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Plasmodium berghei in Malaria Research

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Parassitologia. — Plasmodium berghei *in Malaria Research.* Nota di MEIR YOELI e HARRY MOST, presentata (*) dal Corrisp. G. RAFFAELE.

Historical.—The history of malaria research in the Nineteenth Century and in our present day has been marked by a number of fundamental clinical observations and discoveries in which avian and mammalian plasmodia have played a great and most significant role.

Closely following in the wake of the classical discovery of Laveran in 1880 and the elucidation of the nature and action of the human plasmodia and their periodicity by Camillo Golgi, Marchiafava, Bignami, Bastianelli and Grassi, other scientists and clinicians have shed light on the natural history of the malaria parasites by experimentation on different haemosporidian parasites in nature. Dionisi added important observations on malaria of bats, MacCallum demonstrated the fertilization of the macrogamete and the formation of the zygote (ookinete) in studies on haemoproteus of birds. Ronald Ross and to a great extent Battista Grassi have elucidated the sporogonic cycle of development of the malaria parasites in the different mosquito vectors by using *Plasmodium praecox* of birds. Studies by Danilevsky, the Sergent brothers and others filled the gaps in our knowledge of certain phases of the life history of the plasmodia. Thus, we witness the emergence of the concept of pre-immunity and cellular immunity in malaria and protozoan infections by Talliaffer and Sergent based strongly in experimental work on malaria of canaries and monkeys.

The great epidemics of the First World War brought with them a renewed interest and onslaught of research aimed at a better and deeper understanding of the natural history of the plasmodia and the effect of chemotherapeutic agents on the malaria fevers. It was by investigations on the effect of Plasmochin in avian malaria, later to be followed by studies on the plasmocidal action of Atebrin, that a new era in malaria research was opened. But the elucidation of the complicated life cycle of the malaria parasite and its primary tissue phase was delayed until the 1930's. It was Raffaele's epoch–making discovery on the tissue schizogony of malaria parasites, a stage which precedes erythrocytic schizogony and which may linger on, which has opened up a new horizon and has served as a guide line for later investigations. The work of Brumpt, James and Tate, Huff and others, in chicken malaria (*P. gallinaceum*) has received its impetus from Raffaele's work in *P. elongatum* and *P. praecox*. The subsequent discoveries of Shortt, Garnham, and Bray on the primary tissue phases in the parenchyma cells of the liver in human malaria

(*) Nella seduta del 17 giugno 1965.

were also preceded by experimental research and demonstrated in monkey plasmodia, *P. cynomolgi*, *P. inui*, and others.

Though these discoveries have greatly illuminated many aspects of malaria research, the malaria fevers of man remained and still remain in part a mystery. We know little of the mechanism of relapses. We know nothing of the nature of latency of the infection and the causes of recrudescence. The resistance of plasmodia against modern malaria chemotherapeutic agents and the enhanced resistance of mosquitoes to contact insecticides has focused world attention again upon the global problems of malaria and have greatly frustrated and diminished the possibility of eradication of these ancient scourges of mankind especially in the tropics.

The Discovery of Plasmodium berghei.—During all these years, the dream of malariologists has been to find in nature a mammalian plasmodium easy to handle, easy to transmit to different small laboratory animals, and which could have kinship in its clinical course, pathology, immune response and response to chemotherapy to the human plasmodia. This wish was half realized when Vincke and Lips (1948) discovered *P. berghei* in wild tree rats in forest galleries of the Congo.

The parasite was found to be transmissible by blood inoculation to white mice, hamsters, and albino rats and to a number of other laboratory and wild rodents. However, the early enthusiasm and interest shown to this plasmodium was diminished as it was not possible to transmit it cyclically through laboratory-bred mosquitoes. The natural vector, *Anopheles dureni*, could not be bred in captivity. *A. dureni* was considered too " fragile " and strictly adapted to its own biotope to be able to survive in captivity. Most of the attempts to infect other laboratory species of *Anopheles* with this plasmodium did not succeed.

The trend of research moved by force to other directions and studies of blood induced infections dominated the scene. Research on *P. berghei* in recent years, though manifold and on the rise, found itself on the crossroads. For in spite of the employement of this plasmodium in vast screening projects of chemotherapy and immunology, it was clear to all that the parasite could not aspire to " eminence " as long as part of its life cycle was unknown. These thoughts were upon our minds when we decided to go out to the Congo to observe the parasite in its true habitat and to learn more about its natural history.

