ABERRANT INTESTINALPROTOZOA N PARASITES IN THE TURKEY.

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PLATE 48.

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During a study of tissues from a series of turkeys which had been included in several experiments designed to throw light on the transmission of the protozoan parasite producing the so-called blackhead, the writer came upon a case which furnishes the text for this communication. The history of the animal (No. 98) is given in a recent paper (1).

It was raised with eighteen others in an incubator and brooder. No adult turkeys or poultry had been on the grounds for many years. Nine young turkeys from an infected flock, imported July 8, had been kept confined in a distant enclosure. Hatched about May 15, it was well until July 22, when it appeared quiet, sleepy, and refused food. It was chloroformed in the hope of obtaining fresh tissues for histological examination of the intestines. The autopsy showed an absence of blackhead lesions. There was, however, a distinct increase in mucus in the ceca, and several coccidial cysts, measuring about 24 μ by 17 μ, were found in the feces passed during chloroforming. The contents of the duodenum had a markedly yellowish coloration. The muscular tissue of the body was also tinged yellowish.

Subsequent examination of tissues fixed in Zenker's fluid showed the presence of peculiar bodies in the subepithelial tissues of the villi of the small intestine, which were tentatively diagnosed as the asexual or schizogonic cycle of some coccidium. The apparently unusual position of the bodies in the adenoid tissue of the mucosa, where they were found in very large numbers, contrasted with the scarcity of the same within the epithelial layer where they, as will be shown
later, were probably not within but between the epithelial cells, induced the writer to give this case a more thorough study.

Coccidia have been generally regarded as exclusively parasites of epithelial cells. The older works of Balbiani (2), von Wasselewski (3), Doflein (4), and others take this for granted. Similarly the investigations by Malassez (5), Labbé (6), Pfeiffer (7), Sjöbring (8), Laveran (10), von Wasselewski (11), dealing either with *Eimeria stiedae* of rabbits or the two known species of avian coccidia (*Eimeria avium* and *Isospora lacazei*) refer only to the epithelial cell parasites.

Metzner (12) was the first to call attention to the presence of coccidia in the subepithelial tissues. He "frequently observed the penetration of *Coccidium cuniculi* into the submucous tissue, regularly into the tunica propria of the cecum, the appendix, and the colon, not infrequently of the small intestine." Metzner promised a discussion of these facts in a subsequent paper which has not, however, appeared.

Fantham (13) mentions the finding of coccidia of grouse in the submucosa, though "much more rarely." He further states that "active schizogony and sporogony go on in the ceca, chiefly in the epithelium, very rarely in the submucosa."

The writer (14), in 1910, described the occasional dislocation of sporonts or gametes of *Eimeria stiedae* in the rabbit from the epithelial into the subepithelial tissue. This dislocation was ascribed to the transformation of invaded epithelial cells into multinucleated cells followed by disturbance of normal relationship with the adjacent epithelial cells and the subjacent tunica propria. The parasites were not found in the cells of the latter.

Hadley (15) refers to the invasion of the subepithelial tissues of the ceca of turkeys by coccidia. At that time he identified the protozoan parasite of blackhead, which is a purely connective tissue parasite and which does not enter epithelial cells at any time, as a coccidium.

Gérard (16) in describing a coccidiosis of young chicks takes an advanced position on the basis of material studied by him. He states that the schizogonic stage of his parasite goes on in the epithelial and subepithelial tissues and that sporogony goes on only in the epithelium. One of his figures shows many schizonts apparently in the subepithelial cells, but the drawing is evidently not quite true to nature and the large host cells, in certain cases at least, suggest epithelial cells. However, there seems to be enough evidence in his case to indicate extensive

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1 There seems to be much confusion in the use of the terms mucous and submucous tissue. The writer includes in the mucosa the tissue bounded by the muscularis mucosa. Several authors to be quoted have evidently regarded as submucous the subepithelial portion of the mucosa. Metzner in using tunica propria and submucosa as synonymous is using the latter term in this sense.
invansion of the mucous membrane with a slight simultaneous occupation of the epithelium.

