THE HOEPPLI PHENOMENON IN SCHISTOSOMIASIS

II. Histochemistry

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The Hoeppli phenomenon[1] is an eosinophilic hyaline fringe which occasionally surrounds schistosome eggs entrapped in granulomas or "pseudotubercles" (Fig. 1). It resembles the precipitates observed in some cases of sporotrichosis, phycomycosis, and filariasis.[2,3]

Our earlier study and literature review[4] revealed that Hoeppli precipitates encircle a small proportion of eggs in specific host-parasite combinations and are associated with high egg burdens, most frequently encountered in early stages of bilharzial infection. Immunofluorescent studies showed egg antigen and fixed host globulin in these precipitates; antigen attained highest concentrations in areas adjacent to the eggshell while antibody predominated in the periphery of the Hoeppli fringe.[4] Within the egg itself, the miracidial cephalic glands stained for antigen.[5] This evidence convinced us that the Hoeppli phenomenon is an in-vivo antigen-antibody precipitate arising when certain immunologically critical conditions occur.

Little is known of the composition of this immune precipitate, and some of the earlier information[6-8] is conflicting. Sawada *et al.*[8] showed that both the schistosome eggshell and the Hoeppli phenomenon contain periodic acid-Schiff (PAS) positive material resistant to saliva and hyaluronidase and do not react with Reinhart's colloidal iron. They regarded the eggshell and the "flame-like" substance adjacent to it as identical and composed of a polysaccharide other than glycogen. Andrade and Barka[6] found abundant mucoproteins, acid phosphatase, aminopeptidase, and nonspecific esterase in the miracidial cephalic glands. Coutinho, Magalhaes Filho, and Jampolsky[7] concluded, mainly on the basis of PAS and phosphotungstic acid-hematoxylin (PTAH) positivity, that the perioval deposit was fibrinoid material resulting from the interaction of egg antigen and host antibody. In this study we have utilized a variety of established histochemical techniques to analyze the composition of the Hoeppli phenomenon.[9-12]

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Materials and Methods

Hoeppli phenomena were produced in the colons of 3 baboons, *Papio anubis,* and in the livers of 14 multimammate rats, *Mastomys coucha.* The baboons were infected with 1000 or more cercariae of *Schistosoma mansoni* and sacrificed 7 months later (by Dr. E. Sadun and collaborators at Walter Reed Army Institute of Research), as previously described.[13] The multimammate rats were infected with 400 or more cercariae of the Puerto Rican strain of *Schistosoma mansoni* (supplied by Drs. E. Chernin and C. T. Pan, Harvard School of Public Health) by intraperitoneal injection in our laboratory; the livers were collected after 11–12 weeks of infection, as previously described.[4]

Tissues with many grossly discernible pseudotubercles were fixed in 10% neutral buffered formalin or Baker's formol-calcium, and embedded in paraffin, or were quick-frozen and stored at −20° C. They were serially sectioned at 4–12 μ, as prescribed by each of the staining protocols used. Every fifth section was stained with hematoxylin and eosin and screened for Hoeppli phenomena. Unstained sections interposed between sample sections containing 3 or more Hoeppli phenomena were used for histochemical procedures. The reactions, their reference source, specificity, and the blocking reactions employed are listed in Table I.

Each technique was performed at least twice on each tissue, with strict adherence to the published staining methods; appropriate positive and negative tissue controls were run concurrently. The tinctorial affinities were recorded separately for the inner and outer zones of the Hoeppli phenomenon and 8 additional structures of the miracidium and eggshell defined in detail below. The color intensity of each of these structures was graded on a scale of 0 (unstained sections, negative tissue controls) to 4+ (positive tissue controls), reserving the abbreviation t (trace) for very weak or dubious staining (Table I).

Selected photomicrographs were taken using high-speed Ektachrome B and ADOX KB 14 film, and the Zeiss photomicroscope equipped with apochromatic objectives.

