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Experimental Toxoplasmosis.

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Investigations by others have already shown that certain of the sulfonamides[1,2] have an inhibiting effect on the growth of toxoplasma in the mouse and rabbit, but that penicillin,[3] streptomycin,[4] and certain antiprotozoal[4,5] drugs have no apparent effect. Recently, it has been shown[6,7] that when toxoplasma are inoculated into the 10-day-old chick embryo, death of the embryo regularly occurs 5 to 7 days after inoculation. We have used chick embryos infected in such a manner to determine the therapeutic effectiveness of various drugs on the disease by comparing the survival time of control embryos with infected embryos which had been treated. Additional observations on the infected mouse, rabbit and guinea pig are included in this report.

Methods and Materials. The "R.H." strain of toxoplasma isolated by Sabin[8] from a fatal human case of encephalitis was used in these studies. It was maintained in this laboratory by continuous intraperitoneal or intracerebral passage in mice. Fertile hens' eggs incubated both before and after inoculation at 37°C were inoculated with 0.2 cc of a 10% suspension of either mouse brain or peritoneal exudate into the yolk sac, using a No. 22 needle 1 inch long on a tuberculin syringe. In all instances, the initial inoculation was made in the 10-day-old fertile egg and the drug to be tested was injected in a similar manner 24 hours later. Drugs used were prepared so that the amount to be evaluated was contained in 0.2 cc, the amount injected. The following controls were run with each drug tested: drug control, toxoplasma control, and saline control. All drugs used were from commercial lot preparations and were diluted with sterile saline solution to the proper dosage just before use. After inoculation each egg was candled daily until death or hatching occurred. Chicks that hatched were sacrificed at various ages and 10% suspensions of their livers and brains in physiological saline solution were inoculated into mice to determine the presence or absence of toxoplasma. In the mice, guinea pig and rabbit studies, depending upon the instance, the organisms were either given by mouth or injected subcutaneously, or intraperitoneally.

Results. A. Drug Studies. Sulfathiazol, sulfadiazine, sudfapyridine, sulfamerazine, and sulfathiazol-sulfamerazine (combined) in varying dosages prolonged the survival of the toxoplasma infected chick embryo as shown in Table. I. Penicillin, streptomycin, aureomycin,† and acetarsone had no effect on the survival of the infected chick embryo. In the infected embryos (treated with one of the sulfonamides) that survived and were allowed to hatch, toxoplasma were recovered from the livers by mouse inoculation, in every instance regardless of the age of the chick. In the sulfonamide treated embryos, dosages used below 5 mg per 0.2 cc afforded little or no protection against the toxoplasma; dosages above 20 mg per 0.2 cc appeared too toxic to the embryo to determine its effectiveness.

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† Aureomycin was kindly supplied by the Lederle Laboratories.

TABLE I.
Percentage of Survival of Infected 10-day Chick Embryos with Various Drugs.

No. of eggs	Therapy	Dose	% survival at 21 days*
96	Saline		0
12	Sulfathiazole	.1 mg	0
12	"	1 "	0
12	"	5 "	77
12	"	10 "	71
12	"	15 "	88
10	Sufladiazine	5 "	100
10	"	10 "	88
10	"	15 "	70
10	"	20 "	85
10	Sulfamerazine	5 "	66
10	"	10 "	75
10	"	20 "	77
10	Sulfapyridine	1 "	0
10	"	10 "	33
10	"	15 "	50
10	Acetarsone	.25 "	0
10	"	1 "	0
10	"	10 "	0
16	Penicillin	10 units	0
16	"	100 "	0
16	"	1,000 "	0
6	Streptomycin	10 "	0
16	"	100 "	0
16	"	1,000 "	0
16	"	10,000 "	0
20	Aureomycin	10 "	0
20	"	100 "	0
20	"	1,000 "	0
10	Sulfathiazole Sulfamerazine	6 mg of each	100
10	Sulfathiazole Sulfamerazine	12 mg of each	71

* Based on number of embryos living after subtracting inoculation deaths.

