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The Effect of Burn Injury and Zinc Nutriture on Fecal Endogenous Zinc, Tissue Zinc Distribution, and T-Lymphocyte Subset Distribution Using a Murine Model[1] (42776)

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Abstract. The interrelationship among burn injury, zinc metabolism, and circulating T-lymphocyte distribution was studied using a rat model. Sixty Sprague–Dawley male rats were fed a zinc-deficient (<0.5 ppm) semipurified diet and given daily subcutaneous injections of 1 mg Zn/kg body wt for 14 days. On Day 15, 24 of the rats were subjected to a full-thickness dorsal scald injury of 30% of the total body surface. Half of the burned rats were continued on the zinc supplementation (BS) while the other half were maintained on the zinc-deficient (BD) regimen by injecting physiological saline. Feces and urine were collected for 10 days postburn and subsequently analyzed for zinc content. On Day 10 postburn all the rats were sacrificed. Zinc bound to cytosol proteins in hepatic and intestinal mucosal tissue was determined by gel column chromatography procedures and T-lymphocyte subset distributions were determined by flow cytometry. No significant difference ($P < 0.05$) in total endogenous zinc excretion was seen among treatment groups. A dramatic increase was seen in zinc bound to a 12,000 mol wt protein in hepatic tissue from the BS group only. The only significant ($P < 0.05$) change in T-lymphocyte populations was an increase in T-suppressor cells in the BD group.

Subnormal serum zinc concentrations have been shown to be part of the physiological response to burn injury in humans (1–3). Davies and Fell (4) reported that zinc is excreted in the urine of patients with 10–30% body surface burn at twice the rate observed in normal individuals; excretion rates approaching five times normal were observed in patients with more extensive burns (30–75% of body surface). It has been suggested that enteral supplementation of 10 times the recommended daily normal zinc requirement may be necessary to meet the needs of burn patients (5).

There is a paucity of literature concerning the use of animal models to study zinc metabolism after burn injury. Oh *et al.* (6) have reported the effect of various stresses, including burn injury, on accumulation of zinc bound to metallothionein in the liver and kidneys of weanling male Long–Evans rats. Cold, exercise, and the injection of CCl_4 caused a marked increase in radioactive zinc accumulation in metallothionein from liver cytosol when compared with that of nonstressed controls, while burn injury of about 1% of the total body surface caused only a slight increase over that of controls (6).

Although clinical observations in burned patients and controlled animal studies indicate that thermal injury causes disturbances in zinc metabolism, very little is known about the homeostatic mechanisms involved in the control of zinc nutriture after burn injury. *Powanda et al.* (7) have reported the effect of a burn of 30% of total body surface on redistribution of zinc in male albino rats. That study also included the complication of infection. To obviate differences in food intake caused by burning or infection, rats were fasted following burning and infection with *Pseudomonas aeruginosa.* Control-fasted rats exhibited a gradual decrease in serum zinc without increased accumulation of zinc in the liver over the 6 days of the experiment. The serum concentrations of zinc in both burn-fasted (BF) and burn-fasted-infected (BFI) rats decreased sharply 24 hr after thermal injury, while hepatic zinc

[1] The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

In conducting the research described in this paper, the investigators adhered to the Animal Welfare Act and other Federal statutes and regulations relating to animals and experiments involving animals and with the *Guide for the Care and Use of Laboratory Animals,* National Institutes of Health Publication 85-23.

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concentrations increased. During the next 5 days serum zinc concentrations decreased further in the BFI rats and remained constant in the BF rats, while hepatic levels of zinc increased with time in BFI rats and decreased in the BF group.

This report describes the results of studies of zinc metabolism using a standard rat burn model (8). Because excretion into the intestinal lumen has been shown to be a quantitatively important homeostatic control mechanism of zinc metabolism (9), the present experiment was conducted to determine the effect of a full-thickness burn of 30% of the total body surface on intestinal endogenous excretion of zinc in the burned rat. Standard gel column techniques were used to determine changes in protein bound zinc in hepatic and intestinal mucosal cytosol preparations.