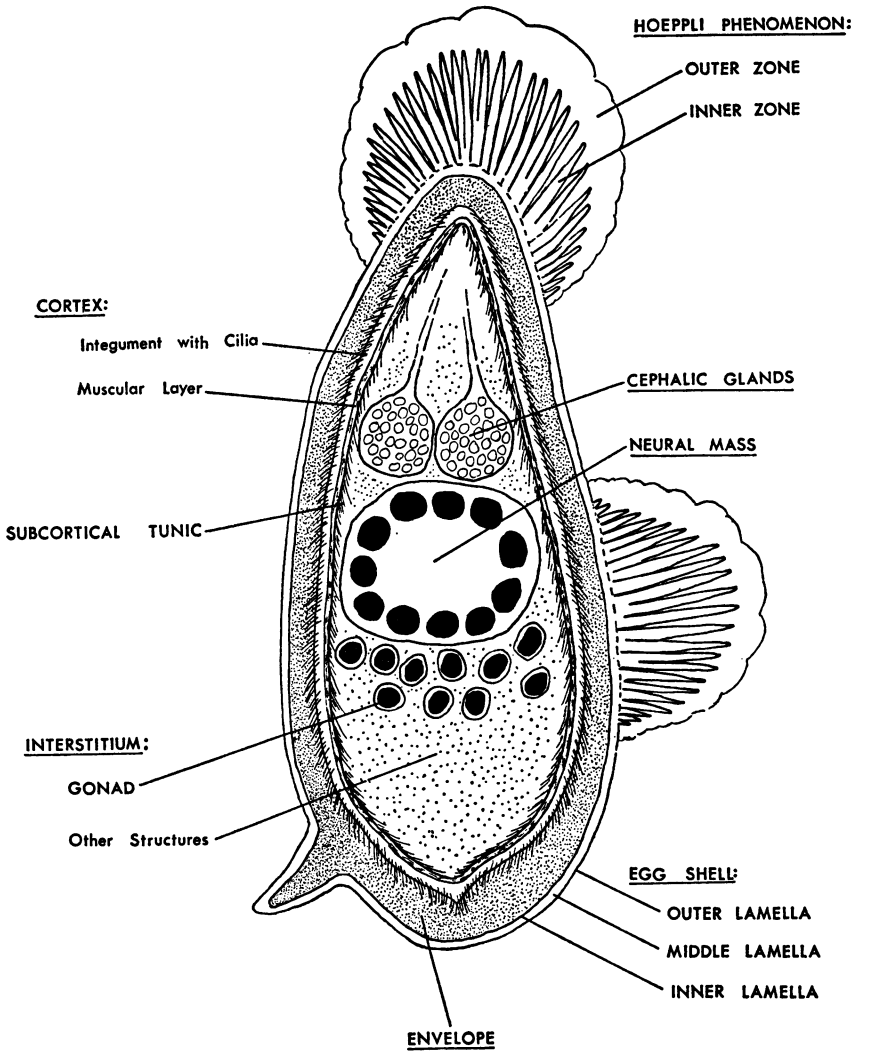
Results

The results will be presented in 3 sections: an outline of the morphology of mature schistosome eggs and Hoeppli precipitates, defining the structures investigated; the tabulated results of the histochemical reactions; and finally, a synthesis of the probable composition of each structure studied.

Morphology

As shown previously,[4] Hoeppli phenomena do not occur in association with immature miracidia, and they undergo progressive deterioration as the embryos disintegrate and pseudotubercules involute. Furthermore, many fine structural details of the embryos cannot be visualized without phase or electron microscopy,[14–16] and are frequently distorted or absent in individual histologic sections. For these reasons, the structures are schematically represented in Text-fig. 1 for systematic observation and reference to the photomicrographs, especially Fig. 2.

The neural mass, a single large cell with numerous peripheral nuclei, occupies the center of the mature embryo. Anteriorly, the cephalic



TEXT-FIG. 1. Schematic representation of *Schistosoma mansoni* eggs with mature embryo and Hoeppli phenomenon.

glands appear as paired globules occasionally displaying small excretory ductules. The miracidial esophagus and gut are seldom visible in histochemical preparations. The interstitium refers to the remainder of the somatic mass, including the gonads, flame cells, and other specialized structures. The miracidial cortex consists of an enveloping integument, a syncytial cytoplasmic layer or "symplasium," which bears the cilia, and a subjacent muscle-cell layer.[16] Beneath the cortex lies the clear-cell subcortical tunic. A viscid fluid miracidial envelope occupies the space between the miracidium and eggshell. The trilaminate eggshell possesses thin inner and outer layers and a broad, refringent middle layer.

At its culmination, the Hoeppli phenomenon shows prominent zonation: Its inner zone consists of spicules perpendicularly radiating from the outer eggshell lamina. The outer zone is a homogeneous and softly contoured material circumscribing the inner corona and interposed between it and the remainder of the pseudotubercle. Comparison of the earlier (11-week) stages in the *Mastomys* with the later (7-month) stages in the baboon establishes that the outer zone appears earliest as a narrow fringe; subsequently the inner zone arises and becomes the larger of the two at maturity. In involuting granulomas, the Hoeppli phenomena progressively shrink, lose zonation, and increase their density (suggesting condensation and homogenization), and eventually disappear as described earlier.[4] Thus, variations from the diagram in Text-fig. 1 are common.

