

UTERINE COCCIDIOSIS OF THE IMPALA CAUSED BY *EIMERIA NEITZI* SPEC. NOV.

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ABSTRACT

McCULLY, R. M., BASSON, P. A., DE VOS, V. and DE VOS A. J. Uterine coccidiosis of the impala caused by *Eimeria neitzi* spec. nov. *Onderstepoort J. vet. Res.* 37 (1), 45-58 (1970).

Four cases of uterine coccidiosis showing readily-detectable macroscopic lesions were collected over a period of two years during which 451 female impalas [*Aepyceros melampus* (Lichtenstein, 1812)] were examined *post mortem* in the Kruger National Park. This report includes descriptions and illustrations of macroscopic and microscopic lesions and various developmental stages of sexual reproduction of the parasite. Because of certain morphological features of the oocysts, the causative organism was determined by the authors to be a new *Eimeria* for which they propose the name *Eimeria neitzi*. This coccidium parasitizes mainly the distal portions of the uterine glands and the adjacent surface epithelium. Another distinctive feature of *Eimeria neitzi* is that sporogony occurs while the oocysts are still within host cells. On the basis of present knowledge, uterine coccidiosis is of very low incidence among impalas in the Kruger National Park and probably of little herd significance under the prevailing veld conditions. Its effect on reproduction was not determined.

INTRODUCTION

Coccidia have been reported in different organs and tissues such as the intestines, lymph nodes, liver and kidneys of warm-blooded animals (Pellérdy, 1965). Subsequently, McCully, Van Niekerk & Kruger (1967) described suspected unsporulated coccidial oocysts in the endometrium of the hippopotamus (*Hippopotamus amphibius* Linnaeus, 1758). This was a hitherto unknown location for coccidiosis. The present report describes various developmental stages of, and lesions caused by a new *Eimeria* sp. in the uterus of another host, the impala, [*Aepyceros melampus* (Lichtenstein, 1812)]. This study was initiated by the macroscopical observation of suspected small intrauterine polyps, which proved to be lesions of coccidiosis microscopically.

MATERIALS AND METHODS

The uteri of 451 apparently normal females were examined and specimens from 100 of these were collected and preserved in 10 per cent buffered formalin for histopathological examination. Among these were placentomes from 36 in various stages of pregnancy. They were studied to determine the presence of coccidia and also to make morphological comparisons with the parasitized tissues of the other animals. The prepuce, glans penis, testes, epididymis and accessory sex glands were examined from ten rams. Smears were made from the vaginal discharge of two ewes.

Two attempts at experimental infection were made. Oocysts were obtained from the parasitized uterus of an impala by excising and mincing bits of mucosa. Suspensions of these oocysts in normal saline were injected into the uterus of one young impala ewe following its exposure by laparotomy and through the cervix of another *per vaginam*.

The formalin-fixed tissues were embedded in paraffin wax in a routine manner. From the embedded blocks of tissue, sections of 3 to 6 microns thickness were cut with a sliding microtome and routinely stained with haematoxylin and eosin (HE). Selected sections of the positive cases were subsequently stained with Giemsa, Gomori's methenamine silver (GMS) (Anon., 1960), Gram's stain (Anon., 1960), Himes & Moriber's triple stain (FSNY)

(Himes & Moriber, 1956) and periodic acid-Schiff (PAS) (Pearse, 1961). Some of the formalin in which positive material had been preserved was centrifuged and oocysts from the sediment were mounted unstained on glass slides for microscopic study. Measurements were made with an ocular micrometer. The illustration of an oocyst [Plate 8 (61)] was drawn with the aid of a side arm drawing tube mounted on a binocular microscope.

RESULTS

Out of the 451 apparently normal impala ewes, specific lesions of the endometrium were detected macroscopically in four non-pregnant ewes. Two of these were suspected because of a fairly copious, slightly turbid vaginal discharge. Examination of smears of the discharge revealed oocysts. The sheath, penis, epididymis, testes and accessory sex glands of males did not have any macro- or microscopic lesions due to the parasite. At necropsy a month after the attempts to transmit the infection experimentally, neither of the two impala ewes had uterine lesions.

Macroscopic findings

The three most affected uteri contained multiple, polymorphous polyps, their diameters ranging from approximately 0.5 to 1.5 cm [Plate 1 (1)]. The protrusions generally had a sessile base and appeared dull greyish white. The impression was gained that the intercaruncular as well as the caruncular areas were involved. Even in a normal contracted uterus both of these may be thrown into slight elevations making distinct recognition of either one or the other difficult. In one case, slightly raised, yellowish-green foci were observed along the edges and on top of some of the elevations that were thought to be caruncles but there were no microscopic polyps.

In one group of 35 gravid impala uteri that were examined macroscopically, 28 contained individual or disseminated small, white firm foci of approximately 1 to 2 mm diameter in the endometrial stroma.

Microscopic findings

Normal histology: As is the case in domestic ruminants, the caruncles proper of the impala uterus are devoid of glands and their openings. Underlying the caruncles,

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however, there are tubular uterine glands deeply situated in the endometrial lamina propria. They open next to the caruncles. Many tubular glands are also present in the endometrium of the intercaruncular areas. The surface epithelium of the caruncular areas is relatively low compared to the tall columnar epithelium found between caruncles. The uterine glands have a thin, very poorly developed basement membrane but the columnar surface epithelium of the intercaruncular areas rests on a well developed basement membrane.

Although not intensive, observations were also made on the histology of the impala placenta, which incidentally resembles that of sheep. The maternal crypts of the placentomes in near-term impala ewes are lined by epithelium of syncytial nature. Our limited studies do not permit us to state if it is of maternal or trophoblastic origin as this is still the subject of considerable controversy in other ruminants, which have been intensely studied in this connection (Davies & Wimsatt, 1966). Diplokaryocytes were observed in the chorionic villi and in the lining of the maternal crypts. These cells in the sheep are known to be trophoblastic in origin (Davies & Wimsatt, 1966). At no stage of pregnancy did the maternal crypts develop a prominent basement membrane.

Uterine changes in parasitized cases

Although four cases of uterine coccidiosis were detected, only three were suitable for significant histopathological study, advanced autolysis being present in the other one. There was considerable variation regarding the extent, stage and type of involvement. The spectrum of changes observed can best be shown if a composite picture of the disease is given.

The well-localized lesions were confined to the inner zone of the endometrium and the areas of most extensive involvement were significantly elevated and polypous. They appeared either to be pedunculated or to have a broad sessile base [Plate 1 (2)]. Some of the larger lesions mushroomed slightly. Near the periphery of such areas, some planes of section gave the impression that the affected tissue was attached by a narrow pedicle. The epithelial cells within the lesions were parasitized and arranged either in a tubular or alveolar pattern and their basement membranes were prominently thickened. It was evident that both the distal portions of the uterine glands as well as the adjacent surface epithelium in these areas [Plates 1 (3) and 2 (7)] were parasitized, probably mainly the former. However, the degree of involvement of the various epithelial cells and zones of the endometrium such as the caruncles and intercaruncular areas could not be determined with any certainty, because of the advanced stage and polypous nature of the lesions. Nevertheless, the impression was gained that the pericaruncular areas and the distal portions of the uterine glands were mostly affected [Plate 2 (9)]. Some of the parasitized uterine glands [Plate 3 (13)] were strongly branched, the laterally-projecting extensions giving them an alveolar pattern.

The parasitized epithelium became both hypertrophic and hyperplastic. Single or multiple layers and syncytial masses were formed [Plate 3 (16, 17 and 18)] in the glands, many cells containing various developmental stages of the coccidium. The syncytia were almost contiguous with the basement membranes in some places but there was usually a narrow space intervening, presumably an artefact due to shrinkage in the preparation of the sections [Plate 3 (14)]. In some of the distal portions of the glands the syncytia were diffusely spread in the central portion of the cavity but still in contact with

the proximal syncytia, both portions of which were parasitized [Plate 3 (17)]. The basement membranes of the parasitized uterine glands were strongly PAS positive [Plate 3 (15)] and in a few places had ruptured which resulted in focal granulomatous reactions with large multinucleated giant cells [Plate 2 (12)]. In the most advanced case, there had been extensive destruction of the basement membrane in some glands, with the result that numerous oocysts escaped into the lamina propria. In response to this there was a more extensive granulomatous reaction [Plate 2 (10)].

