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EXPERIMENTAL CONCOMITANT TOXOPLASMA AND MALARIA INFECTION IN RATS

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Abstract. The effect of concomitant toxoplasma and malaria infection on the reticuloendothelial system was investigated in rats. This was evaluated by the level of plasmodial parasitaemia; humoral antibody response; effect on splenic weight; histopathological changes in thymus and spleen; histopathological and histochemical changes in liver. The parasitaemia appeared after 2 days in single malaria and concomitant infections. The peak was reached after 6 days with single and precedent malaria, and after 10 days with precedent toxoplasma. The clearance of parasitaemia was delayed to 30 days with concomitant infections instead of 14 days with single malaria. Higher than normal malarial antibody levels were reached with precedent toxoplasma, while the toxoplasma antibodies were lower than normal in both concomitant infections. There was a significant increase in splenic weight in both precedent malaria and toxoplasma, followed by a decrease which did not return to normal in case of precedent malaria. The thymus was packed with thymocytes in precedent malaria, while depletion in the cortex occurred in precedent toxoplasma. In the liver, there was glycogen depletion and decrease in succinic dehydrogenase activity in both concomitant infections. Choline esterase activity in precedent malaria was decreased and returned to normal on day 40 while in precedent toxoplasma the activity was normal all through the period. The alkaline phosphatase activity was decreased and returned to normal on day 40 in both concomitant infections.

In the defensive mechanism of malaria there is phagocytosis by reticuloendothelial system (RES) (Globe and Singer 1960), as revealed by a significant proliferation of the lymphoid macrophage elements and an increased number of parasitized erythrocytes within the tissue macrophages (Maegraith 1954). The immune response in malaria is primarily humoral as shown by the increase in IgG, IgM, IgA in primary attacks of malaria (Tobie et al. 1966, Collins et al. 1971). The proven role of serum antibody does not exclude the possibility that cell-mediated immunity dependent upon actively sensitized lymphocytes of thymic origin may play a part in specific acquired resistance to malaria (Cohen and Butcher 1971, 1972, Cohen et al. 1974). The studies of Brown et al. (1968, 1970) and Phillips et al. (1970) indicated that *T.* cells may function in the acquisition and maintenance of protective immunity to animal malaria.

Toxoplasma infection in humans usually stimulates a humoral response. Studies in animals have indicated that the development of cellular immunity is the major factor in slowing the progression of toxoplasma infection in vivo (Jones 1974).

As regards concomitant infection of toxoplasma with other parasites, Ruskin and Remington (1968) demonstrated that mice infected with toxoplasma were resistant to challenge with numbers of *Listeria monocytogenes* and *Salmonella typhimurium* that were uniformly lethal to normal mice. Dumont et al. (1975) reported that splenomegaly induced by toxoplasma infection was superimposed on that induced by schistosomiasis, resulting in marked enlargement of the organ. Mahmoud et al.

(1977) concluded that also toxoplasmosis has a prolonged effect on cell-mediated granulomatous hypersensitivity in vivo, resulting in considerable alleviation of hepatic schistosomiasis. Also Abd-El Wahab et al. (1974) found that malarial infection in mice suppressed granuloma formation around *Schistosoma mansoni* eggs in lungs. There was no significant difference in the level of antibodies specific for *S. mansoni* eggs in the sera of malaria-infected mice as compared to mice infected with *S. mansoni* eggs alone. In previous studies, the histopathological and histochemical reactions on RES after acute and chronic single toxoplasma and malaria infection were reported (Rifaat et al. 1981, 1983). In this study, concomitant infection with these two parasites was studied in two models: rats infected with malaria and 10 days later with toxoplasma (precedent malaria) and rats infected with toxoplasma and 10 days later with malaria (precedent toxoplasma) in comparison with the above two mentioned single infections.