Histochemical Results

The reactivity of the structures described above to the different histochemical procedures is shown in Table I.

Synthesis of Composition

The Hoeppli phenomenon contains proteins, diastase-resistant PAS-positive (Fig. 15) and acid-fast (Fig. 16) material, plus neutral (Fig. 6) and acid lipids (Fig. 8). Calcium is present in small amounts, increasing in older Hoeppli phenomena. Nucleic acids, acid mucopolysaccharides (Fig. 14), glycogen, and lipofuscin are conspicuously absent. Phospholipid (Fig. 7), neutral lipid (Fig. 6), choline-containing lipid, calcium, nonspecific protein (Fig. 1 and 4), and tyrosine are uniformly distributed. Arginine is present in trace amounts. Other components form distinct gradients, predominating in one of the two layers. Sulfhydryl groups—cystine and cysteine—(Fig. 10–12), acid lipid (Fig. 8), acid-fast (Fig. 16), green autofluorescent (Fig. 3), and diastase-resistant PAS-positive (Fig. 15) materials decrease in concentration from the inner to the outer zone. Conversely, tryptophan (Fig. 13) and Biebrich scarlet (a nonspecific histologic stain; Fig. 9) increase. Correlated with the morphologic aging spectrum of the Hoeppli phenomenon, two histochemical variants were observed besides the fully developed, zoned precipitate (Fig. 13): An early pattern displayed a narrow scalloped rim of tryptophan outer-zone material (Fig. 5) with acid lipid-rich substance; a senescent pattern (Fig. 15 and 16) showed a dense amorphous material, with blurred zonation, increased protein concentration and increased calcium. Many substances found in the Hoeppli inner zone are also present in the eggshell—i.e., various proteins, diastase-resistant PAS-positive acid-fast material, various lipids, and calcium. The components cited as absent from the fringe are not present in the eggshell.

Protein is conspicuously less concentrated in the eggshell than in the Hoeppli phenomenon, and specific amino-acid reactions show low intensity and display no differential distribution. Similarly, neutral fat is meager, and acid lipid, moderate. The major eggshell component is a diastase-resistant PAS-positive (Fig. 15), acid-fast (Fig. 16) substance with a strong orange autofluorescence (Fig. 3), in contrast to the bright green of the Hoeppli phenomenon inner zone, and is optically refringent; smaller amounts of protein and acid lipid are admixed with it and are concentrated along its surface in the inner and more especially outer lamella.

The miracidial envelope has a unique composition in the egg, having abundant acid mucopolysaccharide (Fig. 14) and some protein and glycogen, but all other substances assayed could not be detected. The mature miracidium has no acid mucopolysaccharide, but the interstitium of the immature miracidium abounds in this substance.

The miracidium as a whole contains ample protein (Fig. 4); its nuclei, alone, are Feulgen-positive. Some PAS-positive material, especially in the subcortical tunic, digests with diastase, indicating glycogen. Although some embryonic organelles have acid-fast and diastase-resistant PAS-positive material, none possess autofluorescence. The cephalic glands—the most distinctive of the organelles—exclusively display lipofuscin, and possess choline-containing lipid and phospholipid absent elsewhere in the miracidium but present in the Hoeppli phenomenon. Similarly, protein reaches a density comparable only to the Hoeppli fringe. A high concentration of sulfhydryl groups (Fig. 10) appears only here and in the Hoeppli inner zone. Interestingly, tryptophan (Fig. 13) seen in the Hoeppli outer zone also abounds in the cephalic glands (Fig. 5). While these are rich in diastase-resistant PAS-positive material, they lack acid fastness, autofluorescence, acid mucopolysaccharide, and neutral fat. The neural mass (Fig. 2) has ample protein, acid lipid, and acid-fast material. The interstitium is protein-poor relative to the previous two structures, contains both glycogen and some diastase-resistant PAS-positive material, neutral lipid, acid lipid, and some acid-fast material. A detailed analysis of the complex miracidial cortex will not be undertaken here; it contains protein with demonstrable sulfhydryl groups (Fig. 10), neutral and acid lipid, and some glycogen.

Discussion

This discussion is limited to findings concerning the composition and pathogenesis of the Hoeppli phenomenon.