The case which showed the earliest stages of the infection, had various phases of gametogony in the glandular epithelium [Plate 2 (11)] resulting in extensive alterations in the appearance of the epithelium. This frequently made the recognition of the host cell very difficult. In an occasional gland, unparasitized as well as parasitized epithelium was present. Because of involvement of the gland openings near the surface of the endometrium and the resulting obstruction, some of the distal portions of the underlying uterine glands were considerably dilated by cellular exudate, glandular secretion and a few oocysts [Plate 2 (8)].

The lamina propria, especially at the base and adjacent to the polypous lesions was very mildly to heavily infiltrated by lymphocytes, plasma cells and polymorphonuclears [Plate 1 (5 and 6)].

Developmental stages of the coccidium

Schizogony: Because of the many early stages of gametogony described below, it was expected that developing schizonts would still be present. A special staining technique (FSNY) was applied to sections for the purpose of demonstrating mature schizonts which might correspond in appearance to those of other *Eimeria* spp., but not a single one could be identified unequivocally. Some forms of the parasite were observed which could not be specifically identified as gamonts and possibly these may have been either developing or mature schizonts [Plate 8 (62, 63 and 64)] which are different from those described for other *Eimeria*. In the absence of mature schizonts it was not possible to identify with any certainty earlier phases of schizogony which might have been present.

Gametogony: The first evidence of a host cell harbouring a developing parasite was the presence of a single small slightly elongated to oval-shaped structure within a cytoplasmic vacuole. Initially, it was faintly eosinophilic when stained with HE but, subsequently with spherical enlargement, it became a more intensely eosinophilic globule [Plate 4 (19)]. Nuclear material at this stage was not visible. These structures presumably represented early gametocytes. The fact that well-advanced gametogony was also observed seemed to preclude their being trophozoites from the primary infection. As these forms enlarged in the cytoplasmic vacuoles, a small nucleus became visible in some of them [Plate 4 (20 and 21)]. At this stage its specific differentiation into either a macrogamete or a microgametocyte or even a schizont, could not be predicted. The gametocytes became recognizable before it was possible to determine the specific direction of differentiation, either into macrogametes or microgametocytes. With the gradual enlargement of the gamont a reticulated or foamy cytoplasm developed adjacent to the eosinophilic globule. This development eventually displaced the latter toward the periphery [Plate 4 (22)]. The gamont evidently developed within the reticulated cytoplasm adjacent to the eosinophilic globule and was surrounded by a delicate membrane.

Macrogametes [Plate 4 (23 to 28)]: The developing macrogametes were recognizable by certain features, the earliest being the single spherical nucleus which had a round, slightly basophilic nucleolus. Many small eosinophilic globules appeared in the reticulated cytoplasm. These are apparently identical to the plastic granules of macrogametes of other *Eimeria* spp. Since these were more globular than granular, they will be referred to as plastic globules instead of granules. With HE they had a golden yellow tint in contrast to the reddish tint of the large eosinophilic globule referred to previously. Although poorly discernible with HE, there were slightly elongated granules admixed with the plastic globules. The plastic globules did not react with PAS but these granules were PAS-positive and argyrophilic with GMS. The substance in the granules was not digested by diastase; the test thus proved it to be negative for glycogen. These small granules, the plastic globules, the large eosinophilic globule and the single nucleus were specific features of the macrogamete.

The large, peripheral eosinophilic globule was subsequently dwarfed by the enlargement of the macrogamete. It was separated by a membrane surrounding the latter and as the macrogamete developed it became crescent-shaped and less conspicuous. It is apparently analogous to the crescent-shaped bodies found by Hammond, Bowman, Davis & Simms (1946) in developing schizonts of *Eimeria bovis* (Zublin, 1908). Similar bodies were found in early gametocytes of *Eimeria auburnensis* Christensen & Porter, 1939, by Hammond, Clark & Miner (1961). This globule will be referred to as the semilunar body because of its crescent shape and the desirability of giving it a specific name. As the macrogamete enlarged, the nearest adjacent nucleus of the syncytium or nucleus of the parasitized cell was flattened against the cell membrane [Plate 4 (27)]. The plastic globules became more numerous and enlarged as the macrogamete developed. With maturity of the macrogamete, multiple tubelike extensions radiated from the centrally located nucleus and extended into the cytoplasm [Plate 4 (26)]. With FSNY stain the semilunar body, the plastic globules and the radiating tubules stained yellow and the small granules red. The semilunar body and the plastic globules stained brown with GMS. In many of the mature macrogametes the substance between the plastic globules was mildly Gram-positive.

Microgametocytes (Plates 5, 6 and 7): Subsequent to the undifferentiated phase of the organism in gametocytes, microgametocytes could be recognized readily. Multiple nuclei, at first poorly delineated, appeared in the foamy reticulated portion of the gametocyte [Plate 5 (29, 30 and 31)]. The nuclei were frequently situated where the reticular strands crossed [Plate 5 (32 and 33)]. They became more numerous with enlargement of the microgametocyte [Plate 5 (34 to 39)]. A semilunar body similar to those found in macrogametes also occurred [Plate 5 (29, 30, 33 and 37)]. As the number of nuclei continued to increase, they became arranged at the periphery of the developing microgametocyte, the central area having a foamy appearance and being devoid of nuclei [Plate 5 (40)]. Subsequently rosettes of nuclei were formed centrally, apparently by the ingrowth of nuclei from the periphery toward the central area [Plate 5 (41) and Plate 6 (42)]. Secondary rosettes were also formed from primary ones [Plate 6 (43)]. In the lumen of some of the rosettes there were small clumps of nuclei [Plate 6 (43)], the source and ultimate fate of which were not determined. Up to and during the early rosette phase, the

small nuclei had a rather vesicular appearance, there being a dense peripheral rim of chromatin surrounding the clear centre.

The number of nuclei within and between the rosettes increased markedly [Plate 6 (44 and 45)]. This altered the appearance of the microgametocyte as more and more of it was filled by nuclei [Plate 6 (46 and 47)]. With the marked increase in the number of nuclei, they became elongated, less vesicular and stained more uniformly basophilic. As the nuclei became longer, flagella developed [Plate 6 (49) and Plate 7 (50 and 51)] and microgametes ultimately evolved. The microgametes, literally filling the microgametocyte, were frequently arranged in pallisades while other groups appeared in clumps and formed vortices [Plate 7 (50, 51 and 52)]. Mature microgametocytes measured up to 100 microns. After full maturity the wall of the microgametocyte ruptured and the microgametes were released [Plate 7 (53)]. The free microgametes were apparently attracted to the mature macrogametes because frequently they were immediately adjacent to them in the glands [Plate 7 (54)].

Associated with their development, an ever-increasing amount of PAS-positive substance was present within the microgametocytes. This was not removed by prior treatment with diastase. It was either absent or very scarce in some stages that were suspected of being early schizonts. The PAS-positive substance was abundant at the phase of development when the rounded vesicular nuclei of the developing microgametes became more solid in appearance and elongated. It was plentiful thereafter, even being present around the microgametes following rupture of the microgametocytes. With GMS stain there were very fine argyrophilic granules within the microgametocytes.

Stages of gametogony were more numerous in the deeper and more proximal portion of the glands while oocysts were far more plentiful distally. Because of this the glands had a greater diameter near their openings than proximally [Plate 8 (60)]. Conversely the septa were thicker at the base of the glands and thinner between their openings.

Zygotes: Within some of the macrogametes which had radiating tubelike projections from the nucleus, there were some basophilic elongated objects. Whether these were actually parts of microgametes which had entered the macrogamete for fertilization [Plate 4 (28)], or just further extensions of the tubules from the nucleus was not determined. However, the first definite evidence of fertilization and the formation of a zygote was the development of the shell of the oocyst. Refractory to stains, the shell first appeared as a very pale but clearly demarcated structure circumscribing the zygote [Plate 7 (55)]. After formation of the shell, the contents of the zygote appeared rather homogeneous; the plastic globules were no longer present [Plate 7 (56)] and the amount of PAS-positive material increased. Fertilized macrogametes and the early unsporulated stage of the oocysts were mildly Gram-positive.

