A CYSTIC DISEASE OF THE HEART, GIZZARD AND MUSCLES OF YOUNG GRASS PARAKEETS (*PSITTACUS UNDULATUS*) DUE TO A PROTOZOON PARASITE.

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(Plates XIII and XIV.)

Various species of the Order Sarcosporidia, some of which have been studied with great care, infest the muscles of different kinds of birds and mammals. Muscle parasites resembling the one described below do not, however, appear to have been described.

Owing to the failure of attempts to transmit the disease and the limited amount of material these observations are very incomplete, but as the disease is peculiar in the appearance of the cysts and in their distribution, and as no indications have been obtained as to the mode of infection or fate of the parasites, it was thought that a brief description might call the attention of those, who have the opportunity of obtaining material, to its existence.

Two young parakeets which were bred in captivity from apparently healthy parents were sent to me for examination. The birds had never left their nests, and it was thought that their death might be due to neglect by the parents.

On examination parakeet No. 1 showed on the heart wall a large collection of small, closely packed cysts (Pl. XIII, Fig. 5) as well as several smaller groups and scattered single cysts. On teasing portions of the heart muscle numerous cysts were found within the muscle substance. Numerous cysts were also found on the surface and within the muscle of the gizzard, and a few were discerned in the pectoral muscles. No lesions either macroscopic or microscopic were found in the other organs, cavities or blood. In the second specimen (No. 2) large numbers of cysts

were found in the gizzard, but none in the heart or muscles. No other lesions were observed.

Some time later another specimen (No. 3) was received. This bird was very young and the body was somewhat decomposed. In the gizzard very large numbers of cysts, occurring in masses which could be recognised by their yellow colour were found. None were found microscopically in the heart or muscles.

Another specimen showed no cysts.

Macroscopical appearances.

On the surface of the heart, just beneath the visceral pericardium, (No. 1) and on the surface of the gizzard (Nos. 1, 2 and 3) the cysts were found in large and small groups, as raised yellowish areas. Single cysts were also common. Within the muscular substance they were also found in groups, but in this situation there was a tendency for the cysts to be arranged in rows of three or more individuals. With the aid of a needle the cysts could usually be easily separated from the muscle substance, and isolated as small, round, smooth masses, varying in diameter from ·5 mm. to scarcely visible particles.

Microscopical examination of sections.

Sections through the affected areas showed numerous cysts, but except in one instance (Pl. XIV, Fig. 6) no acute inflammatory reaction was ever observed round them. All the cysts were of comparatively large size, and since no young forms were noticed, it is difficult to ascertain whether they were originally formed within the muscle fibres or not. Usually single cysts and groups are surrounded by a delicate zone of fibrous tissue, but occasionally a more extensive fibrous tissue capsule can be seen. Delicate strands of fibrous tissue sometimes break up the muscle fibres immediately surrounding the cysts.

In the larger groups many of the cysts are in contact with one another (Pl. XIII, Fig. 1), and the same may be the case when three or four cysts occur in a row (Pl. XIII, Fig. 4). At other times the members of a group are separated by small quantities of muscle or fibrous tissue (Pl. XIII, Fig. 2).

The conformation of the walls and the contents of the cysts show great variations. In every case the wall of the cyst consists of a thick, smooth membrane in which no radial striation or other structure has been discovered. This membrane, judging by its appearance in collapsed cysts, is very elastic (Pl. XIII, Fig. 4, Pl. XIV, Figs. 8, 9, 10).

I. In some cysts this membrane is complete and closely adherent to the delicate fibrous layer surrounding it. Within the membrane there is often a thin layer of finely granular material. The rest of the cyst is filled with a mass of partially or completely separated spores, apparently imbedded in a small quantity of supporting material.

II. Cysts of the type just described frequently rupture and discharge their contents. Pl. XIII, Fig. 3, (A) shows a cyst in the act of rupture with a large mass of spores passing out. The same figure (B) also shows another cyst which has ruptured. The membrane has become detached from the surrounding muscle tissue and has collapsed. In this case the spores are lying round the collapsed membrane.