The predominantly antigenic [4] inner zone of the Hoeppli phenomenon shares the properties of diastase-resistant PAS positivity, acid-fastness, autofluorescence, and optical refringence with the eggshell; this com-

TABLE I

STAINING PROCEDURES AND HISTOCHEMICAL REACTIONS

Histochemical procedures	*Hoeppli phenomenon*		*Eggshell*			*Miracidium (mature embryo)*				
	Inner zone	*Outer zone*	*Inner*	*Middle*	*Outer*	*Envelope*	*Cortex*	*Cephalic gland*	*Nerve mass*	*Interstitium*
Conventional stains										
Hematoxylin & eosin [10]	2E	4E	4E	E	4E	o	2E	2E	2B	B&E
Masson's trichrome [10]	3S	4S	3A	2S	3S	1A	3S	2A	2S	A&S
Verhoeff's elastic tissue [10]	o	o	2	o	2	o	2	o	1	o
Wilder's reticulum [10]	o	o	4	2	4	o	o	o	2	o
Phosphotungstic acid-hematoxylin [10]	2	2	4	o	4	o	o	o	2	o
Examination for autofluorescence [8]	g	o	or	or	or	o	o	o	o	o
Nonspecific protein reactions										
Performic acid-Schiff [13]	t	t	1	1	1	o	o	o	o	o
Coupled tetrazonium [11,12]	3	3	2	1	2	1	1	2	3	2
Bromphenol blue [11]	3	3	1	2	2	1	o	2	1	o
Indole group reactions										
Dimethylaminobenzaldehyde nitrite [†9]	2	3	1	1	1	o	o	2	o	o
Dimethylaminobenzaldehyde nitrite [†11,12]	2	4	1	1	1	o	o	2	o	o
Postcoupled benzylidene [†13]	2	3	1	1	1	o	o	3	o	o
Xanthydrol [†13]	1	2	t	t	t	o	o	1	o	o
Rosindole [†13]	t	1	o	o	o	o	o	o	o	o
Naphthyl ethylenediamine [†13]	t	t	o	o	o	o	o	o	o	o
Tyrosine reactions										
Diazotization reaction [11,13]	1	1	t	t	1	o	1	1	t	t
Diazotization reaction with tryptophan & sulfhydryl blocks	o	o	o	o	t	o	t	t	o	o
Dinitrofluorobenzene [11,13]	3	3	2	1	2	1	3	1	2	2
Dinitrofluorobenzene with tryptophan & sulfhydryl blocks	1	1	1	o	1	o	t	t	1	1
Sulfhydryl group reactions										
Red sulfhydryl (mercury orange) [†11,13]	1	1	t	t	t	o	t	1	o	t
Dihydroxy-dinaphthyl-disulfide [13] (D-D-D)	3	2	1	o	o	o	2	3	3	2
D-D-D with thioglycollic-acid pretreatment	4	3	1	o	o	o	2	3	3	3
D-D-D with mercaptide	1	1	o	o	o	o	t	1	1	1

D-D-D with iodoacetate	o	o	o	o	o	o	o	o	o	o
D-D-D with iodoacetate & potassium cyanide	2	1	t	o	o	o	t	2	1	1
Performic acid-alcian blue [12]	1	o	o	o	o	t	o	1	o	o
Alkaline tetrazolium [11,12]	4	3	1	o	1	o	3	2	3	2
Arginine reaction (Sakaguchi reaction [13])	t	o	1	1	1	o	t	t	t	t
Nucleic acids (Feulgen reaction [10,12])	o	o	o	o	o	o	o		Nuclei	
Polysaccharides										
Periodic acid-Schiff [10]	3	2	4	4	4	3	2	3	t	2
Periodic acid-Schiff with diastase [10]	3	2	4	4	4	1	1	3	o	1
Alcian blue for acid mucopolysaccharides [11]	o	o	o	o	o	1	o	o	o	o
Hale's colloidal iron [10]	o	o	o	o	o	3	o	o	o	o
Toluidine blue for metachromasia [11,12]	o	o	o	o	o	1	o	o	o	o
Cresyl echt violet for metachromasia [11]	o	o	o	o	o	2	o	o	o	o
Lipids										
Neutral										
Oil red O [10]	1	1	1	1	1	o	o	o	o	o
Sudan black B in propylene glycol [12]	2	2	t	o	o	o	t	o	2	1
Sudan black B in ethanol (70%) [12]	1	1	o	o	o	o	o	o	1	o
Acid										
Nile blue sulfate for acid lipid §[11,12]	1	1	o	o	o	o	1	o	1	t
Baker's acid hematin §[12]	3	o	2	o	1	o	o	o	o	o
Phospholipids										
Plasmal reaction [12]	o	o	o	o	o	o	o	o	o	o
Copper phthalocyanin (Luxol fast blue) [12]	2	2	o	o	o	o	o	1	o	t
Nile blue sulfate for phospholipid [12]	t	t	o	o	o	o	o	o	o	o
Choline-containing lipids (phosphomolybdic acid [12])	1	1	o	o	o	o	o	1	o	o
Acid-fast stain (Ziehl-Neelsen [10])	3	1	4	4	4	o	o	o	2	o
Lipofuscin (Schmorl's stain [12])	o	o	o	o	o	o	o	o	2	o
Calcium (alizarin red S [12])	1	1	t	t	t	o	t	t	t	o

Reactions graded o to 4+, t indicating trace. B indicates basophilic; E, eosinophilic; A, aniline blue; S, Biebrich scarlet; or, orange; g, green.