MATERIAL AND METHODS

Animals. Laboratory outbred albino rats weighing 50–60 gm were used. All animals were fed on a balanced cube diet containing the minimal daily requirement of beans, maize, wheat, barley and water. Groups of 12 rats were used for inoculation in each experiment. For toxoplasma infection, the virulent RH strain was inoculated subcutaneously intrascapularly with a standardized dosè of 5×10^4 extracellular trophozoites. For malaria infection, rats were inoculated intraperitoneally with a standardized dose of 4×10^4 erythrocytes parasitized with *Plasmodium yoelii nigeriense*. The interval between the two inoculations was 10 days. Giemsa-stained blood films from rats were examined on days 2, 4, 6, 8, 10, 12, 15, 20 and 30 after precedent malaria and on days 12, 15, 20, 30 and 40 after precedent toxoplasma as well as on day 14 in single malaria infected rats. Control non-infected rats were examined for comparison of splenic weights and histopathological and histochemical changes.

Sera. The sera were obtained from rats at defined intervals and they were tested with IFAT for toxoplasmosis (Kramář 1963) and for malaria (Sulzer et al. 1969). The antigen was prepared according to Goldman (1957) for toxoplasmosis and Memoranda (1974) for malaria. The fluorescein-labelled antirat globulins were obtained from Burroughs and Wellcome. A Zeiss photomicroscope III, equipped with an Osram lamp HBO W/4, primary exciter filters, BG 12, UG 5, UG 1, and ocular filters, 41/47/53 was used.

Tissue examination. Pieces of spleen, thymus and liver were fixed in acetone for subsequent examination for alkaline phosphatase by the technique of Gomori (1952). Other parts of the same organs were fixed in 10% formaline and paraffin sections were prepared for histopathological study. They were stained by haematoxylin and eosin, Van Gieson for the fibrous tissue and periodic acid Schiff for the liver glycogen (McManus 1946). Fresh frozen sections 20 μm thick were prepared from the liver and examined for succinic dehydrogenase activity (SDH) by Nachlas technique (Pearse 1960) and for choline esterase (Carlton 1967).

RESULTS

The course of parasitaemia in the concomitant infections as compared to control single malaria infection can be seen in Fig. 1. With single and precedent malaria, parasitaemia appeared 2 days after inoculation of the plasmodial parasites and with precedent toxoplasma, parasites appeared also after 2 days from inoculation (day 12). The peak of parasitaemia (mean 21.9 %) was reached after 6 days with single and precedent malaria and after 10 days with precedent toxoplasma at a significantly lower level (mean 2 %). The parasitaemia disappeared after 14 days with single malaria and 30 days with both concomitant infections.

Antibody level. The results of concomitant and single toxoplasma and malaria infections calculated as the average of titres in each group, are presented in Table 1. With precedent toxoplasma, malarial antibodes reached a higher level than with single malaria infection. In both concomitant infections the antibodies were lower than with single toxoplasma infection.

Splenic weights of rats in precedent toxoplasma (Table 2) were increased by day 20 in comparison to the control group. Thirty days after infection, spleens started to decrease in weight but they were still significantly increased and by day 40 the decrease in weight became significant. In precedent malaria (Table 3), the increase in splenic weight was lower than that obtained with precedent toxoplasma. The splenic weights were significantly increased by day 20, then started to decrease in weight by day 30, remaining significantly increased as compared to the non-infected group and persisted as such up to day 40.

