TABLE I.
Rate of Removal of Hemoglobin from the Circulation of Nephrectomized Rats.

| Min. after inj. | Final serum conc. of Hb, mg/100 cc | Wintr. hematocrit | Plasma vol., cc | Total circ. Hb, mg | Hb removed mg | Rate of Hb removal mg/min./ 100 g BW | Serum Prot.-Hb conc., % | Total circ. Prot.-Hb, mg |
|---|---|---|---|---|---|---|---|---|
| | | | Sodium Chloride injections | | | | | |
| 2 | 1492 | 35.3 | 9.5 | 141 | 0 | | 3.54 | 336 |
| 30 | 1398 | 37.9 | 8.9 | 124 | 17 | 0.41 ± .19 | 4.53 | 400 |
| 60 | 1078 | 38.5 | 8.8 | 95 | 46 | 0.50 ± .06 | | |
| | | | Bovine albumin injections | | | | | |
| 2 | 1403 | 33.2 | 10.0 | 141 | 0 | | 6.40 | 640 |
| 30 | 1179 | 33.5 | 10.1 | 119 | 22 | 0.49 ± .16 | 6.56 | 650 |
| 60 | 1135 | 37.8 | 8.8 | 100 | 41 | 0.44 ± .08 | 7.54 | 664 |

36 hours. Control rats in this series had a total circulating protein value (other than hemoglobin) of approximately 350 mg. As the result of injections which introduced approximately 2500 mg of albumin into the circulation during a period of 30 hours (about half of the last injection remained unabsorbed at the time of autopsy), the total circulating protein rose to about 650 mg, nearly twice its previous level. Consequently a minimum of 2200 mg entered and left the circulation during the period of observation. In spite of this, no appreciable change in the rate of hemoglobin removal was detected.

Comparison of the extra-renal rate of removal with the renal excretion of hemoglobin at comparable serum concentrations indicated that, in the rats injected with sodium chloride solution, the renal excretion rate[4] was less than twice the extra-renal removal rate, while in the rats injected with bovine albumin the renal excretion rate was relatively somewhat greater. The difference in ratio depended upon the effect of bovine albumin injections, increasing the rate of renal excretion.

*Summary.* 1. During the hour following intravenous injection of hemoglobin in the rat, about 1/3 of the quantity injected left the circulation by extra-renal routes.

2. Intraperitoneal injection of bovine albumin, with the associated rapid passage of albumin from the circulation, did not affect the rate at which hemoglobin left the circulation.

3. The rate at which hemoglobin leaves the circulation by extra-renal routes is of comparable magnitude to, though less than, the rate of renal excretion.

Received March 30, 1949. P.S.E.B.M., 1949, **71**.

## 17074. Experimental Toxoplasmosis. II. Effect of Sulfadiazine and Antiserum on Congenital Toxoplasmosis in Mice.

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Although toxoplasmosis has been known as an infectious disease of man for over a decade, therapy of the acute condition has remained unsatisfactory. Sabin and Warren,[1,2] testing a wide variety of antimicrobial agents, reported that only the sulfonamides have any beneficial effects on the course of the disease in laboratory infected mice and rabbits. Weinman and Berne[3] extended these observa-

1 Sabin, A. B., and Warren, J., PROC. SOC. EXP. BIOL. AND MED., 1942, **51**, 15.

2 Sabin, A. B., and Warren, J., PROC. SOC. EXP. BIOL. AND MED., 1942, **51**, 19.

3 Weinman, D., and Berne, R., *J.A.M.A.*, 1944, **124**, 6.

tions, and by producing higher blood levels of the drugs with exceedingly large doses of sulfonamides were able to show that if used early in the course of the infection these agents are effective in curing the acute phase of the disease in adult mice. However, this method of therapy did not eradicate the infection; all treated mice remained chronic carriers, retaining virulent organisms in the brain.

