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Myocarditis in Round Heart Disease of Turkeys

A Light and Electron Microscopic Study*

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Summary. Round heart disease in the turkey has been studied by light and electron microscopy. Myocarditis, characterized by focal muscle cell degeneration and mononuclear infiltrates, was present in turkeys from 1 day after hatching through 8 weeks of age. Myocardial injury was most severe from age 5 to 12 days. Virus-like particles, 60–90 mμ in diameter, resembling the avian leukosis viruses, were present in the myocardial cells of all turkeys with myocarditis.

Dilatation and hypertrophy, predominantly of the left ventricle developed after the second week of life. Collagen accumulation beneath the endocardium was evident by electron microscopy in 1- and 2-day-old birds. This progressed to marked endocardial fibroelastosis after 1 month and was often associated with involvement of the mitral valve and its papillary muscles and chordae tendineae. Maturing and adult turkeys demonstrated congestive heart failure with congestion of the lungs and liver, pleural effusions and ascites. Because of the similarity of gross and microscopic findings, round heart disease in turkeys may serve as an experimental model for primary endocardial fibroelastosis in humans.

Introduction

Round heart disease, a cause of stunting and death in the avian species, was first described by Blaxland in 1947. This disease has been diagnosed solely by the postmortem observation of an enlarged round heart. In the turkey, round heart disease presents a spectrum of left ventricular dilatation and hypertrophy associated with endocardial fibroelastosis (Magwood and Bray, 1962; Sautter *et al.*, 1968; Noren *et al.*, 1970). The pathology of round heart disease is similar to idiopathic endocardial fibroelastosis in the human (Hill and Reilly, 1951; Case, 1960). This study was undertaken to describe the natural history including clinical spectrum, and pathology of round heart disease and to relate its features to endocardial fibroelastosis in the human.

Materials and Methods

A flock of small white broad-breasted turkeys with a known high incidence of round heart disease provided the breeding stock for the experimental group. The studies started with incubating 2231 fertilized eggs from the breeding stock in a commercial type incubator.

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As controls for incidence of hatchability and neonatal mortality use was made of statistics from a commercial flock of turkeys in which round heart disease was not a significant problem.

In the experimental group, 1209 poults resulted from this hatching. Of these, 24 were retained as breeding stock while the remaining 1185 birds were subjected to various observations to be described. (By one year of age each of the 24 birds retained showed clinical signs of round heart disease.)

In each of the 1185 birds in the experimental group the heart was examined grossly following death or at sacrifice, the latter at spaced intervals up to 8 weeks of age. Beyond 8 weeks of age, 46 birds remained. Samples of these were sacrificed at monthly intervals up to 12 months. In 97 hearts from the experimental group examination by light microscopy was done and in 26 of these hearts electron microscopic examination of the left ventricular myocardium was done as well.

Material taken for light microscope examination was fixed in 10% formalin and sectioned by standard methods. Duplicate sections were stained with hematoxylin and eosin and counterstained with Van Gieson's connective tissue stain for elastic tissue.

For electron microscopy, small tissue blocks, $2 \times 3 \times 5$ mm, were taken from the left ventricle midway between the apex and atrioventricular ring. The epicardial and endocardial surfaces were included in these blocks. Tissue was fixed in 2% glutaraldehyde plus 2% formaldehyde in 0.1 M cacodylate buffer (pH 7.3) at 4° C (Karnovsky, 1965). One cubic millimeter blocks were washed in 0.05 M cacodylate buffer with 0.2 M sucrose overnight, post-fixed in 1% osmium tetroxide in veronal acetate buffer (pH 7.4) at 4° C, dehydrated in a graded series of ethanol and embedded in Maraglas (Erlandson, 1964). Thin sections were cut on a Reichert OM-U2 ultramicrotome, stained with uranyl acetate and lead citrate and examined in an RCA-3G electron microscope.