B. Congenital Toxoplasmosis in the Guinea Pig. Two gravid guinea pigs approximately one week from delivery were inoculated subcutaneously with 0.2 cc of 1:1,000 dilution of toxoplasma-containing peritoneal exudate. Five days later both guinea pigs delivered 2 living and one stillborn offspring. One of the stillborns appeared to have bilateral cataracts; sections made of most of their tissues for microscopic examination revealed no obvious toxoplasma. The living young were allowed to remain with their mothers until the mothers died about 10 days following the injection of toxoplasma. The offspring were sacrificed at varying periods up to 12 days of age in each instance toxoplasma were either found on stained imprints of body tissues or were recovered by means of inoculation into mice.

A third gravid guinea pig was infected one week before term in the same manner as described above, but before spontaneous labor ensued she was given ether anesthesia and 3 live young were delivered by Cesarian section to prevent the possibility of contamination via the birth canal. Toxoplasma were recovered from the mother's blood at delivery and were demonstrated in imprints of tissues in two of the offspring that died 24 hours later. Toxoplasma also were recovered from the brain of the third offspring that died at the age of 7 days from convulsions. However, we were unable to identify the organisms in sections of tissue from which they were isolated, even after a prolonged search.

C. Recovery of Toxoplasma from the Urine of Rabbits and Guinea Pigs. Two rabbits were inoculated intracutaneously with mixtures of toxoplasma and serum for the routine neutralization test. Two guinea pigs were inoculated subcutaneously with only a suspension of toxoplasma. After 8 days, un-

der ether anesthesia the abdominal walls were opened sterilely and the bladders were aspirated with needle and syringe. In each instance the urine was very concentrated and so was diluted 3-fold with saline solution and then inoculated intraperitoneally into mice. Approximately 7 days later all of the mice became ill with ascites and toxoplasma were found in large numbers.

On 3 occasions mice were likewise infected with toxoplasma, both intracerebrally and subcutaneously, but toxoplasma were not recovered from the urine which was removed sterilely and inoculated into other mice.

D. Failure to Infect Mice with Toxoplasma Given Orally. On 5 occasions 7 mice were fed water containing millions of fresh toxoplasma in suspension, most of which was consumed 24 hours after preparation. None of these mice developed toxoplasmosis during the one month period of observation. Mice were likewise infected with toxoplasma intracerebrally and intraperitoneally and placed in cages with healthy litter mates to ascertain if infection could be acquired by cannibalism. In all instances the infected mice died 4-5 days after inoculation and were consumed by the normal litter mates. Under such circumstances, none of these litter mates developed toxoplasmosis.

E. Failure to Recover Toxoplasma from the Infected-Immune Rabbit. Three rabbits that had been used for the rabbit neutralization test but had recovered from their infection and had a high titer of complement fixing antibodies in their sera were sacrificed. The rabbits were selected so that one had recovered from its infection 1 month, one but 3 months, and one a considerably longer time, namely 19 months. In each instance sections of the brain, liver, and spleen were removed sterilely from the rabbit and made into a 10% suspension with saline. These suspensions (0.5 cc) were inoculated intraperitoneally into each of 3 mice who failed to develop toxoplasmosis after one month of observation.

Discussion. The results of the chick embryo method for testing the effectiveness of various drugs on toxoplasmosis compares favorably with previous studies done in intact animals.[1-5] This technique is relatively simple and non-time-consuming. Although it was thought that acetarsone produced some clinical improvement in acute toxoplasmosis in certain patients,[9] tests in the chick embryo showed no effect of the drug on the organisms. Aureomycin is known to be effective against experimental brucella infection in the chick embryo,[11] however, we were unable to show any inhibiting effect of the drug on the growth of toxoplasma.

While congenital toxoplasmosis was produced in the guinea pig, as proven by isolation of the organism from the brain, examination of the tissue sections microscopically revealed no organisms. This confirms our impression of the difficulty in making a diagnosis from pathologic specimens without the aid of biologic tests.[9]

Since we were unable to infect mice orally with toxoplasma, transmission from animal to animal does not appear to occur via the gastrointestinal tract. The significance of the presence of toxoplasma in the urine of infected rabbits and guinea pigs is not clear at the present time. Apparently, the infected rabbit, as well as the monkey[10] in certain instances, can recover completely from the infection.

Summary. The effectiveness of certain of the sulfonamides and the ineffectiveness of certain antibiotics in suppressing toxoplasmosis in the chick embryo has been demonstrated. Congenital toxoplasmosis was produced in the guinea pig.

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