

Progesterone Rapidly Decreases Brain Edema: Treatment Delayed up to 24 Hours Is Still Effective

ROBIN L. ROOF,¹ REVITAL DUVDEVANI, JOHN W. HEYBURN, AND DONALD G. STEIN

*Brain Research Laboratory, Institute of Animal Behavior, Department of Psychology, Rutgers,
The State University of New Jersey, Newark, New Jersey 07102*

Cerebral edema is a serious side effect of traumatic brain injury. We have previously established that progesterone injections, initiated within 1 h after cortical contusion injury, reduced edema when assessed 3 days later. To determine how rapidly progesterone can reduce edema, male and female rats were given the hormone 1 h after damage to the medial frontal cortex, and edema levels were assessed between 2 h and 7 days postinjury. Progesterone decreased edema within 6 h of the injury and continued to be effective for the duration of treatment. In addition, we assessed whether progesterone injections are effective when delays are imposed between injury and initiation of treatment. Male and female rats received progesterone after postinjury delays of 6, 24, or 48 h. Progesterone was effective in reducing edema when treatment was delayed until 24 h after injury. © 1996 Academic Press, Inc.

INTRODUCTION

Over the past several years, our laboratory has published a series of papers showing that after contusion injury of the medial frontal cortex, posttraumatic treatment with progesterone can relieve edema (20, 21), reduce retrograde neuronal degeneration in the thalamus, and improve the recovery of cognitive performance on a spatial learning task (19). This work stemmed from our initial finding that females have less cerebral edema than males after contusion of the frontal cortex (21). Subsequent experiments showed that this gender difference was due the presence of circulating progesterone in the female rather than less estrogen in the male (20, 21). We have also shown that treatment with progesterone protects brain-injured males as well as females (20) and that progesterone is beneficial when given before, as well as after, the injury (20, 21).

These findings are clinically important considering

the detrimental effects that unchecked edema has in the CNS. Cerebral edema develops rapidly following traumatic brain injury (TBI), becomes more severe over a period of days, spreads outside the primary injury area, and involves increasing amounts of brain tissue (7). Edema can have many consequences, including intracranial swelling, production of free radicals, and, ultimately, neuronal death (1). In a clinical context, the effective control of edema, therefore, would result in long-term improvements in cognitive, sensory, and motor abilities in head-injured patients.

For the most part, current treatments for edema are limited and problematic. For example, mannitol can be effective for short periods of time (24 h) after brain damage, but it is not useful as prolonged therapy for the duration of postinjury edema. When given for more than 24 h, mannitol has been associated with increased edema formation (17). Several corticosteroids, such as methylprednisolone, have also been examined as potential treatments, but they have met with only limited success and are not considered particularly efficacious (6, 11, 24). According to Hall (13), very large doses of methylprednisolone need to be given intravenously, but if the dose is too high, any beneficial effects are lost because the effects of the drug are biphasic (2, 5, 15, 16). Second, the treatment must be given very soon after the injury (i.e., within the first 8 h) to be effective because the uptake of methylprednisolone in injured tissue decreases very rapidly over time (3, 14). Third, because methylprednisolone has a short half-life, repeated doses must be given initially and then be followed by continuous infusion for 48 h (4). In addition, the amounts required to obtain beneficial results are problematic because they cause incapacitating side effects (13), including hyperglycemia, catabolic states, and increased incidence of serious infections (8, 9, 18).

Our initial findings with progesterone have shown that it can reduce edema at doses that are equivalent to those associated with pregnancy in the rat (23). Based on a vast literature examining progesterone effects we are not aware of any serious, negative side effects associated with the levels of progesterone we use. In addition, we have observed significant reductions in

¹ To whom correspondence should be addressed at Department of Psychology, Texas Christian University, Fort Worth, TX 76129. Fax: (817) 921-7110.

edema using daily injections and without the need for continuous infusion (19, 20). Thus, three of the four problems described associated with methylprednisolone are not manifested by progesterone. The fourth problem is the need to administer methylprednisolone within 8 h of injury. Therefore, the purpose of the following experiments are to (1) describe and compare the time course of edema formation following contusion injury in male and female rats (Experiment 1); (2) assess the formation and resolution of edema over time in male and female rats injected 1 h after injury with progesterone (Experiment 1); and (3) to determine whether progesterone injections effectively reduce edema when the initiation of treatment is delayed up to 48 h after injury (Experiment 2).