We also investigated the effects of burn injury and zinc nutriture on peripheral blood T lymphocytes. There exists a considerable amount of evidence to support an interrelationship between zinc nutriture and alterations in cellular immune processes (10–13). On the basis of these reports and the well established immunological consequences of burn injury, we investigated the relationship between circulating blood T-lymphocyte subpopulations and burn and/or zinc restriction.

Methods and Materials. Sixty (n = 60) male Sprague–Dawley rats (Harlan Sprague–Dawley Inc., Houston, TX) weighing approximately 350 g were used in these studies. Rats were housed in individual stainless steel cages, given distilled deionized water *ad libitum,* and maintained on a 12-hr on, 12-hr off light schedule. The rats were fed a semipurified diet (Ziegler Inc., P.O. Box 95, Gardners, PA) designed to meet all the nutrient requirements of the adult rat except for zinc.[1] The rats were fed this zinc-deficient diet (<0.5 ppm) *ad libitum* for 2 weeks and given a daily subcutaneous injection of 1 mg Zn/kg body wt as zinc sulfate. After the 2-week equilibration period, the rats were weighed and assigned to one of the following regimens in a manner that equalized mean body weights among the treatment groups (n = 12):

Burn-sufficient (BS), administered a 30% total body surface (TBS) burn, injected with 1 mg Zn/kg body wt daily and fed *ad libitum;*

Burn-deficient (BD), administered a 30% TBS burn, injected with saline and fed *ad libitum;*

Control-sufficient (CS), injected with 1 mg Zn/kg body wt daily and fed *ad libitum*;

Control-deficient (CD), injected with saline and fed *ad libitum;*

Control pair-fed (CP), injected with 1 mg Zn/kg body wt daily, fed the amount of food eaten in the previous 24-hr period by the burn deficient (BD), weight-paired rats.

The burn injury was administered as described by Walker and Mason (14). Briefly, the animals were anesthetized with pentobarbital (1 mg/25 g body wt, ip). The hair over the dorsum was clipped, and the animals were placed in a mold which exposed 30% TBS to a full-thickness scald burn (95°C, 10 sec).

This procedure has been found to be nonfatal for rats. After recovery from the anesthesia the animals' mobility does not appear to be restricted.

Six rats from each group were placed in stainless steel metabolic cages to facilitate collection of fecal and urine excretion from the 1st through the 10th postburn day when all the rats were killed.

Fecal excreta were ashed at 600°C for 3 hr, followed by 1200°C for 8 hr. The dry ash was solubilized in concentrated nitric acid and diluted to 25 ml with distilled deionized water. Dried aliquots of liver tissue were wet ashed with an acid solution containing 21.5% perchloric acid, 7.0% sulfuric acid, and 71.5% nitric acid. After concentrating to approximately 2 ml on a hot plate, the wet-ashed samples were diluted to 25 ml with distilled deionized water. The diluted samples were aspirated directly into an atomic absorption spectrophotometer (Perkin–Elmer, Model 5000 atomic absorption spectrophotometer, Norwalk, CT) for determina-

[1] Ingredients as a percentage of diet: egg white solids, 20%; sucrose, 31%, corn starch, 31.2%; cellulose powder, 3%; vitamin premix, 0.5%; mineral premix, 4.0%; choline bitartrate, 0.3%; corn oil, 10%.

tion of zinc and copper concentration. The accuracy of the ashing procedures was confirmed using a certified reference standard (National Bureau of Standards standard reference material, bovine liver No. 1577a, Office of Standard Reference Material, Washington, DC).

Urine samples were aspirated directly into the atomic absorption spectrophotometer for zinc analysis. Plasma zinc and copper concentrations were determined as described by Butrimovitz (15).

Hepatic and intestinal mucosal cell cytoplasmic zinc binding proteins were characterized using procedures similar to those described by Richards and Cousins (16, 17). Cytosol preparations from pooled samples within treatments were fractionated on a 2.6 × 50-cm Sephadex G-75 column (Pharmacia Inc., 800 Centennial Avenue, Piscataway, NJ). Fractionation was performed at a flow rate of 0.5 ml/min and at 4°C using a buffer containing 0.9% NaCl, 0.02% sodium azide, and 10 m*M* Tris–HCl (pH 8.6).