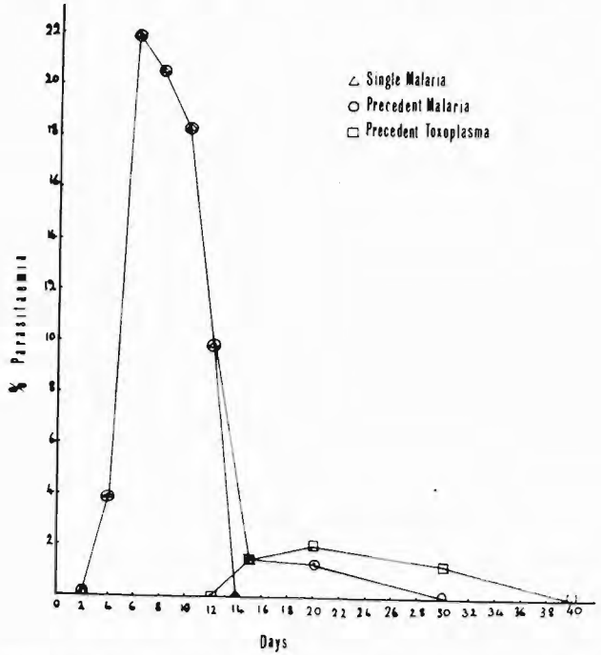

Fig. 1. Course of parasitaemia in rats with single malaria and concomitant infections.

Histopathological study. Spleens in precedent malaria on day 10 showed moderate congestion with reticuloendothelial hyperplasia in the red pulp and active pale germinal centre in the white pulp. On day 20, there was marked lymphocytic depletion of white and red pulp (Table 1, Fig. 1), and heavy pigmentation. On days 30 and 40, while lymphocytic depletion of the white pulp decreased, lymphocytic infiltration in the red pulp became marked. In precedent toxoplasma, through days 20, 30 and 40, there was a moderate degree of vascular congestion and pigmentation. The lymph follicles were large and well developed.

The thymus in precedent malaria showed the cortex and medulla packed with lymphocytes and the epithelial reticular cells slightly hypertrophied on days 20 and 30. On day 40, plasma cells were seen and the reticular epithelial cells regained their normal size. In precedent toxoplasma, there was a massive lymphocytic depletion in the cortex and medulla and numerous plasma cells were seen on day 20 (Plate I, Fig. 2). On days 30 and 40, the cortex and medulla were packed with lymphocytes.

The liver in precedent malaria showed marked vascular congestion and cloudy swel-

Table 1. Antibody level in concomitant and single *Toxoplasma* and malaria infections

Type of infection	Days	Malaria	*Toxoplasma*
Single *Toxoplasma* (Rifaat et al. 1981)	10	—	512
	20	—	4.095
	30	—	ND
	40	—	1.024
Single malaria (Rifaat et al. 1983)	10	128	—
	20	256	—
	30	32	—
	40	8	—
Concomitant infection			
Precedent malaria	20	128	512
	30	256	512
	40	512	512
Precedent *Toxoplasma*	20	128	128
	30	1 024	256
	40	512	512

ND = not done.

Table 2. Weight of spleens (as percentage of body weight) in rats infected with *Toxoplasma* followed by malaria

Group of rats	20 days		30 days		40 days	
	Infected	Control	Infected	Control	Infected	Control
1	1.500	0.590	1.400	0.800	1.600	0.900
2	1.600	0.680	1.587	0.790	1.033	0.890
3	1.630	0.660	1.365	0.990	1.021	0.915
4	1.198	0.611	1.552	0.888	1.772	0.916
Mean	1.532	0.653	1.476	0.867	1.396	0.913
S.D.	1.101	0.041	0.101	0.090	0.391	0.014
+ S.E.	0.050	0.020	0.050	0.045	0.195	0.007
P	P < 0.001		P < 0.001		P < 0.05	

ling of liver cells on day 20 (Plate II, Fig. 1). There was round cell infiltration in the portal tracts and moderate decrease in glycogen content of liver cells. On day 30, the congestion was marked and liver cells showed hydropic degeneration, glycogen depletion and irregular cellular necrosis (Plate II, Fig. 2). The Kupffer cells contained a mild degree of round cell infiltration. On day 40, there was a mild degree of fatty degeneration and cellular necrosis, but some cells had also started regeneration with normal glycogen content. The liver in precedent toxoplasma presented evident vascular congestion and mild degree of hydropic degeneration, some cells with pyknotic nuclei and some cells with absent nuclei and the glycogen content was completely depleted on day 20. On days 30 and 40, except for a mild fatty degeneration, Kupffer cells hypertrophied with fine pigment granules and the liver cells appeared normal.