In man, sulfonamide therapy for toxoplasmosis has so far given striking results in only one of the few cases in which it has been employed.[4-6]

In practice, therapy of the infection is handicapped by the fact that most recognized cases occur in newborn infants who have acquired the infection *in utero*. In consequence, widespread, irreparable, and frequently fatal damage to tissues has occurred even before birth.[7] However, we have recently observed a number of infants who seemingly had acquired the infection shortly before term, so that at the time of delivery there appeared to be little demonstrable tissue destruction. It is for these children that a successful method of treatment would be of particular value.

*Experimental Procedures.* The Toxoplasma strain used was the same RH strain described in an earlier paper.[8] Although it is possible to produce congenital toxoplasmosis in mice similar to that described in man, under the experimental conditions employed only about 30% of the offspring from an infected mother could be shown to harbor Toxoplasma. Of these, about three-fourths died; in the remaining infected animals the disease went on to a chronic carrier state. Because of the relatively small percentage of congenital infections produced by this method, very large numbers of animals would have been required to evaluate adequately the effect of any therapeutic agent. Therefore, in the present investigation an artificial, rather than natural, method of infecting the embryo was employed.

Adult, virgin, female mice were mated with healthy males and as soon as a vaginal plug appeared, a sign of successful copulation, were removed to individual cages. Fourteen days after copulation they were examined for signs of pregnancy. The pregnant animals were anesthetized with ether, the lower abdomen shaved, and a small incision made through the skin and peritoneum to expose the uterus. Aseptic technic was used throughout. The position of the various embryos in the uterus could easily be seen. With some practice, it was possible to insert a very fine hypodermic needle through the walls of the uterus and the fetal membranes so that it just touched the embryo. The infective material was then injected through a 0.5 cc tuberculin syringe attached to the needle. This material consisted of 0.02 cc of a 1:100 dilution of peritoneal exudate harvested from an infected, moribund, adult mouse. Each embryo was individually injected. The peritoneum was then closed with interrupted fine silk sutures, the skin with continuous silk sutures, and the wound covered with a collodion dressing.

As will be seen below, it was possible by this method to produce an infection active at birth in over 90% of the embryos injected. The pathological picture in these animals was indistinguishable from that described for those infected by transplacental transmission, except that in the latter group the degree of parasitization appeared less and the fatality rate was lower.

One hundred sixty embryos were injected on the 14th day in the manner outlined above, and the mothers allowed to give birth, which in almost every case occurred on the 18th and 19th day of gestation. Within 10 hours after birth, the offspring were separated from their mothers, divided arbitrarily into litters of 5, and placed in cages with healthy lactating foster mothers. This was done in order to eliminate the possibility of milk-borne transmission of Toxoplasma during the period of therapy since about 50% of the mothers of the injected embryos developed toxoplasmosis.[8] The litters were then divided into the following 4 groups:

4 Sabin, A. B., *J.A.M.A.*, 1941, **116**, 801.

5 Zuelzer, W. W., *Arch. Path.*, 1944, **38**, 1.

6 Robinson, P., *Ann. Paediat.*, 1947, **168**, 134.

7 Cowen, D., Wolf, A., and Paige, B. H., *Arch. Neurol. and Psych.*, 1942, **48**, 689.

8 Eichenwald, H., *Am. J. Dis. Child.*, 1948, **76**, 307.

TABLE I.
Treatment of Offspring from Non-Immune Mothers.

| Group | Treatment begun 3rd day after birth | No. of newborn mice in group | No. of mice dead of toxoplasmosis | Avg survival period of mice dead of toxoplasmosis, days | Mice surviving experimental period (1 mo.) % | Survivors found chronic carriers, % |
|---|---|---|---|---|---|---|
| A | Sodium sulfadiazine 0.5 mg/g daily, 8 days | 40 | 22 | 6 ± 3 | 45 | 67 |
| B | Antiserum 0.02 cc daily, 8 days | 40 | 28 | 6 ± 3 | 30 | 83 |
| C | Sodium sulfadiazine 0.5 mg/g daily plus antiserum 0.02 cc daily, 8 days | 40 | 5 | 6 ± 2 | 88 | 46 |
| D Controls | None | 40 | 35 | 4 ± 3 | 13 | 60 |

Group A: 40 newborn mice treated 8 days with sodium sulfadiazine, 0.5 mg/g of body weight.

