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THE TRANSMISSION OF *PLASMODIUM FALCIPARUM* TO THE HOWLER MONKEY, *ALOUATTA SP.* II. CELLULAR REACTIONS.*

BY

WILLIAM H. TALIAFERRO AND PAUL R. CANNON.

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In the preceding section a description has been given of the transmission of *P. falciparum* from man to 9 howler monkeys and of the subinoculation from one of these to another monkey (see especially table 3). In this section, the histological study of tissues from biopsies on the spleens of five of them and from necropsies on all of them will be given. This has been carried out in conjunction with an extended study of the cellular responses of various Panamanian monkeys, including the present species, to the natural malarial parasite of monkeys, *P. brasilianum*.

The tissues consisted of the spleen, liver, myocardium, brain, lungs, kidney, intestine, intestinal lymph nodes and bone marrow, the last having been taken from the femur. They were fixed in Zenker's fluid plus 5 per cent neutral formalin without acetic acid, sectioned in celloidin and stained by Maximow's method with eosin-azur II and hematoxylin (Maximow, 1909). The histology of these tissues is of interest when compared with the histology of animals infected with *P. brasilianum*, as described preliminarily by Taliaferro (1932), and may be taken up under the following heads:

1. The macrophage activity.
2. The general lymphoid hyperplasia.
3. Tissue localizations.

No noticeable phagocytosis of parasitized erythrocytes or of pigment was seen in any of the organs in monkeys 156, 68A, 199, 146, and 200, although in monkey 199 many parasites were seen. In monkey 198, which was infected 2¼ days, there were scattered pigment granules in the macrophages of the spleen and Kupffer cells of the

* From the Department of Hygiene and Bacteriology and the Department of Pathology of the University of Chicago and The Gorgas Memorial Laboratory, Panama City, R. de P.

liver, somewhat fewer in the macrophages of the bone marrow and an occasional clump of pigment in the macrophages of the lung, but none in the kidney, brain, intestine and intestinal lymph nodes. The same findings were true of 206 except that the pigmentation was more pronounced and in the case of the spleen and bone marrow a number of parasitized red cells were also seen within the phagocytes.

As pointed out in section I, monkey 171 had probably, and monkey 204 had undoubtedly undergone a crisis. This is reflected in the tissues where much pigment occurred, especially in the macrophages of the spleen, liver and bone marrow. A few pigmented macrophages were found in the lung. Unphagocytosed parasites were not seen in 204, but occurred in considerable numbers in 171, which is what could be expected from the data in table 3. In monkey 171 a large number of pigmented mononuclears (large transitional forms) were seen in the blood vessels of the lung, heart, brain, kidney and in peripheral blood smears, but the actual process of phagocytosis may have taken place in other sites.

The evidence, as far as it goes, indicates that the sluggish phagocytosis by the macrophages of the spleen, liver and bone marrow is correlated in *P. falciparum*, as in *P. brasilianum*, with the death of the organisms during the acute period and is greatly increased at the time of the crisis. Throughout, however, there is less phagocytosis in *P. falciparum* infections. This difference is probably conclusive in spite of the small number of cases and is strikingly brought out by the macrophages shown in plate III from the spleen and liver of monkeys 171 and 204 infected with *P. falciparum* and from monkey 119 infected with *P. brasilianum*. The infection in monkey 119 (fig. 2, prev. sect.) lasted longer than 171 (fig. 1, prev. sect.) but the latter reached a much higher peak in numbers and due to its tertian periodicity probably represented the death of more parasites.

The injection of such large quantities of foreign red cells would suggest an active erythrophagocytosis by the macrophages. None was observed in the spleen and liver of monkeys 156 and 199 which were infected for about a day; a small amount was noted in 198 which died on the 2nd day; and a pronounced amount was evidenced in 206 which lived almost 3 days.

