# Survival Study of Thermally Injured Rats Treated with Piromen®.* (20016)

L. C. GREENE, E. G. STUART, AND JANE JORALEMON. (Introduced by W. F. Windle)
*From the Department of Anatomy, School of Medicine, University of Pennsylvania, Philadelphia.*

In a previous study it was demonstrated that the Arthus phenomenon in rabbits was initially enhanced by administration of the bacterial polysaccharide complex, known as Piromen®, although it was suppressed by administration of cortisone. It was noted at that time that the skin lesions in the Piromen-treated rabbits healed more readily and the scars presented an appearance different from those in the control animals(1). This observation, coupled with other information obtained from studies of the healing of transected spinal cords and inhibition of glial-barrier formation in animals(2,3), lead to the study of the effect of this drug on thermal injuries.

Our initial experiments consisted of spot burns in cats and guinea pigs. The results of this study have been reported in preliminary form(4). Histological aspects of the healing of sublethal injuries of this type are still under investigation. During these studies, the question arose as to whether a bacterial polysaccharide complex might affect beneficially the rate of survival of animals receiving large body-surface lesions. Therefore, it was decided to examine the question of survival from the shock of thermal injuries. The present report deals solely with results of the latter study.

*Materials and methods.* Three hundred and thirty-nine (339) male and female white rats of the Wistar strain, weighing between 150 and 298 g, were used in this study. All the animals were anesthetized with ether; their backs, *i.e.*, 32-35% of the body surface, were then immersed for 45 seconds into water heated to 90°C(5). Not knowing what dosage of Piromen might prove effective, several exploratory experiments, not enumerated above, were carried out. A preliminary experimental series is included in Table I as Section I. The results of these experiments provided certain dosage reference points for definitive and confirmatory experiments. The *control animals* consisted of 157 male and female rats of weights comparable to those of the animals treated with Piromen. They received placebo injections of non-pyrogenic distilled water (30 rats), saline solution (10 rats), or the 1/6 molar sodium r-lactate buffer, which is the vehicle of the drug, Piromen (117 rats). The volume of the placebo, 0.1 to 0.2 cc, equalled that of the drug given to the treated animals. The *treated rats* (182), received Piromen in doses of 0.1 $\mu$g to 0.6 $\mu$g. As shown in Table I, 18 rats received 0.6 $\mu$g; 83 rats, 0.2 $\mu$g; 15 rats, 0.15 $\mu$g; and 55 rats, 0.1 $\mu$g of the drug. They are arranged in the Table in 3 groups according to schedules of administration of the drug. The intraperitoneal route of administration of the drug and of the placebo was used, with one exception in which the intravenous route was chosen for comparison (Group K). The time of the initial injection was immediately after inducing the thermal injury in all cases except Group L, in which the initial injection was delayed until 4 hours afterward. All experimental and control animals were segregated into individual cages 12 hours prior to the experiment and were maintained separately for the survival period of 15 days.

*Results.*† Table I illustrates the results obtained in the present study and shows the marked difference between the Piromen-treated animals and those which received only placebo injections. Under the conditions of the experiments, one can see a dose-weight relationship which was necessary for survival.

In Group A, receiving single daily injections of Piromen at 0.6 $\mu$g per rat, it will be noted that no beneficial results were obtained. All of the animals died within 12 hours, ex-

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† For preliminary report, see (6).

TABLE I. Summary of Experiments.

