Comparative Histopathology of Schistosome Granulomas in the Hamster

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When uniform histologic criteria are applied to staging schistosome egg and granuloma development in the hamster liver, the evolution of the egg foci is shown to be monophasic, albeit with considerable variation of the individual cell response. Both real and artifactual egg-granuloma asynchrony are demonstrable. Alternate granuloma stages occur simultaneously within the same single organ, so that necrosis or fibrous scarring may result in some lesions but not in others. The granulomas of *Schistosoma japonicum*, *S mansoni* and *S haematobium* show both shared and distinctive features. Thus, oviposition is serial in *S mansoni* but clustered in the other two species. Neutrophils are common in *S japonicum* granulomas but are rare in the others. The differential features, listed in detail, will usually permit histologic identification of species during the early stages of infection; subsequently, the species-specific features and the overall intensity of host reaction tend to decline. At comparable egg loads and time spans, the liver pathology of *S japonicum* is the most severe. This is not related to granuloma size, but rather to more exudation and necrosis in early *S japonicum* granulomas, their tendency to encroach on adjacent liver tissue and to more extensive diffuse inflammatory infiltration. Hoeppli phenomena occur around *S japonicum* eggs both in stellate form, and as intraovular "reverse" precipitates. Plasma cells and amyloid deposition are frequent. Conversely, *S haematobium* lesions are less destructive than those of *S mansoni*. These findings can be correlated, to some extent, with current knowledge of the biology of schistosomes and of the antigenic components of their eggs, but several key problems concerning the immunologic host response remain to be solved (Am J Pathol 72:149–178, 1973).

THE CHARACTERISTICS OF LIVER GRANULOMAS produced by *Schistosoma mansoni*, *S haematobium* and *S japonicum* have been reinvestigated in the golden hamster, a host which is susceptible to all three species of human schistosomes. An effort was made to eliminate all experimental variables other than the schistosome species.

In man, *S japonicum* is known to be more severe than *S mansoni*,

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followed by *S haematobium*. The classic experiments of Meleney *et al*[1] suggested this same species ranking of virulence in laboratory animals. Studies on human infection intensity in different schistosome species and endemic foci are few,[2-4] and the reasons for clinical differences are therefore still largely speculative. Moore and Sandground[5] and Moore and Warren[6] have demonstrated the great egg-laying capacity of *S japonicum*, which has generally been regarded as the main reason for differences in virulence.

On the other hand, Meleney[1] and Hsü *et al*[7] have emphasized the destructive character of *S japonicum* granulomas and the presence of neutrophils in them. By contrast, when isolated eggs were injected into mouse lungs, *S japonicum* lesions were the smallest of the three species and resembled foreign body granulomas.[8]

Recent reports on schistosomiasis in chimpanzees[9-11] and in *Aotus* monkeys[12] again noted the severity of tissue lesions in infections with *S japonicum* as contrasted with those seen with the other two species occurring in man.

In the latter study, the mean diameters of granulomas around single eggs differed somewhat between schistosome species, but these differences were less than those in sequential measurements for a single species during long-term infections. Although composite granulomas were frequent in both *S japonicum* and *S haematobium* infections, the far greater mortality with *S japonicum* infections could not be explained on the basis of egg burden or of composite granuloma formation alone. This report illustrates that under controlled conditions for time of observation and egg burdens, the cellular response to eggs of different schistosome species shows significant differences which are related to the ultimate pathogenicity of each species.

Materials and Methods

The details of experimental design for exposures, etc, are being published elsewhere.[13] The groups of animals available for histopathologic study are shown in Table 1.

In the present study, histologic lesions in all hamsters were studied in 6-μ liver sections (six to nine sections/specimen) stained with hematoxylin and eosin. These were examined for the following features: egg morphology and maturation; number and position of eggs per locus; eggs without reaction or with minimal reaction and their developmental stage; stages of granuloma formation and their mutual proportion; cell population at each stage; and presence or absence of neutrophils, central necrosis, Hoeppli precipitates,[15] peripheral fibrosis (concentric, linear, stellate) and residual fibrosis, whether marked or mild. A narrative record of any other distinctive features seen in

Table 1—Experimental Groups*

Species (time of patency)	No. cercariae	No. animals at stated week after onset of patency†					No. Total
		1	3	5	11	19	
S mansoni	15	9	10	10	10	9	
(sixth week)	50	10	10	9	9	4	
	100	10	9	4	2	—	
Species total		29	29	23	21	13	115
S haematobium	35	9	10	7	10	9	
(eleventh week)	100	10	9	9	10	10	
	240	9	10	10	10	13	
Species total		28	29	26	30	32	145
S japonicum	7	10	9	9	8	3	
(fifth week)	15	10	9	10	8	—	
	30	10	8	8	3	—	
Species total		30	26	27	19	3	105
All species		87	84	76	70	48	365

* An analysis of the mortality, number of worms, eggs per gram of liver tissue (EGLT), liver and spleen weight, hematocrit, serum protein, granuloma size and portal pressure in each of these groups is being published elsewhere.[13]

† Animals were examined at 1, 3, 5, 11 and 19 weeks after eggs were first recovered from the feces by the formalin-ether-buffered alcohol technic,[14] to evaluate infections with each species at equivalent periods.

these granulomas was made. The following classifications were adopted for stages of egg development and of granuloma formation:

Stages of Egg Maturation

1. CELL BALL STAGE—the egg contains an undifferentiated basophilic mass
2. NEURAL STAGE—nerve center seen with dense nuclear corona
3. SECRETORY (MATURE) STAGE—eosinophilic, apical glands present
4. DEGENERATE STAGE—pycnosis, homogenization or autolysis of miracidium
5. CALCIFIED EGGS AND EGG REMNANTS

This classification is based on only those features which were recognizable by histology and is analogous to that of Gönnert.[16] Stage identification often required serial sectioning, particularly for stages 2 and 3.

Stages of Granuloma Development

I. MINIMAL REACTION—prominence of vascular endothelium, with or without accumulation of a few inflammatory cells (Figure 1)

IIa. MIXED INFLAMMATORY REACTION—accumulation of lymphocytes, plasma

cells, macrophages, eosinophils and/or neutrophils; no epithelioid cells seen (Figure 2)

IIb. PSEUDOABSCESS—the lesion is almost entirely composed of partially degranulating neutrophils and/or eosinophils (Figure 3)

IIIa. EPITHELIOID CELL GRANULOMA—a prominent epitheloid cell halo directly surrounds the egg, often including some giant cells; lymphocytes, plasma cells, eosinophils are sparse centrally, but there may be a predominantly lymphoplasmocytic infiltrate peripherally (Figure 4)

IIIb. HISTIOGRANULOCYTIC GRANULOMA—similar to IIIa, above, but center of focus shows partly degranulated neutrophils and/or eosinophils (Figure 5)

IIIc. NECROTIC GRANULOMA—similar to IIIa and IIIb, above, but center of focus shows granular, eosinophilic necrosis (Figure 6)

IVa. INVOLUTING GRANULOMA—some features of stage III are retained, but the lesion is smaller, shows fewer epithelioid cells, often prominent giant cells with clumped pigment; no significant fibrosis seen (Figure 7)

IVb. SCARRING GRANULOMA—similar to IVa, above, but there is significant proliferation of fibroblasts and/or collagen deposition resulting in a fibrous scar, either concentric, linear or stellate (Figure 7)

This classification was devised to cover most of the morphologic variants of granulomas seen around single eggs or egg shells of any of the three schistosome species. Like those of Gönnert [17] and Hsü *et al*,[7] it represents an arbitrary selection, disregarding the numerous intermediate and difficult to classify foci frequently encountered. Necrosis and fibrosis were included among the classifying criteria. A comparison of the three granuloma classifications systems is shown in Table 2.

Liver lesions not associated with sites of egg deposition were recorded as follows: portal inflammatory infiltrates and their cell composition; inflammation and endophlebitis of portal radicles and central veins; thrombi, live and dead worms in veins; scattered nongranulomatous intrasinusoidal inflammatory foci; amount and distribution of pigment; condition of the RE cells; liver cell necrosis of the zonal, and of the individual type; and the extent of liver fibrosis and/or amyloid deposition, particularly at later stages of infection. All these features were described and rated as *Mild* (+), *Moderate* (++) or *Marked* (+++).

The following histologic correlations were attempted for all experimental groups listed in Table 1, regardless of species: a) egg maturation vs granuloma stages; b) egg and granuloma stages vs duration of infection; c) overall liver pathology vs infection intensity (EGLT) and d) degree of polymorphism at all levels and stages.

The comparative study of the granulomatous and nongranulomatous liver pathology with the three schistosome species was based on the earliest and latest species groups in which samples with reasonably uniform mean EGLT levels were available.

Results

Correlation of Egg Maturation with Granuloma Stage

The predominating egg stages for each granuloma type are shown in Table 3, together with the whole range of egg forms recorded at least twice per granuloma stage.

A general correlation was evident between egg maturation and

Table 2—Comparison of Three Systems for Classification of Granulomas

Gönnert*[17] (1955)	This study	Hsü et al[7] (1969)
1. Freshly arrived egg	I. Minimal reaction	1. Weakly reactive egg
2. Small cell infiltration	IIa. Mixed inflammatory reaction	
3. Primary abscess	IIb. Pseudoabscess	2. Exudative stage
4. Primary pseudotubercle	IIIa. Epitheliod cell granuloma	4. Productive stage
5. Secondary abscess	IIIb. Histiogranulocytic granuloma	3. Exudative-productive stage
6. Same, with parenchymal necrosis	IIIc. Necrotic granuloma	
7. Early scarring tubercle and 10. dissolving scar	IVa. Involuting granuloma	5. Involutional stage
8. Sclerotized pseudotubercle and 9. hyaline pseudotubercle	IVb. Scarring granuloma	

* Translated and abbreviated from German original.

granuloma stages, suggesting that egg foci evolve along the pathway from stages I through IV. However, in contrast to earlier reports,[7,17] we also noted a variety of patterns in any individual animal, as well as evidence of occasional asynchrony between egg and granuloma stages. Thus, mature (secretory) eggs, which ordinarily are characteristic of the peak stage of granuloma development (III), could be found with minimal cell reactions (Figure 1) at anytime from 1 to 19 weeks postpatency and in any of the other granuloma stages as well, except in old scarred foci. Conversely, fully developed pseudotubercles (stage III) sometimes appeared to contain eggs in the early cell ball stage (Figure 8) and at other times destroyed egg remnants.

Table 3—Correlation of Egg Type and Granuloma Type

Granuloma class	Predominant egg state	Range of egg stages found*
I (minimal reaction)	cell ball, neural (1, 2)	1, 2, 3
IIa (Mixed inflammatory) b (Pseudoabscess)	neural, mature (2, 3)	1, 2, 3, 4, 0
IIIa (Epithelioid cell granuloma) b (Histiogranulocytic granuloma) c (Necrotic granuloma)	mature, degenerate (3, 4)	1, 2, 3, 4, 5
IVa (Involuting granuloma) b (Scarring granuloma)	degenerate, calcified (4, 5)	3, 4, 5, 0

* 0 = No egg structure found in 6 to 9 serial sections of what otherwise appeared to be a single egg lesion

Correlation of Egg and Granuloma Stages with Duration of Infection

In all three schistosome species, specimens taken 1 week postpatency showed a predominance of egg stages 1, 2, and 3 and of granuloma stages I, II and III and their variants. Conversely, at 11 to 19 weeks postpatency, egg stages 4 and 5 and granuloma stage IV predominated. This was evident without performing differential counts, and agreed well with the decreasing mean granuloma volumes recorded for the same experimental groups.[13] However, none of the egg or granuloma stages were completely absent in any animal of any of the groups.