None of the animals showed any marked activation of the lymphoid elements of the spleen or bone marrow. Most of the infections ran too short a course for this to be significant, but in view of our results with *P. brasilianum* we would have expected considerable stimulation in the case of monkeys 171 and 204 which underwent extremely

severe, if short, infections. This did not prove to be the case. The splenic follicles of these animals were compact without pronounced germinal centers and were within the normal limits. The bone marrow was not hyperplastic. Of particular interest was monkey 204 because a biopsy was performed on its spleen before infection. The spleen sections before and after infection were indistinguishable with regard to the hyperplasia of the lymphoid elements. These findings strongly suggest, therefore, that *P. falciparum* does not stimulate the lymphoid system as markedly as the natural parasite. The evidence would be conclusive except for the small number of cases and the variability encountered in studying the monkey-parasite.

The organs showing an invariable concentration of parasites (provided a concentration occurred) were the spleen, liver and bone marrow. Thus, in monkey 199 which was killed after an infection of only one day the blood showed an infection of 63 plasmodia per 10,000 red cells, whereas the spleen and bone marrow showed 223 and 143, respectively. Similarly, monkey 198, after a two-day infection, showed 12 parasites per 10,000 red blood cells in the blood, but 113 and 67 in the spleen and bone marrow, and monkey 171 which was killed on the 6th day (probably at the crisis) showed 21 parasites per 10,000 red blood cells in the blood, but 180 and 106 in the spleen and bone marrow. Monkey 206 showed 57 in the spleen, 85 in the liver and 65 in the bone marrow per 10,000 red cells. (Blood films from this monkey were not made at the time of necropsy.) Interestingly enough, monkeys 156, 68A, 146 and 200, in which the infection quickly subsided, showed no parasites in their blood, spleen, liver or bone marrow. Also, monkey 204 which was infected for 8 days, but which underwent a typical crisis showed at death one parasite in the thick blood film and none in the organs. In other words, this animal had succeeded in eliminating the infection.

One localization of parasites was encountered in monkey 199 in the myocardium. Here, in the larger vessels, comparatively few parasites occurred, but in the capillaries the majority of the red cells were parasitized. This animal (table 3) was killed after an infection of one day.

Discussion.

The foregoing data indicate that the infection with *P. falciparum* can be cleared up with less macrophage activity than is the case with *P. brasilianum* and with practically no demonstrable general lymphoid activation. This suggests to the authors that *P. falciparum* may be so poorly adapted to this abnormal host that it is largely self-limited

and thus necessitates little active resistance on the part of the host. Very possibly the macrophages may be taking up parasitized red cells in which the parasites are already moribund from non-specific factors of the host. Further work will be necessary to ascertain whether *P. falciparum* can be actually acclimated to the monkey.

As would be expected there is conclusive evidence of reproductive activity of the parasites elsewhere than in the peripheral blood. With the exception of one localization in the myocardium, the only general localizations occur in the spleen and to a less extent in the liver and bone marrow. We have interpreted similar concentrations in *P. brasilianum* as being an immune reaction preparatory to phagocytosis, but in view of the lack of marked phagocytic activity and the virtual absence of other localization-sites, we are inclined to believe that they probably represent true reproductive centers in the *P. falciparum* infections.

No gametocytes were found in any of the infections studied—a fact which makes it imperative to examine critically the evidence that the infections described were *P. falciparum*. This can be considered under several headings as follows:

1. *Freedom of experimental monkeys from previous infection.* As stated earlier, only three of the many monkeys from Herrera have been found infected with *P. brasilianum*. Furthermore, the five spleens on which biopsies were performed prior to injection with parasites were negative for parasites and for pigment. Since all of these monkeys were infants, just weened, their spleens would still have shown pigment had they been previously infected. Nor can this be interpreted as indicating that the animals were infected, but still in the incubation period, because not a single infant animal from this locality developed a natural infection after its entrance into the laboratory.

2. *Morphology of the parasites.* As has been shown, the parasites are clearly similar to *P. falciparum* and not *P. brasilianum* of the monkey. It is also noteworthy that no gametocytes, similar to *P. brasilianum*, were found in any of the infections.

3. *Asexual periodicity.* The periodicity of the infections under discussion is clearly tertian, whereas, as has been shown by Taliaferro (1932), that of the malarial parasite of Panamanian monkeys is quartan.

4. *Method of following the infection.* The direct transference of such large numbers of parasites into known uninfected animals precluded the possibility of confusing the resulting infection with a relapse of a preëxisting infection in the monkey.