Sporogony: The next stage encountered was the sporulated oocyst [Plate 7 (58)]. In the microscopic sections from which the above observations were made, an occasional oocyst had been sectioned in a plane which revealed that there were four sporocysts, each containing a pair of sporozoites. Sporulated oocysts were frequently seen within the host cells [Plate 7 (57)] and this is apparently a characteristic feature of this particular coccidium.

Oocysts [Plate 8 (59 and 61)]: In wet preparations prepared from sediments of centrifuged formalin the oocysts were spherical, subspherical or slightly ovoid. The wall was 0.5 microns thick, colourless, smooth without a micropyle and appeared to consist of a single layer. A polar granule was present in about 60 per cent of the oocysts but there were no oocystic residual bodies. Fifty sporulated oocysts measured 29 to 34 microns by 28 to 33 microns with a mean of 31.7 by 30 microns. Their length to width ratios had a mean of 1.06 and ranged from 1.0 to 1.15. The oocysts contained four sporocysts, each containing two sporozoites. The elongated sporocysts were ellipsoidal, tapering slightly towards one end. A Stieda body was present, but only as a thickening of the tapered end of the sporocyst. There was a sporocystic residual body which consisted of large granules that were either dispersed throughout the sporocyst or grouped together forming a more or less compact body. Fifty sporocysts measured 16 to 19 microns in length and 6 to 8 microns in width, with a mean of 17.4 by 7.4 microns. Their length to width ratios ranged from 2.2 to 2.9 with a mean of 2.36.

The sporozoites which could be clearly seen in the sporocysts were thicker and rounded at one end and tapered towards the other. Ten sporozoites which were measured had an average length of 10 microns. One or two refractile globules were present in each sporozoite. The pair of sporozoites were usually oriented so that their rounded ends occupied the opposite poles of the sporocyst.

The histochemical studies revealed that one or both ends of the sporozoites were PAS-positive when stained either with PAS or FSNY and argyrophilic with GMS. The refractile globule was PAS-negative and bright yellow with FSNY. Portions of the sporozoites, excluding the refractile bodies, stained slightly metachromatically with Giemsa and toluidine blue. The tinctorial characteristics of the sporocystic residual body were not determined as this granular structure could not be identified specifically in sections. This was possibly due to shrinkage of the sporocysts during fixation. However, some of the PAS-positive granules which appeared to be within the sporozoites could have been superimposed residual bodies.

The sporulation time could not be determined because sporulation occurred while the oocysts were still in the host tissues.

Lesions due to coccidia in other specimens

The small white foci observed macroscopically in the endometrium of some of the pregnant ewes proved to be tiny mineralized granulomas in the lamina propria [Plate 1 (4)]. In a few of them, oocysts, some containing sporocysts with sporozoites, were recognized. These foci were usually in the lamina propria of the intercaruncular areas.

The following organs were also examined but were not found to be parasitized: ovaries and oviducts from two positive cases and the cervix and vagina from one case.

DISCUSSION AND CONCLUSIONS

The parasite observed in the uterine lesions of a number of impalas is considered to be a previously unknown species of *Eimeria*. *Eimeria* spp. are typically parasitic in the epithelium of the gut. Nevertheless, a number of them has been found developing in or migrating through other tissues during part of their life cycle. A few species have been discovered completing their life cycle in cells other than those of the intestines.

Van Doorninck & Becker (1957) demonstrated the migration of the sporozoites of *Eimeria necatrix* Johnson, 1930, within macrophages in the lamina propria of the intestine of the domestic fowl. Challey & Burns (1959) and Patillo (1959) observed the same phenomenon in *Eimeria tenella* (Railliet & Lucet, 1891). In both species the migration terminates when the sporozoites enter the epithelial cells of the intestinal glands. Hammond *et al.* (1946) described schizonts of *E. bovis* in the endothelium of the central lacteals of cattle. Schizonts of *Eimeria arloingi* (Marotel, 1905) were found in a similar site in sheep by Lotze (1953). Oocysts, macrogametes and microgametocytes as well as schizonts of *E. auburnensis* have been demonstrated in cells of the lamina propria of the intestine in cattle (Hammond *et al.*, 1961; Davis & Bowman, 1962). This implies that this species may complete its life cycle in cells other than those of the intestinal epithelium.

Eimeria stiedae (Lindemann, 1865) and *Eimeria truncata* (Railliet & Lucet, 1891) are species completing their life cycles in sites other than the intestine, the former in the bile duct epithelium of rabbits and the latter in the epithelium of the renal tubules of geese.

Although the reasons for it are poorly understood, there is a tissue specificity shown by *Eimeria* spp. In most instances, infestation is limited to a specific part of the gut or to a specific organ. Development of certain stages has been observed in various aberrant sites. Lotze, Shalkop, Leek & Behin (1964) demonstrated schizonts in mesenteric lymph nodes of sheep. Pienaar, Bigalke, Tustin & Naude (1964) reported oocysts of *Eimeria impalae* Prasad, 1960, occurring in lymphoid tissue of the submucosa of the intestine of the impala. Unidentified schizonts were observed in a similar site in a goat which had a severe mixed infection of *Eimeria* spp. (unpublished personal observation, Basson).

The only previous report indicating that coccidiosis may occur in the uterus was that of suspected unsporulated oocysts in the endometrium of the hippopotamus (McCully *et al.*, 1967). It was not possible to classify the parasite specifically.

Based on the morphology of the oocyst, the parasite in the uterus of these impalas is considered to be an *Eimeria*. Certain of its characteristics, however, are not altogether typical of the genus in mammals. The oocysts become sporulated while still within the cytoplasm of the parasitized cells [Plate 8 (59)] and syncytia [Plate 7 (57)]. This phenomenon is a known characteristic of *Isospora bigemina* (Stiles, 1891) in intestinal epithelium. Microgametocytes of this uterine *Eimeria* are exceptionally large, measuring up to 100 microns in diameter. This is also characteristic of *E. auburnensis* (Hammond *et al.*, 1961). The thin wall of the oocyst ruptures easily, releasing the sporocysts in this manner.

In these impalas the parasitization of the epithelial cells and syncytia of the endometrium by an *Eimeria* sp. appears to be a well-established phenomenon, apparently being the usual host-parasite relationship rather than an example of aberrancy.

The epithelium of the distal portions of the uterine glands and portions of the surface epithelium of both the pericaruncular and intercaruncular areas were apparently parasitized. Observations on the histology of the normal uterus and placentomes of the impala revealed that, as in domestic ruminants, the uterine glands underlying the caruncles do not penetrate the caruncles but open adjacent to them. The impression was gained that these pericaruncular distal portions of the glands were often affected. In some of them, as a reaction to the