* With nitrous acid, benzoylation, performic acid, and iodoacetate blocking.

† With benzoylation and performic acid blocking.

‡ With mercaptide, iodoacetate, thioglycollic acid, and iodoacetate-potassium cyanide pretreatment.

§ With and without pyridine extraction.

bination of characteristics is not found elsewhere in the schistosome egg. Therefore, the major component of the eggshell or its decomposition product forms part of the Hoeppli inner zone. This eggshell material has not been precisely characterized chemically,[17] but remains unaltered upon immersion in weak acids, strong bases, detergents, and lipid solvents in vitro; [18] therefore, when it becomes part of the Hoeppli inner zone, its decomposition must be catalyzed.

In addition to eggshell substance, the inner zone abounds in protein which is sparse in the eggshell. The only structure which approximates the rich endowments of cystine and cysteine, tryptophan, phospholipid, and choline-containing lipid of the Hoeppli inner zone is the miracidial cephalic gland. This organelle differs only in that it lacks eggshell material and contains lipofuscin. Immunofluorescent studies detect antigen in both structures.

The miracidial envelope consists primarily of acid mucopolysaccharide which ostensibly does not arise from the cephalic glands but is related to the interstitium of the immature miracidium. It is doubtful that this material is important antigenically. It is also unlikely that the protein of the Hoeppli inner zone emanates from the host rather than from the miracidium, since this hypothesis would not account for the zonation of the Hoeppli phenomenon, its presence around few, rather than all eggs, its association exclusively with mature miracidia, the paucity of sulfhydryl-rich protein in the outer zone, or the presence of acid lipid in the inner zone which is absent from the cellular portion of the granuloma. Therefore, we conclude that the inner zone of the Hoeppli phenomenon originates from both the eggshell and the miracidial cephalic glands. The outer antibody-containing zone of the Hoeppli fringe [4] possesses scant eggshell substance or sulfhydryl-rich protein, but abounds in tryptophan-rich protein, which may be an integral part of antibody globulins.

Several problems remain: The histochemical reactions utilized demonstrate S-S and S-H groups other than cystine and cysteine [12] and may represent "passenger substances" rather than cephalic-gland antigen. Similarly, the methods employed cannot differentiate between tryptophan, tryptamine, 5-hydroxytryptamine, or 5-hydroxyindole acetic acid; [9,12] therefore localization of "indole" may represent deposition of mast cell products [6] in the Hoeppli outer zone or a rapidly diffusing antigen fraction accompanying the sulfhydryl-rich material of the inner zone. The compound nature of the Hoeppli inner zone implies interaction of the two antigenic moieties in the formation of the Hoeppli phenomenon. The two most plausible mechanisms are: (1) The cephalic-gland antigen may escape the egg via submicroscopic perforations [16,19]

and then form an antigen-antibody complex which results in eggshell decomposition analogous to the mechanism of immune hemolysis. (2) The diffusible antigen may contain an enzyme-catalyzing degradation of shell substance. Localization of acid phosphatase, aminopeptidase and nonspecific esterase in the cephalic glands [6] supports the latter hypothesis and suggests a natural process in the life cycle of schistosomes which facilitates the extrusion of miracidia in an aquatic environment. However, the absence of autofluorescence in circumoval precipitates formed in vitro [4] supports the first alternative. Either explanation outweighs hypotheses which require initial decomposition of eggshell substance, but further discussion without knowledge of the precise chemical nature of the eggshell is premature.

Previous work indicates that antigen release from mature schistosome eggs does not invariably lead to precipitation in vivo; in the majority of hosts antigen is partly diffused and partly sequestered in the pseudotubercle.[4,5] With adequate host sensitization, precipitation occurs, producing the Hoeppli phenomenon. Presumably, this happens soon after maturation of the embryo, after which precipitation ceases, loculating and isolating antigen centrally, while antibody excess persists peripherally. Later on, the precipitate condenses or degenerates and undergoes phagocytosis.

Summary

The pseudotubercle of *Schistosoma mansoni* was studied with a variety of histochemical procedures. The Hoeppli phenomenon displayed zonation into an outer and an inner portion. The chemical composition of both portions, the 3 eggshell lamellae, the envelope, and 5 miracidial structures was described and correlated with earlier immunofluorescent studies.