Blood lymphocytes were isolated by gradient centrifugation with Ficoll–Paque (Pharmacia Fine Chemicals, Division of Pharmacia, Inc., Piscataway, NJ). The proportion of each T-lymphocyte subset was determined using monoclonal antibodies specific for T (W3/13), helper–inducer (W3/25), or suppressor–cytotoxic (OX/8) rat lymphocytes (Pel-Freez Biologicals P.O. Box 68, Rogers, AR). Cells were labeled with goat (Fab'_2, fragments) anti-mouse IgG fluorescein-labeled antibody (TAGO, Inc., Immunodiagnostic Reagents, P.O. Box 4463, 887 Mitten Road, Burlingame, CA). The percentage of lymphocytes positive for each monoclonal antibody was determined using a flow cytometer (Becton–Dickinson, Immunocytometry Systems, Model FACS 400, 2375 Garcia Avenue, Mountain View, CA).

A computer software program (Statistical Analysis System (SAS), SAS Institute Inc., Version 4.10, 1985, Cary, NC) was used to obtain descriptive statistics and perform one way analysis of variance. When the *F* statistic was found to be significant ($P < 0.05$), a Duncan's multiple range test (SAS User's Guide: Statistics, 1982, SAS Institute Inc., Cary, NC) was used to test for differences among treatment means.

Results. The mean weights of the rats placed in the metabolic cages from the burn day until day of sacrifice are shown in Table I. The CS group gained a mean of +10 g over the 10-day period compared to +6, +1, −2, and +1 g in the CD, CP, BS, and BD treatment groups respectively.

Food intake of the BS and BD groups decreased on Days 1 and 2 postburn; however, after Day 3, food intake for both burn groups was comparable to or greater than those of the CS and CD groups for the remainder of the experimental period.

The total obligatory losses of zinc by both fecal and urinary routes of excretion showed no significance ($P < 0.05$) difference due to burn injury when the burned groups were

TABLE I. BODY WEIGHT AND ZINC EXCRETION DATA[a]

Treatment[b]	Start body weight (g)	End body weight (g)	Body weight change (g)	Fecal zinc (μg)	Urine zinc (μg)	Fecal + urine zinc (μg)
BS ($n = 6$)	350 (6)	348 (6)[d]	−2 (3)[d]	2683 (37)[e]	93 (11)[e]	2683 (37)[e]
BD ($n = 6$)	349 (8)	349 (8)[d]	+1 (2)[d]	908 (75)	22 (2)	930 (76)
CS ($n = 6$)	352 (6)	362 (4)[c]	+10 (5)[c]	2892 (71)[e]	90 (13)[e]	2982 (61)[e]
CD ($n = 6$)	353 (12)	358 (10)	+6 (3)	896 (96)	33 (4)	929 (94)
CP ($n = 6$)	351 (9)	352 (9)[d]	+1 (3)[d]	2822 (79)[e]	94 (11)[e]	2916 (72)[e]

[a] Results are expressed as means ± SEM.

[b] Treatment groups: BS, burned, zinc-supplemented, *ad libitum* fed; BD, burned, not zinc-supplemented, *ad libitum* fed; CS, not burned, zinc-supplemented, *ad libitum* fed; CD, not burned, not zinc-supplemented, *ad libitum* fed; CP, not burned, zinc-supplemented, pair-fed to BD group.

[c] Significantly different from BS, BD, and CP groups at $P < 0.05$.

[d] Significantly different from CS group at $P < 0.05$.

[e] Significantly different from BD and CD groups at $P < 0.05$.

TABLE II. PLASMA AND LIVER ZINC AND COPPER CONCENTRATION[a]

Treatment[b]	Plasma zinc (μg/dl)	Plasma copper (μg/dl)	Liver zinc (μg/g)	Liver copper (μg/g)
BS (n = 12)	137 (7)[c]	132 (7)[d]	162 (11)[g]	17 (1)
BD (n = 12)	68 (5)	127 (5)[d]	89 (8)[h]	17 (2)
CS (n = 12)	143 (6)[c]	99 (5)[e]	135 (7)[e]	16 (2)
CD (n = 12)	54 (3)	86 (4)[f]	99 (9)[h]	14 (1)
CP (n = 12)	146 (4)[c]	104 (4)[e]	130 (8)[e]	13 (2)

[a] Results are expressed as means ± SEM.