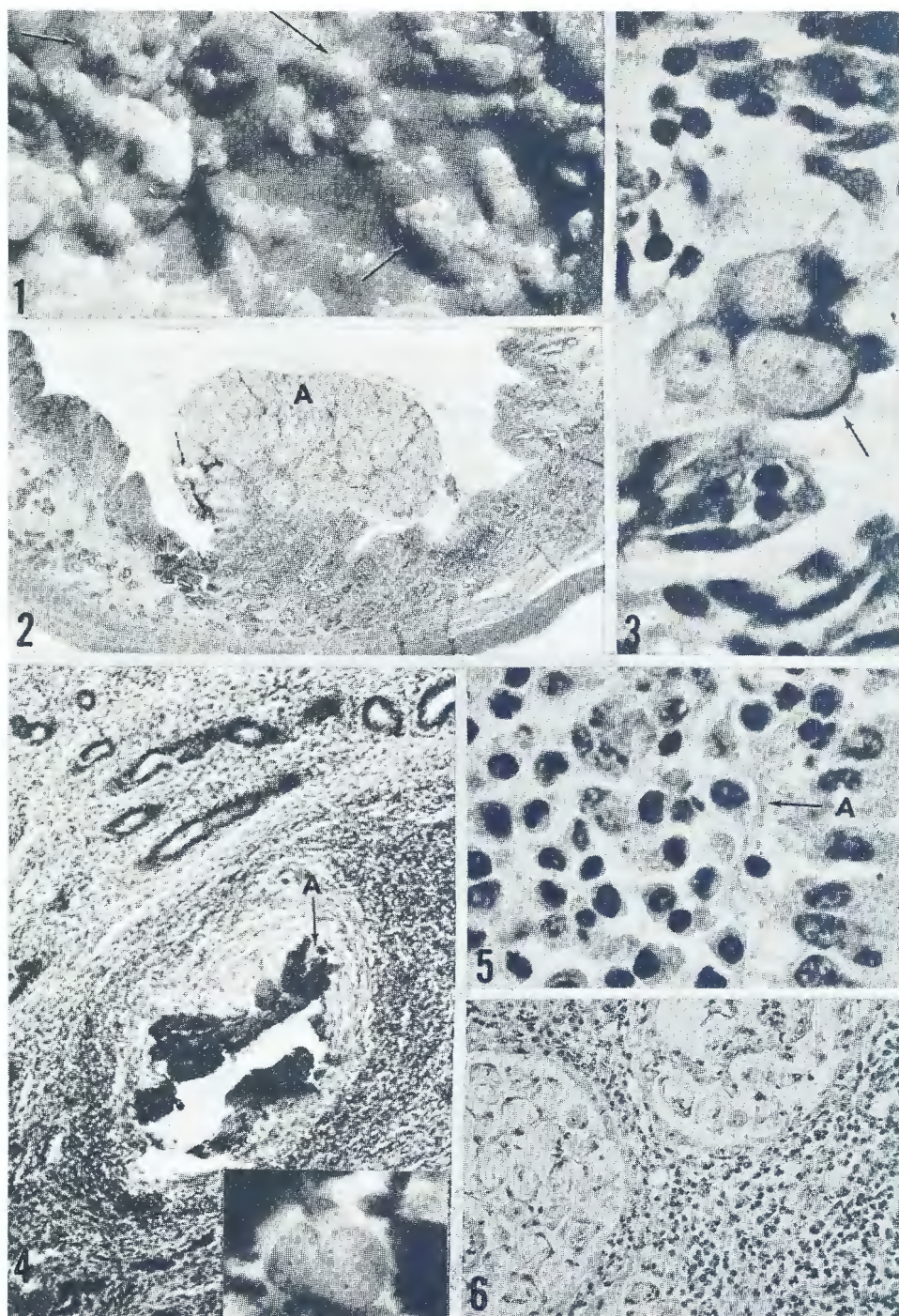


PLATE 1 1. Macroscopic appearance of parasitized endometrium. Notice the multiple polypoid lesions (arrows) of various shapes. 2. Heavily parasitized polypoid area (A). HE. $\times 12$. 3. Parasitized intercaruncular epithelium. Notice the group of three macrogametes (arrow) within tall columnar epithelium. Normal epithelium may be seen above and below the macrogametes. HE. $\times 750$. 4. Photomicrograph of mineralized granuloma in lamina propria of endometrium. With high magnification an oocyst was recognized at the point of the arrow (A) (see insert, $\times 750$.) HE. $\times 75$. 5. Infiltrate in lamina propria of intercaruncular endometrium. A mixture of eosinophils and lymphoid cells predominates. Notice poorly stained, but prominent basement membrane (arrow). HE. $\times 750$. 6. Two uterine glands containing oocysts and surrounding densely infiltrated lamina propria. HE. $\times 300$.

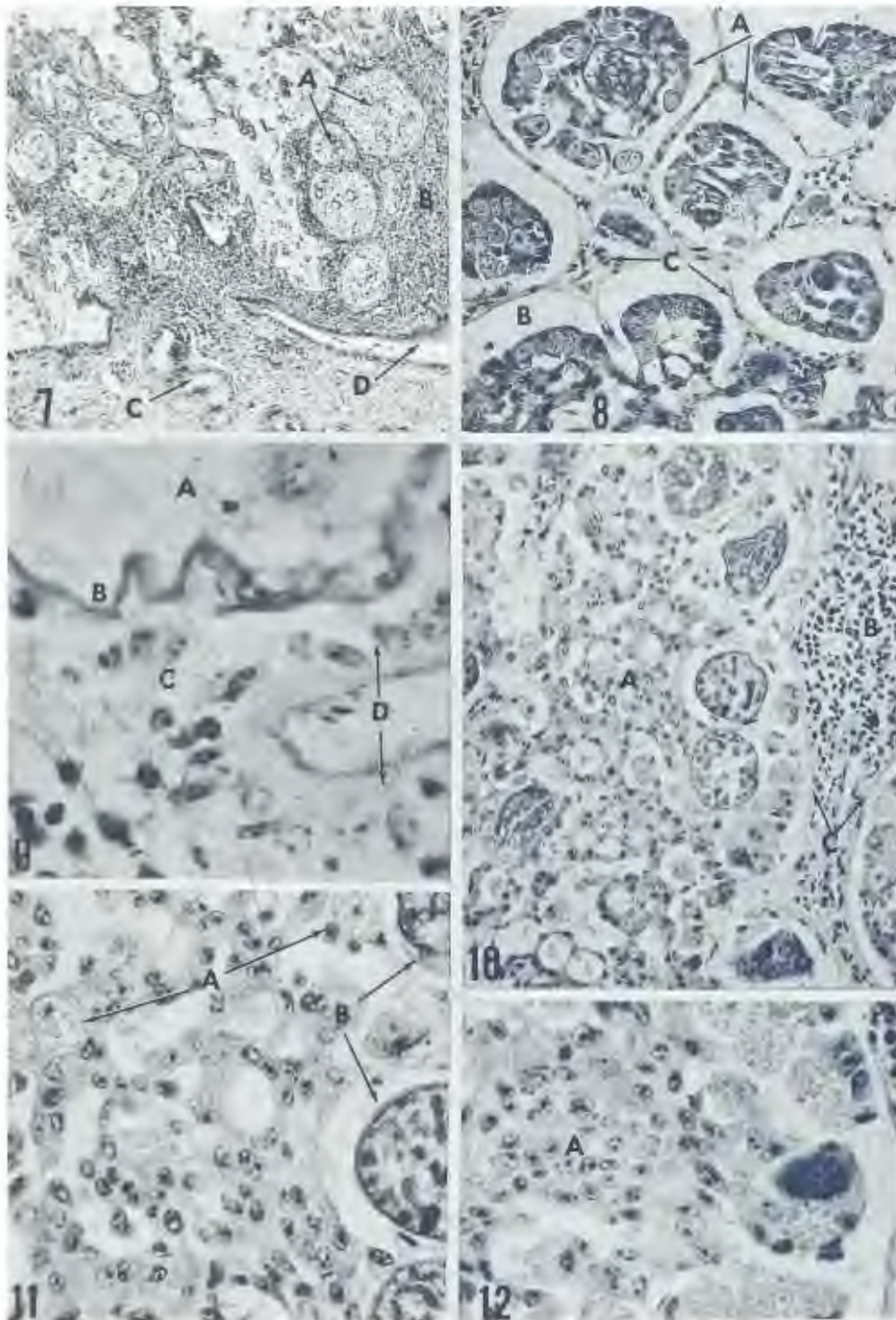


PLATE 2 7. An intercaruncular area of endometrium showing a microgametocyte (A) and the columnar epithelium (B). HE. $\times 750$. 8. Lumen and epithelium (A) of a uterine gland. Notice the inflammatory cells and an oocyst (O) in the lumen. HE. $\times 300$. 9. Junction between intercaruncular surface epithelium (A) and pericaruncular epithelium. Notice the numerous oocysts in the distal portion of a uterine gland in a pericaruncular site (O) and a few oocysts in the lamina propria. HE. $\times 150$. 10. Granulomatous reaction to oocysts in lamina propria of uterus. HE. $\times 150$. 11. Affected uterine gland. Notice the normal epithelium (A), transition to a syncytium (B) and parasitized cells (C). HE. $\times 300$. 12. Multinucleated giant cell in the vicinity of a ruptured basement membrane. HE. $\times 750$.