In histochemical study of the liver, alkaline phosphatase activity was decreased in both precedent malaria and toxoplasma and returned to normal by day 40.

The succinic dehydrogenase activity in precedent malaria showed mild decrease

towards the centre on days 20, 30 and 40, while the peripheral cells showed normal activity. In precedent toxoplasma, the activity was markedly decreased on day 20, followed by a gradual return to normal, especially in the peripheral cells.

The choline esterase activity in precedent malaria showed mild decrease from days 20 to 40. In precedent toxoplasma, its activity was normal all through days 20 to 40.

Table 3. Weight of spleens (as percentage of body weight) in rats infected with malaria followed by *Toxoplasma*

Group of rats	20 days		30 days		40 days	
	Infected	Control	Infected	Control	Infected	Control
1	1.070	0.590	1.150	0.800	1.060	0.900
2	1.300	0.680	1.100	0.790	1.100	0.890
3	1.170	0.660	1.050	0.990	1.030	0.915
4	1.200	0.611	1.200	0.888	1.130	0.916
Mean	1.370	0.653	1.250	0.867	1.160	0.913
S.D.	0.233	0.041	0.158	0.090	0.081	0.014
±S.E.	0.116	0.020	0.079	0.045	0.040	0.007
P	P < 0.001		P < 0.01		P < 0.05	

S.D. = standard deviation, S.E. = standard error.

DISCUSSION

In precedent malaria, the parasitaemia was found similar to that in single malaria infection as regards appearance and peak, but the clearance of peripheral blood was delayed to 30 days instead of 14 days. On the other hand, in precedent toxoplasma, the parasitaemia appeared after 2 days and its intensity was much lower reaching its peak after a longer period (10 days) as compared to single and precedent malaria (6 days). However, it persisted up to 30 days p. i. Strickland et al. (1972) found that mice infected with toxoplasma 7 and 4 days before inoculation of malaria had a persistent high parasitaemia, while groups receiving toxoplasma later did not have higher levels of parasitaemia.

The toxoplasma antibodies in both concomitant infections were relatively lower as compared to levels obtained with single toxoplasma infection (Rifaat et al. 1981), while malarial antibodies with concomitant infection were found relatively higher than with single malaria infection (Rifaat et al. 1983). When toxoplasma was precedent, stimulation of antimalarial humoral response was high, since specific plasmodial antibodies reached a higher peak (1/1024) by day 30, as compared to precedent malaria. In a report by Strickland et al. (1972), antibodies to toxoplasma were suppressed in dually toxoplasma- and malaria-infected mice whichever parasite preceded.

The increase in splenic weight in precedent malaria was lower than that obtained with precedent toxoplasma. The increased splenic weight by the 20th day in precedent toxoplasma coincided with the peak of plasmodial parasitaemia and is in contrast to 10 days and 30 days in single malaria (Rifaat et al. 1983) and toxoplasma (Rifaat et al. 1981), respectively. Cantrell and Elko (1966) found that maximum spleen weight coincided with the peak of parasitaemia and once the parasites were eliminated, the spleen weight declined rapidly. Strickland et al. (1972) found that the infection

with toxoplasma alone caused a moderate increase in size of the spleen, while the malarial infection caused greater splenomegaly, and that the animals with both infections had even larger spleens. Although these results were obtained with acute concomitant infections, yet they parallel those obtained in the present study with chronic concomitant infection.