EXPERIMENT 1: ALTERATION OF EDEMA FORMATION WITH PROGESTERONE TREATMENT FOLLOWING CONTUSION TO THE MEDIAL FRONTAL CORTEX

Methods

Subjects. Fifty-two male and 52 female Sprague-Dawley rats, approximately 90 days of age at the start of the experiment, were subjects. Rats were housed in group cages (4–5 per cage) with a 12-h light–12-h dark reverse light cycle. Males and females were housed separately. Food and water were provided *ad libitum* throughout the experiment.

Surgery and progesterone treatment. Each rat was anesthetized (10 mg/kg xylazine, ip, +50 mg/kg ketamine, ip), the scalp was incised at the midline to expose the skull, and a 6-mm medial craniotomy was made immediately anterior to bregma. The injury was produced using a pneumatically driven piston device, consisting of a 9/16-in. dual-stroke air cylinder containing a 3-mm diameter piston with a 5-mm stainless steel tip. The tip impacted the brain through the craniotomy with a force of 20 psi and a velocity of 2.25 m/s, compressing the cortex to a depth of 2 mm. After impact and once all bleeding was stopped, the fascia and scalp were sutured closed. The superior sagittal sinus is not typically damaged, and any animal with substantial bleeding was eliminated from the experiment. An example of the resulting lesion and extent of degeneration is shown in Fig. 1.

Following surgery, 56 rats were randomly assigned to the treatment group and 48 to the control group. Progesterone treatment began 1 h after contusion. Progesterone (4 mg/ml) was dissolved in peanut oil and the initial injection (4 mg/kg) was given ip to ensure rapid absorption (12, pp. 5–9). The remaining injections (all 4 mg/kg) were given subcutaneously for more gradual absorption at 6 h postinjury and again at 24 h, then repeated once every 24 h until the rats were killed for histology. Control rats received equivalent injections of the oil vehicle at the same times. The rats were

processed in squads of approximately 12 per day of surgery. This was necessary due to the numbers of animals included in the experiment. However, each squad was balanced for gender and treatment.

Edema assessment. At 2 ($n = 22$) and 6 h ($n = 24$) and 1 ($n = 24$), 3 ($n = 24$), or 7 ($n = 10$) days after the contusion, the rats were deeply anesthetized with nembutal (75 mg/kg), decapitated, and, within 90 s, their brain surfaces were exposed. Using a 2-mm diameter cylinder attached to a syringe, four tissue-punch samples were taken from the area immediately surrounding the lesion and four from posterior parietal/occipital areas. The tissue samples included both white and gray matter. These samples were placed in pre-weighed containers, capped to prevent evaporation, then immediately weighed to the nearest 0.01 mg. Containers without caps were placed in a vacuum oven and dried at 60°C, 0.3 atm pressures for 24 h, then recapped and reweighed to determine water content. The average, single punch weight was 13.5 ± 2 mg wet and 2.5 ± 0.4 mg dry. Individual punches weighing less than 1 mg dry were eliminated (a total of 8 of 832 samples). Water content of each sample was calculated as $[(\text{wet wt} - \text{dry wt})/\text{wet wt}]$ and the mean of the four injury area samples was compared to the mean of the four distal samples, such that the final index of edema was percentage increase of water content in injured tissue compared to distal, intact tissue. Surgery, tissue sampling, and analyses were done without knowledge of treatment group. All comparisons of tissue water content between groups were based on samples from identical brain regions.

Results

Edema formation in male and female rats was evident at the earliest time point assessed (2 h), peaked at 1 day, and dropped off significantly by 7 days (ANOVA, main effect of time $F(4, 45) = 23.96$, $P < 0.0001$). In addition, a gender \times time interaction was significant, $F(4, 45) = 3.01$, $P < 0.05$, because edema peaked higher 1 day after injury in males compared to females, $t(10) = 2.80$, $P < 0.05$. These means are shown in Fig. 2.

Progesterone treatment, beginning at 1 hour after injury, altered this pattern of edema formation, and significant main effects of treatment on edema were seen for both male rats [$F(1, 42) = 39.33$, $P < 0.001$] and female rats [$F(1, 42) = 36.22$, $P < 0.001$]. In addition, an interaction of treatment \times time on edema formation was observed for both males [$F(3, 42) = 5.64$, $P < 0.01$] and females [$F(3, 42) = 2.94$, $P < 0.05$]. This effect was due to decreased edema in rats treated with progesterone compared to those treated with the vehicle alone at several time points. Six hours after injury (males $t(9) = 2.045$, $P < 0.05$; females $t(9) = 3.102$, $P < 0.01$), 1 day after injury (males $t(10) = 6.452$, $P < 0.001$; females $t(10) = 3.925$, $P < 0.001$), and 3