Pl. XIV, Fig. 10 shows another cyst in a similar condition. The membrane has collapsed and the rupture is distinctly seen (A). In this case some of the spores are still lying within the membrane, and others round it, but large numbers can also be seen between the muscle fibres (B) at a considerable distance from the remains of the cyst. In some cases the spores have been traced in the connective tissue for long distances and more rarely masses of spores may be seen in the vessels (Pl. XIV, Fig. 7 (A)). In a few cases small extravasations of blood have been seen in which spores are mixed with the blood corpuscles.

III. In other cases the cysts partially or wholly discharge their contents without collapse of the membrane. Such empty cysts are generally filled with blood corpuscles, amongst which a few spores can often be found (Pl. XIII, Fig. 2 (A, B)). Occasionally a row of cysts filled with blood communicate by distinct openings with one another. Round these cysts, especially opposite small openings in the membrane, collections of spores can often be found.

IV. Cysts which do not rupture may undergo other changes, which are difficult to interpret, but apparently end in the contraction of the cysts into small hard irregular masses. From a study of the sections it appears probable that the changes take place in the following manner.

(*a*) The material in which the spores are embedded seems to contract, resulting in the production of irregular slit-like spaces between which the closely packed spores lie (Pl. XIII, Fig. 4 (A), Pl. XIV, Fig. 6) surrounded by a matrix arranged in irregular columns. (*b*) Further contraction leads to the widening of these spaces and partial detachment of the membrane from the surrounding muscle (Pl. XIII, Fig. 2 (C)). A striking example is shown in Pl. XIV, Fig. 9. At this time the spores are very closely packed together and can only be distinguished with great difficulty as darkly staining granules in a

moderately darkly staining matrix, arranged in very irregular columns. (*c*) Later further contraction takes place and the membrane is detached from the muscle over large areas and becomes greatly wrinkled (Pl. XIII, Fig. 4 (D)). As no support is now afforded by the lax membrane the slits collapse and the mass forming the interior of the cyst presents an almost solid appearance, and no spores can be distinguished. (*d*) Still later owing to further contraction the mass becomes almost separated from the membrane being only attached to it by a number of thin trabeculae (Pl. XIV, Fig. 8).

At this stage the spaces seem to be filled by serious exudation.

(*e*) Finally further contraction leads to the formation of irregular, darkly staining bodies surrounded by the distorted remains of the membrane (Pl. XIV, Fig. 11), some of which are reduced to very small dimensions.

Spores.

A large number of smears made from the contents of cysts at various stages were examined after staining by Giemsa's and Leishman's methods.

In some cases masses of minute, irregular, red stained bodies were found imbedded in faintly staining blue material. This condition apparently represents very young spores incompletely separated from one another (Pl. XIV, Fig. 12 (A)). In other cases small, discrete bodies were found each consisting of a small mass of chromatin staining material surrounded by a thin zone of blue staining protoplasm (Pl. XIV, Fig. 12). The chromatin was sometimes dense and rounded in outline, and sometimes irregularly shaped and contained darker staining points. This condition seems to represent an early stage in the separation of the spores. In thick smears small masses of spores sometimes seem to be arranged in groups, but no spore morulae with a definite cell wall as in the pansporoblasts of *Rhinosporidium kinealyi* are present[1]. In other cases the spores consist of rounded or oval masses which stain a deep red colour with Giemsa's stain. Sometimes the staining is uniform, but at other times one or more very minute more darkly staining areas may be present. Some of these bodies are surrounded by a very thin zone of blue, but others show no trace of it (Pl. XIV, Fig. 13). Traces of blue staining material are to be found between these bodies. This appears to be the most advanced stage of spore

[1] J. M. Beattie (1906). *Rhinosporidium kinealyi*; a sporozoon of the nasal mucous membrane. *Journ. of Pathol. and Bacteriol.* Vol. xi. p. 270.

formation met with in these cysts. Although in the preparation of the smears some of the spores became distorted and elongated, in no case were large numbers of elongated or falciform bodies seen.

Fresh preparations showed only minute, round, motionless bodies.

Staining reactions.

The cyst membrane takes on a well marked yellow colour when stained by Van Gieson's method, and a reddish colour by Leishman's stain. It stains very poorly by haematoxylin, carmine or thionin. In sections the spores are stained red by Leishman's stain and can be readily detected in the cysts when stained by haematoxylin or methylene blue. The contents of the contracting cysts take up the common dyes very readily.