5. *Pathology.* Although not as decisive as the foregoing, it is noteworthy that the cellular responses, though qualitatively similar, are quantitatively different from the cellular responses to infection with *P. brasilianum* in the same species of monkey.

Summary of sections I and II.

This study involved the transmission of *P. falciparum* from 9 human beings into 9 monkeys (156, 68A, 199, 198, 206, 146, 171, 204, 167) and its subinoculation from one monkey (171) to another (200) (table 3). After the human infection in monkey 167 had disappeared, it was inoculated with *P. brasilianum,* the quartan malarial parasite of the new world monkeys. The parasites were injected intravenously in such large numbers in all cases that they could be found immediately thereafter and could be studied continuously.

The longest infection was encountered in monkey 204 and lasted 8 days at which time the animal died with a few parasites in its blood. It reached a peak of 187 parasites per 10,000 red cells 92 hours after infection and showed 2 sporulation periods 40 and 88 hours after infection. (Through the balance of the infection parasites were too scarce to ascertain sporulation periods.) (Fig. 3.)

The most intense infection was encountered in monkey 171 and reached a peak of 915 parasites per 10,000 red cells (i.e., every eleventh cell infected) 88 hours after infection. Parasites were moderately numerous in this monkey at the time it was killed, i.e., 5⅔ days after infection. During this time 3 sporulation periods occurred (fig. 1).

The infection in monkey 167 reached a peak of 20 parasites per 10,000 red cells 42 hours after inoculation and exhibited 2 sporulation periods, but disappeared after 4 days (table 3).

The remainder of the infections (table 3) were either extremely transitory (156, 68A, 146, 200) or the monkeys died prematurely (199, 198, 206). Two of these, however, showed one sporulation period (198, 206: table 3).

The infections were characterized by: the occurrence of asexual stages (no crescents were found) morphologically indistinguishable from *P. falciparum* in man (tables 1–2 and plates 1–2); a 48-hour cycle of reproduction during the sporulation of which comparatively few segmenters (more than in man) occurred in the peripheral blood; and a series of marked increases and decreases in the number of parasites terminated by a crisis during which most of the forms disappeared from the blood.

Histological examinations of the tissues showed that pigment oc-

curred in the macrophages of the spleen, liver and to a less extent of the bone marrow by the second day and together with parasitized red cells occurred more abundantly in the macrophages after the crisis. This represented an activation of the macrophages which, however, was not as great as in comparable infections with *P. brasilianum*. No general lymphoid activation was encountered. The sluggishness of the tissue reactions suggests that the parasites were disposed of easily as compared with the natural parasite of the monkey, *P. brasilianum*.

Pronounced concentrations of the parasites were found in the spleen, to a less extent in the liver, and to a still smaller extent in the bone marrow from the first day until the crisis. These concentrations are believed to represent true reproductive centers because of the sluggish phagocytosis and because reproduction of the parasites must take place elsewhere than in the peripheral blood to account for the increases in numbers of parasites in the blood. Only one animal exhibited a localization other than in these sites and that in the capillaries of the myocardium.