Group B: 40 newborn mice treated 8 days with 0.02 cc antiserum per day.

Group C: 40 newborn mice treated with 0.5 mg/g sodium sulfadiazine per day plus 0.02 cc antiserum per day for 8 days.

Group D: 40 newborn mice receiving no treatment.

All treatment was started on the third day after birth. This day was chosen since widespread dissemination of Toxoplasma through the tissues was almost invariably present by this time.

The antiserum was obtained from mice hyperimmunized by the injection of gradually increasing doses of Toxoplasma F strain* at bi-weekly intervals, followed by increasing doses of the more virulent RH strain at weekly intervals until the mice were resistant to at least 100 minimum lethal doses of the RH strain. Two weeks after the last injection, the animals were bled, and the serum frozen at −22°C. Each day the required quantity was thawed and promptly injected intraperitoneally. Enough serum was prepared so that the entire experiment could be performed with one batch. Grace had shown previously that immune serum from rabbits also has a beneficial effect on the course of the infection in adult mice;[9] we preferred to employ mouse serum in order to avoid introducing the additional complicating factor of foreign protein reactions.

Sulfadiazine was injected subcutaneously as a 0.5% solution of the sodium salt dissolved in physiological saline solution. The daily dosage of 0.5 mg per gram of body weight of mouse was divided into 3 doses given at approximately 8 hour intervals. This dosage gives blood levels similar to those achievable safely in man, averaging about 15 mg/100 cc blood one hour after injection, 12 mg after 3 hours and 7 mg after 6 hours, as measured by the methods described by Bratton and Marshall.[10] These levels produced no apparent toxic effects in mice. The results are shown in Table I.

One month after birth, all survivors were examined for the presence of Toxoplasma by microscopic observation of Giemsa-stained material and by subinoculation of tissue suspensions into fresh mice.

Pathologically, the picture presented by the mice treated with sulfadiazine alone dif-

* Obtained through the kindness of Dr. Isabella B. Grace of the Department of Public Health and Preventive Medicine, Cornell University Medical College.

9 Grace, I. B., personal communication, 1947.

10 Bratton, A. C., and Marshall, E., *J. Biol. Chem.*, 1939, **128**, 537.

TABLE II.
Course and Treatment of Offspring from Immune Mothers.

| Group | Treatment begun 3rd day after birth | No. of newborn mice in group | No. of mice dead of toxoplasmosis | Avg survival period of mice dead of toxoplasmosis, days | Mice surviving experimental period (1 mo.) % | Survivors found chronic carriers, % |
|---|---|---|---|---|---|---|
| A | Sodium sulfadiazine 0.5 mg/g daily, 8 days | 40 | 13 | $7 \pm 3$ | 68 | 70 |
| B | Antiserum 0.02 cc daily, 8 days | 40 | 22 | $7 \pm 3$ | 45 | 78 |
| C | Sodium sulfadiazine 0.5 mg/g daily plus antiserum 0.02 cc daily, 8 days | 40 | 4 | $6 \pm 1$ | 90 | 53 |
| D Controls from immune mothers | None | 40 | 26 | $6 \pm 3$ | 35 | 82 |
| E Controls from non-immune mothers | None | 40 | 33 | $4 \pm 2$ | 18 | 71 |

fered considerably from that observed in the animals treated with antiserum alone. In the latter case, active inflammatory lesions were most marked in the brain, and only relatively few scattered, apparently inactive lesions could be found in the other viscera. In the animals given sulfadiazine there were many scattered lesions throughout the liver, lung, spleen, brain, and kidney. These, however, were considerably smaller in both size and number and showed markedly less necrosis and inflammatory reaction than the widespread granulomata noted in the untreated control series. The persistence of active lesions in the central nervous system after treatment with antiserum may, perhaps, be explained best by the slight permeability of cerebral capillary walls to immune bodies, as postulated by Friedeman.[11] It was not feasible to use larger doses of antiserum.