Infection experiments.

The contents of about 100 crushed cysts were injected into the abdominal cavity of an adult parakeet, and another bird ate a large number of cysts. Although carefully watched for eight months these birds never showed any symptoms. Finally they were killed and careful autopsies made. No lesions were found.

So far as I am aware no disease resembling this one has been described. The cysts appear to be produced by a protozoon parasite during a stage in its life history. The constant presence of the cysts in the gizzard suggests that the birds are infected by feeding, and that the other muscles are infected secondarily. On the other hand, since the spores sometimes get into the blood, it is possible that the disease may be conveyed by external blood-sucking parasites. No indication has been obtained as to how the parasites get into the food, if infection occurs through feeding, or of the ultimate fate of the spores which escape from the cysts. It is interesting to note that though only young nestlings died from the disease no young cysts were encountered, and many were found which had become completely contracted.

Though differing in many respects this parasite more closely resembles *Rhinosporidium kinealyi* than any other cyst producing protozoon.

DESCRIPTION OF PLATES.

PLATE XIII.

Fig. 1. A section through the large mass of cysts on the heart of parakeet No. 1 (Fig. 5). Large numbers of cysts can be seen with very little tissue separating them. Some are full of spores, others contain blood and others have degenerated.

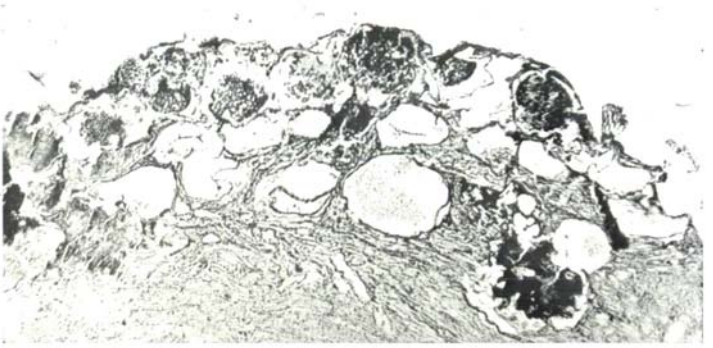

Fig. 1.

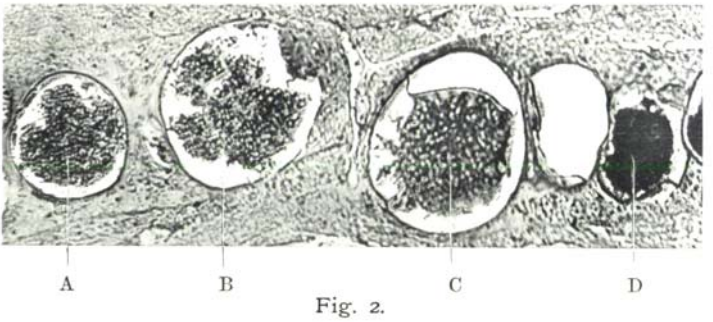

Fig. 2.

Fig. 3.

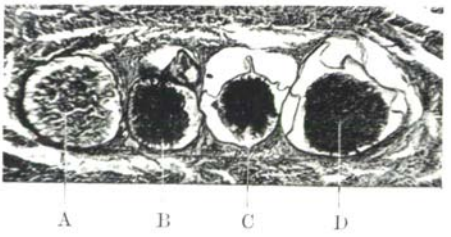

Fig. 4.

Fig. 5.

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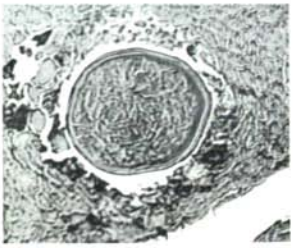
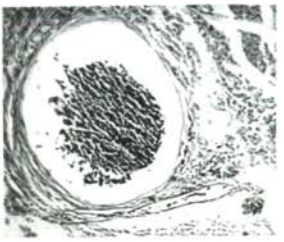


Fig. 6.



A B

Fig. 7.