The histopathological study of the spleen revealed that in precedent toxoplasma the reaction was milder than with precedent malaria, moderate vascular congestion and pigmentation and normal reaction in lymph follicles were found without lymphocytic depletion in white pulp or infiltration in red pulp, which were severe with precedent malaria. In chronic single toxoplasma infection (Rifaat et al. 1981), the lymph follicles were enlarged with packed lymphocytes on days 20, 30 and remained well formed up to day 40, while in chronic single malaria infection (Rifaat et al 1983), the pigmentation was severe, with lymphocytic depletion of follicles on day 30, which then returned to normal by day 40, except for pigmentation.

In the thymus with precedent toxoplasma, there was apparent early lymphocytic depletion in the cortex and medulla, which later returned to normal. This agrees with the results previously obtained in single toxoplasma infection. However, while in precedent malaria no depletion occurred, in single malaria, there was lymphocytic depletion.

The histopathological and histochemical examination of liver showed that degenerative changes were more severe in precedent malaria. These changes, which progressed from hydropic to fatty, were probably due to toxic material liberated from infected cells, causing damage to the organoids. As a result there was also a decrease in succinic dehydrogenase and choline esterase. The decrease in liver glycogen could be attributed to the action of glycolytic enzymes and increase demands for glucose by the parasites. Singh et al. (1956) suggested that the hypoglycaemia which occurs in the terminal stages of malarial infection is not only due to the demand on blood sugars by the parasites, but also to the interruption of glycogen deposition, resulting from the centrilobular necrosis of the liver, which accompanies the disease. The alkaline phosphatase activity in the reticular epithelial cells is probably due to their rapid proliferation.

It can be concluded that toxoplasma infection appeared to confer some resistance against malaria making it run a more chronic course. This is apparent in the low intensity of parasitaemia in precedent toxoplasma and longer period of time taken for its clearance in both precedent toxoplasma and precedent malaria. Remington and Merigan (1968), and Ruskin and Remington (1968) also demonstrated such a resistance to viruses and bacteria. Also in the presence of toxoplasma, stimulation of humoral antibody response against malaria parasites was greater than in single and precedent malaria. Apparently, toxoplasma protected the spleen from the hazardous malarial effect since its size returned to normal in precedent toxoplasma. The histopathological reaction was also milder when toxoplasma preceded since in precedent and single malaria, there was a marked lymphocytic depletion and heavy pigmentation. The depletion of thymocytes in the thymus is probably due to their active sensitisation and migration indicating the involvement of a cell-mediated immune system.

ЭКСПЕРИМЕНТАЛЬНОЕ СОПУТСТВУЮЩЕЕ ЗАРАЖЕНИЕ ТОКСОПЛАЗМОЗОМ И МАЛЯРИЕЙ У КРЫС

М. А. Рифаат, С. А. Салем, М. Е. Азаб, И. А. Ел-Разик, Е. Х. Сафер, С. Р. Бешир и С. Ф. А. Ел-Шенавы

Резюме. Изучали действие сопутствующего заражения токсоплазмозом и малярией на ретикулоэндотелиальную систему крыс. Это действие оценивалось на основе уровня плазмодиальной паразитемии, ответа гуморальных антител, эффекта веса селезенки, гистопатологических изменений тимуса и селезенки и гистопатологических изменений печени. Паразитемия появилась на 2-й день при заражении малярией и при сопутствующем заражении. Максимальное заражение появилось на 6-й день при простом заражении и при сопутствующем заражении с предыдущей малярией и на 10-й день при сопутствующем заражении с предыдущим токсоплазмозом. Заболевание исчезло через 14 дней при простом заражении, тогда как при сопутствующем заражении — через 30 дней. При сопутствующем заражении с предыдущим токсоплазмозом уровень антител против малярии был выше, чем нормально, тогда как уровень антител против токсоплазмоза был ниже при обеих сопутствующих заражениях. При сопутствующем заражении с предыдущим токсоплазмозом и с предыдущей малярией значительно повысился вес селезенки, после того понизился, но в случае предыдущей малярии больше не вернулся к нормальному уровню. Тимус был наполнен тимоцитами в случае предыдущей малярии, тогда как в случае предыдущего токсоплазмоза происходило опустошение кортекса. При обеих сопутствующих заражениях происходило опустошение гликогена и повышение активности сукцинат дегидрогеназы. Активность холинэстеразы в случае предыдущей малярии повысилась и вернулась к нормальному уровню на 40-й день, тогда как в случае предыдущего токсоплазмоза активность этого фермента была нормальной во все время заболевания. Активность щелочной фосфатазы понизилась и становилась нормальной на 40-й день в обеих сопутствующих заражениях.