Results

Clinical Observations

Table 1 shows the results of hatchability and neonatal mortality of the experimental flock compared with a commercial flock. Of 2231 incubated eggs from the experimental flock, 1209 poults resulted (54%). No attempt was made in this study to differentiate between infertility and early fetal death as contributors to decreased hatchability. There was a high neonatal mortality observed in the experimental flock. Fourteen percent of the birds of the experimental flock died within 1 month after hatching. Excluding 489 birds sacrificed in the first month, 168 of 698 birds died spontaneously. The mortality rate was maximum in the first 2 weeks and then fell until the time the majority of the flock was sacrificed at 8 weeks. In the remaining birds no spontaneous deaths occurred. Table 2 shows the number of spontaneous deaths through 1 year.

Table 1. *Comparison of hatchability and neonatal mortality of experimental and commercial turkey flocks*[a]

	Experimental flock	Commercial flock[a]
Number of eggs set	2231	Not available
% Hatched	54%	$>95\%$
Number of poults	1209	2200
% Poults dying in first 4 weeks of life	14%	$<5\%$

[a] Statistics provided by a commercial turkey producer.

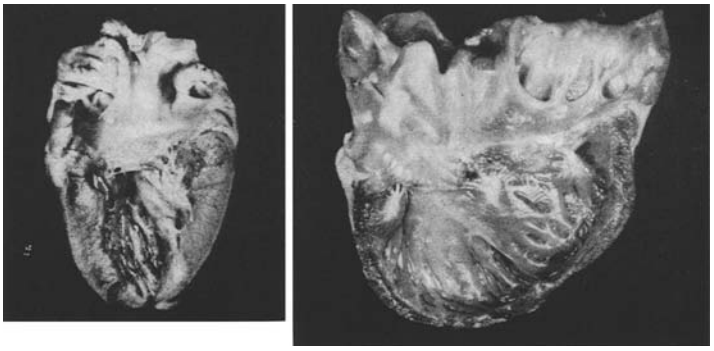

Fig. 1. A Left atrium and ventricle of an unaffected turkey heart, 3 weeks of age. B Heart with round heart disease showing dilatation of left atrium and ventricle, 3 weeks of age. No endocardial fibroelastosis is evident

Table 2. *Summary of mortality and morbidity in experimental flock*

Weeks after hatch	Number of spontaneous deaths	Number sacrificed	Number of abnormal hearts/ Number of hearts examined		
			Gross	Light microscopy	Electron microscopy
1	98	52	5/150	25/45	10/10
2	47	51	18/98	9/9	5/5
3	16	24	5/40	2/2	2/2
4	7	362	106/369	6/11	4/4
5–8	3	479	151/482	3/3	5/5
9–12	0	34	10/34	12/20	
13–52	0	12	12/12	7/7	

The commercial flock of 2200 turkeys surveyed has a greater than 95% hatchability and less than 5% mortality prior to the time of processing. One thousand six hundred hearts were examined grossly at sacrifice (age 14 weeks) and 60 examined by light microscopy. Only one turkey had evidence of round heart disease by gross and light microscopic examination.

Clinically, hatchling turkeys in the experimental flock appeared well until the end of the first week of life, when the most severely affected birds became wasted and died. Death in the maturing birds often occurred following stress and was preceded by dyspnea and low mean systemic blood pressure.

Gross Appearance

In 5 of 150 hearts examined in the first week of life, the myocardium appeared flabby. The others appeared normal. By the third week of age there was a spectrum of biventricular dilatation without endocardial involvement (Fig. 1).

Of 657 turkeys dying or sacrificed in the first month of life, 134 hearts were judged to be grossly abnormal; 106 of these occurred in the fourth week.