The Temperature Factor in Sporogonic Development *of* P. berghei.—In December 1963 we went out to the Congo and made an ecological study of *P. berghei* in the Kisanga forest gallery. *P. berghei* is a seasonal epizootic among the rodents of these small forest galleries situated in the vast savannah of the uplands of Katanga. *A. dureni*, which is a strictly zoophilic mosquito, shade-loving and tree-loving, bites the local rodents and transmits *P. berghei* among them. The sporogonic development, as we have discovered, takes

place under strict temperature conditions. The cool and humid groves keep a static temperature of 21° C–18° C during the day at the height of the transmission season (December to March). During the night the temperature drops to 16° C and lower. The temperature measurements convinced us that laboratory-bred *Anopheles* species exposed to such temperature conditions as prevail in the forest galleries of Katanga during the incubation period of the parasites following infective blood meals would permit normal sporogonic development in the midguts and invasion of the salivary glands by sporozoites. This assumption was found to be true. *A. quadrimaculatus*, *A. stephensi*, and *A. atroparvus* have been experimentally infected with strains of *P. berghei* and cyclical transmissions by mosquito bite or sporozoite inoculation were carried out regularly in mice, hamsters, young albino rats and laboratory-bred tree rats (*Thamnomys*).

The Pre-erythocytic Development of P. berghei.—Having at our disposal viable and invasive sporozoites we proceeded to attempt to solve the problem of the primary development of *P. berghei*. It was a problem of vast proportions for nobody knew the tissue tropism of this parasite. It could have been the reticulo-endothelial system, the hemopoetic system or a new, yet unknown primary development. Methods of search were therefore aimed in different directions.

Sixty sporozoite-inoculated animals were sacrificed during different stages of their prepatency and all their organs searched for the hidden forms. In none was any pre-erythrocytic form found except in a young hamster, number 2385. In the liver of this animal, in histological sections stained in Giemsa collophonium, a single tissue schizont similar in all its morphological details to those described for monkey and human malaria parasites was found. The parasite was large, 36 microns × 28 microns in diameter. It was a mature schizont in process of segmenting. The mature schizont was found 51 hours after sporozoite inoculation. The discovery of this single parasite permitted us to concentrate all our efforts in the search of the pre-erythrocytic tissue growth stages in the parenchyma cells of the liver in different experimentally infected animals. Using sporozoites from *A. stephensi* and the intravenous inoculation route to inject 200,000 viable sporozoites, we were able to find pre-erythrocytic schizonts in abundance. We found them 51–65 hours after sporozoite inoculation. Their diameter varies between 32 × 25 microns, 52 × 35 microns, and as large as 69 × 48 microns. They contain as many as 10,000 to 18,000 merozoites, perhaps even more. The schizonts may by seen in 6–12 consecutive sections each of 4 microns, giving the mature parasite a depth of 36–50 microns.

In most of its aspects the mature pre-erythrocytic schizont resembles *P. cynomolgi* and *P. vivax*. However, smaller and larger forms are encountered. Pre-erythrocytic forms in experimentally infected hamsters, tree rats (*Thamnomys*) and young albino rats have been demonstrated hitherto. But there is no doubt that such forms will be found in sporozoite-induced infection in the white mouse and in other suceptible laboratory animals.

Future Research.—What is the significance of these discoveries ? In our field observations we discovered a general phenomenon and a biological law of temperature governing and affecting the sporogonic development of malaria parasites. This enabled us easily to adapt alien mosquitoes to serve as experimental vectors for *P. berghei*. The discovery of the pre-erythrocytic development of *P. berghei* in the parenchyma cells of the liver anchored this malaria parasite strongly to the plasmodia of man and the primates. Practically, it opened a new horizon for in vestigation in chemotherapy, immunology, and studies of relapses in malaria. It opens to the biologist and to the clinician a gate for new research. The malaria fevers, the oldest and most tenacious adversaries of man, have through centuries of intense study and observation in many different countries been brought to a standstill. There is hope of their decline and control by modern scientific thought and action. *P. berghei* will add to this endeavour greatly because of the facility of large-scale experimentation and the kinship to the plasmodia of man. The amazing quick growth of this plasmodium, from sporozoite to mature pre-erythrocytic schizonts within 51 hours, gives hope of realizing this growth in tissue culture—an aim which has not been realized hitherto for mammalian plasmodia.