The predominantly inner antigenic zone contains diastase-resistant PAS-positive, acid-fast, autofluorescent material identical to the eggshell substance and high concentrations of sulfhydryl-disulfide-rich protein also abundant in the miracidial cephalic glands. Other components of the inner zone are also traceable to the eggshell and the cephalic glands. The outer zone, previously noted to contain high concentrations of antibody, was observed to abound in tryptophan-rich protein. Integrating these facts with the biology of the schistosome egg, it was concluded that at maturation, the miracidial cephalic glands produce an antigenic, cystine- and cysteine-rich protein which leaks through the eggshell (and possibly catalyzes eggshell lysis), evokes an immune response in the host, and precipitates with a tryptophan-rich host globulin to produce Hoeppli phenomena. Thus, the formation of the Hoeppli phenomenon is contingent upon a high degree of host sensitization.

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We are greatly indebted to Miss Kathryn P. Snow for her excellent technical assistance.

[*Illustrations follow*]

LEGENDS FOR FIGURES

FIG. 1. Schistosome egg and small Hoeppli phenomenon (arrow) are centrally located. Peripherally, pseudotubercle is circumscribed by fibroblasts; in between, plasma cells, eosinophils, and macrophages are found. PTAH stain. × 100.

FIG. 2. The Hoeppli phenomenon(h) is nonmetachromatic. Three eggshell lamellae (arrow) and envelope are seen; miracidial cortex (line) with its faintly visible cilia surrounds mature miracidium. Gonadal cells are intensely basophilic. Neural mass (n) and esophagus (e) lie to left. Cephalic glands (g) are unstained. Compare with Text-figure 1. Toluidine-blue stain. × 400.

FIG. 3. Autofluorescence of Hoeppli phenomenon inner zone and eggshell are best seen in areas sectioned perpendicularly. Examination for autofluorescence.[5] × 100.

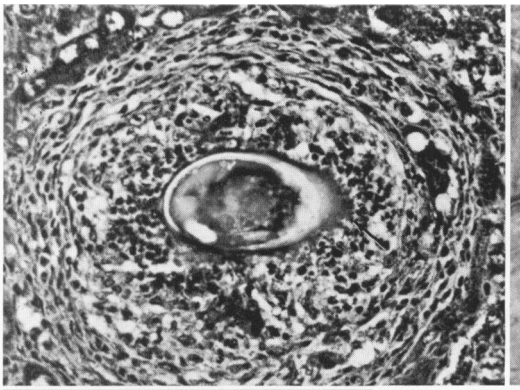
FIG. 4. Hoeppli phenomenon reacts uniformly. Broad middle lamella of eggshell is negative. Miracidium stains uniformly, and discrete organelles cannot be discerned. Bromphenol-blue stain. × 400.

FIG. 5. Miracidial cephalic glands react intensely; inferiorly, early stage of Hoeppli phenomenon also stains deeply. Eggshell is essentially nonreactive. Dimethylaminobenzaldehyde nitrite. × 400.

FIG. 6. Hoeppli phenomenon and outer lamella of eggshell stain moderately showing presence of lipid. Sudan black in propylene glycol. × 100.

FIG. 7. Phospholipid is demonstrated in Hoeppli phenomenon. Copper phthalocyanin. × 400.

FIG. 8. Acid lipid is confined to inner zone; refractile outer zone (arrow) is seen along lower right aspect of egg. Inner and outer eggshell lamellae also contain acid lipid. Baker's acid hematin stain. × 100.



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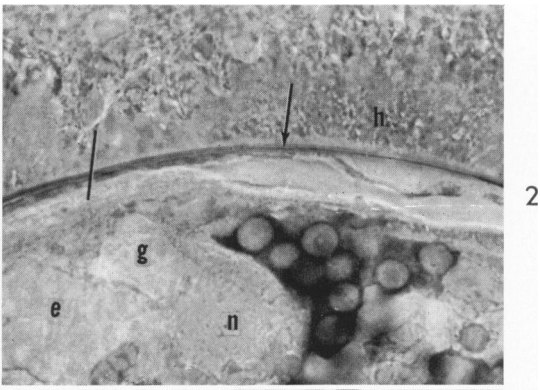

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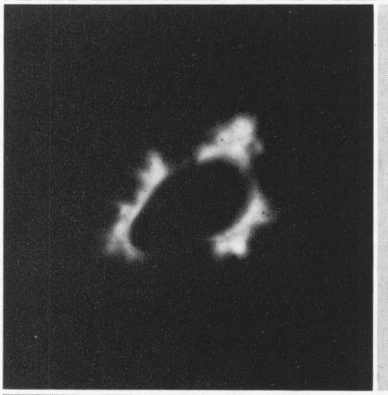