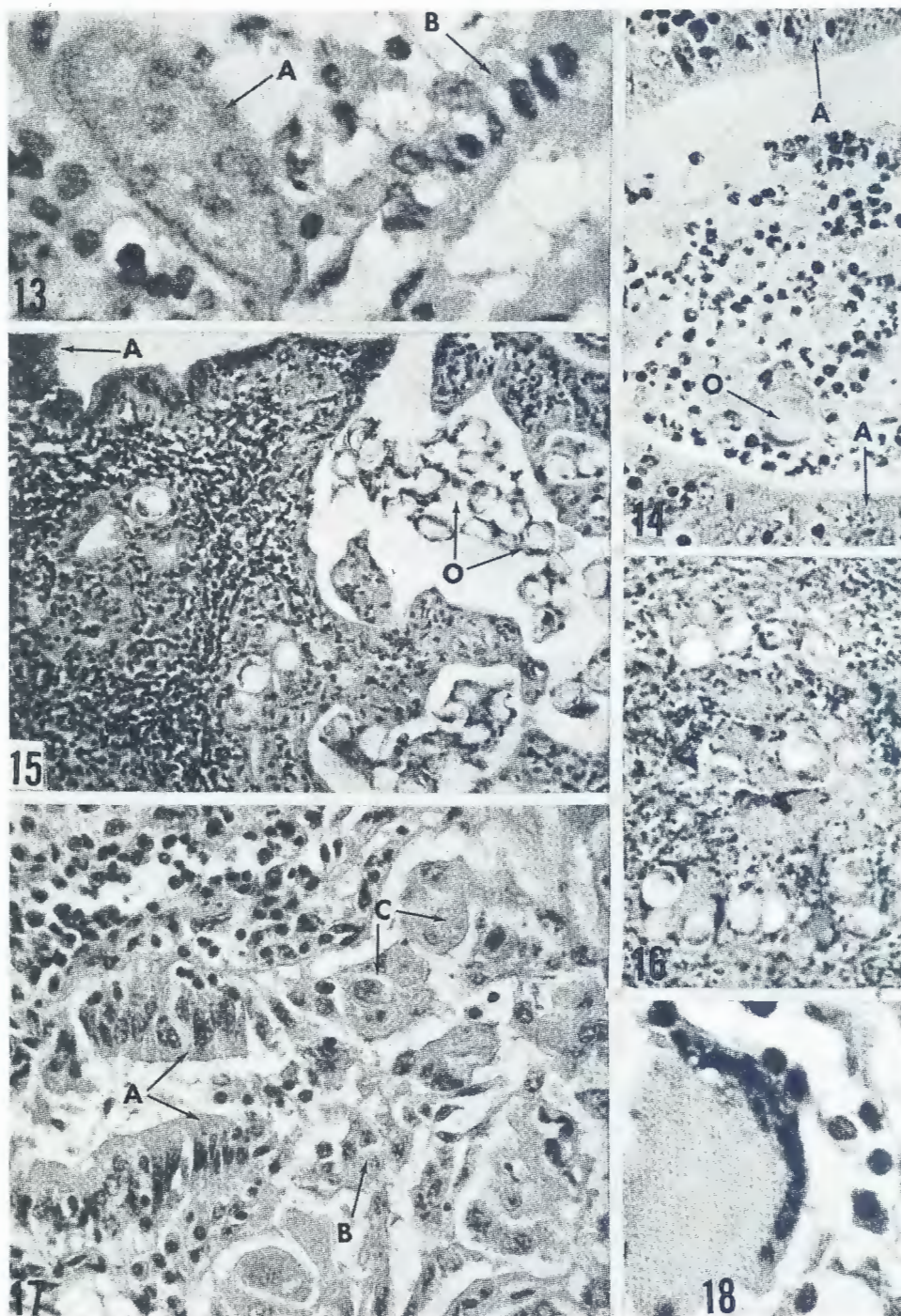


PLATE 3 13. Parasitized distal portions of the uterine glands (A). Notice the densely infiltrated lamina propria (B), the underlying unparasitized proximal portions of the uterine glands (C), the tall columnar surface epithelium (D). HE. $\times 30$. 14. Multiple uterine glands in cross section containing heavily parasitized syncytia (A). Spaces (B) between syncytia and the lamina propria (C) are artefacts. HE. $\times 150$. 15. Parasitized uterine gland (A) separated by a thickened basement membrane (B) from the lamina propria (C). The unparasitized epithelium of the uterine gland (D) does not have a prominent basement membrane. FSNY $\times 750$. 16. Lumen of a uterine gland filled by a syncytium (A) with various developmental stages of the coccidium within the cytoplasm. Lamina propria (B) infiltrated with lymphoid cells separated from the epithelial tissue by prominent basement membrane (C). HE $\times 150$. 17. Higher magnification of syncytium shown in (16) illustrating some macrogametes (A) and microgametocytes (B). HE $\times 300$. 18. Parasitized syncytium within the lumen of a uterine gland. Note concentration of nuclei (A) without accompanying cytoplasmic division. HE $\times 300$.