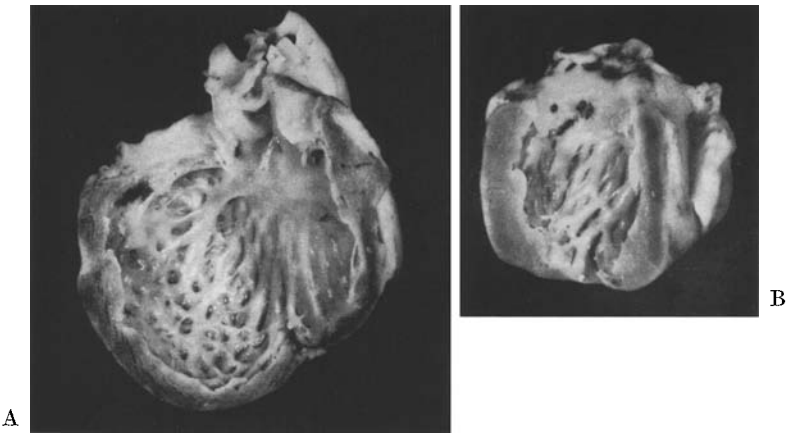

Fig. 2A and B. Round heart at 6 weeks of age. A Dilated left ventricle with endocardial fibroelastosis. B Hypertrophied left ventricle with endocardial fibroelastosis

In maturing birds, age 1 month to 1 year, varying degrees of opacification of the left ventricular endocardium were observed with left ventricular dilatation and hypertrophy (Fig. 2). In some birds, the left ventricle showed focal endocardial thickening or a diffuse gray fibrous change of the entire chamber. The endocardium of the right ventricle was not involved. In some cases, fibroelastic tissue encroached upon the papillary muscles, which were small and displaced toward the mitral valve orifice. These changes are similar to those in human endocardial fibroelastosis described by Moller *et al.* in 1964.

Rarely, there was focal bead-like thickening or a bulbous dysplastic change of the free edge of the mitral valve and chordal thickening was observed (Fig. 3). These changes are compatible with mitral valve insufficiency or endocarditis.

In the most severely affected birds, congestive failure was evidenced grossly by ascites and clear, straw-colored pericardial effusion. The liver and lungs were congested.

Light Microscopy

Of the 45 hearts examined histologically in the first week after hatching, 25 showed abnormalities. This included all 5 of the hearts abnormal by gross inspection and 20 of 40 grossly normal hearts. Similarly 17 of 22 hearts examined in the second through fourth weeks were judged to be abnormal. Histologic examination of these abnormal hearts revealed focal interstitial and perivascular infiltrates of lymphocytes and occasional eosinophils in the myocardium, epicardium and endocardium (Fig. 4). These infiltrates occurred late in the first week of life and persisted until 1 month of age. Adjacent to these, the myocardial fibers had an intense eosinophilic hue. The tinctorial changes and interstitial infiltrates were most striking in turkeys at ages 7 to 9 days.

Light microscopic examination of 8 grossly normal and 22 abnormal turkeys from 6 weeks to 1 year of age failed to show any evidence of the myocarditis as was demonstrated in the younger birds.

Fig. 3. Mitral valve with focal bead-like thickening of the free edge and chordae tendineae, 10 months of age

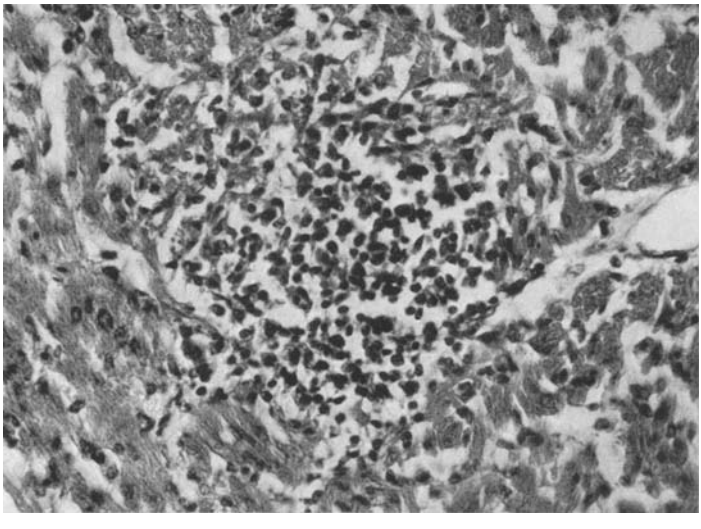

Fig. 4. Myocardium of left ventricle with myocarditis characterized by focal mononuclear infiltrates in the interstitial space, 9 days of age. Hematoxylin and Eosin. × 350

However, in the 28 grossly abnormal turkeys studied after 3 weeks of age there was a gradual deposition of fibroelastic tissue in the endocardium of the left ventricle (Fig. 5). The condensation of this tissue was first noted in the third and fourth weeks of life with an increase in its intensity as the bird matured. In some of the most extreme cases, the endocardium assumed a thick chondroid character which resembled fibrocartilage (Fig. 6).