As indicated on Table I, in the mice treated with combined antiserum and sulfadiazine, approximately half of the survivors had been cured. The remainder continued to harbor parasites, mostly in the brain, and had thus become chronic carriers.[12] The mice that died during treatment showed a widespread parasitization of tisues; in these cases the infection apparently had already been overwhelming before therapy was started.

In order to evaluate the effects of maternal antibodies on the course of toxoplasmosis in the embryo and in the newborn mouse, adult virgin female mice were injected subcutaneously with a sub-lethal dose of Toxoplasma F strain. This produced a transient disease which was followed in about 3 weeks by a strong immunity to a challenge dose of RH strain. Two weeks later the immune animals were allowed to mate with healthy male mice. On the 14th day of pregnancy, the embryos were injected *in utero* as described above. After birth, the litters were again given healthy foster mothers, and divided into 4 groups as before, plus an additional group E consisting of 40 newborn mice born from non-

[11] Friedeman, U., *Physiol. Rev.*, 1942, **22**, 125.

[12] Weinman, D., *J. Infect. Dis.*, 1943, **73**, 85.

immune mothers. This group was given no treatment. The therapy given to the test groups was identical to that used in the previous experiment. The results are shown in Table II.

The data in Table II indicate that antibodies passively acquired transplacentally by the fetus are not sufficient to protect the offspring even when sulfadiazine is given after birth, and that additional antiserum must be supplied to the progeny for optimal therapeutic effects.

Two other drugs† were also tried alone and in combination with antiserum but failed to show any beneficial action. These were "Promizole" (4,2′ diaminophenyl 5′ thiazole sulfone) given to newborn mice in a dosage of 0.5 mg/g per day for 8 days and "Diazone" (disodium formaldehyde sulfoxylate diaminophenyl sulfone) given in 0.1 mg/g per day for 8 days.

*Discussion.* A comparison of the results obtained by the various forms of treatment, as listed in Table I, readily shows that while sulfadiazine and antiserum each have a beneficial action on the course of toxoplasmosis, a combination of these two substances is considerably more effective than the use of either material alone. The reasons for this fact are probably many: one, already mentioned, may be the difficulty encountered in trying to obtain high antibody concentrations in the central nervous system; another may be the fact that humoral antibodies do not appear to have any action on intracellular Toxoplasma,[13] while extracellular organisms are not affected by sulfonamides.[1]

† Obtained through the kindness of Dr. Walsh McDermott of the Department of Medicine, The New York Hospital and Cornell Medical College.

It should be pointed out that the histological structure of the placenta in man and in the mouse is similar if one considers the fact that in both cases only a single layer of cell separates the maternal from the fetal blood.[14] The thickness of this layer is believed to be of importance in transplacental antibody transfer.[15]

We have observed several cases of human congenital toxoplasmosis in whom, when tested a few days after delivery, the mother showed a higher neutralizing titer to Toxoplasma than did the infant. This suggests that perhaps the transfer of antibody from mother to fetus through the placental barrier is not complete, which may be one reason why the embryos from highly immune female mice are susceptible to the infection.

Even though one must be cautious not to apply unselectively data obtained from animal experimentation to human diseases, it would appear that the combined antiserum and sulfadiazine therapy should be given a clinical trial in active human congenital cases.

*Summary.* The effect of sulfadiazine, antiserum and a combination of these two materials on the course of congenital toxoplasmosis in mice was determined. It was shown that while sulfadiazine and antiserum individually had some beneficial action, a combination of these substances was considerably more effective than either used alone.

13 Sabin, A. B., and Feldman, H. A., *Science*, 1948, **108**, 660.

14 Mason, J. H., Dalling, T., and Gordon, W. J., *Path. Bact.*, 1930, **33**, 783.

15 Schneider, L., and Szathmary, J., *Z. Immunforsch.*, 1940, **98**, 24.

Received February 17, 1949. P.S.E.B.M., 1949, **71**.