

The regularity of the size and the morphological structures of these intranuclear spherical bodies seem to suggest a virus-like agent. While no evidence of intranuclear spherical bodies was encountered in the liver of a few healthy strain 661 quails, the significance of the intranuclear spherical bodies remains speculative, since the cause and effect relationship of these particles to tumor development was not determined by this study.

The cytoplasm of the neoplastic reticular cells was largely devoid of organelles and appeared as a faintly stippled homogeneous structure with only a few mitochondria and endoplasmic reticulum. The nuclear ultrastructure, aside from the spherical bodies, was unremarkable.

**DISCUSSION**

An explosive outbreak of a spontaneous reticulum cell tumor in a closed colony of Japanese quails is an unusual occurrence. It is, therefore, of some interest that Bigland et al. (1) reported an incidence of 21.6% of leukemia among 403 Coturnix quail which they examined at random. The authors, however, presented no histopathological evidence to support their observation. In our own study, 91 randomized control quails of Auburn strain 661 at the University's poultry farm were sacrificed and autopsied for histological evidence of reticulum cell sarcoma, but none was observed. Among these controls, 2 birds revealed microscopic foci of lymphocytic infiltrations in the liver, but they did not in any way resemble the tumor found in Auburn strain 571 quails housed in the animal quarters of the Manoa campus. The data presented by Bigland et al. (1) are inadequately documented to support the belief that there exists such an unusually high incidence of spontaneous avian leukemia in a random population of captive Japanese quails. It is possible, but rather unlikely, that they too may have observed an epizooty of quail leukemia. In contrast to that report, the few cases described by Wight (8) were carefully documented and, while one of his quails had abscesses in addition to the tumor, the description of the neoplasm fits the pathological findings observed in the tumor-bearing quails noted in the present study.

The neoplastic cells with a prominent and well-defined cytoplasm and hyperchromatic nuclei containing a large central nucleolus were found in great profusion in the spleen, thymus, liver, caeca, duodenum; often in the bursa of Fabricius; and occasionally in lungs and kidneys. The tumor cells appeared to have arisen from the primitive reticular cells in multifocal sites such as thymus, spleen, lamina propria of the gut, and frequently, but not always, in the bursa of Fabricius. With the progression of the tumor growth, the lymphoid cells were extensively displaced in these tissues and usually obliterated the architecture of the organ. Complete replacement of tissues by the neoplastic reticular cells gave a monotonous, homogeneous appearance which was quite characteristic for the tumor. Special stain disclosed only a slight production of reticulin fibers by these...
cells, and when present in abundance the reticulin was closely related to the vascular adventitia.

The sudden appearance of the tumor in our isolated quails, followed by a progressive and mounting rise in its incidence, suggests that an infectious etiological agent may have been involved. Although the tumor was first observed at 9 months of age, it is not possible to determine the length of the latent period if one were to postulate an infectious etiology. Assuming that a few quails were exposed initially and that the infection progressed “horizontally” from bird to bird, it appears that the latent period may have been moderately long, lasting perhaps several weeks to months. The peak of the tumor incidence occurred at 18 months (Chart 1), which is about 9 months after the first tumor was discovered. The epizooty ended abruptly, and at the time of this writing 8 Auburn strain 571 quails from this colony are still alive and healthy. The tumor has not recurred in the stock colony of Auburn strain 661 at the University poultry farm.

In contrast to our observation in the Japanese quail, the involvement of bursa of Fabricius occurs with regularity and constancy in visceral lymphomatosis of chickens (6). Moreover, Peterson et al. (6) have established that bursectomy in chickens do not develop the disease, while bursectomy performed on birds with established lymphomatosis will result in a remission. In this regard, the dependence of the disease process on the bursa of Fabricius differs significantly in these 2 species. Lymphomatosis of chickens also differs morphologically from the epizootic reticulum cell sarcoma of Japanese quails, and the distributions of the lesions in the viscera do not parallel each other, as already noted. These differences suggest that chicken lymphomatosis is probably not a clinical entity equivalent to the epizootic reticulum cell sarcoma in Japanese quails.

Theilen et al. (7) reported that RE virus (strain T), originally derived from a turkey, induces a reticuloendotheliosis in Japanese quails. Their description of the pathological findings conforms closely with the tumor herein reported. Zeigel et al. (9) described the electron microscopic appearance of the RE virus particles in reticular, endothelial, and connective tissue cells of the infected quails. In their opinion, however, the morphological characteristics of the RE virus were different from other known oncogenic avian viruses, and the agent resembled the RNA-rich, cytoplasmically derived myxoviruses. Nazerian and Burmester (3), but the electron-dense nucleoid in the agent of Marek’s disease differed from that in the spherical bodies observed in the present study. Instead of a nucleoid, a small, eccentrically placed electron-dense dot was seen in the hollow center of the double-walled bodies. It remains highly speculative that these intranuclear virion-like spherical bodies in the epizootic reticulum cell sarcoma are etiologically associated with the tumor.

In the course of this investigation, freshly minced tumor tissues were inoculated into recipients of Auburn strain 661 in an effort to propagate the reticulum cell sarcoma. The study describing the transmissibility and serological tests to determine the possible involvement of several established oncogenic viral agents will be the subject of a separate report.

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REFERENCES

Epizootic Reticulum Cell Sarcoma

Fig. 1. The external appearance of enlarged thymuses.
Fig. 2. Enlarged thymuses exposed.
Fig. 3. The spleen on the left is from a normal adult quail; the one on the right is infiltrated with tumor cells.
Fig. 4. The duodenum is infiltrated with pale gray tissue. The dark center in the infiltrating mass represents a focus of hemorrhage. The gut segment is also diffusely thickened and edematous.
Fig. 5. The histological appearance of anaplastic reticular cells. Note the homogenous pattern with a slight variation in the size and shape of the neoplastic cells. Large, intranuclear inclusion-like bodies are present in many of the cells. X 550.
Fig. 6. Liver. Focal infiltrations of tumor cells are present. Note the invasion of portal vein. X 125.
Fig. 7. A diffuse proliferation of reticulum cells is observed in the thymus. Note Hassel’s corpuscles and the obliteration of corticomedullary demarcation. X 125.
Fig. 8. Electron micrograph of a liver cell containing 2 nuclei. Arrows, intranuclear spherical bodies. Note their double walls and the eccentric, nucleoid-like dots within the spherical bodies. X 23,000.
Fig. 9. Electron micrograph of tumor cells. Arrow, intranuclear spherical body. X 9000.
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