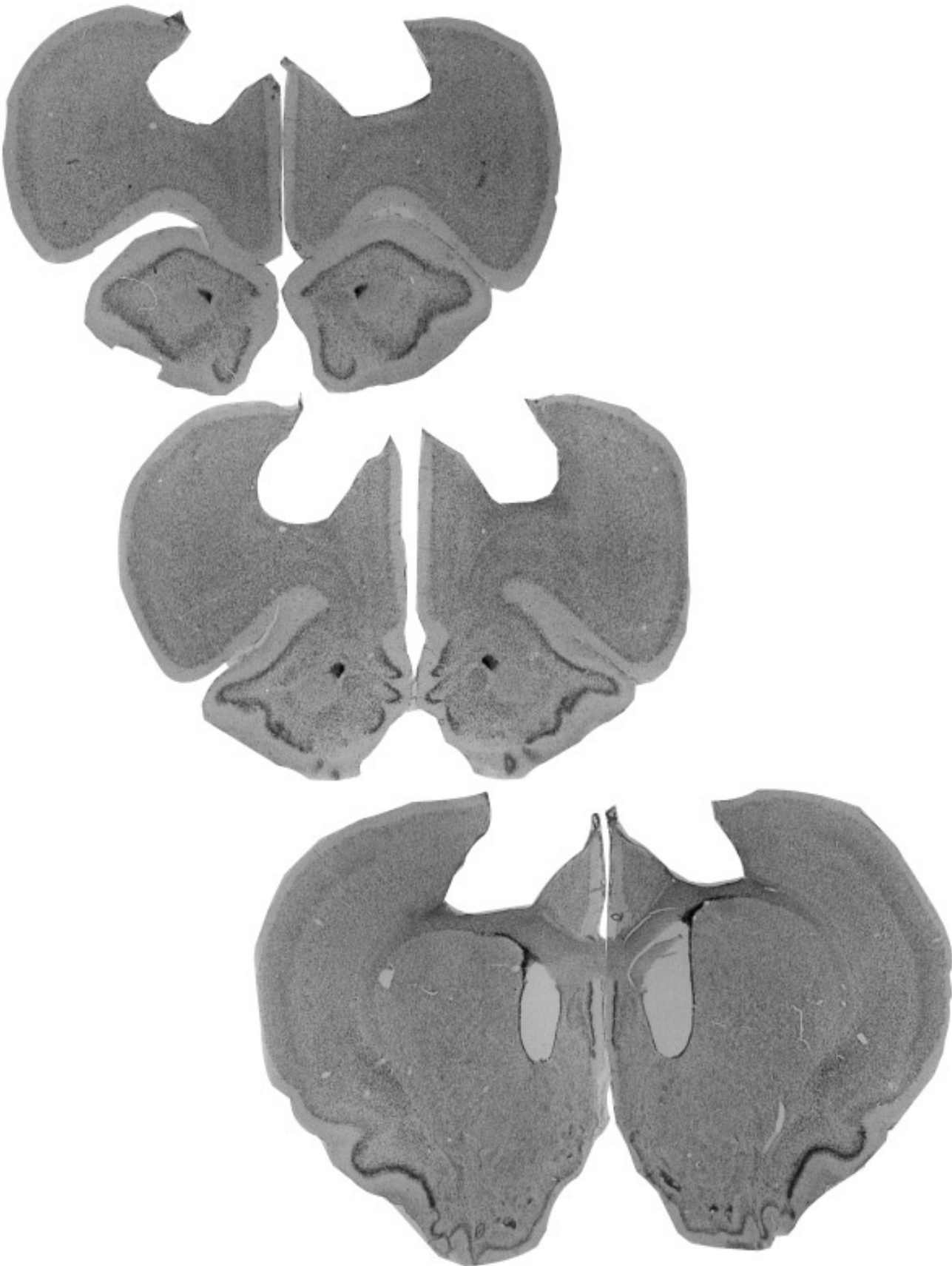


FIG. 1. Example of contused brain 28 days after injury at levels 3.7, 2.7, and 1.7 mm from bregma.

