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SUSCEPTIBILITY OF THE MARMOSET, *MARIKINA GEOFFROYI* AND THE NIGHT MONKEY, *AOTUS ZONALIS*, TO EXPERIMENTAL INFECTION WITH *TOXOPLASMA*

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SUSCEPTIBILITY OF THE MARMOSET, *MARIKINA GEOFFROYI*, AND THE NIGHT MONKEY, *AOTUS ZONALIS*, TO EXPERIMENTAL INFECTION WITH *TOXOPLASMA*

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Relatively few primates have been tested for susceptibility to *Toxoplasma gondii* and those tested have usually shown a high degree of resistance. Negative results were obtained in the following species, *Cercopithecus patas* and *Papio sphynx* by Levaditi and co-workers in 1929, *Cercopithecus sabaeus* and *Papio doguera* by Cowen and Wolf in 1945, *Cynocephalus babuin* by Levaditi and Schoen in 1933 and *Macacus cynomolgus* and *Macacus sinicus* by Nicolle and co-workers in 1909 and 1913. No symptoms were produced and no toxoplasmas found in the tissues by histological examination in the few instances where this was attempted. Only very small numbers of animals, usually one of each species, were used in these experiments. *Macaca mulatta* has been tested more extensively than the other species (Levaditi and co-workers, 1929; Sabin and Olitzky, 1937; Sabin and Ruchman, 1942; Cowen and Wolf, 1945). Levaditi and co-workers obtained totally negative results in a rhesus monkey inoculated intracerebrally. Sabin and co-workers produced self-limited febrile reactions associated with parasitemia and local lesions in *M. mulatta* inoculated by various routes. Cowen and Wolf reported the first fatal infection in a 7-month old rhesus inoculated intracerebrally. No evidence of infection was obtained in 8 others except for a changing serology in 4.

It may be of interest, therefore, to report the high susceptibility to toxoplasmosis of 2 species of primates native to Panama, the marmoset, *Marikina geoffroyi*, and the night monkey, *Aotus zonalis*. In both species *T. gondii* produces a rapidly progressive fatal infection with parasites abundantly present in most of the tissues and organs at the time of death.

MATERIALS AND METHODS

The strain of *Toxoplasma* used was one recently isolated in mice from a spontaneous infection in an infant *Cebus* monkey. It had received two passages in mice prior to being tested in the marmoset. This strain has been described in another report (Rodaniche, 1954). Morphologically, in animal pathogenicity, and in physiological properties it corresponds with strains described by other authors. In the early mouse passages the strain showed low virulence for this rodent, producing only slowly developing chronic infections. After the first passage in the marmoset, however, the virulence was greatly increased for mice and acute, rapidly fatal infections were produced.

A total of 31 marmosets and 15 night monkeys was employed in tests of susceptibility. The marmosets were juvenile or adult animals weighing from 340 to 555 grams. All but 5 showed spontaneous infection with microfilariae or tryp-

anosomes of little or no pathogenicity. Such infections are very common in marmosets in Panama (Clark, 1931). No difference was observed in the course of the toxoplasmic infection in those animals free of blood parasites and in those harboring them. The night monkeys also were juvenile or adult animals, weighing from 535 to 925 grams. Repeated blood smears revealed microfilariae in one of the animals. All the others were free of blood parasites.

Inocula consisted of peritoneal fluid or tissue emulsions of infected mice or monkeys. The infection could be transferred readily from mice to monkeys and vice versa. Various routes of inoculation were tried. Both marmosets and night monkeys were tested by the intraperitoneal, intracerebral, intradermal, intranasal, ocular and oral routes. Marmosets alone were tested by the subcutaneous and intrapleural routes. Rectal temperatures and thick blood films were taken daily. Animals either were allowed to die spontaneously or were sacrificed when moribund. A postmortem examination was made of each animal and Giemsa-stained smears of the body exudates and viscera examined. In a few instances, histological sections stained with hematoxylin and eosin also were prepared.