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In memoriam: K. M. Ryzhikov

We seem only recently to have commemorated the important 70th anniversary of Konstantin Minayevich Ryzhikov, the Corresponding Member of the USSR Academy of Sciences and Director of the Helminthological Laboratory of the USSR Academy of Sciences in Moscow. In deep sorrow today we remember this outstanding Soviet scientist-helminthologist, who died on 6 August 1983.

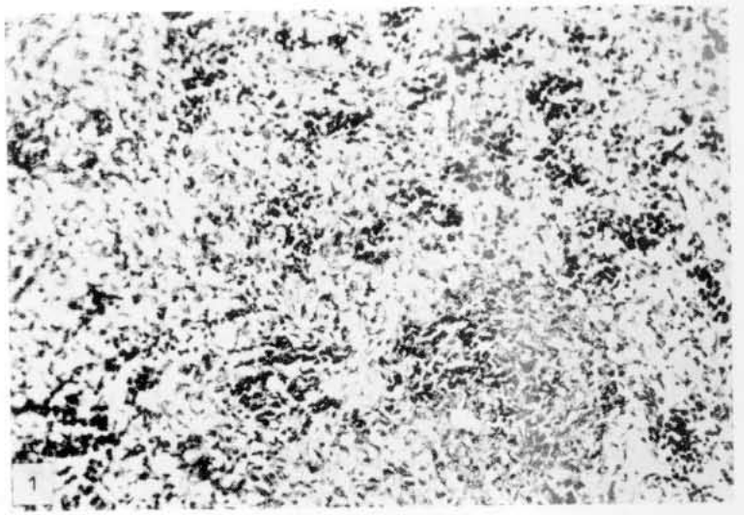
K. M. Ryzhikov was born on 26 September 1912 at Smolensk. His professional career and scientific achievements were appreciated in Folia parasitologica No. 4/1982. Being one of the organizers of the Soviet helminthology he was for a long time head of a department of the Helminthological Laboratory of the USSR Academy of Sciences and since 1972 its director. He devoted great efforts to the training of young scientific cadres, the students, post-graduate students and junior scientific workers. He was deputy head of the General Biology Department of the USSR Academy of Sciences, editor-in-chief of the journal "Parazitologiya" and chairman of the helminthological division of the Scientific Board for zoological problems.

K. M. Ryzhikov served as member of the editorial board of the journals Folia parasitologica and Helminthologia, as well as member of the Soviet-Czechoslovak committee for scientific cooperation and was honorary member of the Czechoslovak Parasitological Society.

Czechoslovak and world parasitology lost an eminent scientist and kind friend. His passing will be mourned by parasitologists the world over.

We honour his memory.

RNDr. J. Prokopič, D.Sc.