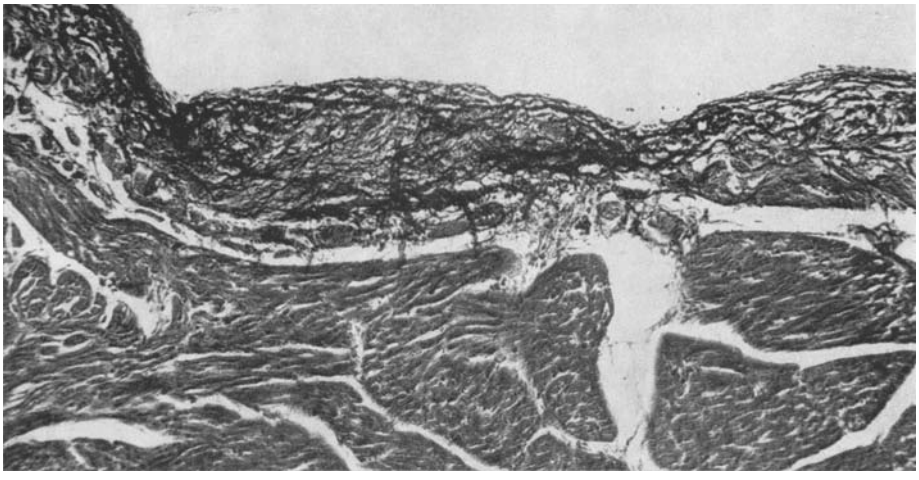

Fig. 5. Endocardium of left ventricle with moderate deposition of fibrous and elastic tissue in the subendocardial space, 6 weeks of age. Elastic Van Gieson. × 530

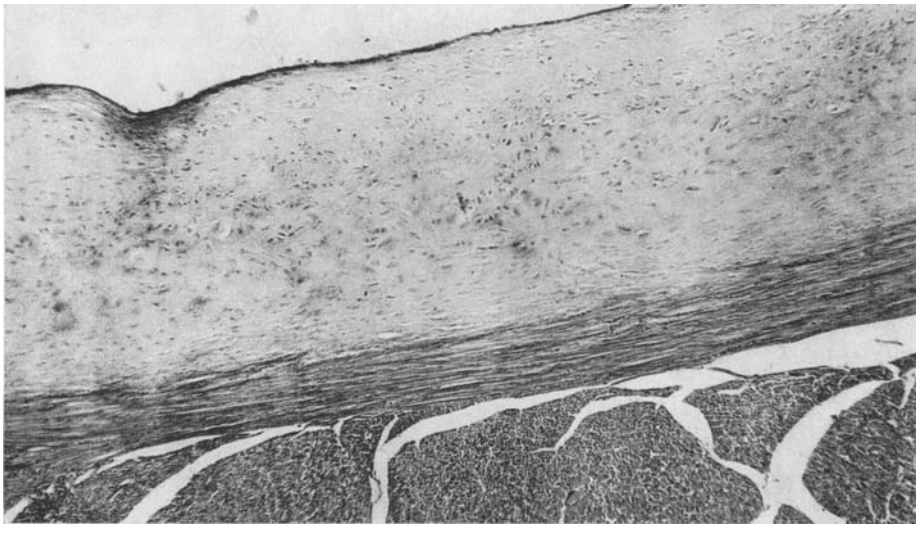

Fig. 6. Endocardium of left ventricle which resembles fibrocartilage due to fibrosis and extreme thickening of subendocardial space, 3 months of age. Hematoxylin and Eosin. × 85