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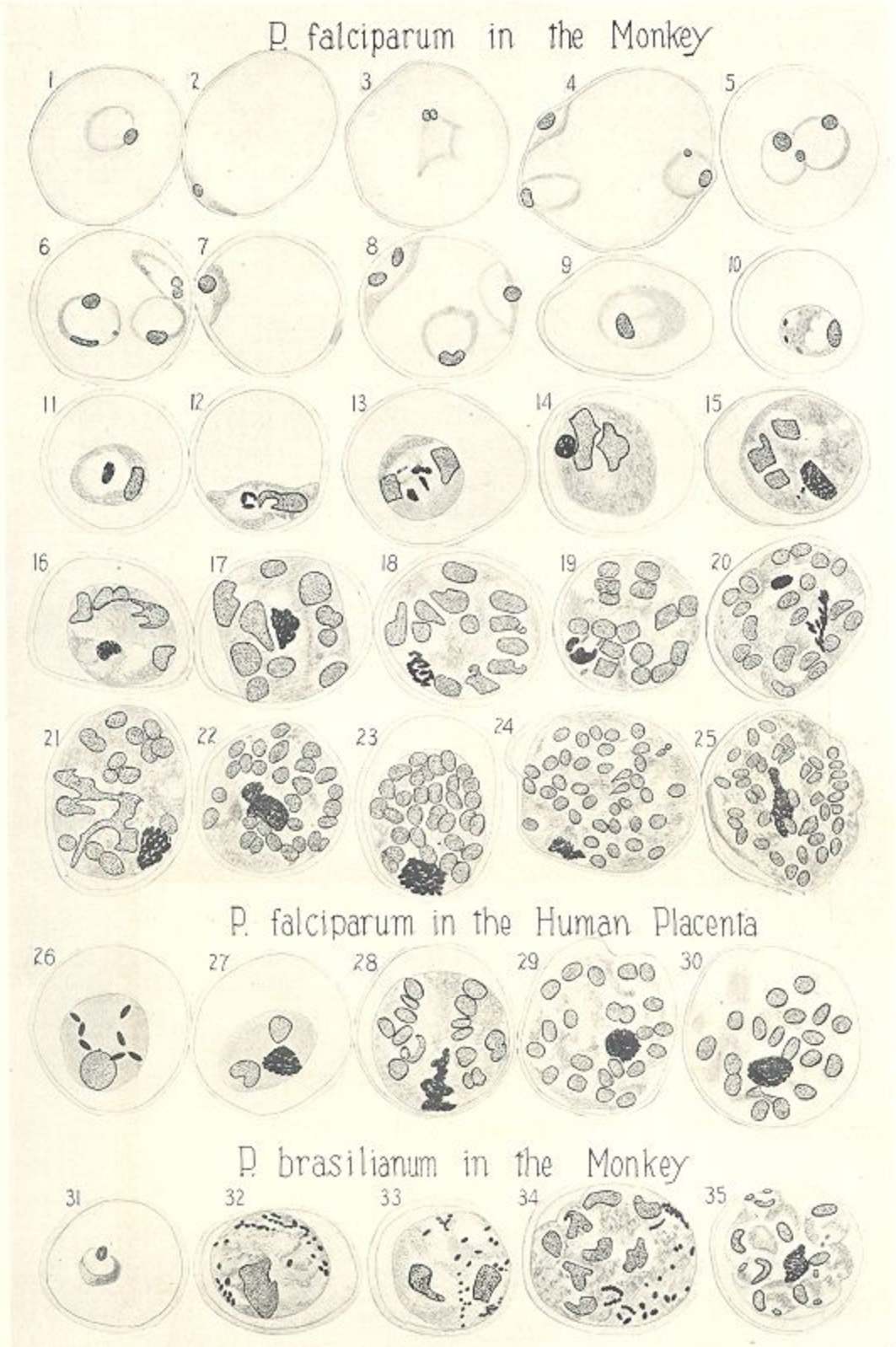
EXPLANATION OF PLATES.

PLATE 1. 1–25, *P. falciparum* in the monkey; 1–3, young rings; 4–8, larger rings; 9–12, young schizonts showing the development of pigment; 13–25, segmenters; 26–30, *P. falciparum* in the human placenta; 31–35, *P. brasilianum* in the monkey. × 3000.

PLATE 2. 1–10, *P. falciparum* in the monkey; 1–2, young rings, showing typical sub-tertian morphology (after second sporulation in monkey); 3–4, young schizonts (the red cells show Maurer's dots); 5, schizont, undivided as yet; 6–10, segmenters. Photographs × 2500.

PLATE 3. Comparative pigmentation of the macrophages of the spleen and liver in *P. brasilianum* and *P. falciparum*. 1, macrophages of spleen in monkey 119 after crisis with *P. brasilianum;* 2, Kupffer cell of liver in monkey 119; 3, macrophages of spleen in monkey 204 after crisis of *P. falciparum;* 4, isolated macrophages in monkey 171 after probable crisis of *P. falciparum;* 5, Kupffer cell from liver of monkey 171. *Ery*, erythrocyte; *K Lit*, Kupffer cell; *Lit*, littoral cell; *Liv*, liver cell; *Mac*, macrophage; *Par*, malarial parasite; *Pig*, malarial pigment; *Poly*, polymorphonuclear leucocyte; *Sin*, sinusoid; *S Lym*, small lymphocyte. Drawings × 1500.