Correlation of Liver Pathology with Infection Severity

An overall positive correlation was evident between the mean EGLT, the number of eggs and granulomas observed in histologic sections and the degree of architectural liver abnormality in each experimental group. Some individual discrepancies were noted, as has been the case in other work in which histologic and digestion results have been compared.[2–4] The mean granuloma volume did not significantly correlate with the tissue egg burdens at the time intervals studied with the exception of *S mansoni* infections at 3 weeks postpatency.[13]

Although not subject to objective quantitation, the following features were augmented with increasing infection intensity in all of the species: proportion of composite granulomas and of scarring associated with large egg foci; central necrosis and (in *S japonicum*) Hoeppli precipitates;[15] degree of portal and intrasinusoidal inflammatory infiltration not associated with sites of egg deposition (Figure 9); portal endophlebitis (Figure 9), adult worms seen in portal veins; amount of pigment; infarct-like eosinophilic liver cell necrosis; degree of scarring and/or amyloid deposition. Thus, infection intensity had a marked influence on the overall degree of liver damage.

Polymorphism of the Lesions

The heterogeneity and asynchrony of granuloma stages (described above) made scanning of multiple sections necessary for an accurate identification of schistosome species, particularly at lower EGLT levels. Since differential features were most apparent in early and in mature epithelioid granulomas, the difficulty in species identification increased as infections progressed in duration.

Within each experimental group, considerable individual varia-

Table 4—Characteristic Features in Liver Sections of Hamsters with Schistosomiasis*

Criterion	S mansoni	S haematobium	S japonicum
Egg morphology (stage)	3	1, 2, 4	3 (bright apical gland)
Egg deposition	Serial	Clustered	Clustered
Proportion, multiovular foci (estimated)	2–7%	35–67%	20–54%
Giant granulomas (20 + eggs)	—	+	+
Mean granuloma volume† (cu mm × 10^{-3})	6.21	5.87	5.93
Predominant granuloma type	IIIa, IIa	IIIa, IIa	IIIb, IIb
Neutrophils	±	±	+++
Central necrosis	+	+ (Giant granulomas)	++
Hoeppli phenomenon	—	—	+
Peripheral hemorrhage, edema	±	—	++
Granuloma border	Sharp	Fairly sharp	Encroaching
Scarring pseudotubercles (LS)	+	+ (Giant granulomas)	++
Portal endophlebitis	++	+	+++
Dead worms, thrombi (LS)	—	—	+
Centrolobular endophlebitis	—	—	+
Plasma cell infiltration	+	+	+++
Zonal liver cell necrosis	+	±	++
Councilman bodies	+	—	++
Portal fibrous bridging (LS)	+	±	++
Amyloidosis (LS)	11%	0	44%

* 1 week postpatency; LS = later stages, ± = rare or minor; + = mild or few; ++ = moderate; +++ = marked or frequent; — = absent.

† Mean volume of single egg granulomas for all exposure levels.

tion was noted, regarding the intensity of focal or periportal inflammatory infiltration, and the proportions of lymphoid, plasma cells, or eosinophils in the infiltrates. Some hamsters showed follicular lymphoid aggregates which were absent in the others (Figure 10). A few animals had sharply punched-out intralobular defects containing stellate fibroblasts and scattered lymphocytes which were interpreted as depopulated follicular lymphocytic aggregates. In addition, unexplained variations in the degree of liver cell damage (especially necrosis, fibrosis and amyloid deposition) occurred, but these may have been partly due to sampling error.

Description of Characteristics Differentiating Species

The main criteria useful in differentiating species are summarized in Table 4. Additional observations are as follows:

S mansoni

The typical egg lesion was a large classic epithelioid granuloma or "pseudotubercle" (Type IIIa) with a single central egg and a prominent pale or vacuolated epithelioid cell center, sharply demarcated by a lymphoid cell halo, or by concentric fibroblasts (Figure 3). Earlier lesions were usually mixed inflammatory foci containing many more eosinophils than neutrophils (Figure 2). Diffuse and portal vascular lymphocytic infiltration and liver cell necrosis were usually moderate but became significant at higher infection levels. Late scarring was generally moderate and linear or stellate in form. Egg calcification, seen at later stages, tended to be concentric (Figure 14).

S haematobium

Aside from their smaller size, *S haematobium* single-egg granulomas resembled those of *S mansoni,* but many seemed to involute without significant scarring (Figure 11). The most typical finding was the occasional "giant" composite granuloma containing over twenty centrally clustered eggs of similar maturation (Figures 12, 13 and 14) and often exhibiting central eosinophilic necrosis (Figure 6). These foci tended to be portal and elongated or sausage shaped.

Egg development in the *S haematobium*-infected hamster liver seemed somewhat atypical when compared to the other schistosomes. Some eggs containing a single or a few germinal cells surrounded by vitellaria resembled intrauterine stages prior to cell ball formation. These formed clusters inside portal lumina (Figures 10 and 14). A large proportion of eggs at all stages were either immature, degenerate or fragmented, and there was marked egg-granuloma asynchrony (Figure 8). Egg calcification was irregular and extensive (Figure 14). "Potato particles" (vitelline conglomerates)[18] were numerous.

Diffuse liver pathology other than mixed inflammatory cell infiltration was mild, and fibrosis was largely limited to sites of giant granulomas. By the nineteenth week postpatency, the parenchyma between egg foci showed only scattered pigment clumps and inflammatory cell aggregates (Figure 6).

S japonicum

The bright eosinophilia of the cephalic glands in mature eggs contrasted with their pale-staining miracidial parenchyma (Figure

15). Egg deposition was clearly of the clustered type (Figure 16). Presumably, in the liquefied environment of the typical histiogranulocytic granuloma (IIIb) or pseudoabscess (IIa), eggs were easily displaced and were therefore haphazard in distribution; in some lesions, eggs did not appear in the available serial sections or were close to their border [11] (Figure 5).

Neutrophils were the most conspicuous cell type in granulomas (Figures 3, 5 and 17) and were often partly degranulated when in contact with the egg shell or where Hoeppli precipitates were present (Figures 5 and 15). On slides bearing eggs with a radiating Hoeppli corona (Figures 5 and 16), other eggs had lucent eosinophilic precipitates *inside their shells* which formed bars and droplets (Figures 15 and 16) and were suggestive of an intraovular immune precipitate or a "reverse Hoeppli phenomenon." Dying liver cells or granular eosinophilic central necrotic areas were likewise frequent in these exudative granulomas and pseudoabscesses, and a peripheral epithelioid cell layer was often absent or poorly developed (Figures 5 and 16). Occasionally, eggs were sheathed by a single radiating layer of dark cuboidal epithelioid cells which were embedded in a broad halo of inflammatory infiltrate resembling granulation tissue.

At 1 week after patency, the periphery of egg foci often exhibited focal hemorrhage or edema (Figure 3), or, sometimes, a fringe of stellate fibroblasts and capillary buds embedded in pale ground substance. These inflammatory structures infiltrated between and encroached upon adjacent liver cells (Figure 17) which often showed focal necrosis. Thus, although single egg granulomas were actually smaller than in *S mansoni* infection (Table 4), total liver enlargement was greater in *S japonicum,* and the calculated ratio between inflammatory tissue and liver parenchymal tissue measured on histologic slides was higher.[13] By 11 weeks after patency, however, most granulomas were delimited by fibroblastic rims and were less encroaching. Edema or hemorrhage was then rare, but stellate or linear scarring was common. At all stages, there was considerably more disturbance of the parenchymal liver pattern than was evident with the other two species.

Portal veins were diffusely cuffed by infiltrates with many plasma cells, which were numerous everywhere (Figures 9 and 17). Some veins contained egg foci implanted on their intima (Figure 17) or thrombi in addition to widespread endophlebitis with subendothelial edema. Focal endophlebitis of central veins near encroaching egg

lesions or necrotic foci was an exclusive feature with this species. Zonal infarct-like eosinophilic liver cell necrosis and scattered Councilman bodies were frequent (Figure 9). By the eleventh week, some portal fibrosis was observed, and occasional bridging enclosed islands of liver parenchyma. In addition, 4 of 9 hamster livers then exhibited moderate to marked diffuse amyloid deposition (Figure 18). Thus, S *japonicum* at comparable levels of egg deposition had a more deleterious effect on the host liver than did the other two species.

Table 4 includes both qualitative and quantitative differences which will be the basis for discussion below. The order of severity of lesions was S *japonicum* > S *mansoni* > S *haematobium*, which corresponded well with the mortality data.

Discussion

Methods

In this study host age, sex, breeding stock, housing and feeding conditions, and tissue egg burdens were kept reasonably uniform. Since schistosome species develop and lay eggs at different rates, we attempted to adjust our observations to their biologic timetables by timing necropsies at intervals after patency rather than after exposure; this was complemented with serial sacrifices. Exposure levels were varied with the aim of achieving comparable EGLT levels among the species. By human standards, even the lowest of these levels would represent severe infections.[19] Mortality proved to be higher with S *japonicum* and S *mansoni* than with S *haematobium* infections, both absolutely and relative to exposure and EGLT levels. Therefore, in contrast to the random selection available 1 week postpatency, only the less heavily infected survivors could be studied at the later stages of infection.

Even under the best conditions and with the use of serial sections, histologic assessment of egg stages is poor as compared with *in vivo* oograms.[20] Regardless of the classifications used, granuloma staging is at least partly subjective. Part of the asynchrony of egg-versus-granuloma stages, noted in Table 4, arises from fused or composite foci which cannot be distinguished from single egg lesions without scanning an unreasonable number of serial slides. For all these reasons, precise correlations between egg and granuloma development remain beyond the power of routine histology.

Egg-Granuloma Correlations

Our observations, unlike those of Gönnert,[17] are consistent with a variable but monophasic evolution of granulomas for all three schistosome species. Most granulomas reached their maximum development at the peak of miracidial maturity and secretory capacity and thereafter declined with miracidial death and degeneration (Table 3). This interpretation is consistent with the successive decrease in mean granuloma volumes observed during the course of the infection [13] and has also been observed with injection of isolated eggs into mouse lungs.[21,22] However, in both experimental settings, there was evidence of a real, not just an artificial, egg-granuloma asynchrony. This was caused partly by variations in stages of egg maturation at the time of their deposition, by variations of shell integrity and by differing egg survival span and antigenic load.[21] The occurrence of abnormal oogenesis in normal schistosome infections has been documented.[18] All of these factors and others may have contributed to the variations in the granulomatous process as recorded in Table 3.

Although the sequence of individual lesions cannot be accurately reconstructed, our findings strongly suggest the possibility of alternate granuloma development when eggs are deposited within the same animal and organ. Thus, some egg lesions might become necrotic or result in scarring, while others might not. This would also agree with the apparently haphazard distribution of neutrophil aggregates, Hoeppli phenomena and many other histologic features in granulomas. Heterogeneity in granulomas is a common feature in many other chronic infections and can be defined only by statistical means.

