EXPERIMENTAL BLASTOMYCOSIS IN MICE[1]

JOSEPH M. HITCH, M.D.

Raleigh, N. C.; Instructor in Dermatology and Syphilology, Duke University School of Medicine

WITH THE TECHNICAL ASSISTANCE OF JANE S. SHARP, M.S.

(Received for publication November 25, 1941)

INTRODUCTION

In humans, an infection with *Blastomyces dermatitidis*, whether localized or systemic, is of great import; for, although the disease may advance slowly, there is to date no universally satisfactory treatment. The combination of physical, biological and chemical therapy perhaps offers the best treatment but even this intensive combined approach leaves much to be desired.

It would seem justified, therefore, to search for some agent lethal for the fungus but innocuous for the host. The nature of such an experiment precludes use of the human subject. Because the mouse (1, 2, 3) has been shown consistently susceptible to blastomycosis, it was employed in the experiments to be reported in this paper.

A culture of *Blastomyces dermatitidis* (Duke Strain no. 2), originally obtained in 1934 from a patient with systemic disease (case 5 in the paper by Martin and Smith (4)), was available and, in the past, had repeatedly been lethal to mice by intraperitoneal injection in 20 to 30 days. Throughout this work a seven-day blood agar culture, grown at 37°C., was used. When thus grown this strain of blastomyces maintains the budding, yeast-like form and does not develop mycelia.

In all cases, the intravenous injection of organisms or chemical was accomplished through the tail vein. The mouse was first heated over an incandescent lamp, which produces venous dilatation and was then etherized and placed in a confining wire mesh "stall," which prohibits movement but allows access to the tail, around the base of which a rubber band was placed. With a tuberculin syringe and hypodermic needle, intravenous injections were made rather easily after some practice.

TESTING INFECTIVITY OF BLASTOMYCES DERMATITIDIS (DUKE STRAIN NO. 2) FOR MICE

Several animals were injected intravenously with various concentrated suspensions of the organisms. This procedure always produced immediate death

[1] From the Section of Dermatology and Syphilology of the Department of Medicine, Duke University School of Medicine.

and was abandoned. It is believed that this lethal effect was due to the organisms' acting as emboli.

A series of mice were then injected intraperitoneally with 0.5 cc. of a heavy suspension of an unmeasured quantity of the organisms. Death intervened in all of these in 10 to 21 days. At autopsy each showed extensive invasion of the viscera with small blastomycotic abscesses.

In an effort to find the minimal lethal dose of this strain of blastomyces, a series of dilutions were made using weighed amounts of the culture in normal saline. The total dose was kept at 0.5 cc. and the dilution varied from 1:10 to 1:200. All animals injected with 1:10 or 1:50 dilution died of blastomycosis in 20 to 30 days, while those receiving higher dilutions lived for several months and at autopsy the organism could not be recovered.

Realizing that this method of introducing the infection was gross and did not parallel natural infections in man, an attempt was made to modify it. Accordingly, four groups of 12 mice each were inoculated with a heavy suspension of blastomyces as follows: group 1—approximately 0.1 cc. was introduced intradermally in the abdominal wall; group 2—the abdomen was shaved, painted with a heavy suspension of the organisms and then subjected to multiple dermal puncture in the painted area; group 3—the abdomen was shaved, vigorously scarified with sandpaper and painted with the suspension; group 4—the abdomen was shaved, deeply scarified with sandpaper and allowed to remain uninoculated for twenty-four hours. The wounds then showed some granulation and crusting. The crusts were washed off and the area painted with the suspension of blastomyces.

In all of the groups (48 mice in all) no definite evidence of either local or systemic blastomycosis developed.