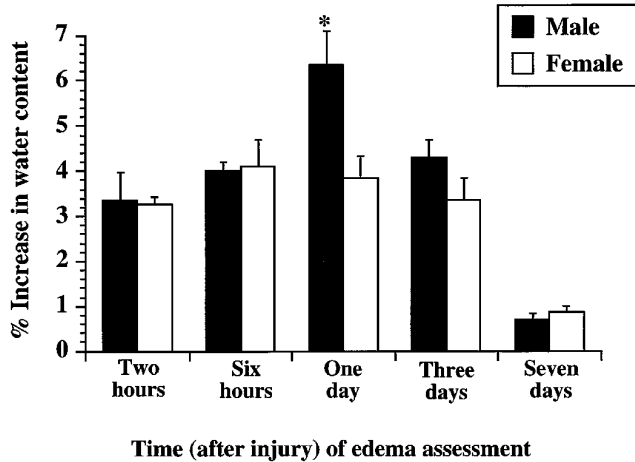


FIG. 2. Edema formation in male and female rats after contusion of the medial frontal cortex. Asterisk indicates gender difference at that time point.

days after injury (males $t(10) = 4.973$, $P < 0.001$; females $t(10) = 4.598$, $P < 0.001$), progesterone-treated rats had less edema than those receiving only the oil vehicle. There was no interaction between gender and treatment on edema formation; i.e., the treatment was equally effective in both genders. The means for males and females are shown in Figs. 3A and 3B, respectively.

Discussion

The contusion injury used in these experiments produced significant edema evident as early as 2 h after impact. These results are consistent with our previous findings (10) and fit with our observations of progesterone's potent effects on edema. At the peak of edema formation, males had significantly more edema than females. This finding is also consistent with our previous work (21). The hormone dramatically altered the extent and development of edema formation. When given 1 h after injury, reductions in edema were seen 6

h later and then again at 1 and 3 days after cortical contusion. By 3 days, the level of edema observed in progesterone-treated male and female rats was equivalent to that of nontreated, contused rats at 7 days after injury. This is very important because it implies that after TBI, progesterone treatment can reduce the duration of edema by nearly half. No apparent effect of progesterone was observed when the tissue samples were taken at 2 h after injury, which is not surprising because in these rats the progesterone had been in their system for only 1 h.

EXPERIMENT 2: EFFECT OF DELAYED PROGESTERONE INJECTIONS ON EDEMA FORMATION

Methods

Subjects. Forty-eight male and 48 female rats, approximately 90 days of age at the start of the experiment, served as subjects in this experiment. Housing and handling were the same as in Experiment 1.

Surgery and progesterone treatment. Surgery and treatment were the same as in Experiment 1, excepting time of treatment initiation. In Experiment 2, one-fourth of the rats were given injections beginning at 1 h postinjury, one-fourth at 6 h postinjury, one-fourth at 24 h postinjury, and one-fourth at 48 h postinjury. Equal numbers of male and female, progesterone and oil-treated, rats were included at each time point. As in Experiment 1, the rats were processed in balanced squads of approximately 12 rats per surgery day.

Edema assessment. Three days postinjury, the rats were anesthetized, decapitated, and assessed for edema using the methods described for Experiment 1.

Results

The amount of edema reduction produced by progesterone treatment depended on when the treatment was

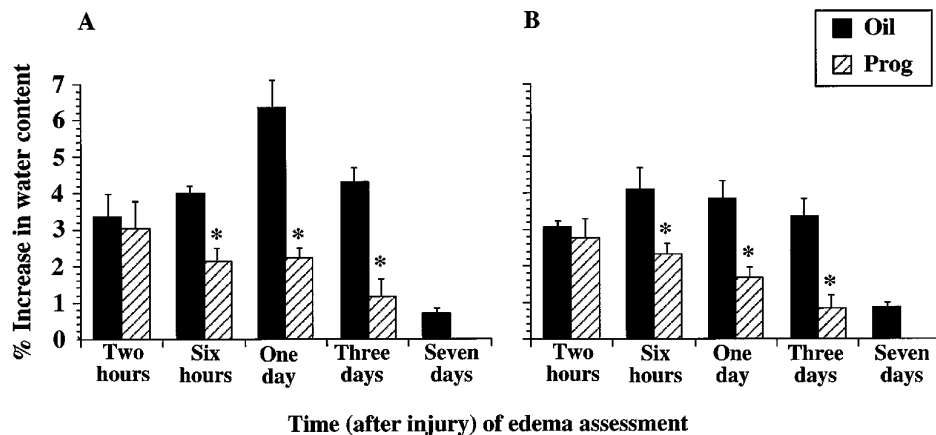


FIG. 3. Edema formation in (A) male and (B) female rats treated with progesterone or oil after contusion of the medial frontal cortex. Asterisks indicate significant difference from oil-treated rats at that time point.