The inner line of the apparent double line in each figure of Plate I denotes the contour of the red cell. The outer line is a false shadow.


P. falciparum in the Monkey
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P. falciparum in the Human Placenta
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P. brasilianum in the Monkey
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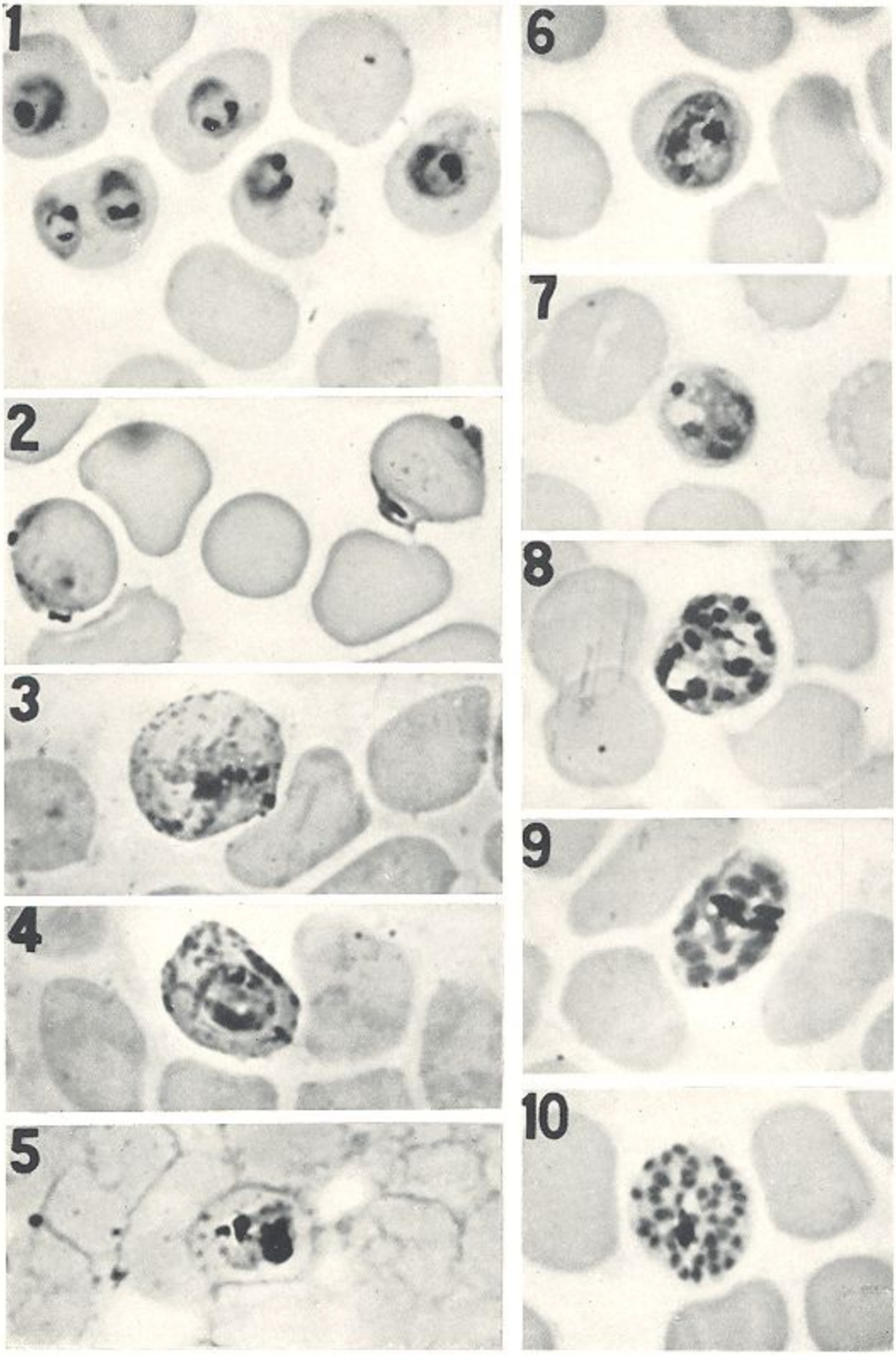
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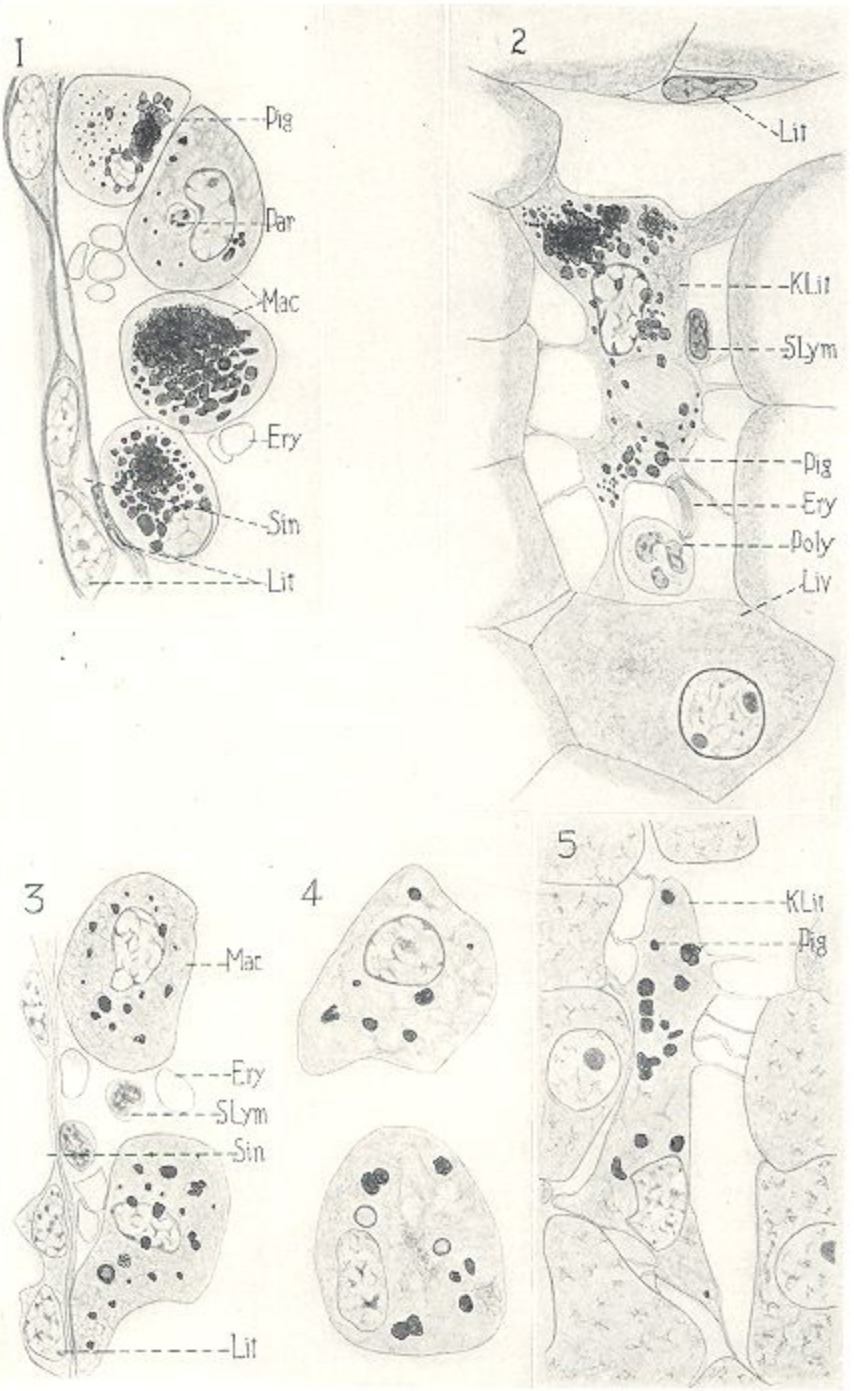
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