The course of the infection in both *Marikina* and *Aotus* on the whole was similar. Temperature changes in the marmosets were variable, some animals showing only a terminal drop in temperature and others one to three days of fever, usually shortly prior to death. In the night monkey, on the other hand, fever was a constant feature, temperatures of 104°F. or higher being registered for 1 to 4 days prior to death. Both marmosets and night monkeys remained otherwise asymptomatic until about one day prior to death when they became inappetent, sluggish and tended to maintain a crouching position. Marmosets sometimes developed a cough shortly prior to death. Thick blood films in both species became positive for *Toxoplasma* within 12 to 36 hours of death. The toxoplasmas were never very numerous in the blood, usually about one to every 2 to 10 fields. Their presence in thick films could be interpreted as indication of a fatal outcome within 36 hours.

Gross pathological changes in the internal organs depended partly on the route of inoculation and partly on the duration of the infection. The lungs always showed some degree of involvement, except after intracerebral inoculation, varying from congestion to extensive bronchopneumonia. Massive edema of the lungs was a common finding. Small amounts of pleural fluid were often present. The spleen was usually enlarged, the liver congested. In marmosets intraperitoneal inoculation resulted in the formation of relatively small quantities of a yellowish rapidly coagulating peritoneal fluid. In the night monkey 5 to 10 ml. of fluid was present at the time of death and there were fibrinous adhesions between the omentum and the viscera. Local changes observed after intradermal and ocular inoculation will be described in connection with results obtained by inoculation by these routes. In animals dying of the infection, toxoplasmas were always found in very large numbers in impression smears of the spleen, liver and lungs, in smaller numbers in blood, kidneys and mesenteric lymph nodes. In the other viscera and exudates they were sometimes encountered and sometimes not, being observed at one time or another in smears of the bronchi, trachea,

esophagus, stomach, pancreas, bile, urinary bladder, testes, ovary, uterus, brain, meninges, peritoneal fluid, urine, ocular and nasal exudates, sputum and on one occasion in the milk of a lactating night monkey. There was not much correlation between the route of inoculation and the postmortem distribution of the parasites, except that they were observed in the brain only after intracerebral inoculation, in the peritoneal fluid only after intraperitoneal inoculation or subcutaneous inoculation in the inguinal area, in the skin only after intradermal inoculation and in the conjunctiva and ocular exudates only after ocular administration. Toxoplasmas were never encountered in smears of the feces or smears or sections of the intestinal mucosa. The protozoons were usually observed as plump, actively dividing forms, free or in an intracellular position, single or in small or medium-sized clusters. Pseudocysts were encountered only in the conjunctivae of 2 marmosets inoculated by the ocular route.

A summary of results of the inoculation of *Marikina geoffroyi* by various routes is presented in Table 1 and of *Aotus zonalis* in Table 2. With the exception of one *Aotus* accidentally destroyed in 3 days, all marmosets and night monkeys receiving parenteral injections of *Toxoplasma* suffered a rapidly progressive, uniformly fatal infection, with these parasites abundantly present in smears of the various tissues and organs. Death occurred in 3 to 11 days.

Intranasal inoculation, performed by dropping 2 to 5 drops of the inoculum in each nostril, resulted in a fatal toxoplasmic infection in 2 of 3 marmosets in 10 and 11 days respectively. The third marmoset died of intercurrent infection in 6 days. Toxoplasmas were not encountered in tissue smears of this animal. One of 2 night monkeys similarly inoculated succumbed to the infection in 13 days, the other was accidentally killed in one day.

Oral administration, performed by allowing one to two ml. of the inoculum to

TABLE 1

Susceptibility of Marikina geoffroyi *to inoculation with* Toxoplasma *by various routes*

Route of Inoculation	No. of Monkeys Used	No. Dying	Days of Death	No. Showing *Toxoplasma* in their Tissues Postmortem
Intraperitoneal	4	4	4, 5, 5, 6	4
Intrapleural	2	2	6, 10	2
Subcutaneous	4	4	3, 6, 8, 10	4
Intracerebral	2	2	5, 5	2
Intranasal	3	3	6,* 10, 11	2
By feeding	3	3	4, 4, 10	3
By stomach tube administration	4	2	1,* 13	1
Intradermal	3	3	7, 7, 11	3
By instillation into the eye	5	5	8, 9, 10, 11, 12	5
By instillation into the vagina	1	0		
Total	31	28		26

* Indicates the animal dying on that day did not show toxoplasma in the tissues, death being due to accident or intercurrent infection.