Electron Microscopy

Twenty-six hearts were examined by electron microscopy. Ten specimens, from turkeys age 1 to 4 days, were normal by both gross and light microscopic examination. Sixteen hearts were studied from birds age 1 week to 2 months and of these, 6 were normal by gross inspection but abnormal by light microscopy. By electron microscopy, each of the 26 hearts had abnormalities, which included

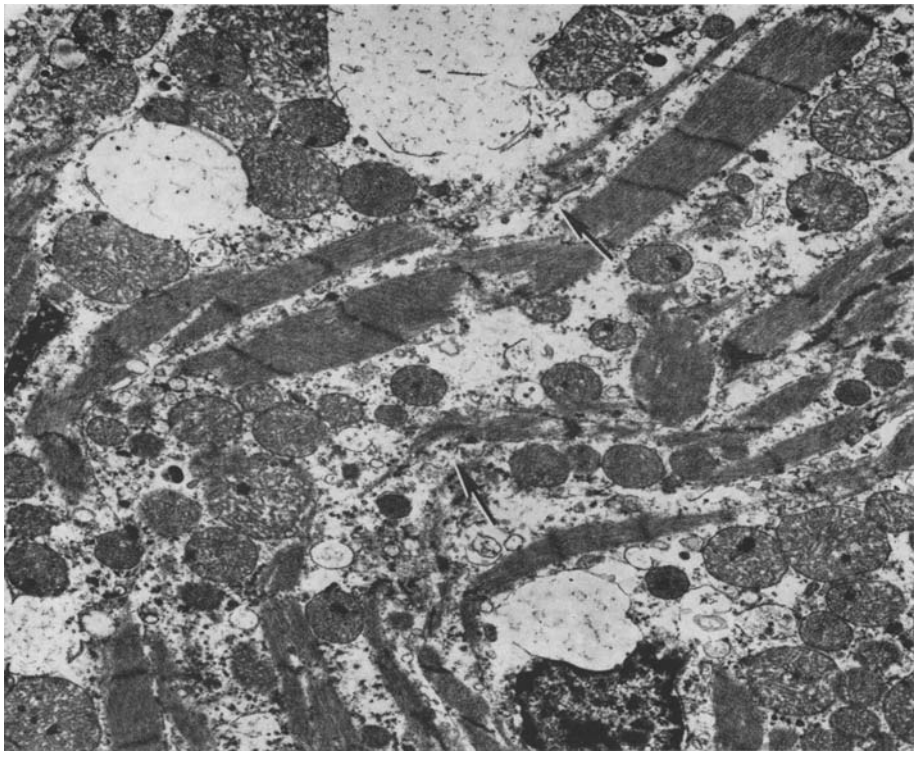

Fig. 7. Myocardium of left ventricle which shows intracellular edema and fragmentation of the plasma membrane (arrows) of myocardial cells. Mitochondria are swollen and contain electron-dense granules. Nucleus shows clumping and peripheral margination of chromatin. 6 days of age. Uranyl acetate and lead citrate. × 8800

injury of the myocardial cells and deposition of collagen and elastic tissue within the subendocardial space. In 1- to 4-day-old birds, in many regions of the myocardium, only isolated single cells showed evidence of damage. This injury would not be noted with light microscopy. However, by 6 days of age and later, injury was more extensive and evident by light and electron microscopy. Myocarditis was defined as the injury to the myocardial cell detectable by electron microscopy.

Fine structural alterations within the myocardium first noted in 1- and 2-day-old birds, consisted of the following changes. Myocardial cells showed either severe intracellular edema or condensation of the cytoplasm and dilatation of the sarcoplasmic reticulum and nuclear envelope. In the nucleus, chromatin clumping and peripheral margination of chromatin were present. In all injured cells, mitochondria showed variable swelling of the matrix. By 6 to 8 days in the most severely affected birds, the plasma membrane of muscle cells was broken or absent but the basement membrane remained. The mitochondria were swollen, fragmented and contained large, amorphous, electron-dense granules (Fig. 7). Myofibrils showed severe contraction bands and in some cells, were fragmented into a homogeneous mass of myofilaments. Cellular debris and mononuclear cells were present