Effect of Infection Intensity

A close correspondence was noted in our material between the degree of granulomatous and nongranulomatous liver pathology and the increasing infection intensity. It is of interest that the diffuse lesions were associated with increased granuloma number, rather than size. Moreover, while single egg *S japonicum* foci tended to be smaller than those of *S mansoni*, the overall liver pathology, at equivalent EGLTs, was more severe in the former. Although it has been shown that part of the soluble antigen generated by schistosome eggs is sequestered during granuloma formation, some of it diffuses out [23] and may therefore be implicated in stimulating the inflammatory and degenerative changes seen away from sites of egg deposi-

tion. The pathogenesis of these lesions still remains to be fully explained.

Differences Between Schistosome Species

It has been shown herein that under reasonably constant conditions, the same host will exhibit both shared and distinctive patterns of reaction when infected with different schistosome species. In analyzing the distinctive features, one must consider: a) host susceptibility and parasite development, b) species peculiarities of schistosome migration and oviposition and c) species differences intrinsic to schistosome eggs.

Host Susceptibility and Parasite Development

Although the golden hamster was an adequate host for all three schistosome species, worms and egg recoveries were lower for S *haematobium*,[13] worm location was somewhat abnormal, patency onset was somewhat delayed compared with optimal conditions, and mortality was low. Histologic findings suggested that S *haematobium* egg development in the hamster liver was somewhat impaired or their life-span shortened, although this may not be the case in the intestine.[8] Furthermore, the slower and more gradual onset of patency in S *haematobium* infection may stimulate the host's immunologic reactivity less than the more sudden and intense challenge which occurs with the other two species. Similar observations have been made in mice,[1] *Aotus* monkeys,[12] chimpanzees [10] and in experimental egg injections in mice, rats and guinea pigs.[8,24] Therefore, differential host susceptibility probably was less important in producing the differences than were other factors.

Species Differences in Migration and Oviposition

In man, mice, chimpanzees and *Aotus* monkeys, S *mansoni* lesions have a dispersed pattern of distribution in contrast to the localized, massed or "nesting" pattern with S *haematobium* [10] and S *japonicum*.[11] This difference is evident only in end organs such as the bladder or colon. Egg deposition patterns in the liver are produced mainly by random embolization from distant sites into portal radicles.[17] The contrast between serial oviposition in S *mansoni* and clustered oviposition in S *japonicum* and S *haematobium* had already been noted by Faust and Meleney,[26] but it is of interest that egg clustering is still evident in an embolic site, the liver. This might be due to

rheologic factors alone, to adhesiveness of newly laid eggs, or to uterine contents of the female worm expelled with the clusters, as suggested by Meleney *et al*;[1] further studies on this point would be useful. The finding of early S *haematobium* egg clusters in the single and early dividing germ cell stage outside the worm uterus in host tissue had been noted in the chimpanzee,[10] but it is emphasized here for the first time (Figure 12). This finding suggests a physiologic difference in oogenesis in S *haematobium*, and since early thin-shelled eggs may be more vulnerable, this might also account for the greater number of destroyed eggs and of asynchronous egg forms found in S *haematobium* granulomas.

Nevertheless, it seems unlikely that clustered oviposition or the formation of composite granulomas could account for differences in species virulence. With relatively uniform EGLT levels, one of the cluster-laying species, S *haematobium*, was less virulent; the other, S *japonicum*, was more highly virulent than the serial egg-laying S *mansoni*.

Intrinsic Egg Properties

In this study, granuloma size was not directly related to the degree of pathogenicity of each species. The total aggregate granuloma volume per liver would be largest when eggs are dispersed as individual units; thus, if virulence were a function of granuloma volume, this would make S *mansoni* more virulent than S *japonicum*, whereas the opposite was true. Indeed, it is questionable whether granuloma measurements are relevant parameters, since they consider only the distance between the borders of focal inflammatory cell aggregates and disregard any peripheral hemorrhage, edema, diffuse infiltration or cell damage around these foci. Clearly, the degree of *destructiveness* of the lesions must also be considered. In S *japonicum* granulomas, there was more central necrosis and neutrophil accumulation, and more severe liver cell and vascular inflammatory damage around their periphery and throughout the liver. When the total ratio of intact versus damaged liver tissue was measured by low power field planimetry,[13] it was shown that the aggregate tissue damage was indeed greatest for S *japonicum* and least for S *haematobium*. Similar destructive effects have been described with S *japonicum* infection in the chimpanzee,[7,11] the *Aotus*,[12] the dog[26] and the rabbit.[15,27] In fact, as early as 1953, Meleney *et al*[1] stated that the eggs of S *japonicum* usually produce more severe lesions than do the other two species.

Pathogenesis

Differences in the destructive effects of schistosome eggs could be due to variation in their cytotoxic components (such as the lysophosphatides demonstrated in S *mansoni* eggs) [16,28] or to antigenic variation. Reactions should be relatively uniform throughout a wide range of host species in the former but not in the latter case, since even single defined antigens will evoke variable responses when different animal species are challenged.[29] A review of host-species influence on schistosome granulomas is beyond the scope of this paper, but in mice [8] and chronically infected humans,[30] S *japonicum* develops normally, yet produces far less intensive cell responses than seen here or in the other hosts cited. Conversely, S *mansoni* can produce necrotic granulomas and Hoeppli phenomena during the acute stage of infection in a variety of susceptible hosts;[31,32] pseudoabscesses may occur in the intestine of macaques at the beginning of S *mansoni* oviposition, and in the human and chimpanzee bladder [10] in early S *haematobium* infection.

Therefore, it is likely that, in addition to shared antigenic determinants, each schistosome egg species possesses unique antigens or at least higher proportions of certain egg antigens, which could account for the differential responses which concern us. Preliminary data indicate that the soluble egg antigen of S *mansoni* is separable into complex fractions,[33] and it is known that S *haematobium* lacks an acid-fast component present in eggs of S *mansoni* and S *japonicum*.[34]

A second problem is whether the observed variations of host cell response are related to delayed hypersensitivity, which is known to augment the basic granulomatous response to schistosome eggs in various experimental systems,[22,35] or whether humoral responses may also be involved.

In the S *japonicum*-infected hamster we noted frequent Hoeppli phenomena [32,36] as well as what has been interpreted as an intraovular or reverse form of immune precipitates (Figures 15 and 16); the close relationship of these with degranulating neutrophils or with central necrosis, and their apparent stage dependence on egg maturity and miracidial secretion suggest antigen-antibody interaction and activation of complement. The striking plasmocytic infiltration found at 1 week postpatency in S *japonicum* infection versus the predominantly lymphocytic infiltrate in S *mansoni* and the greater frequency of amyloid deposition in the former are also noteworthy, since plasma cell proliferation is linked with antibody and immunoglobulin

production,[37] of which amyloid may be a modified product.[38] Similar considerations apply to nongranulomatous liver changes (such as vasculitis, intrasinusoidal cell aggregates and liver cell damage) which accompany egg deposition and contribute to the ultimate sequelae of infection,[11] and yet are difficult to account for on the basis of delayed hypersensitivity alone.

Evidence that both cellular and humoral responses are evoked by schistosome eggs has been summarized in an earlier publication,[35] but further confirmation and definition is required. Thus, it might be informative to investigate the intraovular precipitates in S *japonicum* eggs at the histochemical and ultrastructural level and compare them with the classic radiating Hoeppli phenomenon. It is important to study the respective roles of complement in the nondestructive, as well as in the destructive, granuloma variants observed, a feat we were unable to accomplish in earlier experiments performed in *Mastomys coucha.*[32,36] Perhaps most importantly, immunochemical analyses and experiments with the artificial granuloma system[39,40] should be applied to S *japonicum* and S *haematobium* eggs, in addition to those of S *mansoni,* in order to learn more about the different antigenic structures involved. The difference in the antibody formation evoked by various natural and artificial schistosome granuloma models also requires further elucidation.

Our studies confirm that the cellular responses to eggs and the qualitative species differences among schistosome species were most dramatic during the early acute stage of infection, and that these subsequently declined, leaving mostly quantitative differences in the degree of scarring and overall liver damage. This agrees with numerous observations in a variety of animal models,[7,41] as well as in man,[42] and suggests the existence of a phenomenon of "endogenous desensitization,"[41] "accelerated antigen sequestration,"[35] or, perhaps, establishment of a new immunologic equilibrium involving an "enhancing" antibody which still remains to be fully defined. Indeed, in prolonged single worm pair S *japonicum* infection of mice, an apparent amelioration of the liver pathology and a decreased granulomatous response have been reported.[43] There is a greater need now for pursuing these clues experimentally than for further exploration of comparative schistosome pathology at the morphologic level.

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Acknowledgments

This paper is dedicated to the memory of Dr. Henry E. Meleney, pioneer in the comparative pathology of infection and one of the founding fathers of tropical medicine in the United States.

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Legends for Figures

All photomicrographs are of hamster liver tissue, embedded in paraffin, sectioned at 6 to 8 μ and stained with hemotoxylin and eosin. The shrinkage artifact around schistosome eggs seen in some figures is to be disregarded. KEY: *Sm* = *S mansoni*, *Sj* = *S japonica*, *Sh* = *S haematobium*; Wk– = number of weeks after patency of infection; E– = egg stage(s); G– = granuloma stage.

Fig 1—*Sm*; Wk 1; E 3; G 1. Although the egg is mature, cell reaction is minimal and limited to a few leukocytes and swollen endothelial cells. Note fine pigment granules in the Kupffer cells (× 530).

Fig 2—*Sh*; Wk 1; E 4; G 2a. Moderate sized, mixed inflammatory cell focus surrounding an egg in an early degenerative stage. Eosinophils are numerous (× 530).

Fig 3—*Sj*; Wk 1; E 3; G 2b. Pseudoabscess, with numerous partly degranulated neutrophils crowding around a mature egg. There is peripheral red cell extravasation and edema (pale areas); leukocytes invade the liver parenchyma along its border (× 330).

Fig 4—*Sm*; Wk 1; E 3; G 3a. Classic epithelioid cell granuloma; a core of pale, discrete epithelioid cells is bordered by a concentric rim of fibroblasts and lymphoid cells (× 530).

Fig 5—*Sj*; Wk 1; E 3; G 3b. Histiogranulocytic granuloma. A mature blunt-spined egg with a radiating Hoeppli corona along its left border rests excentrically on a group of epithelioid cells below. Elsewhere, it is surrounded by crowded neutrophils. This poorly demarcated focus is part of a larger composite granuloma (× 530).

Fig 6—*Sh*; Wk 19; E 4, 5; G 3. Large composite granuloma containing degenerate eggs and egg rests, with central eosinophilic necrosis and cholesterol clefts (an unusual feature). Around this and the contiguous smaller foci, dense portal lymphoid cell infiltration and mild stellate scarring are seen. The clumped pigment and minimal parenchymal damage are characteristic of this late stage (× 130).

Fig 7—*Sh*; Wk 19; E 5; G 4a, 4b. The calcified egg on the left has induced mild stellate scarring; the small granuloma with egg shell next to it is involuting without fibrosis. Note again the paucity of parenchymal lesions (× 330).