In the past, malaria research has greatly influenced the medical and the biological sciences. Many a medical, clinical discipline were borne out of the struggle and effort to solve an unknown aspect in malaria. The science of clinical haematology owes its birth to the staining of the malaria parasites and Romanovsky's description of the properties of methylene blue, eosin and azur. Golgi's classical description of the effect of a parasitic rhythmic growth upon its mammalian host is the basis of a new science of biological clock mechanisms in nature and in cells. The geographical pathology of diseases is a child of observation over centuries of the ebb and flow of the malaria fevers.

The malaria parasite still keeps within itself, hidden and obscure, a world of its own. Intracellular and for short periods free, it moves from a poikilothermic vector to the liver and blood of a mammalian host. Its behaviour within the body, its growth, its metabolism, its biochemical and enzymatic pathways may be followed closely. The effect of chemotherapeutic agents can be studied on the blood forms and with the new tool of easy access of pre-erythrocytic forms also on the stages, hidden and protected in the liver. Future research could employ *P. berghei* as a many-sided biological tool for measuring bio-phenomena and to bridge the unknown in many fields.

Thus, we can envisage that the plasmodia, one of the most ancient and formidable enemies of mankind, can be harnessed like the wind and the wave, the sunray and the atom for research and for the use of welfare of man.

RIASSUNTO. — Le grandi scoperte fatte nel passato in campo malariologico ed i successivi progressi scientifici, medici e biologici, sono stati possibili in seguito agli esperimenti sui plasmodi degli uccelli e dei mammiferi. Le nostre conoscenze del ciclo di sviluppo del parassita malarico nella zanzara vetrice e nell'ospite vertebrato, l'azione delle sostanze chemio-

terapeutiche ed il concetto di immunità nelle infezioni causate da protozoi, non sarebbero state possibili senza le indagini sperimentali sui plasmodi degli uccelli e delle scimmie.

Plasmodium berghei, il parassita dei roditori selvatici del Congo, che fu scoperto da Vincke e Lips nel 1948, ha portato alle ricerche sulla malaria e sulle malattie tropicali uno strumento preciso, di grande utilità. La facilità di trasmissione di questo plasmodio agli animali comuni da laboratorio – il topo bianco, il criceto dorato, il ratto albino – e la possibilità di sperimentare su grande scala, fanno sì che si possa considerare *P. berghei* come il plasmodio essenziale per le indagini scientifiche dell'avvenire.

Il comportamento clinico della malaria causata da *P. berghei* nei diversi ospiti sperimentali si svolge in un modo simile alle febbri malariche nell'uomo; ma soprattutto la scoperta fatta recentemente dagli Autori del ciclo pre–eritrocitico nelle cellule del parenchima epatico, con forme assai simili a quelle trovate nella malaria delle scimmie e dell'uomo, porta alla possibilità di nuovi sviluppi nel campo della malaria sperimentale. Le ricerche sulla chemioterapia e sulla chemioprofilassi medicamentosa contro i parassiti resistenti, gli studi sulla immunità contro gli sporozoiti e le forme tissulari del parassita, protette e nascoste nel fegato, potranno essere eseguiti per mezzo di *P. berghei*.

Questa scoperta del ciclo esoeritrocitico di *P. berghei* e l'osservazione di una legge biologica circa l'effetto della temperatura sullo sviluppo sporogonico, che ha permesso di introdurre facilmente trasmissioni cicliche in laboratorio, aprono un nuovo orizzonte non solo alla scienza della malaria sperimentale, ma anche ad altri campi della biologia e della medicina, formando uno strumento versatile a futuri studi basilari.

DESCRIPTION OF THE PLATES I–IV

PLATE I.

Fig. 1. – Mature oocyst with sporozoites of *P. berghei* on the midgut wall of *Anopheles stephensi*, unstained, oil immersion, × 900.

Fig. 2. – Mature oocyst of *P. berghei* in midgut of A. *quadrimaculatus*, stained oil immersion, × 900.

PLATE II.

Fig. 3. – Sporozoites of *P. berghei* from the salivary glands of experimentally infected *A. stephensi*, unstained, × 825.