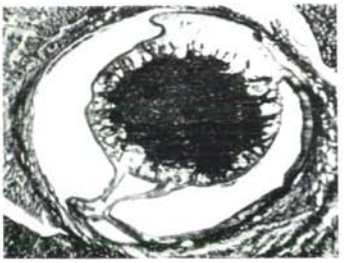


Fig. 8.

Fig. 9.

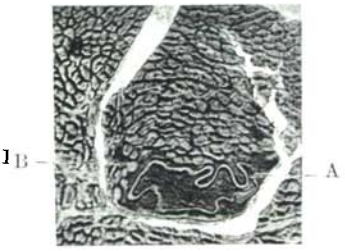


Fig. 10.

Fig. 11.

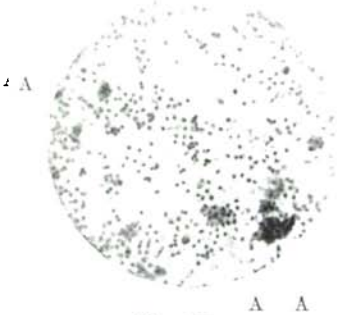


Fig. 12.

Fig. 13.

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Fig. 2. A row of cysts from the gizzard (Bird No. 1). Two (A and B) contain blood corpuscles and a few spores; another (C) is in an early stage of degeneration and shows slit-like spaces in its contents. Over a quarter of its circumference the membrane has become detached from the muscle. Cyst D is in an advanced stage of degeneration and contains a solid black mass. × 150.

Fig. 3. Cysts from the gizzard (Bird No. 2) during and after rupture of the membrane. Cyst A is in the act of discharging a mass of spores. Cyst B has ruptured and the membrane is distorted. The spores are lying round the distorted membrane. × 150.

Fig. 4. A row of cysts from the gizzard (Bird No. 1). Cyst A is in an early stage of contraction. It shows slit-like spaces, but the membrane is only wrinkled and is not detached from the muscle. Cyst D is in a slightly more advanced condition of contraction. The slits are much smaller, and the membrane has become detached over large areas. Cyst B shows an intermediate condition between A and D. Cyst C is still more contracted. × 90.

Fig. 5. Photograph of a water colour drawing of the heart of bird No. 1. It shows one very large collection (A), two medium sized collections (B) and several small groups (C) of cysts.

PLATE XIV.

Fig. 6. A cyst in an early stage of contraction in the pectoral muscle (Bird No. 1). The irregular slit-like clear spaces are well seen. Some inflammatory cells can be seen round the cyst. × 150.

Fig. 7. A mature cyst containing a mass of spores (heart, Bird No. 1). In the small vessel (A) an elongated mass of spores can be seen. A large collection of spores (B) is also to be seen between the muscle fibres. × 100.

Fig. 8. A contracting cyst from the gizzard (Bird No. 2). The contents show narrow irregular slits in the centre and the main mass is connected to the membrane by numerous fine trabeculae. The membrane has become detached from the muscle over large areas. × 135.

Fig. 9. A contracting cyst from the gizzard (Bird No. 1) showing a peculiar condition. The central mass has become separated from the membrane over half the circumference of the cyst. The contents have undergone contraction resulting in the production of numerous large irregular spaces. Spores could still be distinguished with difficulty. × 150.

Fig. 10. Remains of a ruptured cyst in the pectoral muscle (Bird No. 1). The membrane has collapsed and shows a rupture (A). Large numbers of spores are seen within and immediately round the membrane, but small masses (B) have been forced some distance from the cyst. × 100.

Fig. 11. A cyst in an advanced condition of contraction from the heart (Bird No. 1). The distorted remains of the membrane can be seen round the dark contents. × 200.

Fig. 12. Spores in a smear preparation of the contents of a cyst. The majority of the spores are separate, each showing a round or slightly irregular mass of chromatin surrounded by pale protoplasm. Several masses (A) of partially separated spores can be seen. Stained by Giemsa's method. × 1000.

Fig. 13. Spores in a smear preparation of the contents of a cyst. In this case the spores are completely separated and appear as oval or round almost uniformly staining chromatin masses. Some remains of the supporting material can be seen. Stained by Giemsa's method. × 1000.