| Group | Wt, g | No. of animals | Drug | Dose, μg | Deaths 0-4 hr | Deaths 4-12 hr | Deaths 12-24 hr | Deaths 1-14 days | Survivors at 15 days | Initial wt of survivors, g |
|---|---|---|---|---|---|---|---|---|---|---|
| I. Single daily inj. for 15 days | | | | | | | | | | |
| A | 280-298 | 16 | Pl. | | 7 | 8 | 0 | 1 (2 days) | 0 | — |
| | | 18 | P. | .6 | 6 | 12 | 0 | 0 | 0 | — |
| B | 263-287 | 6 | Pl. | | 2 | 4 | 0 | 0 | 0 | — |
| | | 9 | P. | .2 | 0 | 0 | 0 | 0 | 9 | 263-287 |
| C | 185-207 | 14 | Pl. | | 6 | 7 | 1 | 0 | 0 | — |
| | | 14 | P. | .1 | 0 | 0 | 0 | 0 | 14 | 185-205 |
| II. Inj. 3 times daily for 15 days | | | | | | | | | | |
| D | 280-290 | 23 | Pl. | | 4 | 18 | 0 | 1 (2 " ) | 0 | — |
| | | 23 | P. | .2 | 0 | 2 | 0 | 0 | 21 | 282-287 |
| E* | 150-158 | 10 | Pl. | | 2 | 8 | 0 | 0 | 0 | — |
| | | 10 | P. | .1 | 0 | 0 | 0 | 2 (2 " ) | 8 | 150-158 |
| | 190-220 | 13 | Pl. | | 4 | 7 | 0 | 2 (7 " ) | 0 | — |
| | | 17 | P. | .1 | 0 | 0 | 0 | 3 (2 " ) | 14 | 190-218 |
| III. Single inj. immediately after thermal injury | | | | | | | | | | |
| F | 154-210 | 22 | Pl. | | 4 | 18 | 0 | 0 | 0 | — |
| | | 22 | P. | .2 | 5 | 17 | 0 | 0 | 0 | — |
| | 269-298 | 20 | Pl. | | 6 | 12 | 2 | 0 | 0 | — |
| | | 29 | P. | .2 | 1 | 9 | 1 | 0 | 18 | 269-287 |
| G | 246-278 | 13 | Pl. | | 4 | 9 | 0 | 0 | 0 | — |
| | | 15 | P. | .15 | 0 | 4 | 2 | 0 | 9 | 250-260 |
| H | 237-248 | 6 | Pl. | | 1 | 5 | 0 | 0 | 0 | — |
| | | 11 | P. | .12 | 0 | 1 | 0 | 0 | 10 | 237-248 |
| J | 237-248 | 2 | Pl. | | 1 | 1 | 0 | 0 | 0 | — |
| | | 2 | P. | .1 | 0 | 2 | 0 | 0 | 0 | — |
| | 154-170 | 4 | Pl. | | 2 | 2 | 0 | 0 | 0 | — |
| | | 4 | P. | .1 | 0 | 1 | 0 | 0 | 3 | 154-170 |
| K | 190-196 | 4 | Pl. | | 3 | 1 | 0 | 0 | 0 | — |
| | | 4 | P. | .1 | 0 | 1 | 0 | 0 | 3 | 190-192 |
| L† | 160-235 | 4 | Pl. | | 1 | 2 | 0 | 1 (8 " ) | 0 | — |
| | | 4 | P. | .1 | 0 | 0 | 0 | 0 | 4 | 166-235 |

P. = Piromen; Pl. = Placebo treated.
* Initial inj. was .1 μg; .2 μg, dose was started 24 hr after thermal injury.
† Piromen admin. 4 hr after thermal injury.

cept one of the controls which lived 2 days. The animals of Group B, receiving 0.2 μg of Piromen, survived for a period of at least 15 days. The same was true of the animals in Group C, receiving 0.1 μg of the drug. It is to be noted, however, that there was a considerable difference in weight between the latter 2 groups. Under *Section II*, it will be noted that the administration of Piromen 3 times daily, instead of once daily, did not materially alter the survival rate. The first of 3 injections was given immediately after thermal injury, the second was given 4 hours later, and the third was given 8½ hours afterward. The initial injection appears to have been the important one, for the total daily amount (0.6 μg Piromen) which was optimum for survival in Group D animals, was equal to the amount of the single injection (0.6 μg) which was too large for survival of the animals in Group A. In Group F, it should be noted that 0.1 μg of Piromen per rat was given 3 times daily for the first day but, on subsequent days, the dosage was increased to 0.2 μg 3 times a day. Other experiments had indicated that a single initial dose of 0.2 μg per rat of this weight group failed to bring about survival. It was noted that after an initial optimum dose of 0.1 μg of Piromen, it made no difference that the dose was increased to 0.2 μg on subsequent days. *Section III* represents a large series of experiments performed to determine the efficacy of different dosages of Piromen in relation to different weights of rats, by single initial administration immediately after thermal injury.