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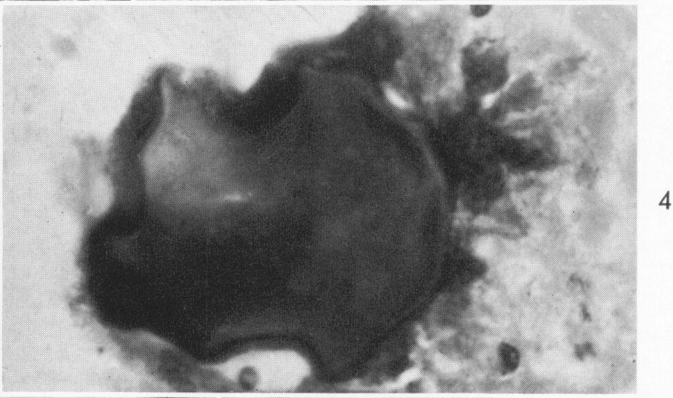

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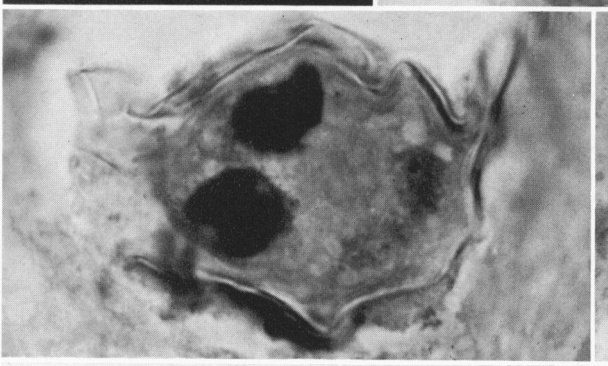

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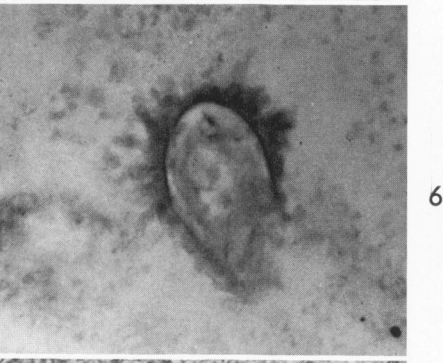

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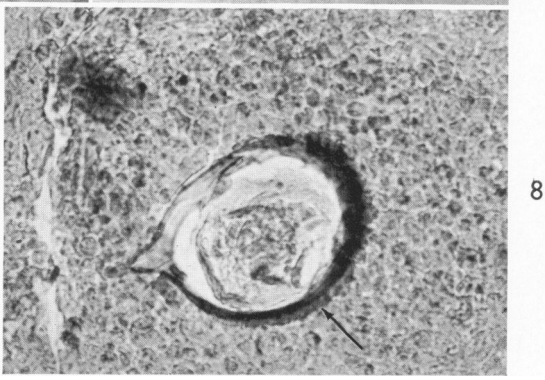

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FIG. 9. Hoeppli phenomenon is fully developed and surrounds most of egg. Note blue inner lamella of eggshell in transverse section; remainder of shell and Hoeppli fringe stain intensely with Biebrich scarlet, more intensely in periphery. Masson's trichrome stain. × 400.

FIG. 10. Blue tinge seen in miracidial cephalic glands and inner zone of Hoeppli phenomenon indicates high concentrations of sulfhydryl groups while red of Hoeppli outer zone signifies lower concentrations. Dihydroxy-dinaphthyl-disulfide reaction. × 400.

FIG. 11. Both disulfide and sulfhydryl groups are demonstrated in high concentrations in Hoeppli phenomenon. Note faint staining of eggshell. Dihydroxy-dinaphthyl-disulfide reaction with thioglycollate reduction. × 400.

FIG. 12. High concentration of disulfide is indicated by bluish pink color of inner zone. Dihydroxy-dinaphthyl-disulfide reaction after iodoacetate blocking of sulfhydryl groups followed by potassium cyanide (which produces a thiocyanide and reactive sulfhydryl group from each disulfide group [12]). × 400.

FIG. 13. Dense staining of outer zone of Hoeppli phenomenon contrasts with less reactive inner zone. Dimethylaminobenzaldehyde nitrite (Adams, 1960 modification). × 100.

FIG. 14. Miracidial envelope stains deeply, indicating acid mucopolysaccharides. Note nonreactive Hoeppli phenomenon at left. Hale's colloidal iron stain. × 100.