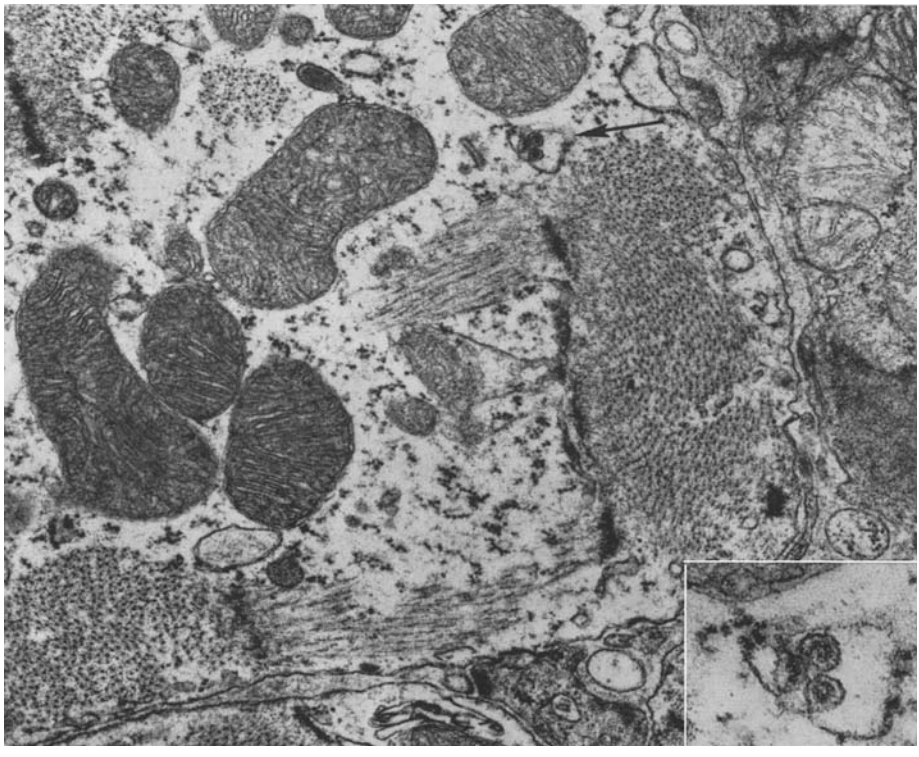

Fig. 8. Myocardial cell with small portion of endoplasmic reticulum containing several virus-like particles (arrow). Inset of endoplasmic reticulum shows details of the particle structure. Each particle has an outer envelope and a central core. 1 week of age. Uranyl acetate and lead citrate. × 24500. inset × 64300

in the extracellular space. By 1 month, these changes had diminished; a few necrotic cells containing only small, electron-dense particles and mitochondria were present. Macrophages, mononuclear cells, fibroblasts and collagen were prominent in the extracellular spaces.

Examination of the endocardial region in 1- and 2-day-old birds showed small accumulations of collagen with fibroblasts beneath a single layer of endothelial cells. This deposition of collagen increased progressively after 2 weeks of age and by 7 weeks a thick band of fibrous and elastic tissue was present between the endothelial cell layer and the underlying Purkinje fibers and muscle cells. The endothelial cells of the endocardium showed no changes. At all stages, Purkinje fibers were injured to the same extent as ventricular muscle cells. Collagen accumulation around these fibers within the myocardium was prominent by 1 month.

Injured and morphologically normal ventricular muscle cells of all 26 hearts examined contained circular or oval virus-like particles singly or in groups of 5 to 6 within dilated vesicles of the endoplasmic reticulum (Fig. 8). These particles, 60–90 mμ in diameter, possessed an outer membrane with a central electron-dense core, 30–60 mμ in diameter.