Looking at the present study as a whole, it will be seen that the extent and degree of the

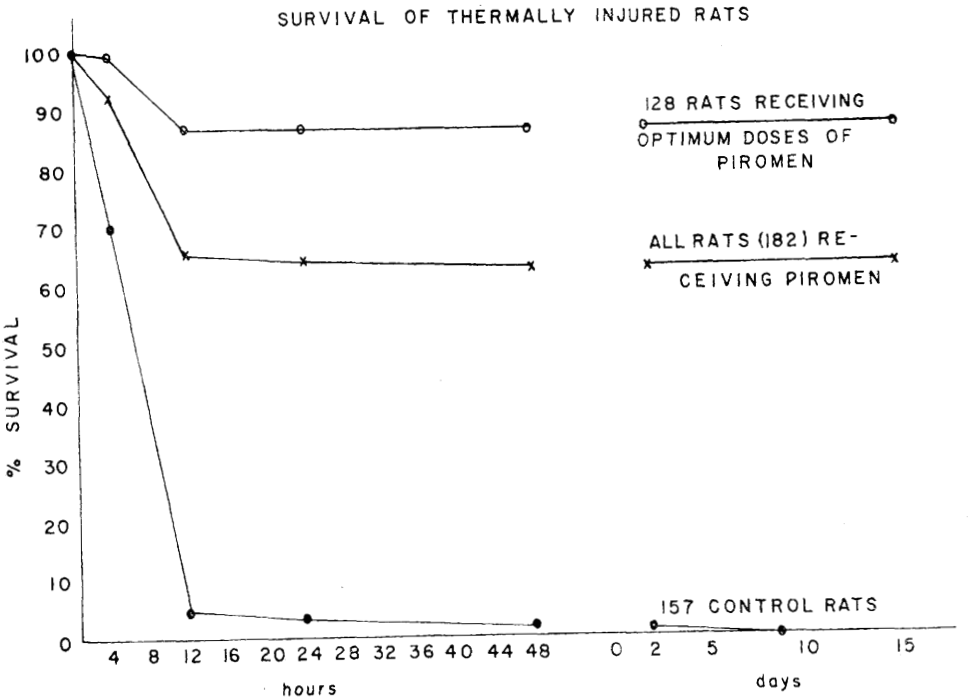


FIG. 1.

thermal injury administered to the placebo-treated animals was 100% lethal in less than the period of 15 days allowed for survival. Approximately 95% of the placebo-treated animals died between 0 and 12 hours. No animal survived longer than 8 days.

The survival of Piromen-treated animals is expressed in Fig. 1. Taking the entire series, irrespective of the dosage of drug administered or route of administration, 62% of the animals survived at least 15 days. Again, the mortality peak occurred between 0 and 12 hours. The experiment was terminated arbitrarily at 15 days, although some animals were not actually killed until 20 days or more after the initiation of the experiment.

The study contains certain experiments in which the size of the dose of the bacterial polysaccharide in relation to the weight of the animal burned was obviously larger or smaller than optimum. Eliminating from calculation certain animals of Group A and parts of Group F and J, and leaving only those to which we consider that an optimum dose of Piromen was administered, the survival rate increases to 86%. Again, the peak of mortality occurred between 0 and 12 hours.

One of the differences between the treated and non-treated animals was that of general prostration which was greatly reduced in the Piromen-treated groups. These animals were more active during the critical 0 to 12 hour period after thermal injury than were the placebo-treated groups.

We are unprepared at the present time to describe in detail the histological changes that occur during the healing of thermal lesions in animals treated with Piromen. A number of sublethal thermal injuries were administered to rats to serve in the histological study. Comparison of these with Piromen-treated animals of the present studies reveal significant differences. The apparent decrease in edema formation, increase in vascularity, and inhibition of scarring in the lesions under the influence of Piromen therapy after sublethal as well as lethal injuries will be discussed in some detail in an article currently in preparation.