It should be stated here that Rivolta (17), as early as 1873, described coccidia-like cysts in the submucosa of fowls which were relatively large and visible to the naked eye as white points, the size of poppy seeds. In 1878 he (18) again referred to similar white points about 0.5 mm. in size, in the same situation in fowls.

In 1893 the writer (19) published a brief account of similar cysts in the villi of the ileum in calves. These were situated near the free end of the villus, 0.3 to 0.4 mm. in diameter, and filled with mobile and immobile merozoites.

Two genera of sporozoa, very different from one another, yet both connecting the coccidia with the gregarinida, should here be mentioned, Léger and Duboscq's (20) Selenococcidium and Tyzzer's (21, 22) Cryptosporidium. In the former the schizont is a free-living nematode-like organism entering cells only during the periods of asexual and sexual reproduction. The latter passes its entire life cycle attached to, but not in epithelial cells.

Returning to the case of the young turkey, I wish at present to call attention to certain features only, for the sparse material and the absence of a sexual stage make any definite correlation with earlier work and any interpretation premature.

Sections of the intestinal tract were available from the upper, middle, and lower small intestine and the ceca. Conditions as regards the degree of invasion were practically the same throughout the small intestine, but over lymphoid tissue the parasites were scarce.

The epithelium was still present but lifted off from the subjacent core of the villus. The intervening space was filled with a network of circular lines of a precipitate made up of fine granules. The parasites were visible under a low power as vacuoles arranged in an almost continuous band near the margin of the villus core. Occasional bodies were nearer the central axis of the villus (Fig. 1). Under high powers these vacuole-like bodies were found to be but partially empty. A few were well filled. They consisted of some host cell whose cytoplasm had been moulded into a shell (or ring in section) with the much flattened nucleus against this shell. The contents were a very fine lining membrane within which were roundish bodies of various diameters, 2 μ and more, staining feebly reddish and with or without a mass of chromatin. Frequently a body contained two chromatin masses situated at opposite poles, as if division had taken place. Those bodies which were full of the small spheres, contained
about sixteen or more of more or less uniform size (Fig. 2). The vacuolated appearance under low power was due to the disappearance of some or all of the parasitic contents of the host cell. Prolonged search for the characteristic products of asexual multiplication—falciform bodies—brought to light only two or three parasites containing them. It is not to be denied that these may have been moulded into crescent shape by the pressure of the other growing and segmenting members in the same membrane. One of these crescents is shown in Fig. 3.

In addition to the parasites in the core of the villus, a certain number of bodies were found free in the space between villus core (tunica propria of Stoehr) and epithelium. If, as is claimed by most histologists, this space is an artefact, the free parasites must have been embedded in the adenoid tissue at the base of the epithelial cells and set free when the core was pulled away by the shrinking action of the fixing fluid. These bodies differ from those embedded in the core of the villus in having a more condensed cytoplasm, staining more intensely with eosin. They also contain relatively large and more numerous clumps of a substance staining deeply with nuclear dyes and presumably chromatin (Fig. 1 on the left above). These compact clumps are very irregularly grouped and of various shapes, many of them broadly oval or biconvex. No form which may be regarded as normal was detected, no two being precisely alike.

Relatively few parasites were found embedded in the epithelial layer itself. In the one shown in Fig. 2 there is considerable chromatin flattened against the periphery of the body as if it were a host cell nucleus. If so, the body is in a cell lodged between epithelial cells and not in the epithelial cell itself.

The same parasites were also found, but in relatively small numbers, in the mucous membrane of the ceca.

Assuming for the moment that all the parasites were striving toward the formation of merozoites of characteristic form, the great variation in the size of the products of division and the irregularity as regards chromatin, lead the writer to infer that they were largely degenerating forms. This theory is supported by the abortive attempts at repeated multiplication within the primary cysts and the partial disappearance of their contents.
As stated above, it is impossible from this case alone to determine whether the parasite belongs to one or the other of the two well known species of avian coccidia, or whether it is a foreign, aberrant type which fails to survive in the accidental host. The relative smallness of the schizonts, which measure 10 microns, and of the merozoites (the only one that could be found and measured being 5 \( \mu \) long) led the writer to assume that it is a foreign species. The species described by Gérard has schizonts up to 30 \( \mu \) by 42 \( \mu \), and merozoites 14 \( \mu \) long (16).