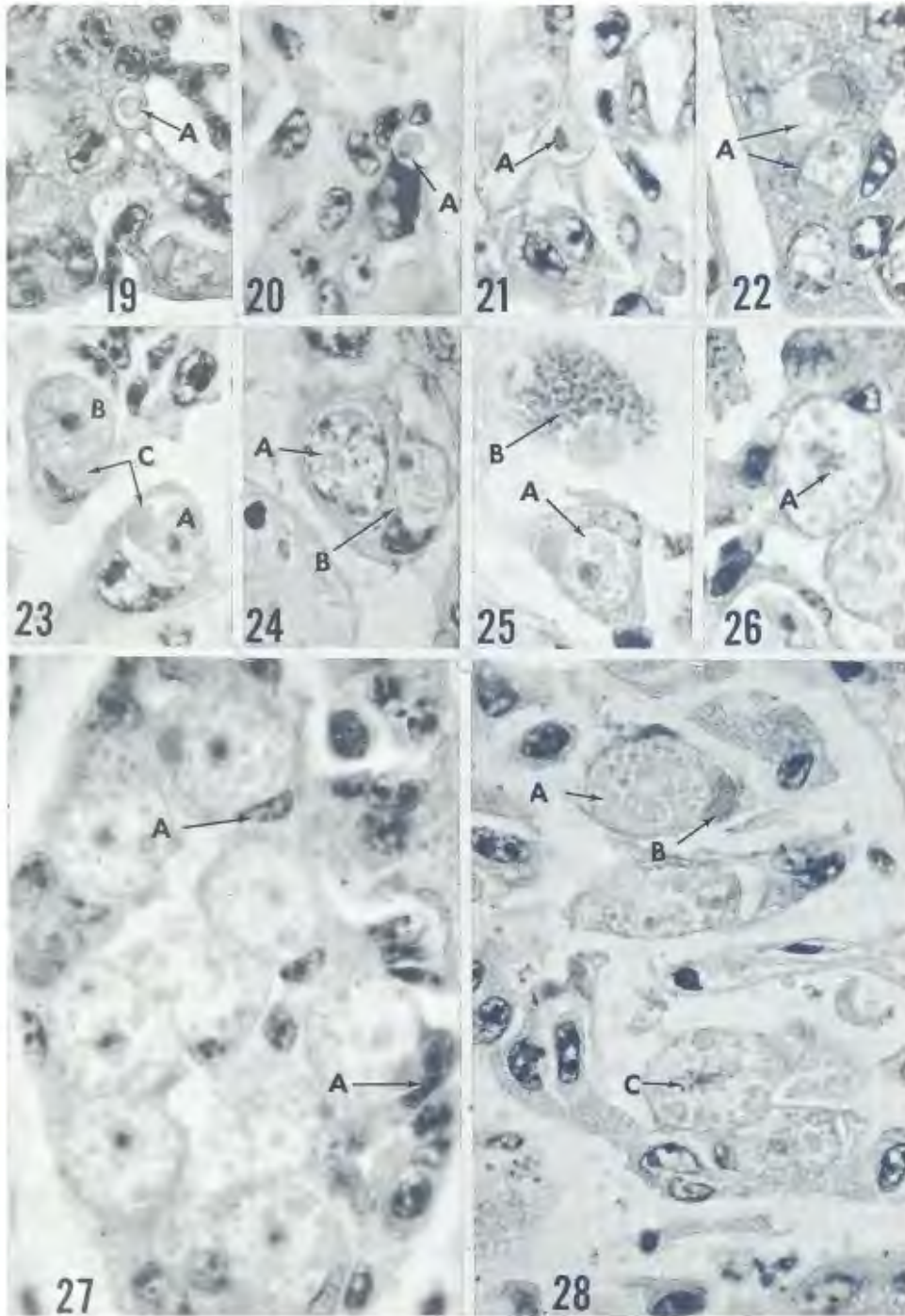


PLATE 4 Photomicrographs of gametogony. HE \times 750. 19. A single small parasitic form within a cytoplasmic vacuole (A). 20. An eosinophilic globule closely associated with nuclear chromatin (A) is apparent. 21. Cytoplasmic vacuole filled by an enlarging globule with an eccentric nucleus (A). 22. Early gametogony (A). 23. A very early (A) and a more developed macrogamete (B). Notice semilunar bodies (C) and a single nucleus in each macrogamete. 24. Early phase of a microgametocyte (A) and a macrogamete (B). Notice multiple clumps of nuclear chromatin in the microgametocyte and single nucleus of the macrogamete. 25. For comparison, a later phase in the development of a microgametocyte (top) with many nuclei (B) and the mononuclear macrogamete (bottom) with plastic globules forming in the cytoplasm (A). 26. Large macrogamete (A) with many plastic globules in the cytoplasm and multiple tubular extensions radiating from the nucleus. 27. Large group of mature macrogametes. Notice the eccentric position of some of the host nuclei (A). 28. Several mature macrogametes. Notice the size of the plastic globules (A), the semilunar body (B) and the rod-shaped objects (C) adjacent to a nucleus which has radiating tubular extensions.

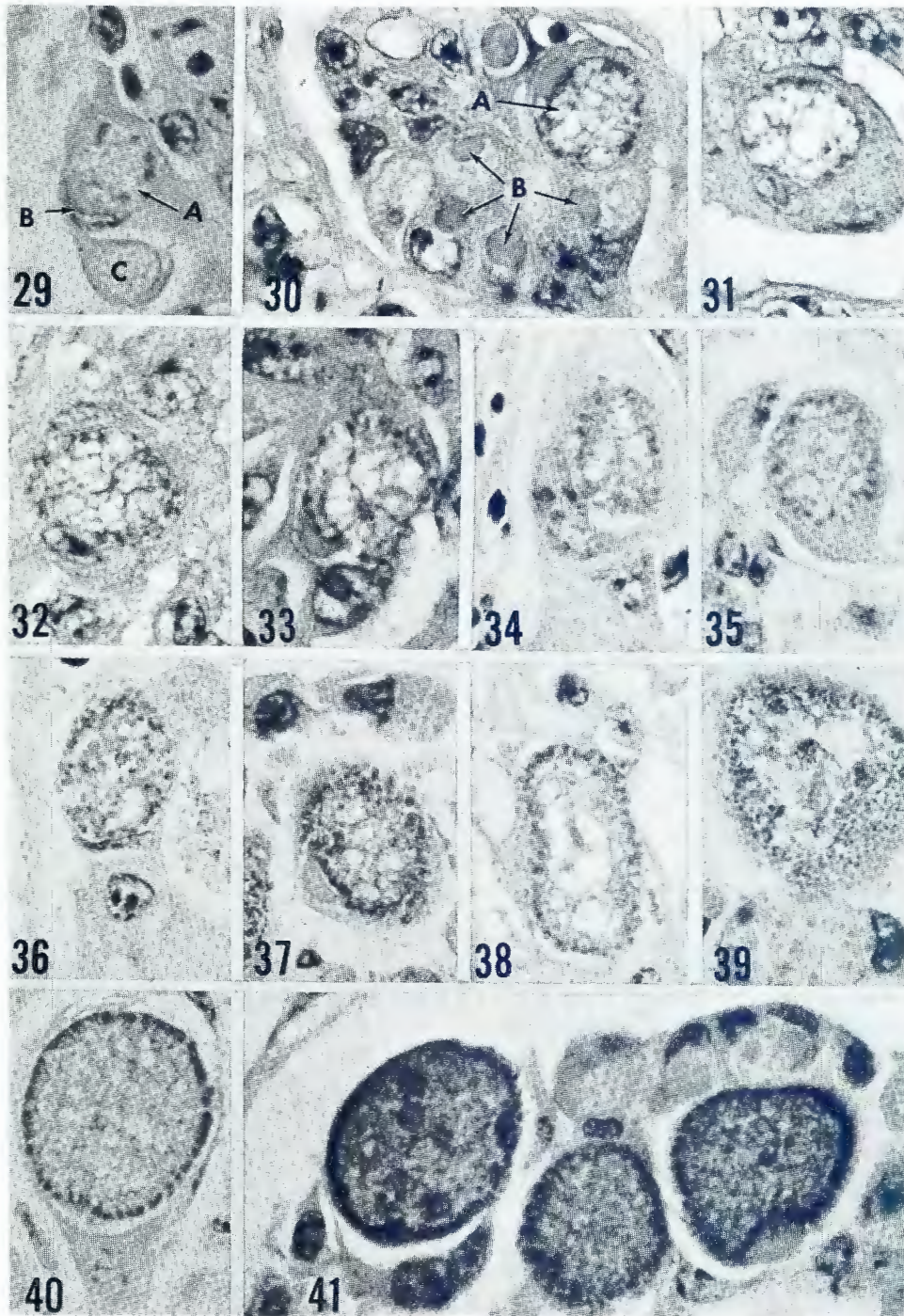


PLATE 5 Photomicrographs showing the development of microgametocytes. HE \times 750. 29. Early stage showing the reticulated appearance of the gametocyte cytoplasm (A) and multiple clumps of chromatin adjacent to the semilunar body (B). An even earlier phase is present below (C). 30. Semilunar bodies of multiple gametocytes (B) and an early phase of a microgametocyte (A). 31 to 41. Nuclei have gradually become arranged at the periphery of enlarging microgametocytes, the central portion having a foamy appearance. Notice the rosettes (39) and in the microgametocyte on the left in (41).