[b] Treatment groups: BS, burned, zinc-supplemented, *ad libitum* fed; BD, burned, not zinc-supplemented, *ad libitum* fed; CS, not burned, zinc-supplemented, *ad libitum* fed; CD, not burned, not zinc-supplemented, *ad libitum* fed; CP, not burned, zinc-supplemented, pair-fed to BD group.

[c] Significantly different from BD and CD groups at $P < 0.05$.

[d] Significantly different from CS, CD, and CP groups at $P < 0.05$.

[e] Significantly different from BS, BD, and CD groups at $P < 0.05$.

[f] Significantly different from BS, BD, and CP groups at $P < 0.05$.

[g] Significantly different from BD, CS, CD, and CP groups at $P < 0.05$.

[h] Significantly different from BS, CS, and CP groups at $P < 0.05$.

compared to their respective nonburn control groups (Table I). The pair feeding regimen did not cause a significant difference in zinc loss when compared to that of the zinc sufficient nonburn control group.

The zinc restriction regimen significantly ($P < 0.05$) lowered plasma zinc concentration in both the BD and the CD groups, compared to that of the supplemented groups (Table II). Plasma copper concentration was significantly elevated in the BS and BD groups when compared to that of the three nonburn control groups. Hepatic zinc concentration was significantly ($P < 0.05$) increased in the burn-injured rats that were supplemented with zinc. No significant differences were found between the BD and the CD treatment groups.

Fractionations of cytosol preparations from the BS and CS groups are shown for hepatic tissue in Fig. 1 and for intestinal mucosal tissue in Fig. 2. Burn injury caused a sharp increase in zinc bound to a 12,000 mol wt protein in the hepatic cytosol of rats supplemented with zinc when compared to that of the control rats. Elution profiles of the burned rats maintained on the zinc-deficient regimen were similar to the control groups. There was no increase in zinc bound to low-molecular-weight proteins in intestinal mucosal cytosol in any of the treatment groups; however, there was an increase in zinc bound to a protein that eluted in the void volume in the burned rats that were supplemented with zinc.

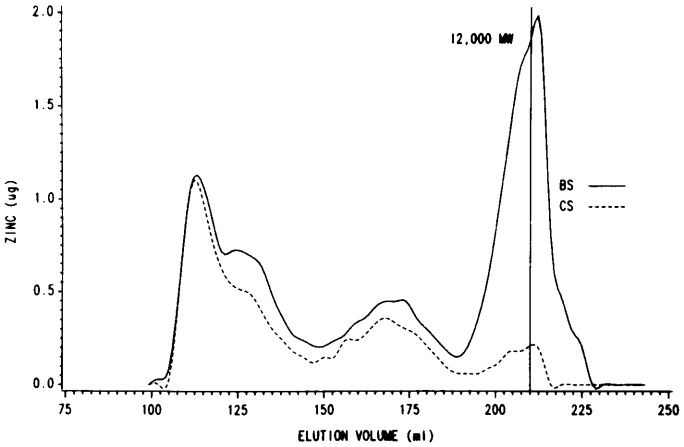

FIG. 1. Zinc bound to hepatic cell cytosol proteins separated by gel column chromatography (2.6 × 50 cm column packed with Sephadex G-75 at a flow rate of 0.5 ml/min at 4°C) for the 30% burned, zinc-supplemented, *ad libitum* fed rats (BS) and the nonburned, zinc-supplemented, *ad libitum* fed rats (CS).