Fig 8—*Sh*; Wk 3; E 1; G 3a. Egg granuloma asynchrony: the miracidial embryo is a dense intensely basophilic cell ball lacking neural differentiation; the granuloma is a classic "pseudotubercle", as in Figure 4 (× 530).

Fig 9—*Sj;* Wk 1. Diffuse portal infiltration and endophlebitis. There is endothelial damage and subendothelial edema. Many of the mononuclears are plasma cells. Note Councilman body at right margin and pigment clumps nearby (× 530).

Fig 10—*Sh;* Wk 3; E 1; G 1. A cluster of very immature ova with minimal reaction occupies the portal venule on the right. (Compare with Figure 12). A sharply defined follicular cluster of lymphocytes is seen to the left (× 330).

Fig 11—*Sh;* Wk 19; E 5; G 4a. The egg shell material within this giant cell has been reduced to wavy basophilic strands and clefts. A few pigment flecks are present. Sparse fibroblasts and lymphoid cells surround the giant cell, but there is no significant scarring (× 530).

Fig 12—*Sh;* Wk 1; E 1; G 2a. Egg cluster inside a portal branch with endophlebitis and early granulomatous transformation. The vacuolated cells inside the thin-shelled ova are vitellaria; the basophilic cells, early embryonic division stages. Note mitosis in upper left embryo. Compare with the later stage, seen in Figure 8 (× 530).

Fig 13—*Sh;* Wk 5; E 3b. Subcapsular giant granuloma, typically portal and sausage shaped, elicited by a cluster of mature eggs. Note the virtual absence of lesions elsewhere in this field (× 130).

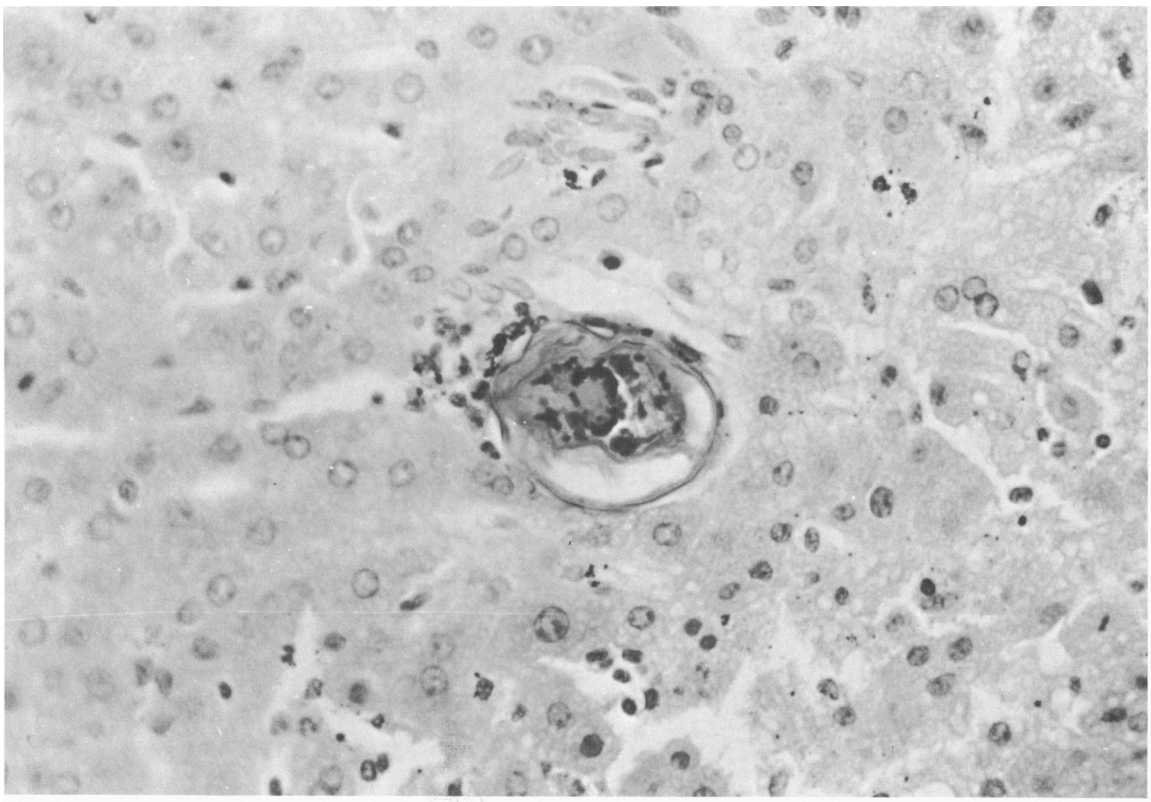
Fig 14—*Sh;* Wk 19; E 5; G 4b. Healing stage of a lesion similar to that of Figure 13. It shows both concentric (right) and diffuse (left side) miracidial calcification, with persistence of nuclear fragments, egg shell deformity and foreign body giant cell reaction (× 530).

Fig 15—*Sj;* Wk 1; E 3; G 3b. Immediately below the cilia at the left tip of this miracidial cross-section are the paired apical glands, shown dark grey—*ie,* markedly eosinophilic in color. Rightward, the tangentially sectioned nerve center and a basophilic gonadal cell are seen. Note the pale miracidial stroma. The bar and droplet-shaped deposits just inside the lower egg shell contour ("reverse Hoeppli phenomenon") show the same color shade as the apical glands. Degenerating neutrophils cover the egg shell, surrounded by epithelioid cells. This lesion is part of a larger, composite granuloma (× 1200).

Fig 16—*Sj;* Wk 1; E 3, 4; G 3b. The stellate form of the Hoeppli phenomenon (left egg) and its reverse form (right) are seen in an egg cluster within the same composite granuloma (× 530).

Fig 17—*Sj;* Wk 1; E 2; G 2b. Maturing egg embedded in a dense cluster of neutrophils, which has implanted on the wall of a portal branch with extensive endo- and periphlebitis. Note encroachment of inflammatory infiltrate on the liver trabeculae. (× 330).

Fig 18—*Sj;* Wk 11; E 5; G 4a. On the right, a portal space, densely infiltrated by lymphoid and plasma cells, with a single involuting granuloma is seen. Only small islands of parenchyma remain, surrounded by massive amyloid deposits (× 130).



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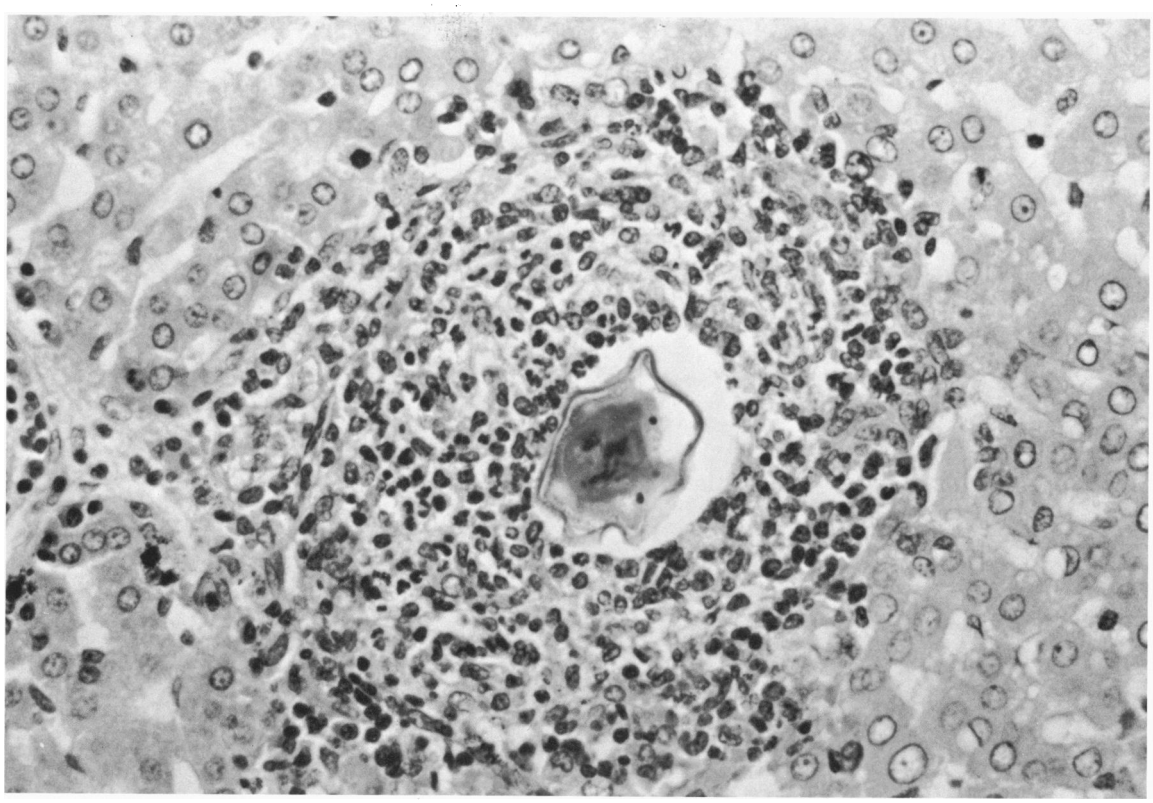

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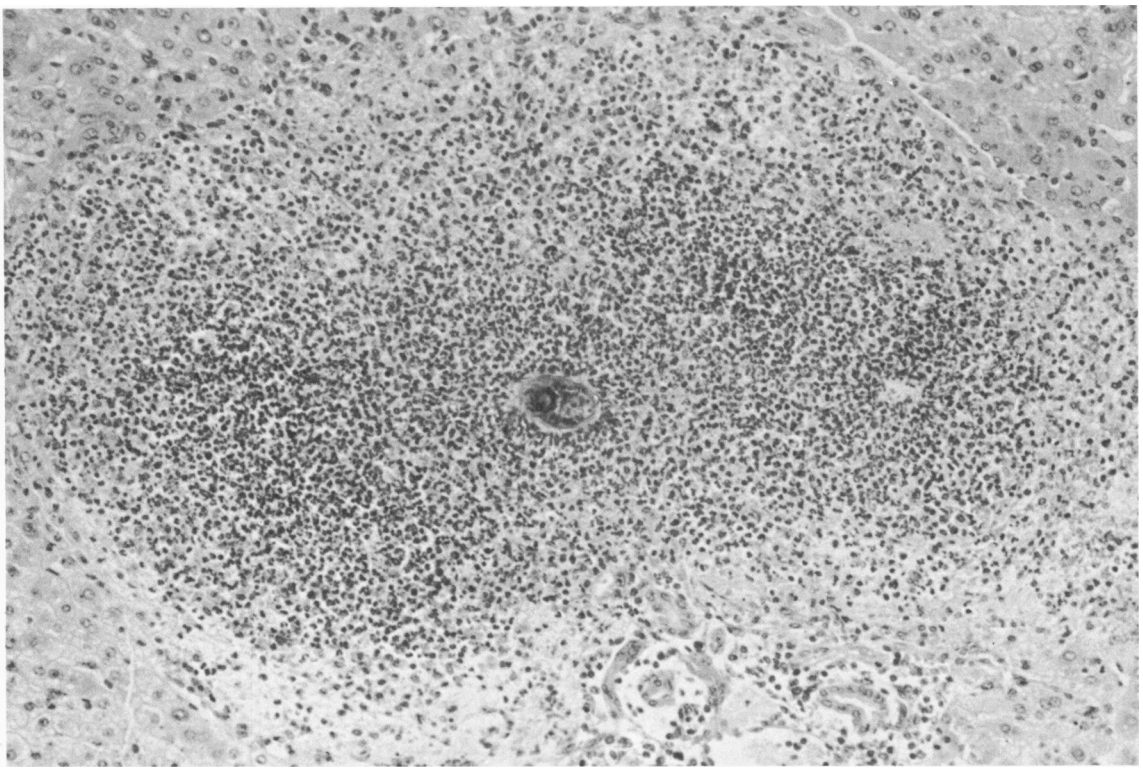