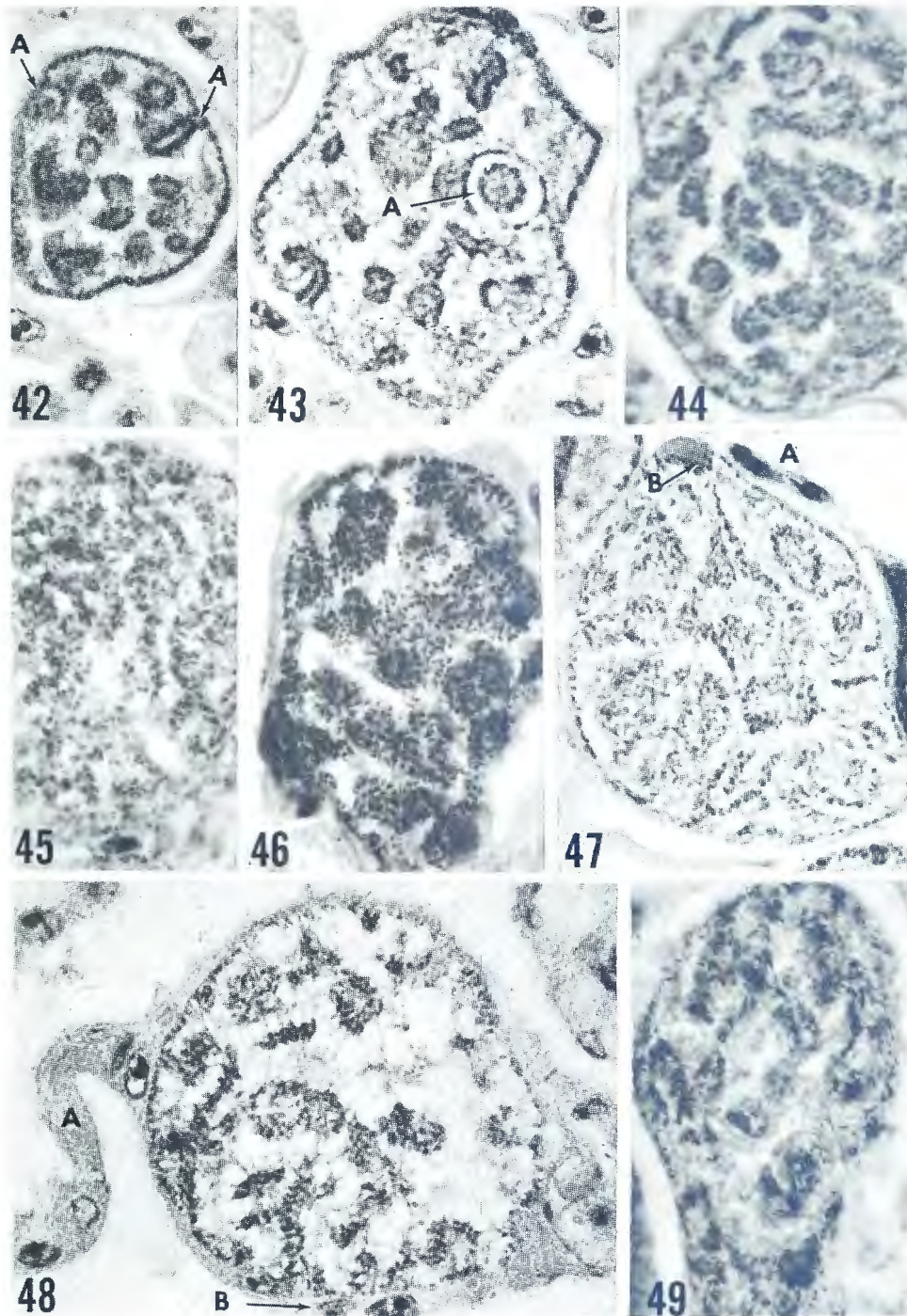


PLATE 6 Continuation of photomicrographs showing the development of the microgametocytes. HE \times 750. 42. Multiple small rosettes of nuclei in the central area. Notice that some appear to be formed by an invagination (A) of the peripheral row of nuclei. 43. Enlarged rosettes. Notice that there is a small group of nuclei (A) within the centre of one rosette. 44 to 46. Large number of nuclei in the immediate vicinity of and between the rosettes. 47. In the enlarged microgametocyte small nuclei and bits of accompanying cytoplasm become more prominent. Notice the persisting semilunar body (B) adjacent to one of the nuclei (A) of the syncytium. 48. Quite advanced phase of microgametocyte formation. Notice continuation of the syncytium to the left (A) and one of the nuclei below (B). 49. Maturing microgametocyte. Notice microgametes have developed from the many nuclei. The microgametes contain flagella and groups of them are arranged as pallsades or in vortices.

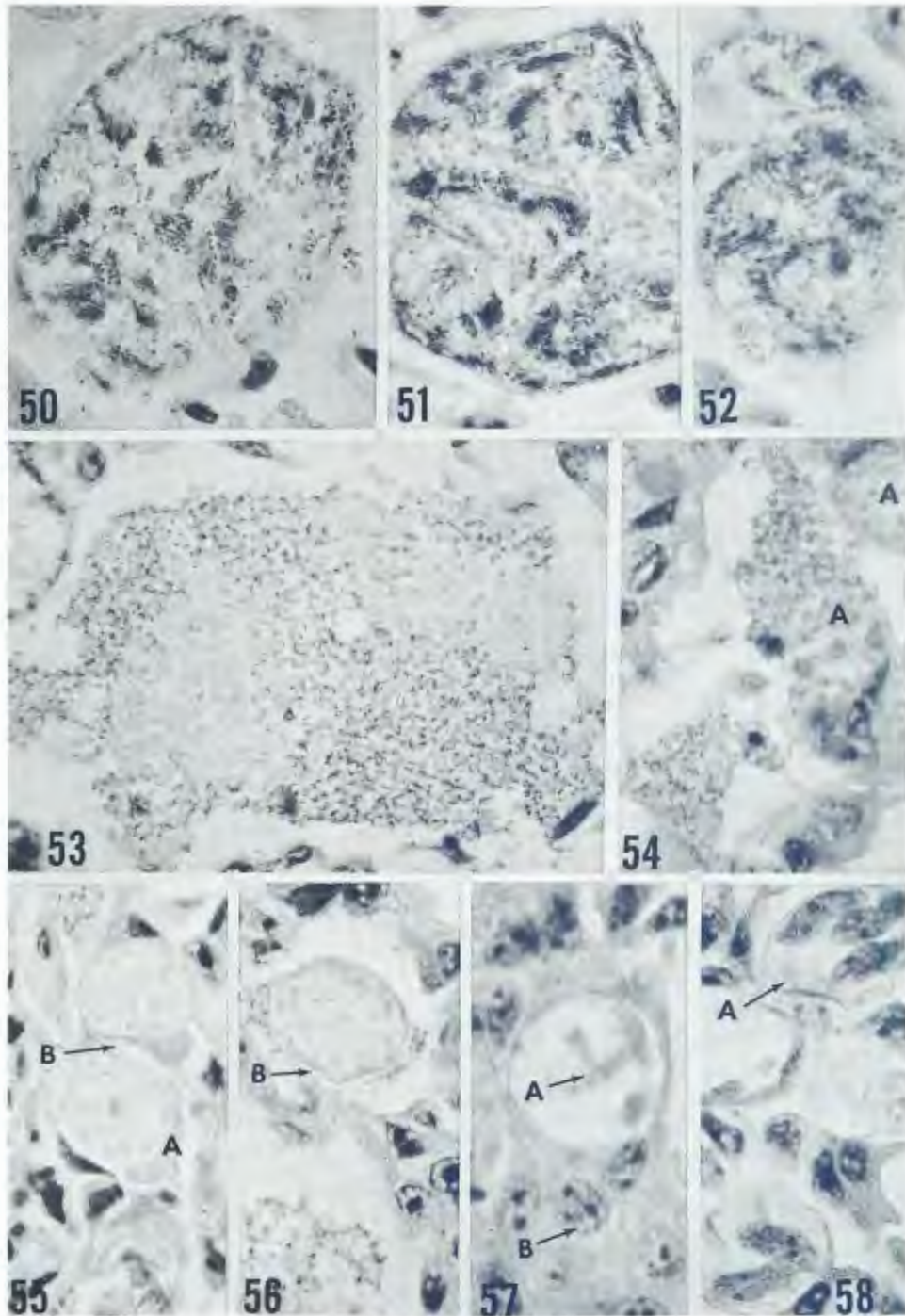


PLATE 7 Photomicrographs. HE \times 750. 50 to 52. Very late phases of microgametocytes. Notice pallisades and vortices of the microgametes. Notice also the complete dissolution of the wall on one side of the microgametocyte in (52). 53. Microgametes free in lumen of a uterine gland. The pale amorphous material was strongly PAS-positive with the appropriate stain. 54. Numerous microgametes immediately adjacent to macrogametes (A). 55. Early phase of the development of a shell. Notice the distinct, unstained zone here (B), the presence of plastic granules and the semilunar body (A). 56. Formation of a distinct shell (B) around a zygote. Note that the plastic granules, as such, have disappeared, and that the accumulation of granular (PAS-positive) material prevents the observation of inner changes. 57. Sporulated oocyst within the syncytium of a uterine gland. Notice the sporocysts (A) and the nuclei of the syncytium (B). 58. Sporulated oocysts. In the uppermost oocyst the fourth pair of sporozoites could not be photographed on the same level as the other three, but an indication of it is just visible (A).