TABLE 2

Susceptibility of Aotus zonalis *to inoculation with* Toxoplasma *by various routes*

Route of Inoculation	No. of Monkeys Used	No. Dying	Days of Death	No. Showing *Toxoplasma* in their Tissues Postmortem
Intraperitoneal	3	3	5, 6, 7	3
Intracerebral	2	2	4, 5	2
Intranasal	2	2	1,* 13	1
By stomach tube administration	2	1	8	1
Intradermal	4	4	3,* 6, 8, 11	3
Conjunctival	2	1	13	1
Total	15	13		11

* Indicates the animal dying on that day did not show toxoplasma in the tissues.

run into the mouth from a pipette while the animal was maintained under ether anesthesia, resulted in death of all 3 marmosets so inoculated in 4, 4, and 10 days respectively. Toxoplasmas were readily identified in various tissues postmortem. However, administration of 2 to 4 ml. of the same inoculum by stomach tube without anesthesia produced infection in only 1 of 4 marmosets and 1 of 2 night monkeys. These two animals died in 13 and 8 days respectively and showed the parasites widely distributed throughout their tissues. One marmoset died in 24 hours of other causes. Two marmosets and one night monkey survived throughout a 2-month observation period. Complement-fixation and intradermal neutralization tests against toxoplasma were conducted with their blood at 30- and 60-day intervals with negative results. One of the marmosets and the night monkey were sacrificed at the end of the 2-month observation period. Toxoplasmas were not observed in direct smears or histological sections of their tissues nor were the parasites recovered by subinoculation of emulsions of brain, spleen, kidney and liver tissue in mice. The other marmoset was reinoculated with *Toxoplasma* by the intraperitoneal route and developed an acute infection which proved fatal in 4 days indicating that it had developed no immunity.

Intradermal injection of 0.1 to 0.2 ml. of body fluids or tissue suspensions containing variable numbers of viable *Toxoplasma* produced an acute infection in all of 3 marmosets and 3 of 4 night monkeys, with death in 6 to 11 days. The fourth night monkey was lost to intercurrent infection in 3 days. A raised red papule developed at the site of inoculation in 4 days. On the fifth day a central vesicle filled with nonpurulent fluid was formed. These vesicles remained small in the marmoset, but in the night monkey achieved a diameter of 8–12 mm. Later there was ulceration of the center and scar formation. Moderate numbers of *Toxoplasma* were found in the vesicular fluid during life and in the skin and subcutaneous tissue postmortem. The severity and rapidity of development of the skin lesions depended directly on the number of *Toxoplasma* injected, 25 or fewer organisms producing a minimal reaction in 7 days and 250–25,000 organisms a maximum reaction in the same period.

This local reaction could be neutralized by positive monkey serums in tests essentially similar to those devised by Sabin and Olitsky (1937), employing the rabbit as experimental animal. The following technique was employed. Mouse peritoneal fluid was diluted serially with saline solution to contain from 2,500 to 5,000; 25,000 to 50,000; and 250,000 to 500,000 *Toxoplasma* per ml. To 0.1 ml. of each dilution was added 0.1 ml. of immune monkey serum. The mixtures were allowed to remain one hour at room temperature. Then 0.1 ml. of each dilution was injected intradermally in the shaved abdominal skin of paired marmosets. As a control, serial dilutions of *Toxoplasma* were similarly combined with negative monkey serums and injected. Failure to develop a papule 6 mm. or more in diameter with necrotic center within 6 to 8 days of inoculation was interpreted as a positive reaction, provided typical lesions were observed in the control sites.

