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Effect of tetracycline on experimental Venezuelan equine encephalitis.

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Abstract. The effect of tetracycline on experimental Venezuelan equine encephalitis was studied using three groups of embrionated chick eggs: one group was innoculated with viruses, a second group was innoculated with viruses and two hours later injected with tetracycline and a third group which received the tetracycline injection prior the innoculation with the viruses. It was observed that the group which did not receive tetracycline showed 98,8% mortality, while in the groups injected with the antibiotic, the mortality was only 71.5%. No differences were observed between the prophylactic and therapeutic administration of the antibiotic.

For many years different authors have had the opinion that illnesses produced by small virus are not affected by antibiotic treatment.

Coriell affirms: "The equine encephalitis viruses are not affected by penicillin, streptomycin, aureomycin, terramycin and chloromycetin" (3). However, Negrette affirms to have obtained good therapeutic results with the use of tetracycline hydrochloride during an encephalitis outbreak (9). The same author has demonstrated the therapeutic affect of tetracycline on the experimental infection of albino mice by Venezuelan equine encephalitis virus (10).

In order to confirm those previous findings we decided to use chick embryo as experimental animal, for it is known the effectiveness of the viral multiplication and the distribution of the virus in the several cavities and organs of the embryonated eggs (1, 2, 6, 8). Is also well known the pathogenesis of the Venezuelan equine encephalitis virus in the chick embryo (7).

The purpose of this communication is to report the effects obtained with the therapeutic and prophylactic administration of tetracycline on the infection of Venezuelan equine encephalitis in chick embryo.

MATERIAL AND METHODS

Virus. The Guajira strain of Venezuelan equine encephalitis virus was used. Suckling mice brain suspension was inoculated at a dose of 10 chick embryo lethal dose 50 (CELD50) via allantoic cavity of the embryonated eggs. Viral suspensions were made 0.75% bovine albumin in phosphate buffer 0.02M. Viral titration was made according to Reed and Muench (11).

Tetracycline. The embryonated eggs received 0.03 ml of an aqueous solution containing 0.56 mgs of pirrolidine-methyl-tetracycline per ml.

Periodicity of inoculation. The best effect of the drug was noted to be when administered every 6 hours. Any other rythm of inoculation was not advisable due to the rapid effect of the virus on the embryo which is present in 24-48 hours.

Toxicity of tetracycline in chick embryo. In order to test the toxicity of the drug, groups of 10 eggs each were inoculated in the allantoic cavity with 2, 2.5, 3 and 4 mgs of tetracycline distributed in four daily doses. Tetracycline proved to be innocuous up to 3 mgs daily for 88 hours. After that, 10% of mortality was observed with such dose. With 4 mgs daily tetracycline was toxic with 100% mortality at 88 hours.

pH of tetracycline. It can be thought that the acid pH of tetracycline determine some grade of inhibition on the virus. In order to test this hypothesis 0.9% saline solution was prepared with a similar pH to that of the tetracycline solution (pH 3.25). Two groups of 55 embryonated eggs were set. Both received 10DL50 of Venezuelan equine encephalitis virus via allantoic cavity. One group served as control while the other received the pH 3.25 saline solution at 6 hours interval by the same cavity.

Mortality in both groups were similar in three different experiments, which demonstrated that the pH of tetracycline does not hinder the viral action. (Fig. 1).

Embryonated eggs. Chick embryos were supplied by a comercial firm. At the begining of the experiment the eggs had 11 days of incubation. During the experiment they were kept at 37 oC with adecuate conditions of humidity. They were revised by ovoscopy every 6 homs in order to look the following signs of lethality.

a) Disappearance of the large capillary vessels and the capillary net. The egg took a yellowish colour different to the pink shown by the living embryo.

b) Immovability of the embryo. When these eggs were opened the embryos were found to be hemorrhagic. Embryos so dying were pooled and inoculated into chick embryo fibroblasts where a cytopathic effect was observed.

Using the data obtained regarding the infecting capacity of the virus, therapeutic utility of tetracycline we decided to perform three experiments for which we made 5 groups of 30 embryos each. The

groups were used as follows: A: Healthy controls; B: Tetracycline control which received the given dose of drug. V: Virus control which received 10LD50 of VEE virus. P: Prophylactic inoculation of tetracycline, that is administration of tetracycline every 6 hours, 24 hours before giving the viral inoculation and then each 6 hours thereafter and T: Therapeutic inoculation of tetracycline which means starting administration of the antibiotic 2 hours after 10LD50 VEE virus was given and then each 6 hours thereafter.

RESULTS

In the first experiment mortality started 24 hours after inoculation of the virus, in the three experimental groups being higher in the groups not injected with tetracycline; 36 hours after inoculation of the virus those embryos receiving tetracycline had a survival of 27% while those which did not receive the drug had 100% mortality.

In the second experiment mortality started again at 24 hours, being 4 times greater in the group inoculated only with the virus than in the other groups. 42 hours later mortality was 77% in the tetracycline groups against 100% in the non protected one.

In the third experiment, 24 hours after the inoculation, no mortality was found in the group previously injected with tetracycline, it was four times greater in the non protected group than in the one which received the antibiotic 2 hours after inoculation with the virus. At the end of the experiment, 72 hours after inoculation, mortality was 97% in the group without tetracycline and 63% and 67% respectively in the groups with prophilactic and therapeutic administration of tetracycline.

No mortality was encountered in the control groups, non inoculated with virus.

