COMPARATIVE ACTION OF AN EXTRACT OF BRAIN TISSUE AND PENICILLIN ON STAPHYLOCOCCUS AUREUS INFECTIONS

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Carefully controlled clinical studies have been made (Chain *et al.*, 1940; Lyons, 1943; Robinson, 1943) of the value of penicillin and the sulfonamides in various infections, and the production and use of these antibiotics has attracted considerable attention to other potential sources of antibiotic agents (Waksman, 1945).

For a number of years our laboratories have investigated the effects on bacterial growth of certain substances obtained from plant and animal sources. Some extracts have been found to stimulate, other to inhibit, growth (Sperti *et al.*, 1937; Fardon and Sullivan, 1938–1939; Cook *et al.*, 1941; Schroeder and Hollencamp, 1941). A substance obtained from both human and beef spleen has a germicidal action against *Streptococcus pyogenes, in vitro* (Nutini and Kreke, 1942). A factor extracted in a similar manner from beef spleen, heart, kidney, and brain produced a conversion *in vitro* of the original yellow S form of *Staphylococcus aureus* to a white R configuration, the latter showing altered biochemical features (Nutini and Lynch, 1945). The converted organism retained its altered morphologic and biochemical characteristics *in vivo*, producing no evidence of virulence when injected into mice (Nutini and Lynch, 1946). The extracts of beef spleen, heart, kidney, and brain were effective against *Staphylococcus aureus* infections induced subcutaneously, intravenously, and intraperitoneally (Nutini and Lynch, 1946). The mortality in experimental animals receiving *S. aureus* and the brain extract subcutaneously was 0.9 per cent in the prophylactic series of 223 animals and 3 per cent in the therapeutic series of 116 animals, mortality in the control series being 75 and 87 per cent, respectively. The healing time for the small, dry, atypical lesions in the prophylactic series averaged 7 days and ranged from 3 to 14 days; for the typical suppurative lesions the healing time in the control series was 18 days (range 9 to 30). The brain extract was effective orally as well as subcutaneously against the infections. It is relatively nontoxic, as much as 400 mg, or 2 per cent of the body weight of the experimental animal, having been given daily for 10 days without ill effect in mice.

The present paper is a report of the comparative value of brain extract and penicillin as therapeutic and prophylactic agents against *S. aureus in vivo*.

The virulent strain of *S. aureus* was obtained from ATCC 6636, and 48-hour broth cultures were used for subcutaneous inoculations of 0.5 ml (1.5 LD_{50}) into the ventral abdominal region of the test animals, which were brown black mice of the BBC strain, 3 to 6 months old.

The brain extract, prepared as previously described (Nutini and Lynch, 1946), was administered subcutaneously in the ventral abdominal region in doses of 50

mg daily. In the prophylactic experiments the first injection was given 2 hours before inoculation with the *S. aureus*. In the therapeutic experiments treatment was begun on the third day following inoculation with the infecting organism, at which time there were typical suppurating lesions. The control animals received 0.25 ml of saline daily.

The penicillin was the commercial sodium salt manufactured in the Cheplin Laboratories. The dosage was 750, 1,000, and 2,000 Oxford units per day, given subcutaneously in divided doses at 6-hour intervals in the ventral abdominal region. The procedure for inoculating the mice with the infecting organism was the same as that for the animals treated with brain extract. When the experiments were repeated, the injections of penicillin were given at 12-hour intervals. No difference was observed in the response from that of animals treated at shorter intervals. The dosage levels were 5 to 10 times greater than

TABLE 1

Comparison of the prophylactic action of brain extract and penicillin on subcutaneous Staphylococcus aureus infections in mice

GROUP	NO. OF ANIMALS	FREQUENCY AND TYPE OF ABSCESS	MORTALITY	TIME OF HEALING FOR SURVIVORS, AVG RANGE
		per cent	*per cent*	*days*
Control	20	100 severe	90	23 (23)
Brain extract, 50 mg/day	20	80 needle point 20 moderate	0	7 (4–9)
Penicillin, 750 u/day	10	20 moderate 80 severe	70	13 (10–17)
Penicillin, 1,000 u/day	20	100 moderate	25	11 (8–16)
Penicillin, 2,000 u/day	10	20 none 80 moderate	10	9 (6–14)

Staphylococcus culture, ATCC 6636; dose used, 1.5 LD_{50}.

the maximal amounts used by Robinson (1943) in the treatment of staphylococcic infections in mice.