Fig. 4. – Giemsa stained sporozoites of *P. berghei*, oil immersion, × 900.

PLATE III.

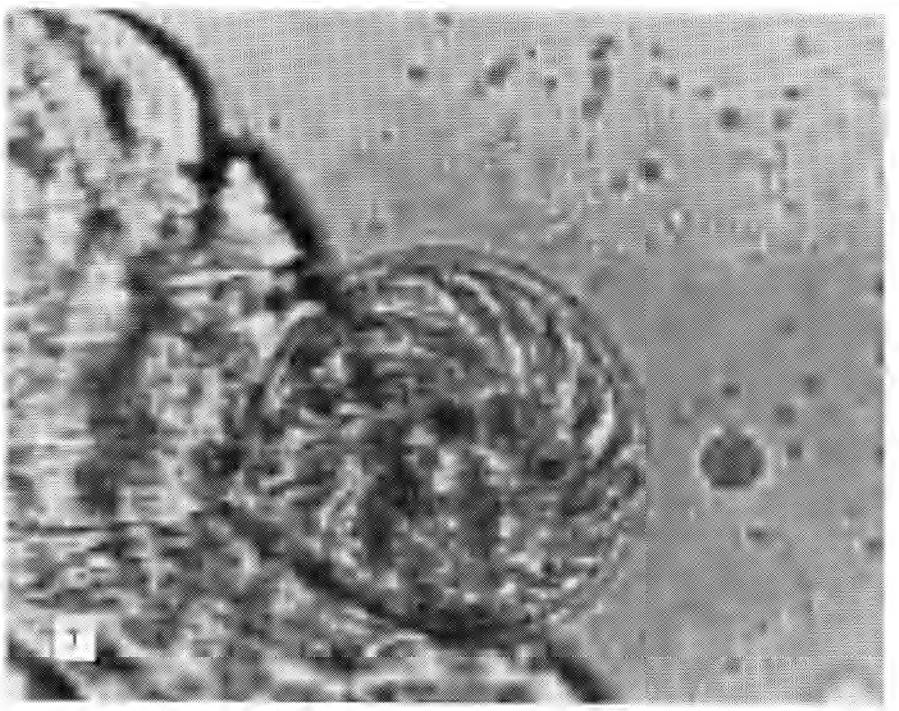
Figs. 5, 6. – Pre-erythrocytic schizonts of *P. berghei* approximately 40 hours old in liver parenchyma cells of experimentally infected young albino rat, Giesma-collophonium staining section, 4 microns thick, oil immersion, × 900.

Figs. 7, 8. – Nearly mature schizonts of *P. berghei* in experimentally infected tree rat hours after intravenous sporozoite inoculation, × 900, oil immersion.

PLATE IV.

Fig. 9. – Pre-erythrocytic schizont of *P. berghei* in liver parenchyma cell approximately 42 hours after intravenous sporozoite inoculation. Observe nuclear division in process. Baby rat 411, oil immersion, × 900.

Fig. 10. – Nearly mature schizont of *P. berghei* measuring 58 × 57 microns, 51 hours old, tree rat 134, oil immersion, × 900.



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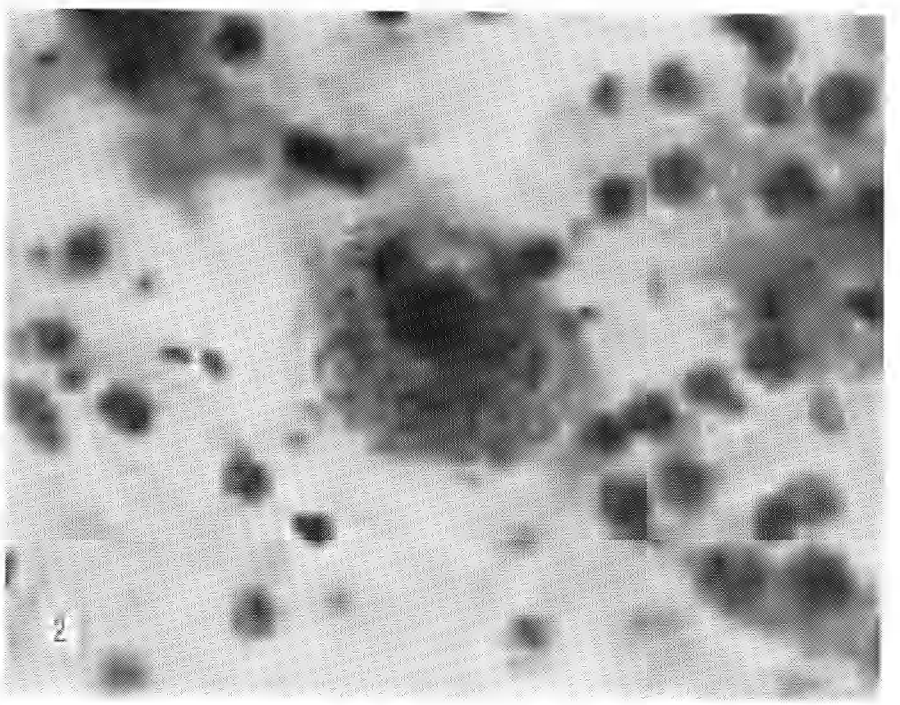