*Discussion.* Death in our experimental animals was associated with shock mechanisms which are not fully understood. We can par-

tially evaluate certain factors which appear to have played a role in the shock, one of which was fluid shift. In previous studies of cats, rats, and guinea pigs, there was a marked tendency towards prevention of the initial edema when Piromen therapy was employed (4). This same apparent inhibition of a fluid shift was associated with survival of the treated animals in the present study. As yet we do not know the nature of the permeability changes effected. When more is known about the role of bacterial polysaccharides in the physiology of connective tissue(7-9) it will be easier to visualize the mechanism by which Piromen influenced the survival rate.

The question of adrenal participation must be considered, since it plays a major role in connection with the shock process. Effects of Piromen on adrenal function have been reported(10-12). The nature of relationship is not entirely clear, but experiments in animals have shown that chronic bilateral adrenalectomy very markedly alters the sequence of events that can be observed when Piromen is administered to an intact animal. Hypophysectomy has been shown to impair the animal's response to Piromen administration much less than adrenalectomy(13). It is tempting to differentiate between a response of connective tissue *per se* to Piromen and a response mediated through or permitted by the adrenal glands. Certain work by Dougherty(14) in eliciting local tissue changes in response to Piromen after adrenalectomy are suggestive of this dualistic action. Whatever the role of the adrenals in response to Piromen administration, it is apparent that the entire pituitary-adrenal mechanism need not be brought into play, and that it is not characteristically a "stress" response.

Analysis of the present data reveals a certain optimum dose-weight relationship. It also suggests that the initial dose of the polysaccharide was the critical one. This optimum dose-weight relationship must be carefully regulated if one is to obtain maximum effects. Calculation from the data in Table I shows an effective range of only 0.5 to 0.75 $\mu$g/kg. So far, the dose-weight relationship is applicable only to conditions of the present experiment. It would appear that, as we employ larger, heavier animals and different species of animals, or as we alter the experimental conditions of the thermal injury, such as increasing the area of the body surface involved, a new dose-weight relationship for each particular case may have to be established.

*Summary.* A bacterial polysaccharide complex, Piromen, was tested for its effectiveness for promoting survival for an arbitrary period of 15 days of rats receiving fatal thermal injuries. An over-all survival of 62% of the rats was observed in this present experiment, regardless of the route of administration, the regimen of administration, and the size of the dose of the drug. No placebo-treated animal survived longer than 8 days and 95% of them died in 12 hours. Results of the present experiments indicate a dose-weight relationship necessary for survival. When such a relationship was recognized and optimum dosage of Piromen was administered to rats, 87% of the treated animals survived.

1. Stuart, E. G., *Fed. Proc.*, 1951, v10, 133.
2. Windle, W. F., and Chambers, W. W., *J. Comp. Neurol.*, 1950, v93, 241.
3. Windle, W. F., Clemente, C. D., and Chambers, W. W., *J. Comp. Neurol.*, 1952, v96, 359.
4. Greene, L. C., *Am. J. Physiol.*, 1951, v167.
5. McCarthy, M. D., *J. Lab. Clin. Med.*, 1945, v30, 1027.
6. Greene, L. C., *Am. J. Physiol.*, 1952, in press.
7. Stuart, E. G., *Am. J. Physiol.*, 1950, v163, 754.
8. Windle, W. F., *Ann. New York Acad. Sci.*, 1952, v14, 159.
9. Stuart, E. G., *Am. J. Physiol.*, 1952, in press.
10. Mitchell, S. Q., *Am. J. Physiol.*, 1951, v167.
11. ———, *Am. J. Physiol.*, 1952, in press.
12. Soylemezoglu, B., and Wells, J. A., PROC. SOC. EXP. BIOL. AND MED., 1951, v77, 43.
13. Stuart, E. G., and Mitchell, S. Q., 1952, unpublished data.
14. Dougherty, T. F., 1952, personal communication.

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