TESTING INHIBITORY ACTION OF GENTIAN VIOLET ON BLASTOMYCES DERMATITIDIS (DUKE STRAIN NO. 2)

In 1927 Sanderson and Smith (5) reported upon the inhibitory action of gentian violet on cultures of pathogenic blastomyces. It was decided to repeat this experiment, using the present strain of organisms. A 1:100,000 dilution of gentian violet was made by diluting the 1 per cent alcoholic solution (National Chemical Company) with distilled water. This was added in varying amounts to blood infusion agar to make 1:250,000; 1:500,000; 1:750,000; 1:1,000,000 and 1:2,000,000 dilutions respectively. Divided plates were poured, using untreated blood infusion agar on one side and the various dilutions of gentian violet agar on the other. All the plates were then streaked with *Blastomyces dermatitidis* (Duke Strain no. 2) and incubated at 37°C. Complete inhibition of growth occurred on the treated sides of the plates containing the 1:250,000 and the 1:500,000 dilutions. There was partial inhibition in dilutions up to 1:1,000,000 but none in the plates containing 1:2,000,000 gentian violet. These results were in complete accord with those of Sanderson and Smith.

Because Sanderson and Smith (5) suggested that gentian violet might be an effective agent in the treatment of human blastomycosis, and Smith (6) has

found that in man an intravenous dose of 0.005 gram per kilogram is safe and the results encouraging, it was decided to investigate this chemical first.

In an effort to find the maximum tolerated dose of gentian violet in the mouse, various dilutions and amounts were given intravenously to a large group of animals. It was eventually determined that the most adaptable dilution was 0.15 per cent (table 1).

Thus it appears that the mouse tolerates a dosage of 0.005 gram per kilogram—the same that has been suggested for humans. Saline solutions are perhaps slightly less toxic. In working out the dosage of gentian violet, it was found that any appreciable amount of the drug causes a transient cyanosis and occasionally respiratory difficulty. In lethal amounts death is accompanied by convulsions. It was also noted that the intravenous injection of as much as 1 cc. of solution, no matter how dilute, produces signs of severe discomfort. Because both saline and distilled water dilutions of alcoholic gentian violet are

TABLE 1

Comparative tolerance to varying amounts of gentian violet in saline and in distilled water

GENTIAN VIOLET SOLUTION	AMOUNT INJECTED	NUMBER OF MICE INJECTED	LIVED	DIED	MORTALITY	DOSE PER KILO ON BASIS OF 30 GRAMS
	cc.				*per cent*	*grams*
0.15% saline	0.5	3	0	3	100	0.025
0.15% water	0.5	3	0	3	100	0.025
0.15% saline	0.25	4	0	4	100	0.012
0.15% water	0.20	21	3	18	86	0.010
0.15% saline	0.20	10	2	8	80	0.010
0.15% water	0.15	21	8	13	62	0.0075
0.15% saline	0.15	6	3	3	50	0.0075
0.15% water	0.10	5	5	0	0	0.005
0.15% saline	0.10	6	6	0	0	0.005
0.15% water	0.05	5	5	0	0	0.0025
0.15% saline	0.05	6	6	0	0	0.0025

accompanied by crystallization of the drug on standing, all solutions should be freshly made before injection.

With this preliminary work as a basis, the study of the therapeutic value of gentian violet for mouse blastomycosis was undertaken. Accordingly, six groups of mice (four to seven animals in each group) were treated as will be described. All blastomyces suspensions (Duke Strain no. 2) were given intraperitoneally, and the gentian violet intravenously.

Group I, consisting of six animals, received *Blastomyces dermatitidis* only and acted as control. The first fatalities occurred fourteen days after inoculation and all were dead by the twenty-second day.

Group II was injected with the blastomyces and gentian violet 0.1 cc. simultaneously. This group was made up of seven animals. The first mouse died fourteen days later and all were dead in twenty-one days.

Group III, composed of six animals, was injected with the same dose of gentian

violet one week after infection with blastomycosis. The first death occurred on the ninth day of the infection and all were dead on the twentieth.

Seven mice made up Group IV and were treated similarly except that the dose of gentian violet was withheld until the fourteenth day after infection. One animal died on the eleventh and two on the twelfth day of the disease. Thus there were but four to receive the gentian violet. These four were all dead by the twenty-ninth day, having suffered the first mortality on the twentieth day of the infection.