initiated [treatment \times delay, $F(3, 80) = 6.59$, $P < 0.001$]. There was no interaction of gender with delay, so male and female data were analyzed separately. An interaction of treatment \times delay was significant for males, $F(3, 40) = 2.958$, $P < 0.05$. Progesterone was effective in reducing edema in males when given 1 h after injury [$t(10) = 5.32$, $P < 0.0001$ compared to oil-treated], when given 6 h after injury [$t(10) = 3.39$, $P < 0.01$, compared to oil-treated], and when given 24 h after injury [$t(10) = 3.69$, $P < 0.001$, compared to oil-treated]. Progesterone injections were not effective when treatment was delayed 48 h. An interaction of treatment \times delay was also observed for females, $F(3, 40) = 3.94$, $P < 0.05$. As with the males, progesterone was effective in reducing edema in females when given 1 h after injury [$t(10) = 5.26$, $P < 0.001$ compared to oil-treated], when given 6 h after injury [$t(10) = 3.35$, $P < 0.01$ compared to oil-treated], and when given 24 h after injury [$t(10) = 3.75$, $P < 0.001$ compared to oil-treated]. The means for males and females are shown in Figs. 4A and 4B, respectively.

Discussion

Progesterone treatment was effective in reducing edema in both male and female rats when given as late as 24 h post-TBI. This is in contrast to methylprednisolone, for which administration must be initiated within 8 h to be effective (3, 14). This suggests that progesterone would be useful even in those cases in which it is not possible to begin treatment immediately after cerebral trauma.

CONCLUSION

The results of these experiments can be taken to demonstrate that progesterone is an effective treatment for edema after TBI, reducing the severity of its

formation within hours of injections. Progesterone continues to be effective when given over a number of days and reduces the duration of edema perhaps by half. When combined with our observation that progesterone is effective when given in moderate, daily injections the current data support progesterone's potential as an effective treatment for TBI.

We are currently working to determine the mechanism of action by which progesterone reduces edema. Since a causal factor in vasogenic edema is the breakdown of the blood-brain barrier, we are examining the effect of progesterone injections on the integrity of the blood-brain barrier and have found preliminary evidence that progesterone treatment after injury may result in a more intact barrier. In addition, we hypothesize that progesterone may reduce membrane breakdown by interfering with free radical-induced lipid peroxidation. That oxygen radical-induced lipid peroxidation leads to blood-brain barrier breakdown has been demonstrated by Smith *et al.* (22). In addition, Smith *et al.* (22) reported attenuation of the blood-brain barrier damage with tirilazad mesylate treatment, a 21-amino steroid lipid peroxidation inhibitor. The reduction of blood-brain barrier breakdown and edema formation may lead to the sparing of neurons that would ordinarily die as a result of the injury. Such neuronal sparing after progesterone treatment has been shown to be associated with improvements in cognitive and behavioral function which are severely disrupted after cortical contusion (19).

ACKNOWLEDGMENT

The research reported in this publication was supported by the Centers for Disease Control (CDC) (R49/CCR208836). Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the CDC. Publication No. 678, Institute of Animal Behavior.

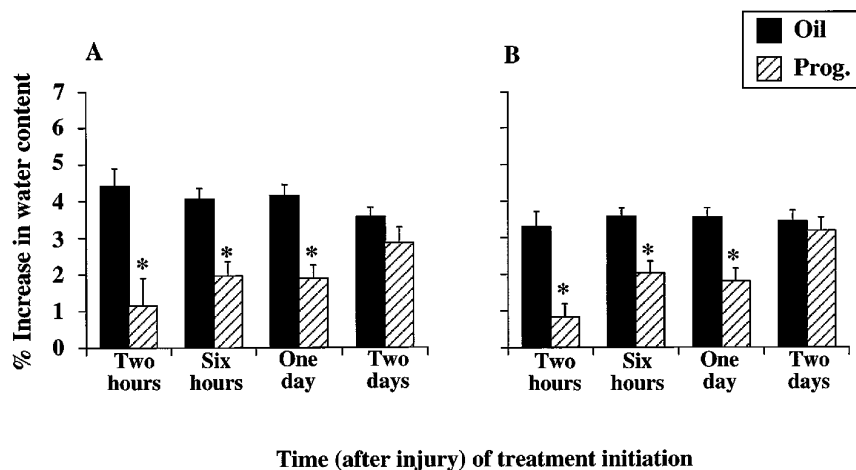


FIG. 4. Edema formation in cortically contused (A) male and (B) female rats treated with progesterone or oil when treatment was delayed. Asterisks indicate significant difference from oil-treated rats at that time point.

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