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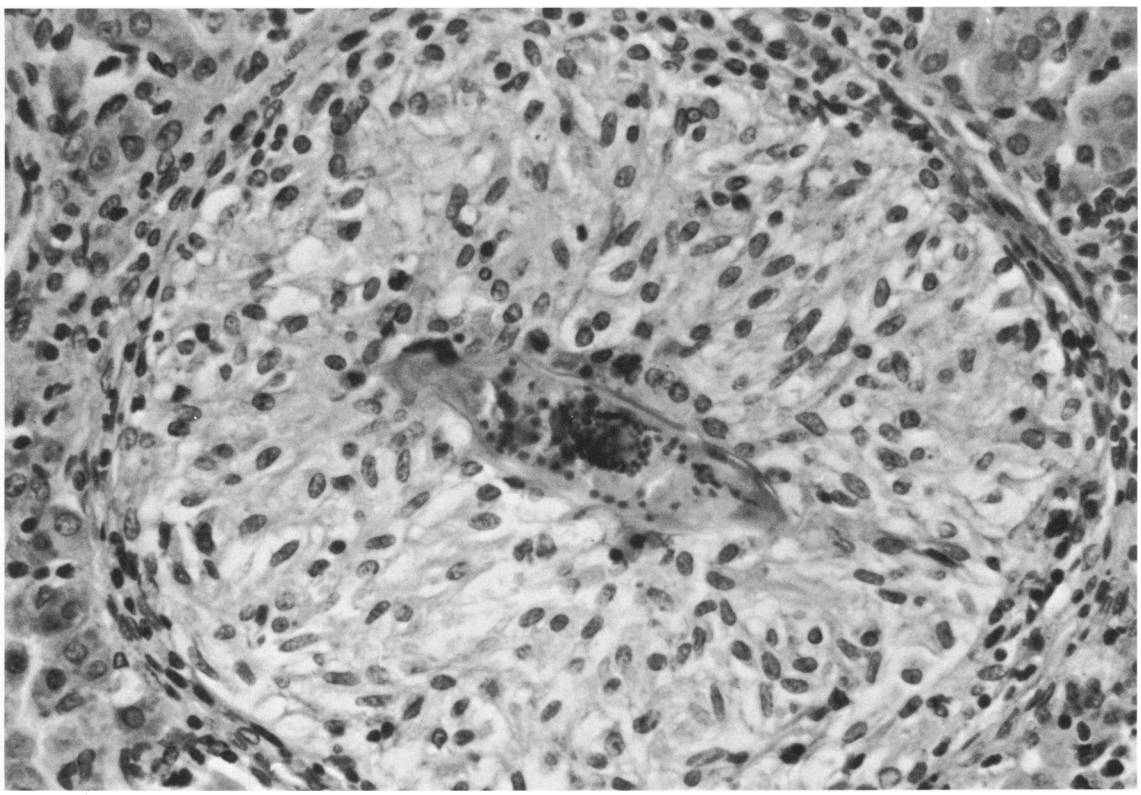

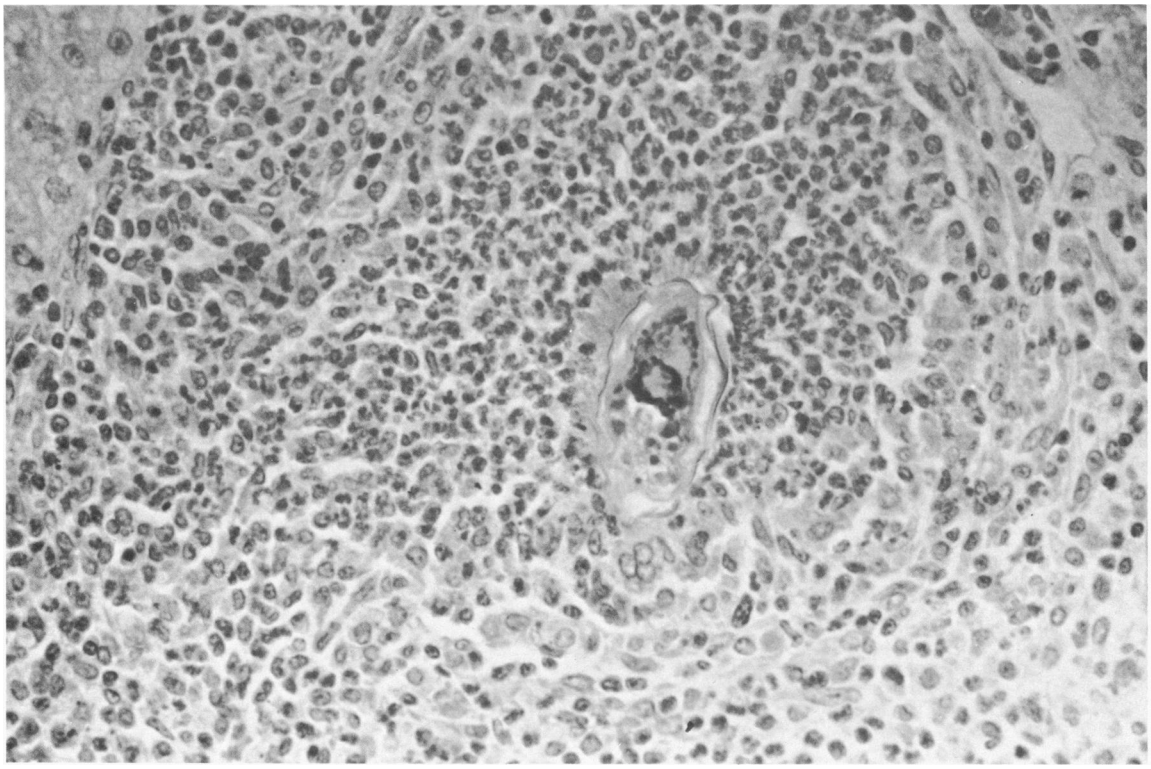

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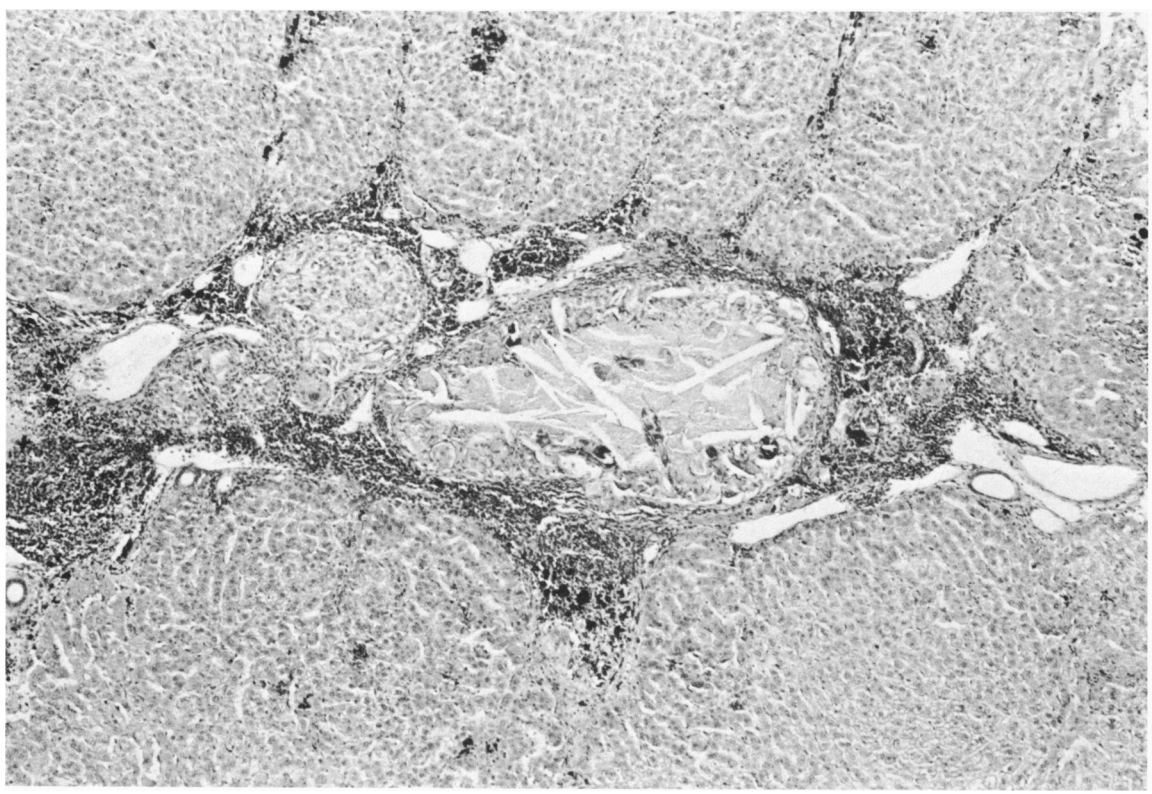