In Group V, an attempt was made to determine the protective value of the drug. This group, composed of four animals, was given fifteen doses of gentian violet over a period of eighteen days prior to injection with the blastomyces. The doses varied from 0.1 to 0.5 cc. of 0.15 per cent solution. No drug was given after inoculation with the blastomyces. The first death occurred on the sixteenth day of the disease and all were dead on the twenty-fifth day.

There were four mice in Group VI. This group was given both prophylactic and therapeutic gentian violet. Each animal received fifteen injections of the drug in doses varying from 0.1 cc. to 0.5 cc. of 0.15 per cent solution over a period of eighteen days before inoculation with blastomyces and 12 injections of 0.3 cc. each over a period of fourteen days immediately following infection. The first mouse died on the fourteenth day, the last on the twenty-fifth.

In all groups, those animals which died under the anesthetic or from injection procedure are omitted from the summary above.

It is seen then that under the conditions of this experiment, gentian violet fails to offer any appreciable degree of protection against blastomycosis in the mouse. In the control group, death occurred in from sixteen to twenty-two days while an average of all the groups treated with the dye showed a 100 per cent mortality in from fourteen to twenty-four days. Group IV was the only one which showed a noticeable variation. In this group, gentian violet was given two weeks after inoculation and those animals which survived to the time of medication did not die until from the twentieth to the twenty-ninth day of the disease. It is believed that this variation is too small to be of significance.

COMMENT

These studies have shown a technical method of approach to the study of therapeutic agents in mouse blastomycosis. Gentian violet was chosen to initiate these experiments because other workers found that this chemotherapeutic agent offered some promise. This experiment fails to show that this chemical has any protective or curative value for blastomycosis in the mouse. However, it is fully realized that conclusions from these experiments cannot necessarily be expected to apply to the disease in man. Obviously, in these experiments, the number of blastomyces and the mode of inoculation bears no resemblance to the natural human infection and, although primary cutaneous blastomycosis has been produced in monkeys (2), to date this has not been done in the mouse. There may be great differences between the mouse and man in the rate of spread of the infection, development of biologic resistance factors, chemical changes in

gentian violet after introduction into the body, speed of elimination of the chemical and other factors. Therefore, it is not proposed that this work indicates the abandonment of gentian violet therapy studies in humans.

Work with other chemical and biologic agents in mouse blastomycosis is in progress.

SUMMARY

Studies on experimental blastomycosis in mice are presented with determinations of the minimum lethal dose of *Blastomyces dermatitidis* when given intraperitoneally.

An unsuccessful attempt to produce primary cutaneous blastomycosis is described.

Evidence of the in vitro toxicity of gentian violet for *Blastomyces dermatitidis* is shown.

The maximum tolerated dose of gentian violet in mice is determined and a technique for intravenous injections in these animals described.

Studies of the protective and therapeutic effect of gentian violet on blastomycosis in mice are presented and found to be nil by the method used.

REFERENCES

(1) Spring, D.: Comparison of seven strains of organisms causing blastomycosis in man. J. Infect. Dis., **44:** 169, 1929.

(2) De Monbreun, W. A.: Experimental chronic cutaneous blastomycosis in monkeys: Study of the etiologic agent. Arch. Dermat. & Syph., **31:** 831, 1935.

(3) Baker, R. D.: Comparison of infection of mice by mycelial and yeast forms of *Blastomyces dermatitidis*. J. Infect. Dis., **63:** 324, 1938.

(4) Martin, D. S., and Smith, D. T.: Blastomycosis. Am. Rev. Tuberc., **39:** 2, 1939.

(5) Sanderson, E. S., and Smith, D. C.: Effect of gentian violet on the organism of blastomycotic infection. Arch. Dermat. & Syph., **16:** 153, 1927.

(6) Smith, D. C.: Personal communication.