In the first of the prophylactic series of experiments, 40 mice were used, 10 serving as infected control animals, 10 receiving 50 mg of brain extract per day, and 20, in groups of 10, receiving penicillin in daily doses of 750 and 1,000 Oxford units, respectively. Daily treatments were continued until the lesions were healed as judged by the scabs dropping off and leaving the smooth new skin beneath. When the experiment was repeated, a dose of 2,000 Oxford units per day of penicillin was substituted for the 750-Oxford-unit dose per day.

In the therapeutic experiments subcutaneous inoculations were made with 0.5 ml(1.5 LD_{50}) of the virulent 48-hour broth culture of *S. aureus*, and 3 days later, after the development of typical lesions, the animals were divided into 4 groups, each containing mice with infections of similar severity. One group of 10 served as infected, untreated control animals, and the other groups received, respectively, 50 mg of brain extract per day, and 1,000 and 2,000 Oxford units of the sodium salt of penicillin per day in divided doses at 6-hour intervals until healing

was complete. In the second experiment the doses of penicillin used were 750 and 1,000 Oxford units per day.

RESULTS

Prophylactic experiments (table 1). All of the control mice developed suppurating lesions that were typical in size and appearance for staphylococcic infections. In those animals receiving the brain extract 2 hours before inoculation with the *S. aureus*, needle-point lesions at the site of the injection developed in 80 per cent of the animals; in 20 per cent there were atypical, small, dry, nonsuppurating lesions, which healed within 4 to 9 days. In the animals treated with penicillin, 100 per cent of those that received 750 and 1,000 Oxford units per day and 80 per cent of those that received 2,000 Oxford units per day developed suppurating lesions that were smaller than those in the control animals but were larger than the nonsuppurating lesion characteristic of the animals receiving the brain extract. As shown in table 1, the average healing time for the

TABLE 2

Comparison of the therapeutic action of brain extract and penicillin on subcutaneous Staphylococcus aureus infections in mice

GROUP	NUMBER OF ANIMALS	MORTALITY	AVERAGE TIME OF HEALING FOR SURVIVORS
		per cent	*days*
Control	20	95	27 (27)
Brain extract	20	0	7 (4–11)
Penicillin, 750 u/day	10	80	23 (21–26)
Penicillin, 1,000 u/day	20	45	17 (11–19)
Penicillin, 2,000 u/day	10	10	19 (18–22)

Staphylococcus culture, ATCC 6636; dose used, 1.5 LD_{50}.

groups of animals receiving penicillin was 13, 11, and 9 days, the interval being the shortest with the highest dosage of penicillin.

Therapeutic experiments (table 2). In the animals treated with brain extract, the suppurating lesions typical of staphylococcic infections in mice apparently began to dry up as early as the second day following initiation of treatment, the lesions developing a dry, hemorrhagic appearance with the crust dropping off on the sixth to the ninth day. In the groups of animals receiving penicillin, suppuration continued for 3 to 9 days, with the first signs of healing appearing on the seventh day. None of the animals receiving brain extract died. In the groups receiving penicillin the mortality was 80, 45, and 10 per cent, the lowest mortality occurring in the animals receiving the highest dosage. Healing required, on an average, 23, 17, and 19 days in the survivors of the penicillin-treated animals.

The results of experiments with penicillin-resistant strains of *S. aureus* and on the possible development of resistance to brain extract by these organisms are to be published in a separate communication.

CONCLUSIONS

It is apparent from the evidence presented that there is in brain tissue a factor that functions effectively against *Staphylococcus aureus in vivo*. Whether the extract is used as a prophylactic or therapeutic measure, it is superior to penicillin in the dosages used for *Staphylococcus aureus* infections in these experiments.

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