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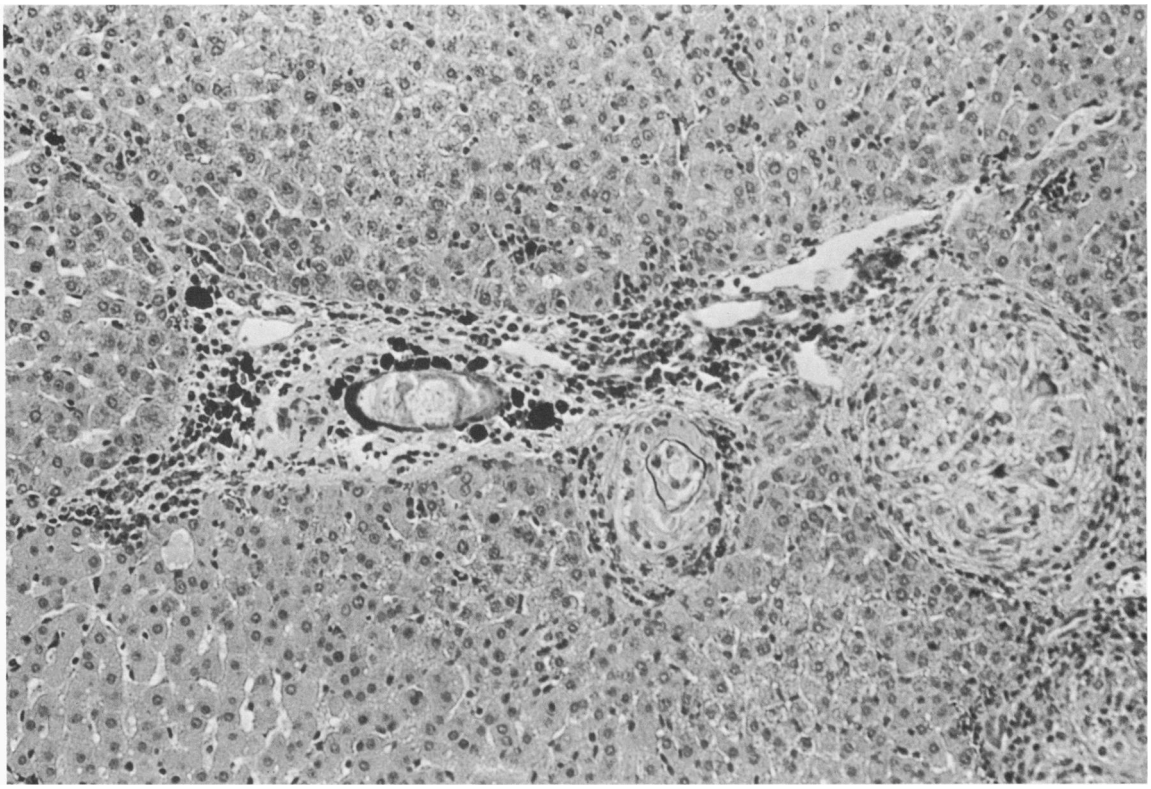

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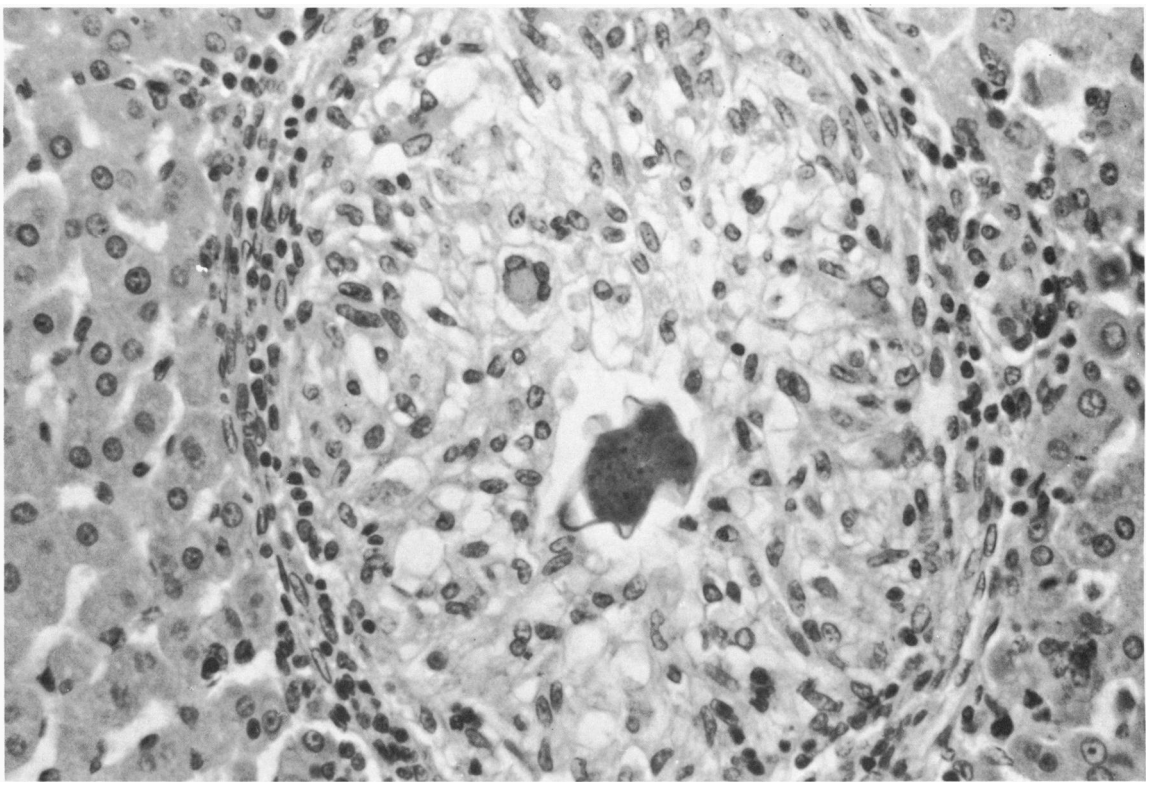

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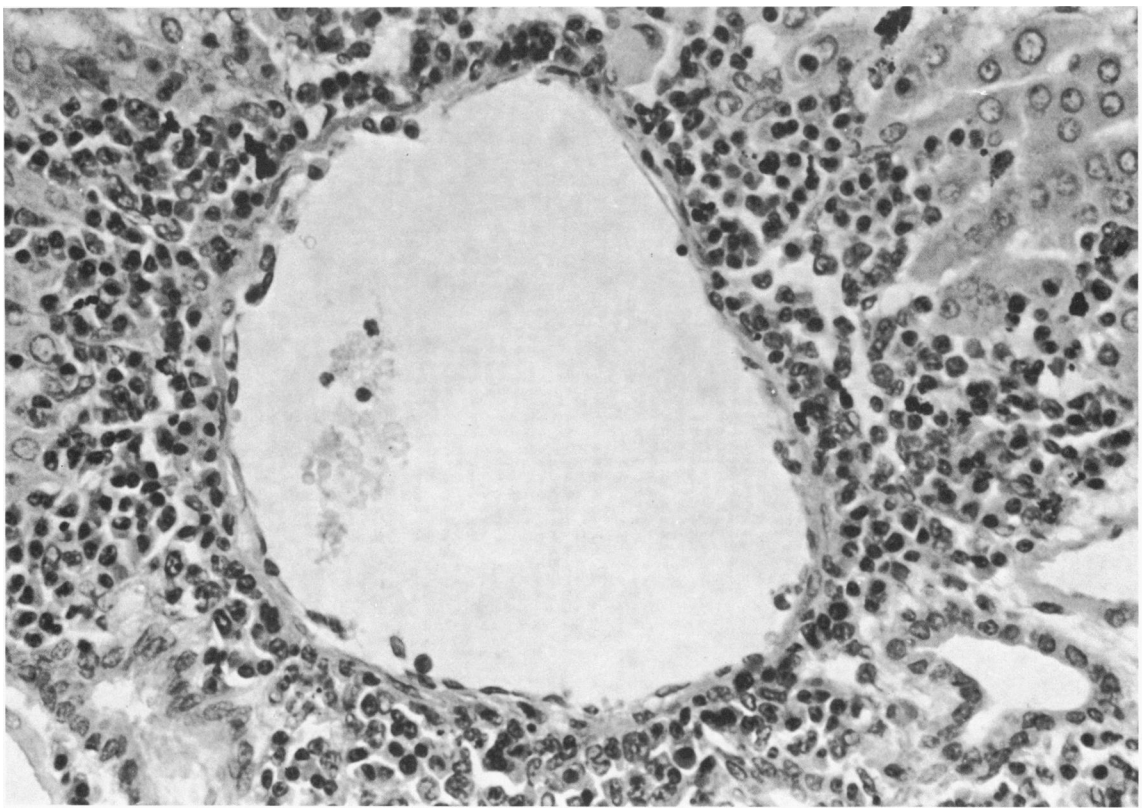

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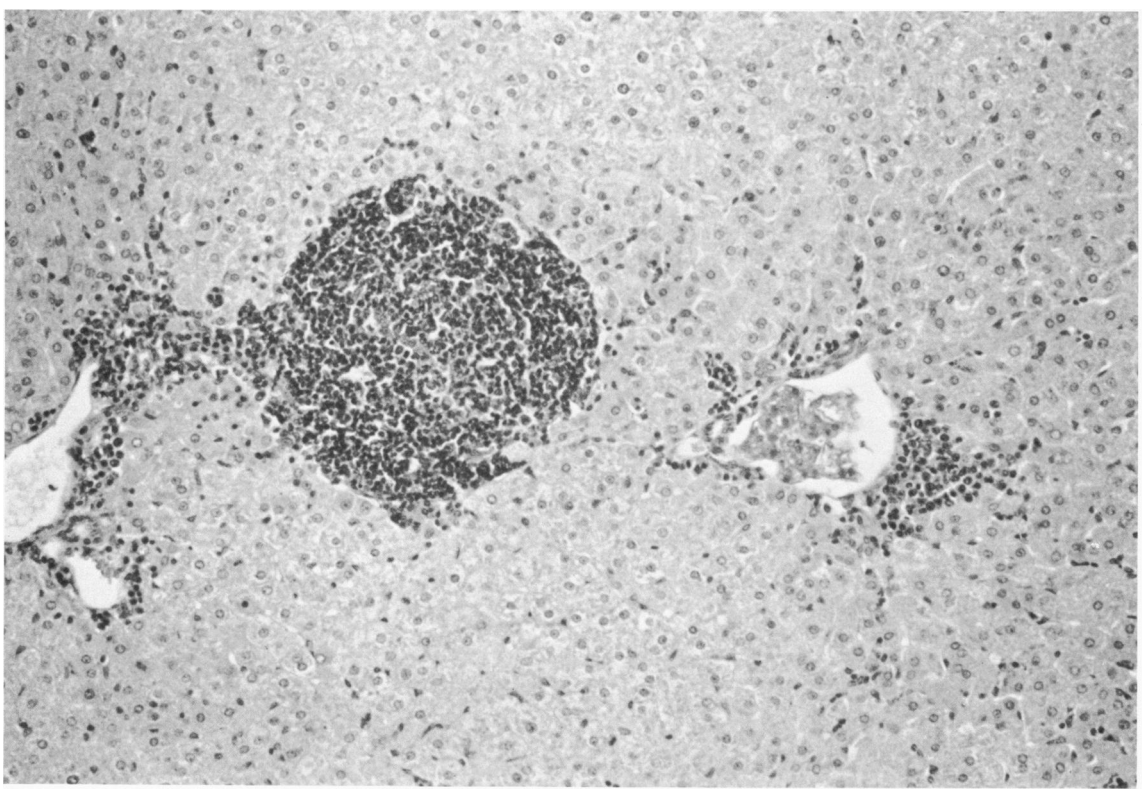