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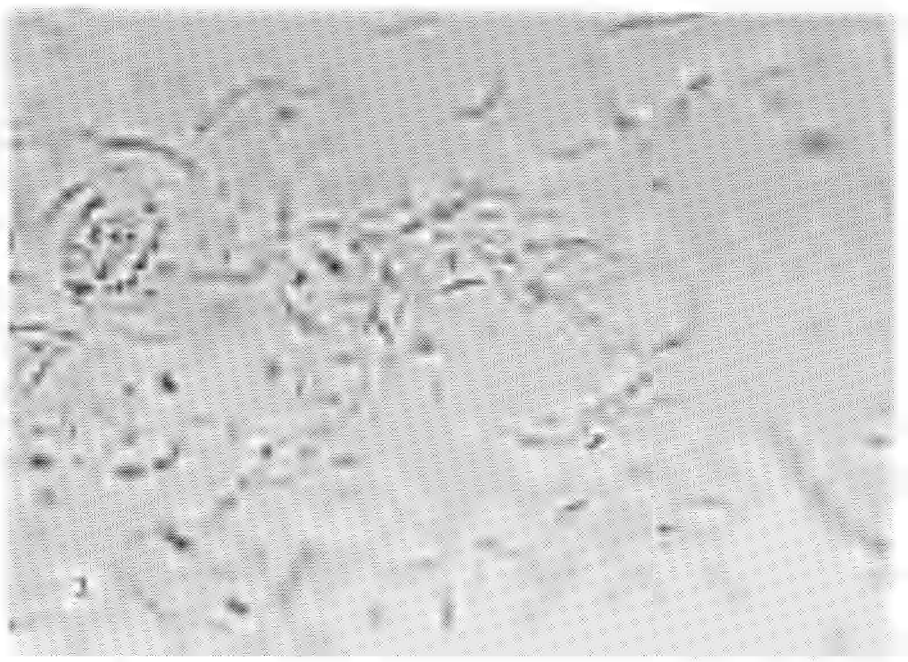