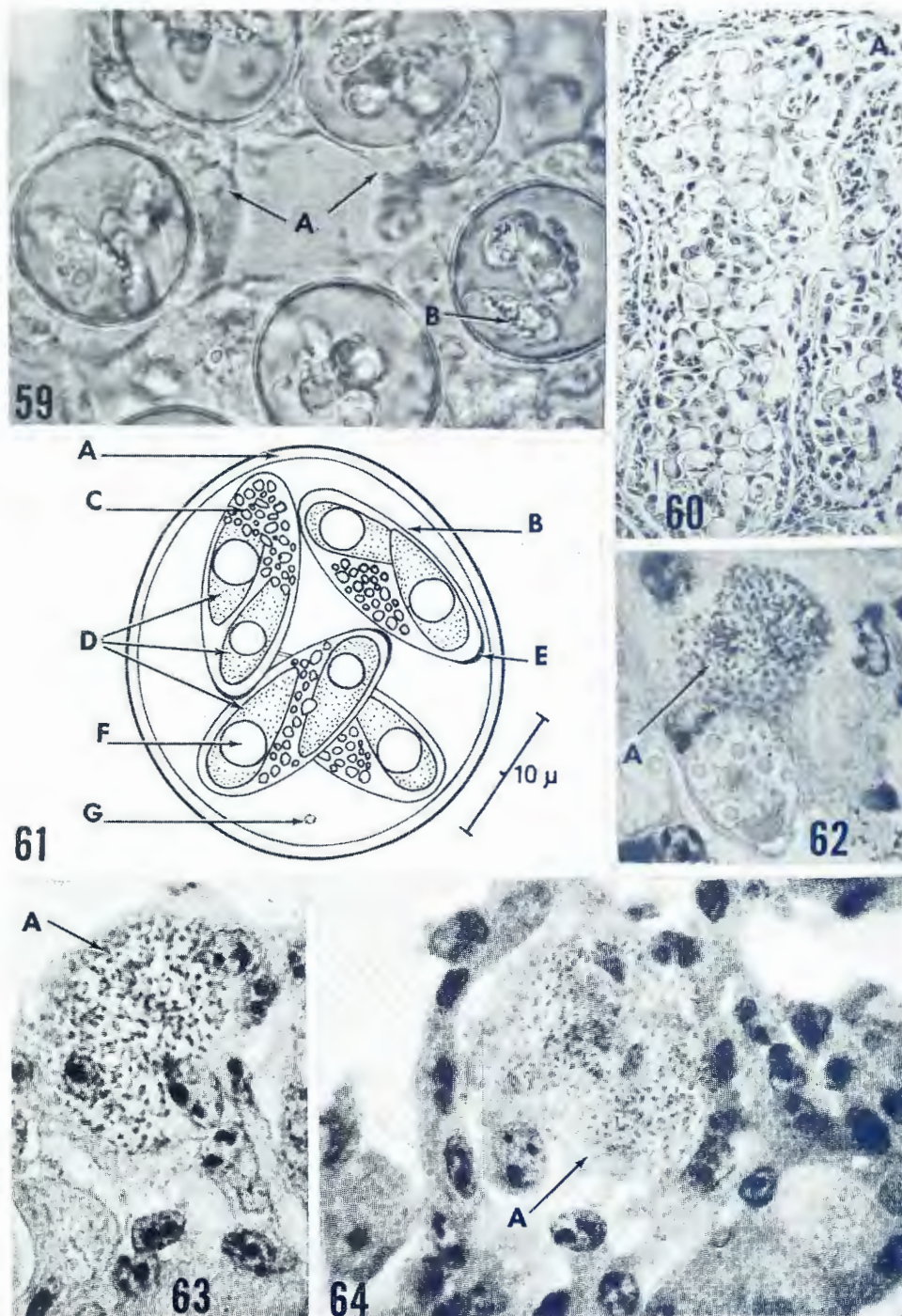


PLATE 8 59. Photomicrograph of an unstained preparation showing a group of sporulated oocysts. Notice that the oocysts are still surrounded by the cytoplasm (A) of the parasitized cells. Notice also the highly refractile granules of the residual body (B) of the sporozoite. $\times 750$. 60. Lumens of some uterine glands filled with oocysts. Notice the proximity of one to the surface at (A). HE $\times 75$. 61. Drawing of sporulated oocyst. A. Oocyst wall; B. Sporocyst; C. Sporocystic residual body; D. Sporozoites; E. Stieda body; F. Refractile globule; G. Polar body. 62 to 64. Photomicrographs of some of the parasitic forms (arrows) about which there was some question as to specific identity. Both schizonts and microgametocytes were considered. The individual organisms in (64) resemble piroplasms very closely because of their shape and may be merozoites. HE $\times 750$.

parasitization, the epithelium was markedly altered and in some places syncytial proliferations had occurred. The basement membrane of the gland, normally rather poorly developed, became quite prominent.

In the placentomes of a number of wild and domestic ruminants the maternal or placental crypts are lined to various degrees by epithelial syncytia. The syncytia have been proved to be of trophoblastic origin in some species, e.g. the sheep (Assheton, 1906; Wimsatt, 1950). In Baker's roan antelope (*Hippotragus equinus bakeri* Heuglin, 1863 = *Hippotragus bakeri* Heuglin, 1863) the syncytia have been shown by Sedlaczek (according to Wimsatt, 1950) to be maternal. Wimsatt (1950) moreover stated: "It is obviously important to determine the origin of the syncytium in every species of ruminant which possesses one".

As we have made limited observations on the placentomes at different stages of pregnancy, we are not prepared to state definitely that the syncytia are of trophoblastic origin although they appear to be. The characteristic binucleate cells (diplo-karyocytes) which have been extensively studied in ruminants (Wimsatt, 1950) are present in the placentomes of the impala. The overall pattern of the papillomatoid lesions led us to consider two possibilities. The first was that the uterine glands, especially their distal portions and openings in the pericaruncular areas, become parasitized and under the influence of the parasite the epithelium forms syncytia and the basement membranes thicken. The second possibility was that the parasitized syncytia represent proliferations from rests of trophoblastic epithelium remaining in maternal crypts after termination of pregnancy. There was some similarity in appearance between the distal portions of the uterine glands containing the syncytial proliferation of the epithelium and the caruncular portion of a ruminant placentome. Some ruminants apparently lack a distinct basement membrane between the maternal septa and maternal crypt of the placentome viz. the sheep, but others, such as the fallow deer [*Dama dama* (Linnaeus, 1758)] have a prominent one (Hamilton, Harrison & Young, 1960). Our observations on placentomes of the impala indicate that at no time during gestation is there a prominent basement membrane between the maternal crypts and septa. This was one of the findings which convinced us that the parasitized cells were uterine epithelium which had become abnormally hyperplastic and in some places formed syncytia.

As there were no cases of pregnant animals with involvement of the uterine glands the possibility of detecting parasitism of the chorionic epithelium in such a case was not afforded to us. Should chorionic involvement occur, the shedding of the placenta at birth would be one way of disseminating the oocysts. Another possibility of transmission is coitus though we have no direct evidence that supports it.

In the most severely affected specimen, the host response was a cellular infiltrate composed of lymphocytes, plasma cells and eosinophiles. In one specimen some of the basement membranes were occasionally completely destroyed. In these foci there were granulomatous reactions with multinucleated giant cells. In the endometrial stroma the calcified granulomas represented foreign-body reactions to oocysts.

The incidence of significant changes due to this parasitism appears to be very low. The presence of oocysts in small calcified foci of a number of pregnant impala may be an indication of a higher incidence of a very benign form of the disease. Although serious deleterious effects

on reproduction are thought to be doubtful, this aspect requires further investigation.

This is believed to be a previously unknown entity due to a new species of the genus *Eimeria* for which the authors propose the name *Eimeria neitzzi* in honour of Dr. W. O. Neitz a protozoologist of reknown and a world authority on tick-borne diseases. In addition to his many contributions to protozoology, he has also contributed significantly to the advancement of virology and rickettsiology and through his active interest in diseases of game animals he has furthered progress in this field of veterinary concern as well.

SUMMARY

Four cases of uterine coccidiosis, a previously unknown entity of impalas, are described. It was determined that the parasite belongs to the genus *Eimeria* and the authors propose the name *E. neitzzi* for it. This parasite apparently inhabits mainly the distal portions of the uterine glands, especially those that open pericaruncularly, and also the adjacent surface epithelium. Stages of sexual reproduction of the coccidium are described and illustrated with photomicrographs. One of the characteristic features of *E. neitzzi* is the occurrence of sporogony while the oocysts are still in the host cells. Overt disease from infection with this interesting coccidium appears to have a very low incidence. It is not considered on the basis of present knowledge that the disease in the majority of infected ewes has any serious deleterious effects on reproduction, except perhaps in individual animals.

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