FIG. 15. Eggshell and inner zone react intensely while outer zone of Hoeppli phenomenon fades peripherally. This is not altered by diastase digestion. Pseudotubercle is senescent, as indicated by giant cells and condensing Hoeppli phenomenon. PAS stain. × 100.

FIG. 16. Eggshell is intensely acid-fast. Note that inner zone is also acid-fast but that staining fades toward periphery. Ziehl-Neelsen stain. × 400.

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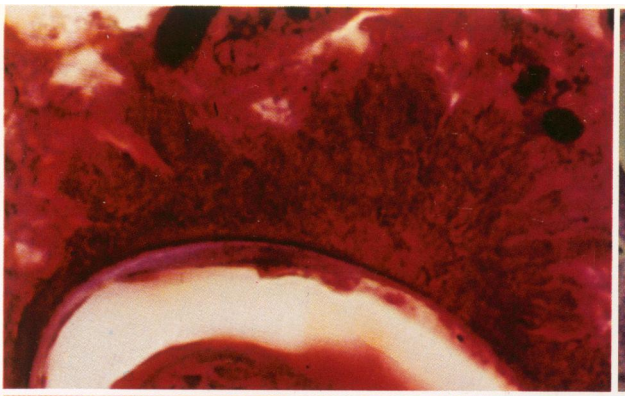

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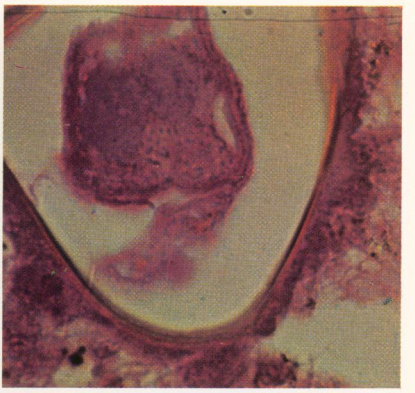

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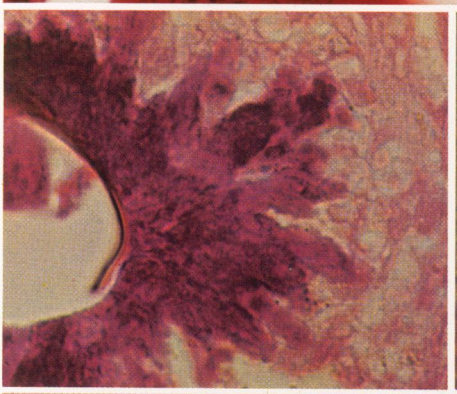

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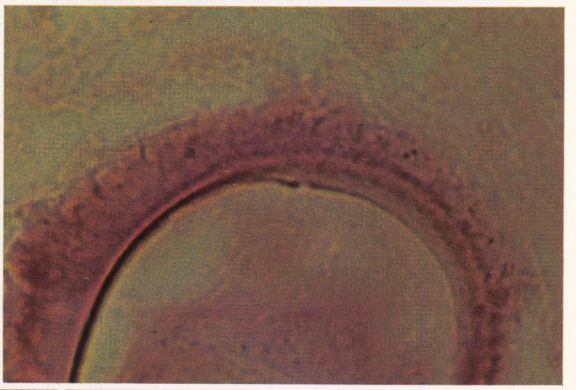

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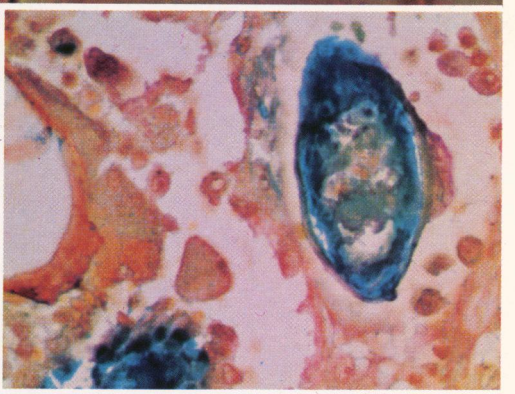

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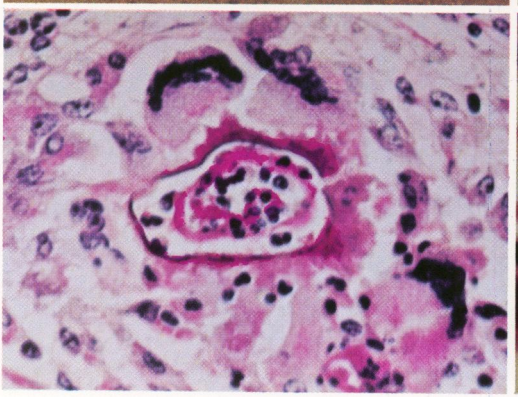

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