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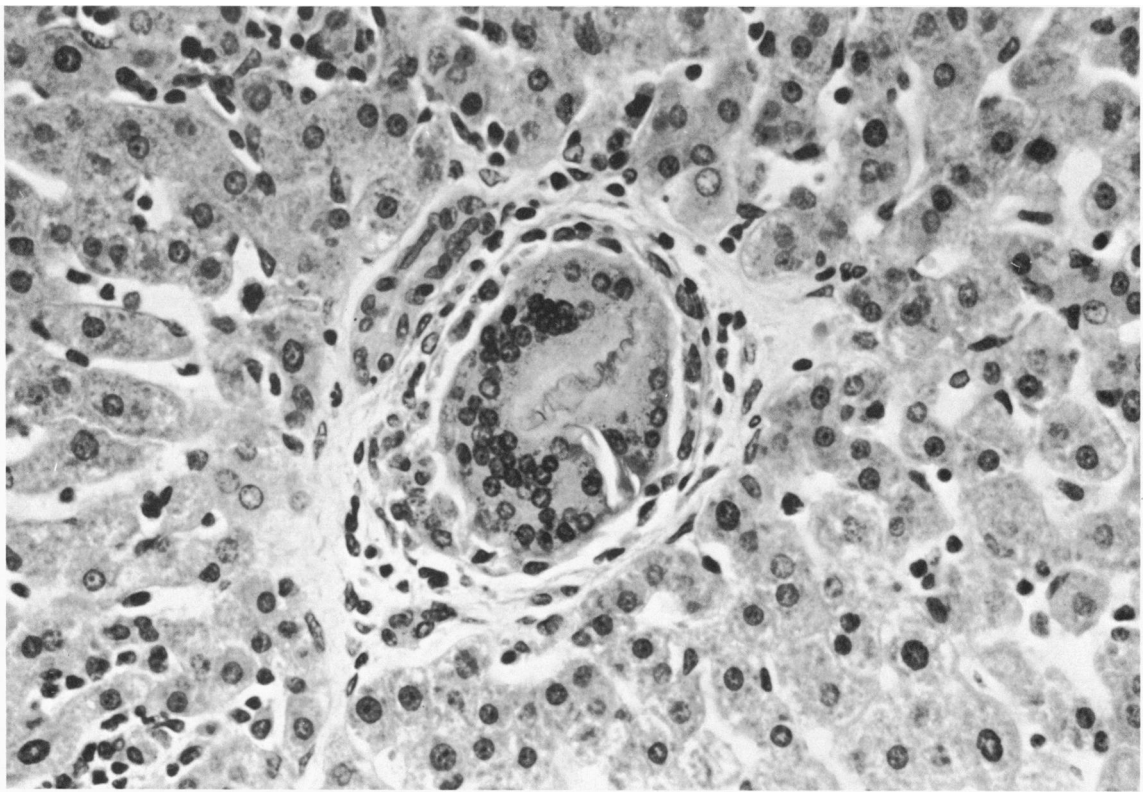

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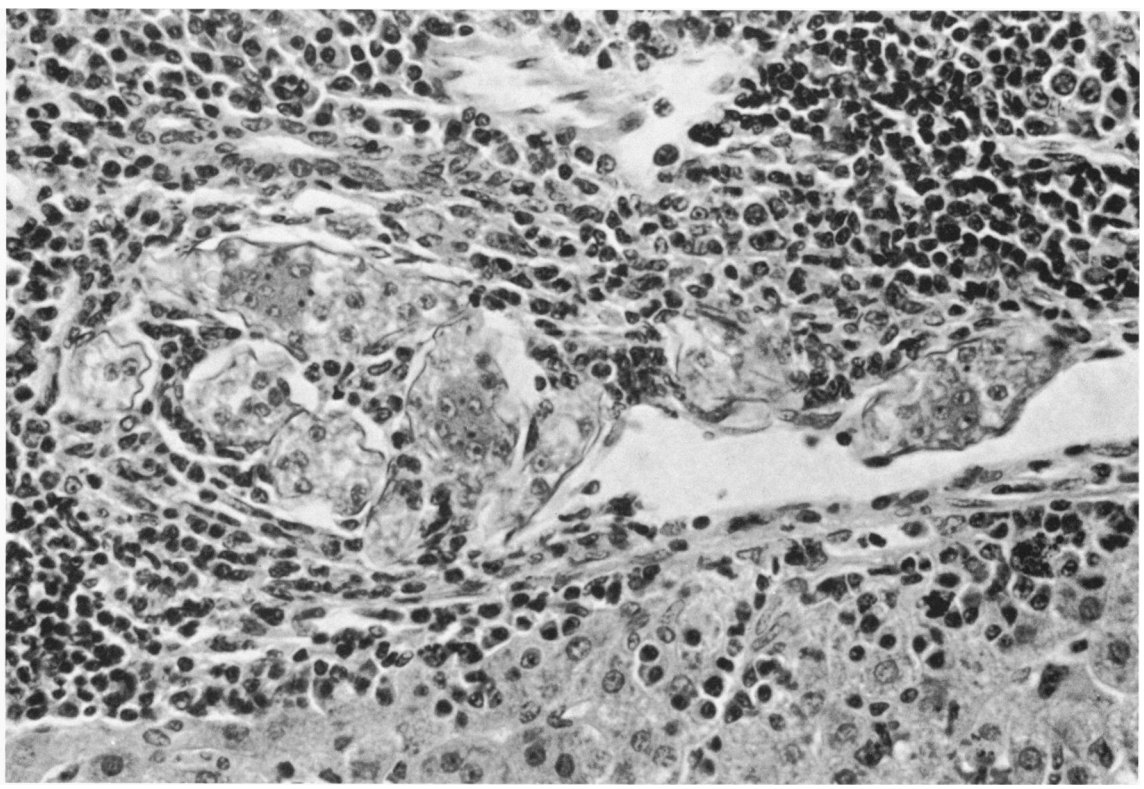

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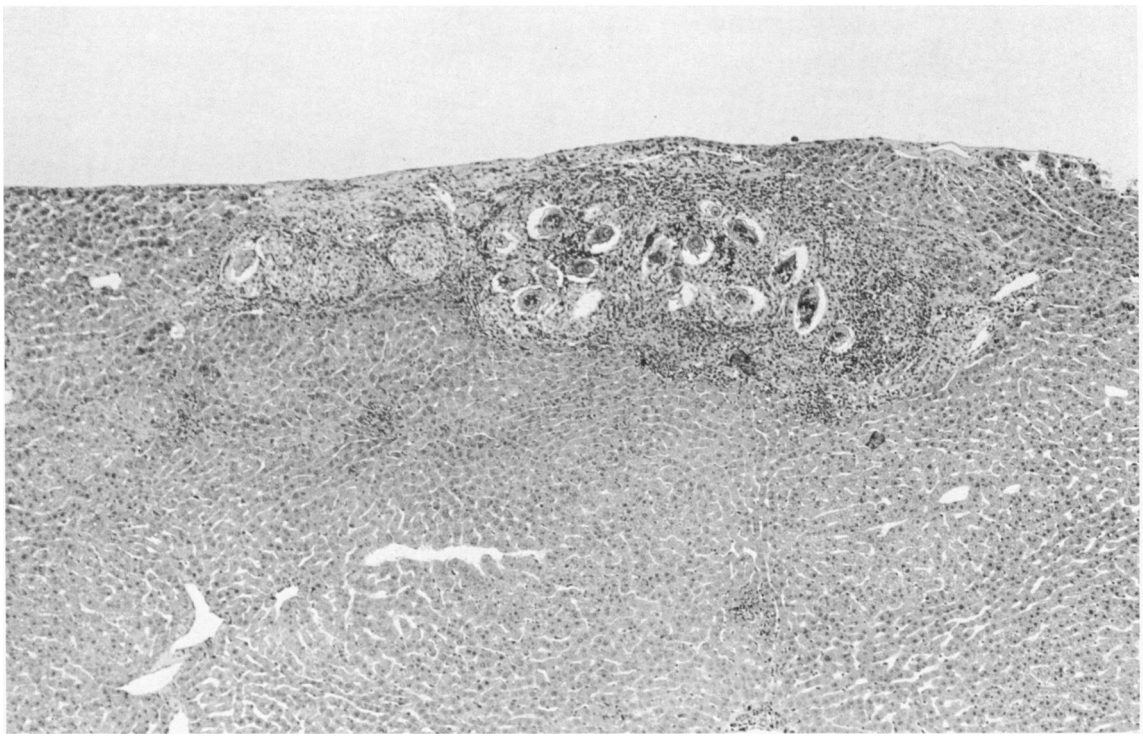

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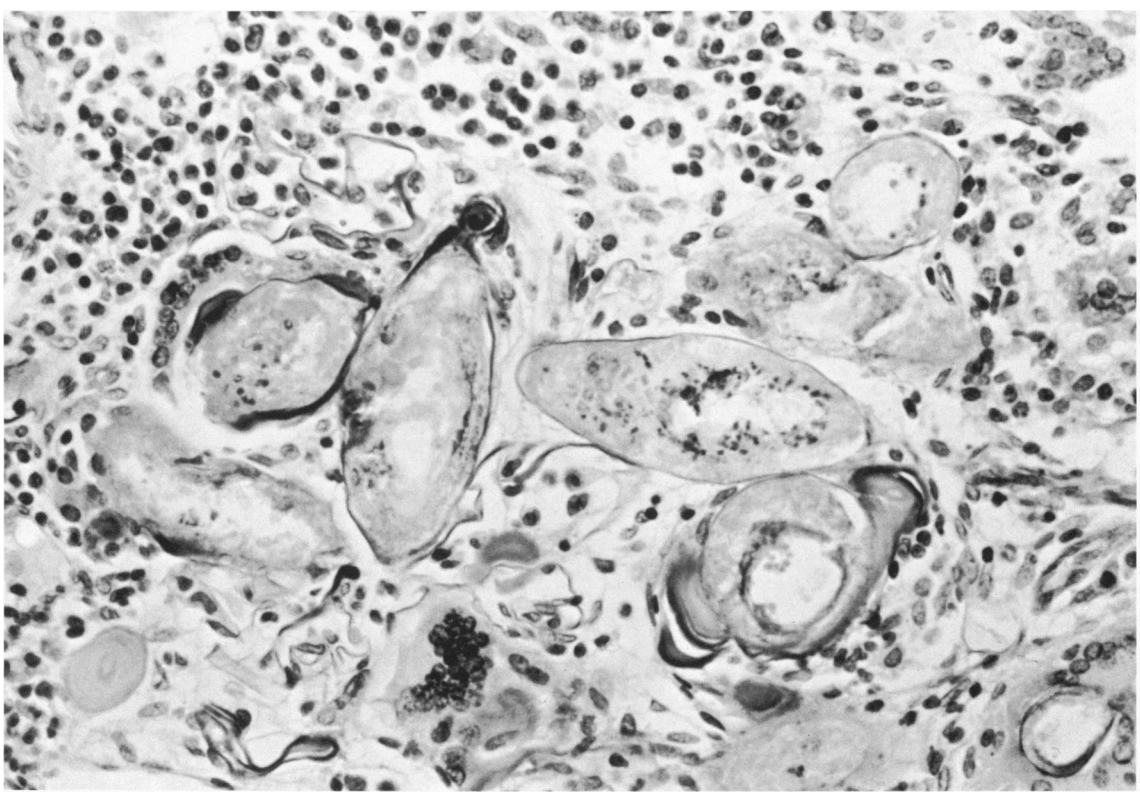