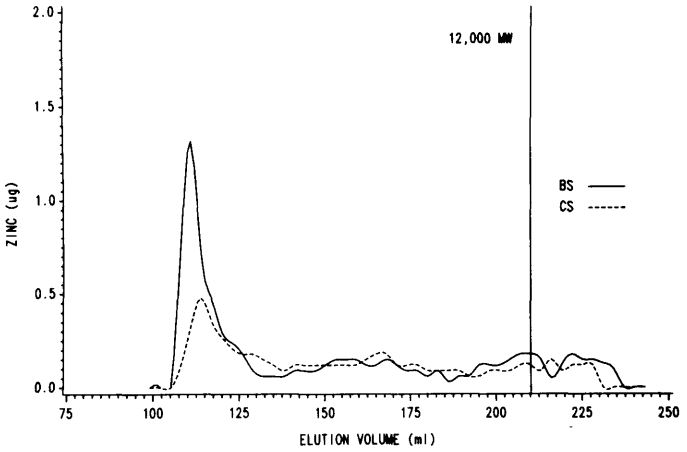

FIG. 2. Zinc bound to intestinal mucosal cell cytosol proteins separated by gel column chromatography (2.6 × 50 cm column packed with Sephadex G-75 at a flow rate of 0.5 ml/min at 4°C) for the 30% burned, zinc-supplemented, *ad libitum* fed rats (BS) and the nonburned, zinc supplemented, *ad libitum* fed rats (CS).

The peripheral leukocyte blood cell data are shown in Table III. Burn injury caused a significant leukocytosis, irrespective of zinc supplementation. Flow cytometry (Table III) revealed no significant differences among the treatment groups in the percentages of T cells or T-helper cells. There was a significant ($P < 0.05$) increase in T-suppressor cells in the BD group over the other four treatment groups. This was true whether the data were expressed as percentage positive cells or as cells per milliliter of whole blood.

Discussion. Previous studies have shown that a 30% total body surface burn caused sequestration of zinc in hepatic tissue (7), even during deficient zinc intake. To create the zinc deficiency in those studies the rats were subjected to a fasting regimen during the postburn injury period, making it difficult to separate the effect of total malnutrition from burn injury. Our results show that rats allowed to maintain nutrient intake and given adequate zinc supplementation sequester zinc in hepatic tissue subsequent to burn injury, while zinc deficient but otherwise well-nourished rats had significantly lower levels of hepatic zinc concentration when compared with those of nonburned *ad*

TABLE III. PERIPHERAL BLOOD CELL DATA[a]

Treatment[b]	WBC (cells/ml)	% Lymphocytes	% T-cell	% T-helper	% T-suppressor	Absolute t cell (cells/ml)	Absolute t helper (cells/ml)	Absolute t suppressor (cells/ml)
BS $n = 12$	6100 (472)[c]	68 (2)[d]	66 (2)	44 (2)	22 (2)	2766 (279)	1845 (199)	948 (171)
BD $n = 12$	6035 (385)[c]	71 (3)[d]	66 (2)	40 (1)	32 (1)[f]	2896 (310)	1728 (152)	1430 (177)[f]
CS $n = 12$	4255 (293)	75 (3)	70 (2)	45 (2)	21 (1)	2298 (224)	1423 (157)	696 (88)
CD $n = 12$	3778 (332)	82 (3)[e]	67 (4)	43 (3)	22 (2)	2219 (312)	1424 (213)	723 (111)
CP $n = 12$	4625 (490)	80 (2)[e]	73 (1)	46 (1)	25 (1)	2789 (397)	1689 (223)	913 (129)

[a] Results are expressed as means ± SEM.

[b] Treatment groups: BS, burned, zinc-supplemented, *ad libitum* fed; BD, burned, not zinc-supplemented, *ad libitum* fed; CS, not burned, zinc-supplemented, *ad libitum* fed; CD, not burned, not zinc-supplemented, *ad libitum* fed; CP, not burned, zinc-supplemented, pair-fed to BD group.

[c] Significantly different from CS, CD, and CP groups at $P < 0.05$.

[d] Significantly different from CD and CP groups at $P < 0.05$.

[e] Significantly different from BS and BD groups at $P < 0.05$.

[f] Significantly different from BS, CS, CD, and CP groups at $P < 0.05$.

libitum or pair-fed zinc sufficient control rats.

In order to further characterize the changes that occur in zinc metabolism during recovery from a burn injury, we analyzed the protein bound zinc in mucosal intestinal and hepatic cytosol, using gel column chromatography. Other studies (18) using similar procedures suggest an important role of metallothionein, a low-molecular-weight protein, in the homeostatic control of zinc metabolism. This protein elutes at the 12,000 mol wt range and is synthesized in both liver and intestinal tissue (19). Most such research has used high levels of zinc or cadmium intake to induce metallothionein synthesis in experimental animal models. Other reports have described the effects of bacterial lipopolysaccharides (20) or hypersensitivity (21) on metallothionein induction, however, relatively little research has been reported on the role of this protein in zinc metabolism during disease or after trauma.