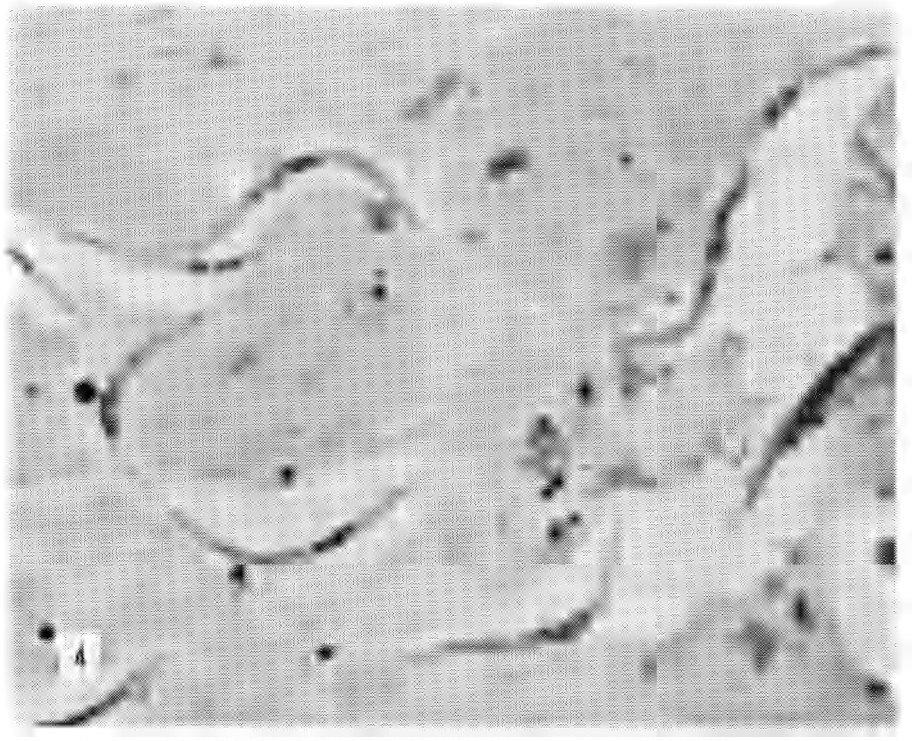

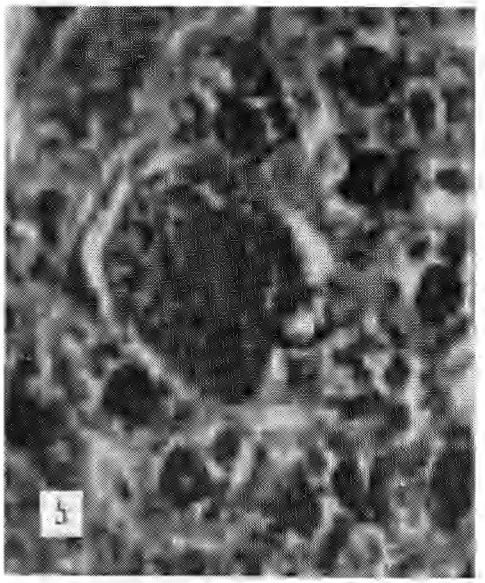
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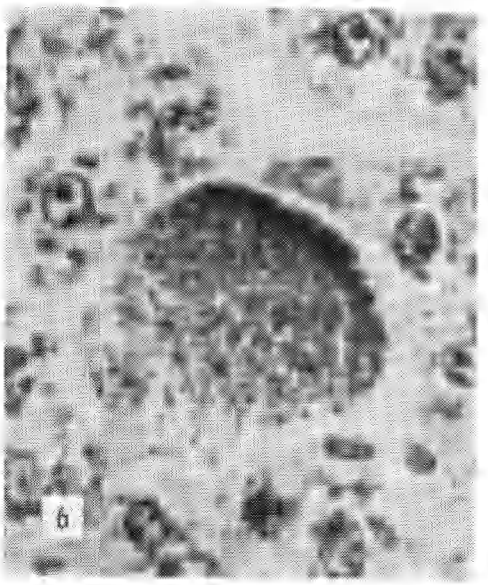
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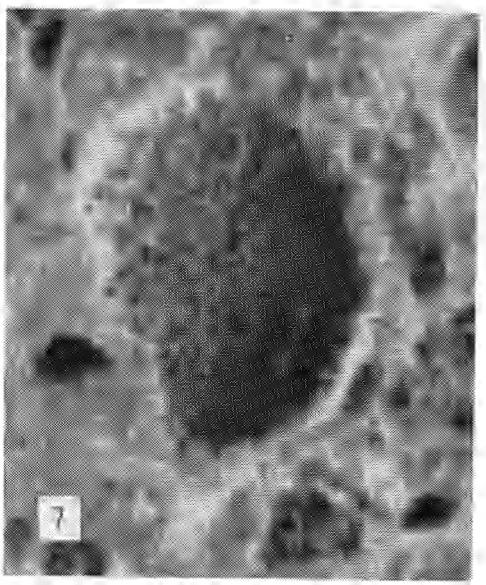