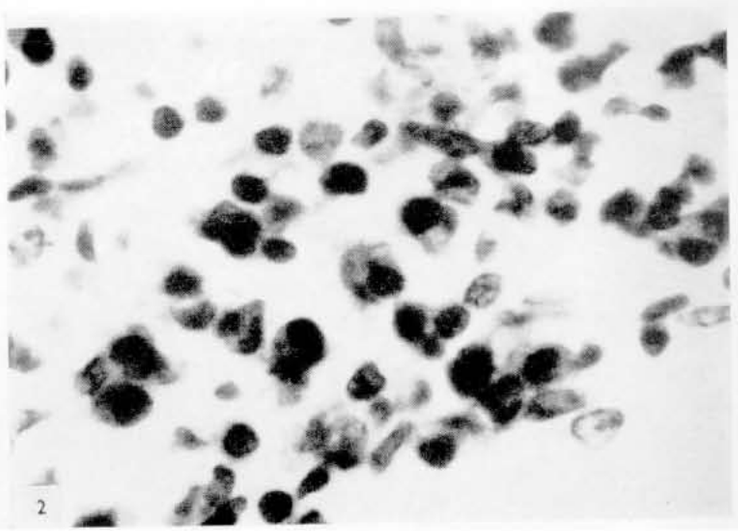
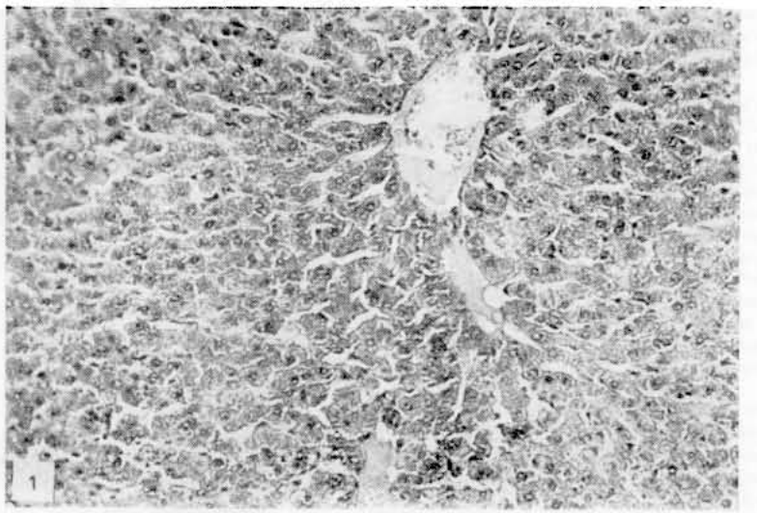


Fig. 1. Concomitant malaria and toxoplasma infection. Spleen, 20 days, showing depletion in the red pulp and white pulp. H & E, (× 120). **Fig. 2.** Concomitant toxoplasma and malaria infection. Thymus, 20 days, showing plasma cells. H & E, (× 1000).



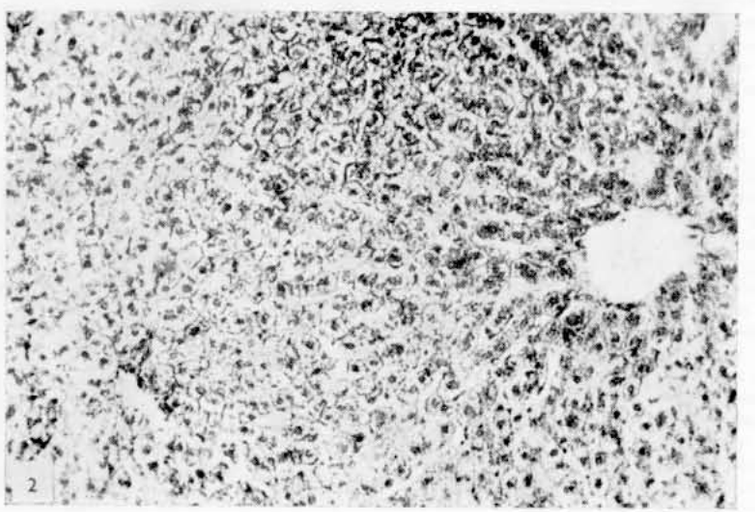


Fig. 1. Concomitant malaria and toxoplasma infection. Liver, 20 days. Liver cells showing cloudy swelling with some necrotic cells. H & E (× 120). **Fig. 2.** Concomitant malaria and toxoplasma infection. Liver, 30 days. Liver cells showing hydropic degeneration and irregular cellular necrosis. H & E (× 120).