Table I and figure 2 show a synthesis of the three mentioned experiments. As it can be seen, there was a statistically significant difference between the groups ($p < 0.01$).

DISCUSSION

As shown in the results, the mortality of chick embryos, inoculated with Venezuelan equine encephalitis virus, started earlier and

TABLE I

Group	12h	24h	30h	36h	42h	48h	54h	60h	66h	72h
A	0/90	0/90	0/90	0/90	0/90	0/90	0/90	0/90	0/90	0/90
B	0/90	0/90	0/90	0/90	0/90	0/90	0/90	0/90	0/90	0/90
V	0/90	24/90	52/90	72/90	78/90	84/90	86/90	89/90	89/90	89/90
P	0/90	5/90	26/90	50/90	53/90	57/90	59/90	62/90	64/90	64/90
T	0/90	9/90	28/90	43/90	52/90	57/90	60/90	64/90	65/90	65/90

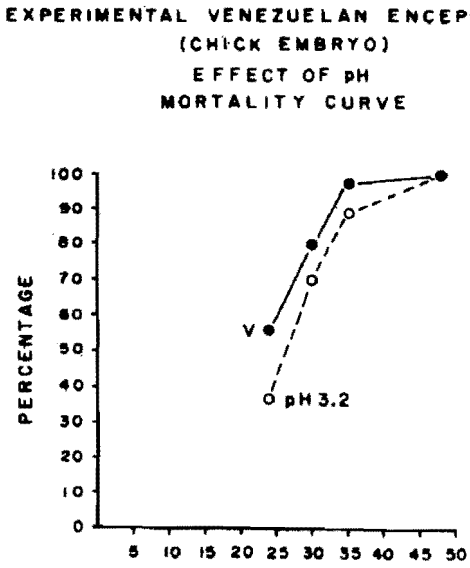
EXPERIMENTAL VENEZUELAN ENCEPHALITIS
(CHICK EMBRYO)
EFFECT OF pH
MORTALITY CURVE
PERCENTAGE
100
90
80
70
60
50
40
30
20
10
0
V
pH 3.2
5 10 15 20 25 30 35 40 45 50
HOURS

Fig. 1

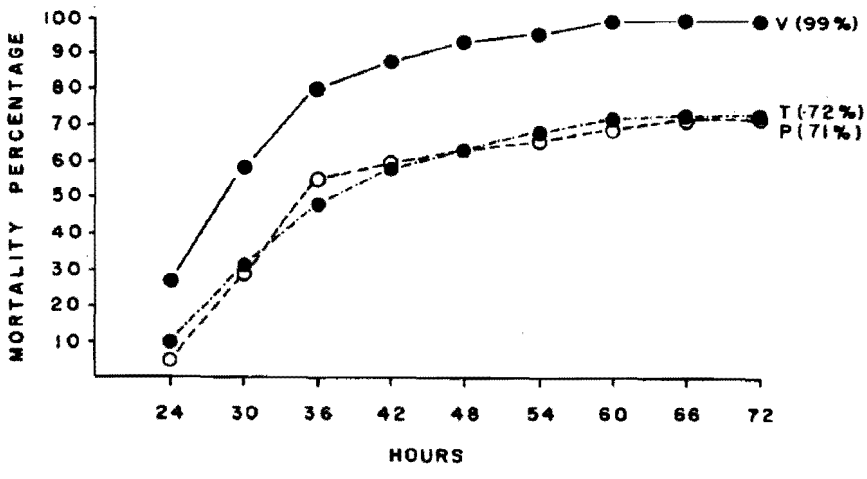
MORTALITY PERCENTAGE
100
90
80
70
60
50
40
30
20
10
V (99%)
T (72%)
P (71%)
24 30 36 42 48 54 60 66 72
HOURS

Fig. 2

reached a higher percentage (98,8%) than in the embryos which received tetracycline prior or after inoculation with the virus (71.5% mortality).

In a previous report, we have shown, that a better response to tetracycline is obtained by using adult mice (protection: 50%). In the present work we have been able to demonstrate that very sensitive animals such as chick embryo can also be protected from the viral action by tetracycline, although to a lesser degree. However, we do not know whether the action on the virus is direct or has an inhibitory effect on its reproduction, as other authors have pointed out(13).

Coriell (3) tested several antibiotics, and none of them proved to be efficient against Venezuelan equine encephalitis, however he did not use tetracycline. The mechanism of action of this antibiotic on the virus is not known; nevertheless, as Forsgren (5) demonstrated tetracycline does not interfere with the natural defenses of the organism, therefore, making it recomendable in clinical practice.

Some authors (12) refer to the diverse inhibitory effects of antibiotics on the synthesis of proteins, preventing in this manner the proliferation of bacteria. It is possible that a similar mechanism exists when tetracycline is used to detain viral multiplication. Zhdanov (13), working with puromycin, demostrated its inhibitory effect on the reproduction of Venezuelan equine encephalitis virus. Chang (4)) used novobiocin to inhibit the cytopathogenic effect of virus (herpes, vaccinia, polio, coksackie A9, B4, 6, 1) on cellular cultures, and demonstrated greater effect of the drug under low pH. Our results agree with these findings.

Although the therapeutic action of antibiotics on small viruses is doubted, we believe that our clinic experience (9) and experimental results (1, 13, 10) established the basis for the aplication of antibiotics in the treatment of diseases produced by small viruses.

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