On the basis of the fractionation data from the rats maintained on a sufficient zinc regimen, a 30% total body surface burn caused an increased binding of zinc to a protein of approximately 12,000 mol wt in liver cytosol tissue. This phenomenon was not seen in burned, zinc-deficient rats. The burn injury in the zinc-deficient rats caused neither an increase in hepatic zinc concentration nor a redistribution in protein bound zinc.

It is important to note that the pair-feeding regimen did not cause an increase in hepatic zinc concentration or in zinc bound to low-molecular-weight proteins. Food restriction alone has been shown to induce metallothionein zinc binding (22). This may account for the discrepancy between earlier studies (7) and the present results. It is possible that the combination of burn injury and the fasting state in the earlier studies induced metallothionein synthesis and increased zinc binding in hepatic tissue.

It should be pointed out that our experiments only measured the amount of zinc bound to proteins eluted at varying molecular weights by gel column chromatography. It would be of interest to use a radioactive-labeled amino acid pulse concurrent with radioactive zinc experiments to determine simultaneous changes in protein synthesis and zinc binding in burned/zinc-deficient animals.

The metabolic mechanisms causing an increase in zinc bound to low-molecular-weight protein in hepatic tissue in the burned/zinc-sufficient rats failed to induce similar effects in intestinal cytosol. Cousins (18) has proposed a role for intestinal metallothionein in the excretion of zinc that is in excess of metabolic requirements. Intestinal metallothionein is induced in response to zinc loading, binds excess zinc, and accumulates in the mucosal cells. Subsequently, the zinc bound to metallothionein is lost when cells are sloughed into the lumen, thereby increasing fecal endogenous zinc excretion. Consistent with this suggested role of intestinal metallothionein, it could be hypothesized that the lack of an increase in metallothionein binding of zinc in the intestinal cytosol of the burned rats would ensure unobstructed zinc absorption and decrease obligatory loss of fecal zinc. Our results support this hypothesis in that total endogenous fecal zinc excretion for the 10 days postburn did not differ significantly between the burned rats and their respective control group.

Along with defining changes in the zinc metabolism following burn injury, we also investigated the effects of an interaction between zinc nutriture and burn injury on peripheral blood T lymphocytes. Based on monoclonal antibody labeling and flow cytometry analysis, zinc restriction during recovery from burn injury caused a significant increase in T-suppressor lymphocytes. This phenomenon was not seen in the burned/zinc-sufficient, nonburned/zinc-deficient, or pair-fed zinc-sufficient rats.

Increased-suppressor cells and suppressor-cell activity has been described in a number of clinical studies of burns and other trauma (23–25). It is difficult to assess these studies for possible nutritional effects on circulating lymphocytes because dietary intake and vitamin and mineral supplementation were not controlled. Some recent animal studies suggest increased suppressor activity due to burn injury (26–28), but again, nutritional support was not described.

Although impairment of immunological function in zinc-deficient animals has been well documented, there has been little defin-

itive evidence at the molecular level to indicate that zinc is directly involved. Recently it has been shown that the biological activity of thymulin, a nonapeptide hormone produced by thymic epithelial cells, is dependent on the presence of zinc in the molecule (13), and this finding may, in part, explain the relationship.

In this model, (30% total body surface, nonlethal scald burns) sufficient and deficient zinc intake, did not place the rats in additional zinc deficit, relative to the appropriate controls, when obligatory fecal and urinary zinc losses and circulating plasma zinc concentrations were used as assessment criteria. Burn injury caused an increase in total liver zinc concentration and zinc bound to liver cytosol protein of approximately 12,000 mol wt in rats maintained on an adequate zinc regimen but not in rats maintained on a zinc-deficient regimen. However, a significant ($P < 0.05$) increase in circulating blood T-suppressor lymphocytes occurred in burned animals maintained on a zinc-deficient regimen during a 10-day period postburn. Although this suggests an interrelationship among immunocompetence, burn injury, and zinc nutriture, it remains to be shown how these changes relate to infection or mortality associated with burn injuries. Future experiments will use functional tests of the immune system in order to relate the observed morphological changes to immune function.

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