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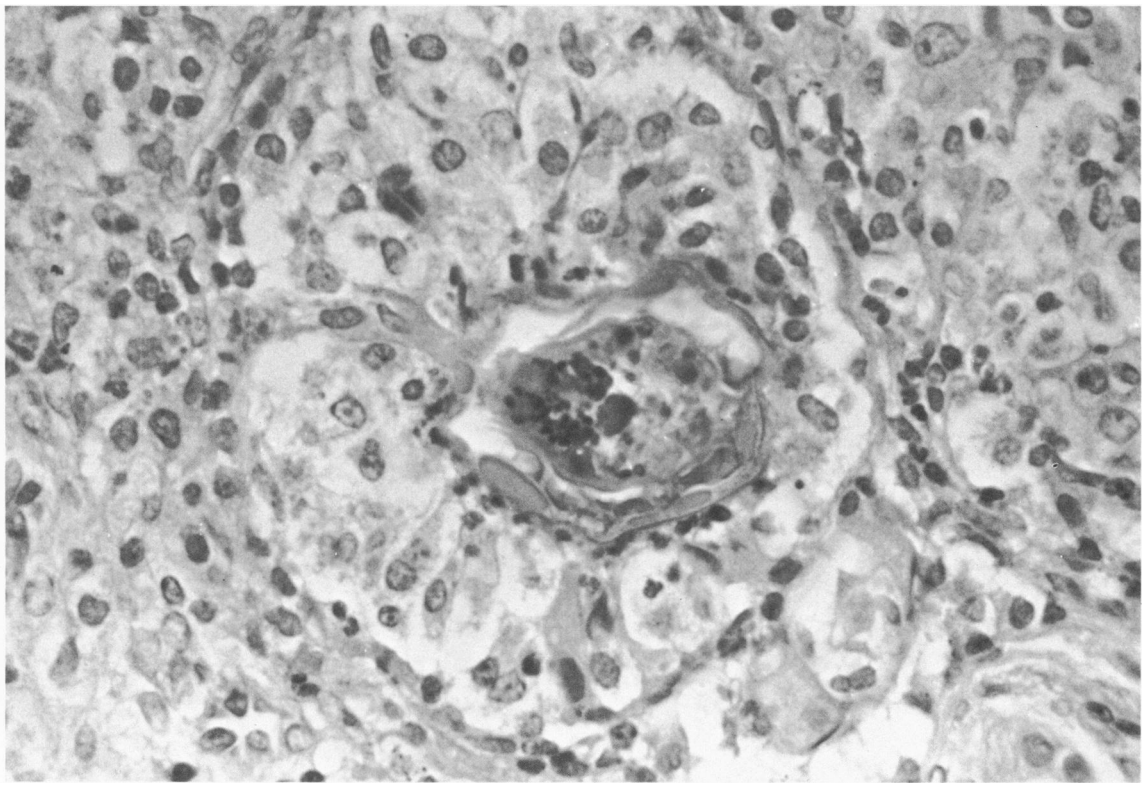

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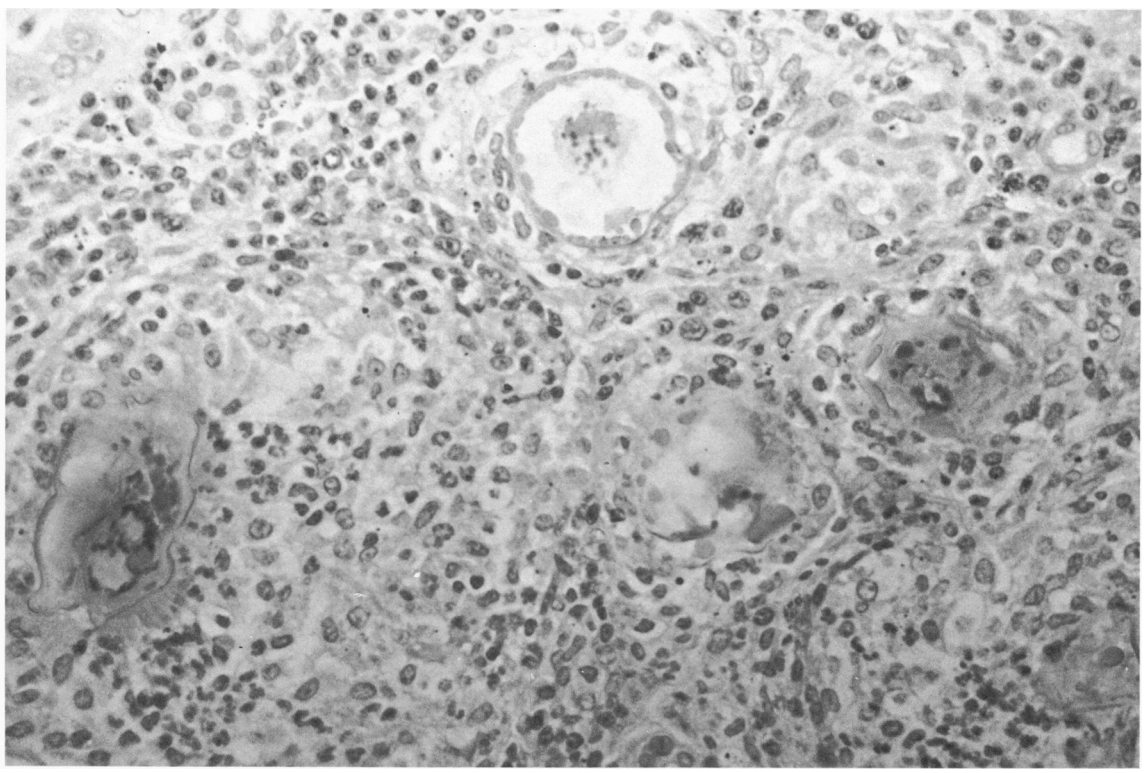

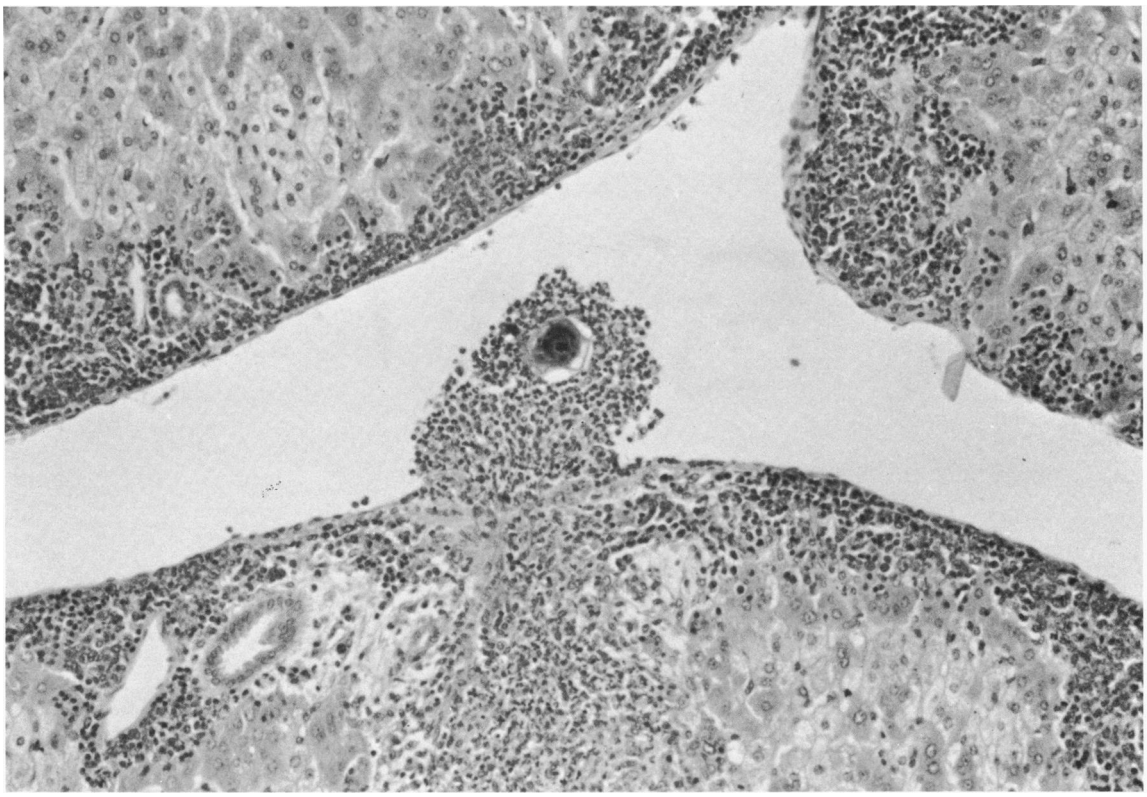

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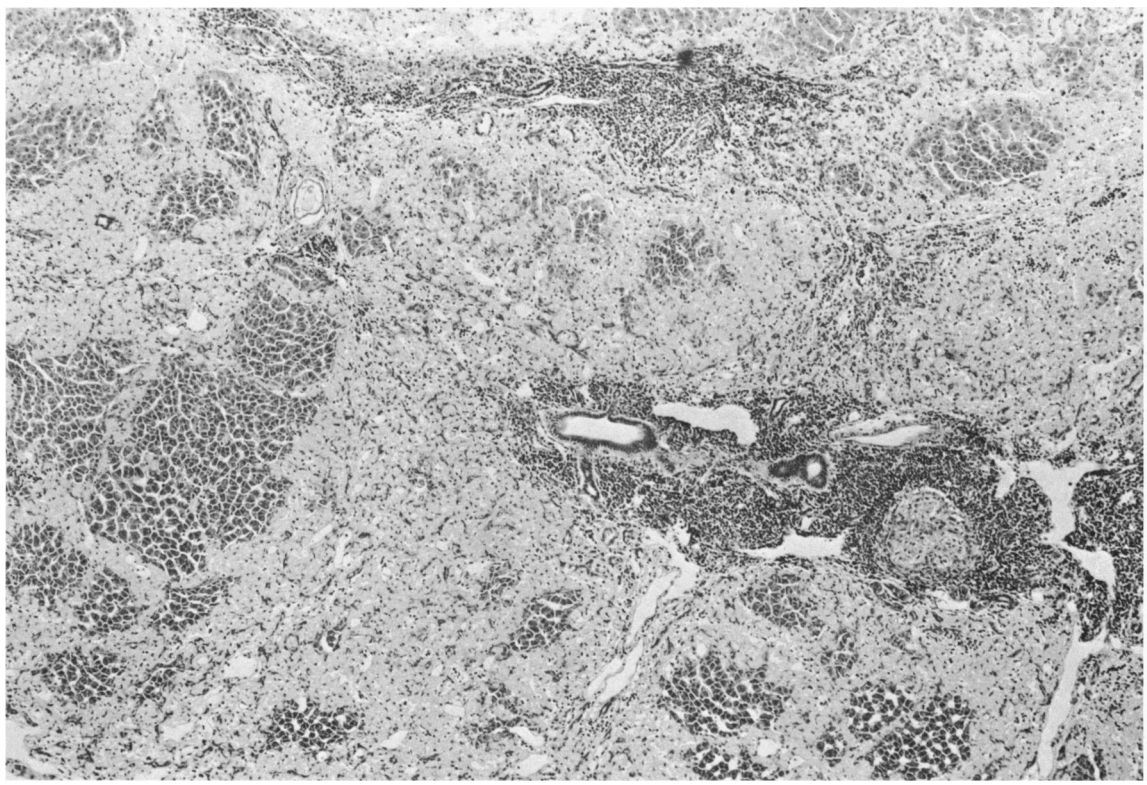

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