Discussion

Round heart disease in the avian species has been reported sporadically over the past few decades in a number of countries (Kilian *et al.*, 1964; Shishkov, *et al.*, 1968). A constant feature and, indeed, the criterion for its diagnosis in the mature bird, has been an enlarged round heart often associated with congestive heart failure (Blaxland and Markson, 1947; Magwood and Bray, 1962; Sautter *et al.*, 1968). Only limited descriptions of microscopic changes have been given (Sautter *et al.*, 1968). There is some question whether the same etiologic factors are operational in all instances of this disease.

The present study describes the pathogenesis of round heart disease. Histologically, a wide spectrum of focal myocarditis was evident in all birds within the first week after hatching and increased in severity by 2 weeks of age. At this time mortality in our experimental flock paralleled the severity of histologic findings, with 85% of deaths occurring within the first 2 weeks. Some birds that survived this acute stage of the disease showed a persistent, chronic myocarditis and by 2–3 weeks biventricular dilatation. Endocardial fibroelastosis became prominent after 1 month of life and progressed thereafter in severity. This chronic process predominantly affected the left ventricle and occasionally involved the papillary muscles, mitral valve and chordae tendineae. From age 1 month to 1 year, a few birds showed evidence of congestive heart failure. This coincided with a continuing, although low, rate of mortality through the first year of life.

The virus-like particles present in the myocardial cells of turkeys in this study resemble the viruses of the avian leukosis group (Haguenau and Beard, 1962). Indeed, the association of these particles with the myocarditis suggests that there may well be an etiological relationship. This hypothesis is supported by both the light and electron microscopic pattern of injury (Sanders, 1963; Rabin and Jenson, 1967; Sohal and Burch, 1969). Experimental myocarditis produced in ovo in the chicken by mumps virus has resulted in the development of left ventricular endocardial fibroelastosis in the older bird (Noren *et al.*, 1968). Endocardial fibroelastosis following viral myocarditis in the adult has also been documented (Fruhling *et al.*, 1962; Carstens, 1969). It has been speculated that so-called primary endocardial fibroelastosis in the child is secondary to intra-uterine viral myocarditis (Noren *et al.*, 1963; St. Geme *et al.*, 1966; Noren *et al.*, 1968).

In man, as in the turkey, the clinical features of interstitial myocarditis are variable. Subtle EKG alterations or premature ventricular contractions may be the only evidence that the heart is involved. In the turkey poult during the second week of life, EKG identification of round heart disease may be made by a frontal plane axis shift (Jankus *et al.*, in press). On the other end of the spectrum is the development of conspicuous congestive heart failure with cardiomegaly, as many turkeys with round heart disease demonstrate.

The presence of myocarditis in the very young bird suggests that the etiologic agent may be present and transmitted in ovo. In addition, genetic susceptibility or resistance to this disease may be present with variable expression in different inbred turkey flocks. This is suggested by the difference in hatchability, neonatal mortality and incidence of round heart disease, in the experimental inbred turkey flock as compared to the commercial flock, also an inbred strain. In the commercial flock, 0.06% of turkeys examined grossly showed changes of round heart

disease and by light microscopy, 1.6%. In the experimental flock, 26% of turkeys showed gross changes while 66% showed light microscopic changes. In the diseases of the avian leukosis-sarcoma complex in the chicken, a genetic pattern has been observed (Waters and Fontes, 1960; Burmester, 1967). By selective breeding, susceptible and resistant strains have been developed.

The turkey thus presents an intriguing experimental model of myocarditis with the subsequent development of endocardial fibroelastosis. Of special interest is the variable response in this host to viral infection of the heart, ranging from an essentially normal heart to pronounced endocardial fibroelastosis, and the non-specific fibroelastic reaction of the injured endocardium and subendocardium in a dilated, high pressure, pulsatile chamber.

References

Blaxland, J. D., Markson, L. M.: Toxic heart degeneration or "round heart disease" of poultry. Brit. vet. J. **103**, 401–405 (1947).

Burmester, B. R.: Current status of the avian leukosis complex. U.S. Department of Agriculture Research Service, ARS44–195, November, 1967, pp. 2–16.

Carstens, P. H. B.: Postnatal mumps virus infection. Arch. Path. **88**, 399–401 (1969).