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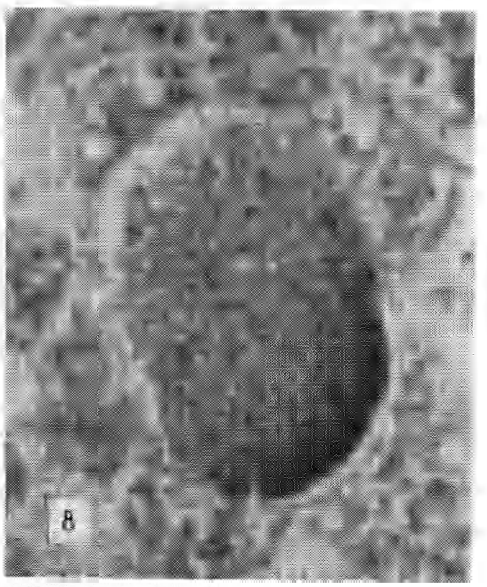
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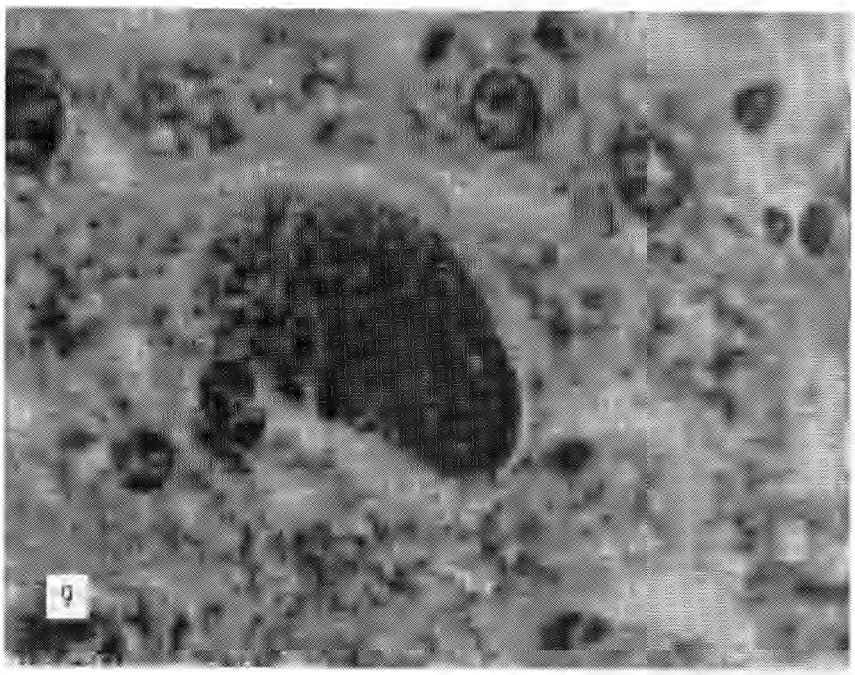
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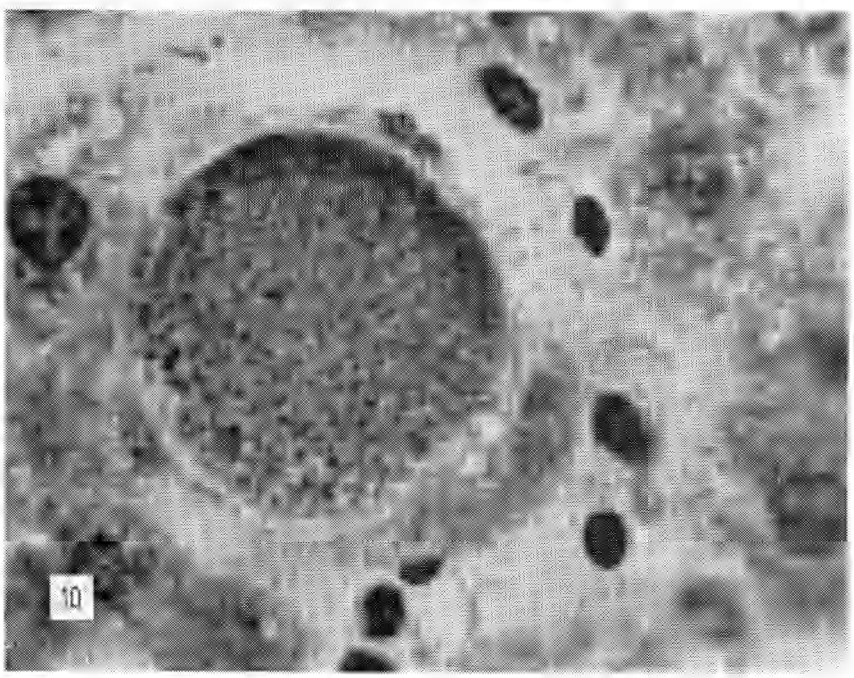
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