The location of this parasite within cells of the villus indicates that the former may have actively invaded the cells and that phagocytosis did not play a part. The nature of the cells invaded the writer is unable to state.

In general, the invasion of the substance of the villus is of more than ordinary interest to the pathologist. A relatively large amount of foreign proteid is brought within the reach of the absorbents and the disintegration of the parasites may well account for the symptoms produced. Several other young turkeys died within a week after the case under consideration had been chloroformed, and the writer attributed these deaths to arsenic and lead spray. The symptoms in these cases were somnolence, indifference to food, and diarrhea, and the lesions were confined to hyperemia and swelling of the mucous membrane of the small intestines. Coccidia were not found in the intestinal contents or in scrapings of the mucous membrane. Unfortunately tissues of these cases were not saved for microscopic examination. Similarly there was slight temporary indisposition and drooping among other members of the flock, which may have been due to the unknown parasite. The presence of pheasants, sparrows, and song birds in the territory surrounding our experimental grounds may account for the infection, for the young turkeys were allowed to feed over a certain territory outside of the screened shelter each day (1).

Toward the end of the study of this case, the writer came upon two small areas in sections of the ceca of the same case within which nearly every epithelial cell contained minute protozoa which on further study were diagnosed as true coccidia (Fig. 4). The parasites were in an early stage, either as schizonts or sporonts. Putting all the
facts together, the writer is inclined to regard these epithelial cell parasites as belonging to a species distinct from that in the subepithelial tissue, and perhaps the same as the species represented by the oocysts found in the contents of the large intestine at autopsy. To all appearances we have in these parasites true coccidia to deal with.

The invasion of the subepithelial territory of the mucous membrane raises the question concerning the frequency of this phenomenon among coccidia. Is it that this early stage, coming as it does before or with the earliest symptoms, has been overlooked? The negative evidence the writer has cited above from the writings of others should not count too heavily, for much of the work was done by those for whom the life cycle of the parasite was the chief object of pursuit. It may now be desirable to examine the intestines of young healthy animals and of those in which symptoms are just appearing, to determine to what extent early schizogony goes on in the subepithelial tissues, whence direct invasion of epithelial cells for the sporogony may take place from the base of the cells.

There are so many points of difference between the blackhead parasite and the unknown parasite of the mucous membrane as encountered in this single case, that any attempt to present them would require an elaborate restatement of what is now published. Assuming that they are different, we are confronted with the fact that, even after the blackhead parasite shall have been eliminated, the outlook for raising turkeys without some losses due to avian coccidia and perhaps other still unknown protozoan parasites is not very encouraging. Fortunately the mortality due to these aberrant parasites was low. In any case the specific sources of coccidia and other parasites must be found and dealt with.

BIBLIOGRAPHY.

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ABERRANT INTESTINAL PROTOZOAN PARASITES


EXPLANATION OF PLATE 48.

Fig. 1. Section through a villus. The main axis is from above down. Only a few nuclei of the epithelium appear on the left below. The core of the villus is surrounded by a bubbly coagulum under the epithelium. On the right, the marginal zone of the villus contains a row of faintly outlined parasites (from above down). The largest, below, appears as a vacuole containing a few remnants of the schizogenic division. On the left margin of the villus near the bottom of the figure several parasites are faintly outlined.

In the subepithelial (bubbly) space on the left, above, a parasite is seen staining more intensely and with six or more clumps of chromatin.

Fig. 2. A parasite within the epithelial layer. The nucleus of the host cell is shown only in a small part in the photograph. The nucleus of an epithelial cell is seen in the invaded territory crowded towards the free border of the epithelial layer. The progeny of the schizogenic (?) division are faintly outlined. Only one shows a nucleus indistinctly.

Fig. 3. A schizont showing what appears to be a merozoite with a nucleus near one extremity. The schizont is situated in the space between the epithelium and the villus core and is evidently attached to the latter. A row of parasites situated in the marginal zone of the villus is faintly indicated.

Fig. 4. Epithelium of one cecum, showing nuclei near the base of the cells and parasites nearer the free border. The parasite farthest to the right contains four nuclei, but only two are in focus.
(Smith: Aberrant Intestinal Protozoan Parasites.)