Intravaginal inoculation in one marmoset failed to produce infection. One ml. of a heavily infected monkey liver suspension was introduced into the vagina without trauma with a blunt pipette. The animal survived throughout a 12-week observation period when it was sacrificed. Direct smears, histological sections and subinoculations into mice of brain, spleen, liver, lung and kidney tissue failed to reveal the parasites. *Toxoplasma* were not observed in scrapings of the vaginal or uterine mucosa or impression smears of the ovaries.

Ocular inoculation proved to be of especial interest. Five marmosets and 2 night monkeys were inoculated by dropping 1 to 4 drops of the inoculum in the eyes under ether anaesthesia while gently manipulating the lids with gloved fingers. This route of inoculation produced an acute fatal infection in all 5 of the marmosets with death in 8 to 12 days, and in one of the 2 night monkeys which died in 13 days. In the marmosets, edema of the face was noted one or two days prior to death and a watery exudate filled the eyes. About one day prior to death the blood smears became positive and *Toxoplasma* were present in the ocular exudates and nasal secretions. A persistent cough was noted. Postmortem, the lungs showed the most severe changes. *Toxoplasma* were identified in large numbers in smears of the conjunctiva, liver, lung and spleen and in lesser numbers in the edema fluid present in the subcutaneous tissue of the face, in the nasal exudates, trachea, kidney and mesenteric lymph nodes. The *Toxoplasma* in smears of the conjunctivae were found free, in small intracellular groups and in two instances in large pseudocysts, the only occasions when pseudocyst formation was observed in *Marikina*. In the night monkey which succumbed to the infection there was slight opacity of the cornea, but no edema of the face or exudation of the eyes was observed. There was a bronchopneumonia involving all lobes of the lung with water-logging. *Toxoplasma* were relatively scarce in smears of the conjunctivae, but extremely abundant in smears of liver, lung and spleen. They were present also in throat and nasal swabbings. No *Toxoplasma* were found in smears of the brains of any of these monkeys.

DISCUSSION

The marmoset, *Marikina geoffroyi*, and the night monkey, *Aotus zonalis*, should prove useful experimental animals in the investigation of the numerous

unsolved problems in the field of toxoplasmosis. Both species are highly susceptible and develop an acute fatal infection after inoculation by a number of routes. No evidence has as yet been obtained of a chronic form of the disease in *Marikina* or *Aotus*, at least with the strain employed here.

Of especial interest is the susceptibility of these monkeys to infection by feeding or by instillation of the inoculum into the eyes or nose without trauma. The *Toxoplasma* are capable of direct invasion of the conjunctiva where they multiply and from which they spread producing a generalized infection and death. The marmoset is more highly susceptible to this route than the night monkey. This finding suggests the possibility that the eye may be a natural portal of entry of the disease. Local multiplication in the nasal mucosa was not noted after intranasal instillation of the inoculum. Infection by feeding is probably effected through the upper respiratory tract as only 2 of 6 monkeys showed evidence of infection after feeding by stomach tube. Irregular results by stomach tube administration were also obtained in rabbits by Wolf, Cowen and Paige (1940) and in guinea pigs by Kozar and co-workers (1952).

Our failure to infect one marmoset by a single intravaginal instillation of *Toxoplasma* obviously has a limited significance. Cowen and Wolf (1950) succeeded in infecting pregnant mice by repeated intravaginal instillations. The effect of repeated instillations in a large number of marmosets in various physiological states remains to be ascertained. *Toxoplasma* are commonly found in smears of the uterine mucosa of both night monkeys and marmosets dying of the disease although not in such large numbers as in other organs.

Preliminary experimentation according to the technique described indicates that the local reaction provoked by the intradermal injection of *Toxoplasma* may be neutralized by immune monkey serum. Further standardization is required before results with human serums can be interpreted accurately. *Marikina* and *Aotus* should prove superior to the rabbit as experimental animals for this type of reaction because resistance to infection in these animals in nature must be rare, if it occurs at all. To date we have used a total of 60 marmosets and night monkeys without encountering a single immune one.

CONCLUSIONS

The marmoset, *Marikina geoffroyi*, and the night monkey, *Aotus zonalis*, are highly susceptible to experimental infection with *Toxoplasma gondii*. They uniformly develop acute fatal infections after inoculation by a wide variety of routes.

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