Case, Q. C.: Primary endocardial fibroelastosis. U.S. armed Forces med. J. **11**, 1408–1414 (1960).

Erlandson, R. A.: A new Maraglas, D.E.R. 732, embedment for electron microscopy. J. Cell Biol. **22**, 704–709 (1964).

Fruhling, L., Korn, R., Larillaureix, J., Surjus, A., Foussereau, S.: La myo-endocardite chronique fibro-élastique du nouveau-né et du nourrison (fibro-élastose). Ann. Anat. path. **7**, 277–303 (1962).

Haguenau, F., Beard, J. W.: The avian sarcoma-leukosis complex; its biology and ultrastructure. In: Tumors induced by viruses: Ultrastructural studies (A. J. Dalton, F. Haguenau eds.), p. 1–59. New York: Academic Press 1962.

Hill, W. T., Reilly, W. A.: Endocardial fibroelastosis. Amer. J. Dis. Child. **82**, 579–586 (1951).

Jankus, E. F., Good, A. L., Jordan, K. A., Saxena, S. K.: Electrocardiographic study of round heart disease in turkey poults. Poultry Sci. (in press.)

Karnovsky, M. J.: A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy. J. Cell Biol. **27**, 137A–138A (1965).

Kilian, J., Babcock, W. E., Dickinson, E. M.: A report on round heart disease in Oregon chickens. Avian Dis. **8**, 56–61 (1964).

Magwood, S. E., Bray, D. F.: Disease condition of turkey poults characterized by enlarged and rounded hearts. Canad. J. comp. Med. **26**, 268–272 (1962).

Moller, J. H., Lucas, R. V., Jr., Adams, P., Jr., Anderson, R. C., Jorgens, J., Edwards, J. E.: Endocardial fibroelastosis. A clinical and anatomic study of 47 patients with emphasis on its relationship to mitral insufficiency. Circulation **30**, 759–782 (1964).

Noren, G. R., Adams, P. A., Jr., Anderson, R. C.: Positive skin reactivity to mumps virus antigen in endocardial fibroelastosis. J. Pediat. **62**, 604–606 (1963).

— Jankus, E. F., Staley, N. A., Stevenson, J. E.: A new experimental model of myocarditis (Abstract). Pediat. Res. **4**, 377 (1970).

— Peralta, J. H., Farais, N. E., St. Geme, J. W., Jr.: Myocardiopathic effect of mumps virus in the chicken embryo (Abstract). Circulation **38**, 148 (1968).

Rabin, E. R., Hassan, S. A., Jenson, A. B., Melnick, J. L.: Coxsackie virus B_3 myocarditis in mice. An electron microscopic, immunofluorescent and virus-assay study. Amer. J. Path. **44** 775–797 (1964).

— Jenson, A. B.: Electron microscopic studies of animal viruses with emphasis on *in vivo* infections. Progr. med. Virol. **9**, 392–450 (1967).

St. Geme, J. W., Jr., Noren, G. R., Adams, P. A., Jr.: Proposed embryopathic relation between mumps virus and primary endocardial fibroelastosis. New Engl. J. Med. **275**, 339–347 (1966).

Sanders, V.: Viral myocarditis. Amer. Heart J. **66**, 707–713 (1963).
Sautter, J. H., Newman, J. A., Kleven, S. H., Larsen, C. T.: Pathogenesis of round heart syndrome in turkeys. Avian Dis. **12**, 614–628 (1968).
Shishkov, N., Obreshkov, C., Enchev, S.: Round heart disease of the domestic fowl (Gallus gallus) in Bulgaria. Path. Vet. **5**, 41–50 (1968).
Sohal, R. S., Burch, G. E.: Ultrastructural lesions of the myocardial cell in Coxsackie B_4 infected mice. Virchows Arch. path. Anat. **346**, 361–373 (1969).
Vogt, P. K.: Avian tumor viruses. Advanc. Virus Res. **11**, 293–385 (1965).
Waters, N. F., Fontes, A. K.: Genetic response of inbred lines of chickens to Rous sarcoma virus. J. nat. Cancer Inst. **25**, 351–357 (1960).

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