Studies in Burns:

XIV, Healing in Burn Wounds Treated With Ethyl Linoleate Alone Or in Combination With Selected Topical Antibacterial Agents

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Studies of the efficacy, in terms of burned wound healing, of a mixture of Ethyl Linoleate (ethyl 9-12 (cis, cis) octadecadienoate) with α-l-histidine, α-tocopherol, and TBHQ (hELate) was undertaken in 12 swine. The species was selected so as to study an animal with skin anatomy similar to the human. Statistically significantly greater healing occurred in 730 C/7sec contact burns (20% BSA) treated with hELate than in untreated burns in pigs. Further, there was no contracture noted in the hELate treated lesions, while marked contracture occurred in the untreated burns. Additionally, we noted that there was a proportional increase in weight gain amongst swine studied as their burn lesions epithelialized. In order to evaluate the compatibility of hELate with selected, currently-used topical antibacterial agents, 154 rabbits with 20% 730 C/7 sec contact burns were studied. The lipid was applied (0.01 ml/cm² burn) at 1 hour postburning; the topical agent was applied at 2 hours post-burn and every 24-hours. All animals were washed once daily. hELate was applied only once. We found no statistical difference in the number of subjects healed or in the mortality between animals treated with hELate alone and those treated with the agent plus Gentamycin® cream, Neosporin® cream, and silver sulfadiazine 1% in Unibase USP (compounded at Medical College of Georgia specifically and only for this study.) We suggest that Ethyl Linoleate agent (hELate) may be used safely in combination with selected antibacterial substances. Further, these selected combinations seem to be non-toxic and appear to allow the calorie-saving and healing effects of the lipid to proceed unimpeded.

EARLIER, we demonstrated that the naturally-occurring cutaneous lipid, Ethyl Linoleate (ethyl,9-12(cis, cis) octadecadienoate) possessed certain unique properties *vis á vis* burned surfaces. When applied in a single topical dose comprising not more than 0.01 ml/cm² burned surface, a highly statistically significant normalization occurred in metabolic heat production. Simultaneously a proportional diminishing and normalization occurred in evaporative water loss from the same surface. Furthermore, re-epithelialization, often with re-growth of hair, occurred over the majority of the treated area in the majority of the subjects treated.[4,5]

In order to establish that the material would be efficacious on a species with skin similar to human, we evaluated the lipid on contact burns in swine.

Additionally, since it was the ultimate aim of our studies to develop a material that could be used in the treatment of human burn injury (and since Ethyl Linoleate is not, in itself, an antibacterial substance), it was necessary to evaluate the compatibility of Ethyl Linoleate with the currently-used topical antibacterial agents. This study of healing and toxicity was conducted in burned rabbits.

Materials and Methods

All experimental burns were contact lesions created by the 7 second application at 83.51±7.38 gm pressure of a pyrex beaker heated to its annealing temperature, 730 C, over a pre-measured 20% of the body surface. The same technician who has been performing this procedure for 10 years in our laboratories created these burn lesions. The Ethyl Linoleate used comprised the 75% pure material to which was added 0.1 mM α-l-histidine, Tertiary Butyl

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Hydroquinone (TBHQ) 5 ppm, and α-tocopherol 5% (w/w). The latter materials have been previously defined as effective antioxidants which prolong the efficacy of the lipid.[3,5] We have named this mixture of Ethyl Linoleate and antioxidants "hELate"™.

The lipid was applied to the burned surface of the animal at one hour after burning. This was the *only* application of hELate. In the swine, 0.16±0.06 gm/kg were used (comprising $1.45\times10^{-3}\pm0.53\times10^{-4}$ ml/cm²). In the rabbit, a standard dose of 6.68×10^{-3} mg/cm² (0.01 ml/cm²) was applied.

Swine

Twelve Landrace breed swine weighing 19.05±4.70 kg were used in the study. The animals had been clipped, bathed with hexachlorophene-based cleanser, and wormed prior to use. Anîmals were housed in separate stalls in an outdoor Vivarium, protected only partially from the elements by a roof but no walls. They were fed standard hog feed, and, except for the burning itself were studied unanesthetized. Under pentobarbital anesthesia, a 10% burn was inflicted on each flank of each pig. The total burn was 20%. At random, the right or left side was painted with hELate as described. The animals were allowed to waken and were returned to their cages. There was no control of activity, feeding habits, or ambient thermal gradients over the 35-day duration of the study. Animals were weighed at the beginning of the study and at days 7, 11, 14, 21, and 35. They were observed daily with specific regard to the quality of the wounds and evidences of healing. From photographs taken daily, and using a standard diagram, the area re-epithelized was assessed daily.

Rabbits

One hundred fifty-four New Zealand male albino rabbits were employed in the study of the effects of hELate used with various other topical agents. Using techniques previously described, a 20% burn lesion was applied to the flanks of each rabbit. Animals were divided into 14 groups. One group, "controls." received as their only therapy a daily bath in Betadine Whirlpool Concentration® (1:8 H_2O). *All* animals, in fact, were washed daily using the solution indicated.

hELate was used as the only therapy in one group, and was used in combination with Betadine ointment®, Garramycin cream®, Neosporin cream®, Silvadene cream®, (MCG) silver sulfadiazine cream, Sulfamylon ointment® in one group each. These topical preparations were used alone (without hELate) in one group each (Table 3). The (MCG) silver sulfadiazine was compounded at the Medical College of Georgia only for this study and was made up into a 1% w/w preparation using Unibase USP as the vehicle.

TABLE 1. *Percent Healing (X±1SEM) With and Without hELate in 730 C/7 sec Contact Burns in Swine*

Day	% Healed: hELate Treated Side (N=12)**	% Healed: Non-Treated Side (N=12)
14	17.17±5.28[a]	2.92±1.74
21	30.00±5.96[b]	8.08±4.56
35	89.25±3.78[c]	81.42±4.41†
14	7 of 12 healed > 3% (28.71±4.89)	9/12 healed 0-1%
21	9 of 12 healed > 9% (39.44±4.52)	7/12 healed 0-1%
35	10 of 12 healed > 81% (94.60±1.33) With no contracture	7/12 healed > 81% ($\bar{X}$ and SEM not valid since all had severe contracture)

*hELate is a mixture of 75% Ethyl Linoleate with α-tocopherol, α-l-histidine and TBHQ. Burns are 7 sec, 730 C 20% BSA contact lesions.

**Treatment comprised one application of 0.16±0.06 gm/kg to the "treated" side at 1 hour post-burn. This was the only treatment. Area treated was 713.99±176.04 cm². Control, untreated area was the contralateral side, and was the same size as the treated area.

†8 of 12 had marked contracture.

With the exception of hELate, all other topical agents were applied daily in the manner recommended by the manufacturer and were washed off daily. The application

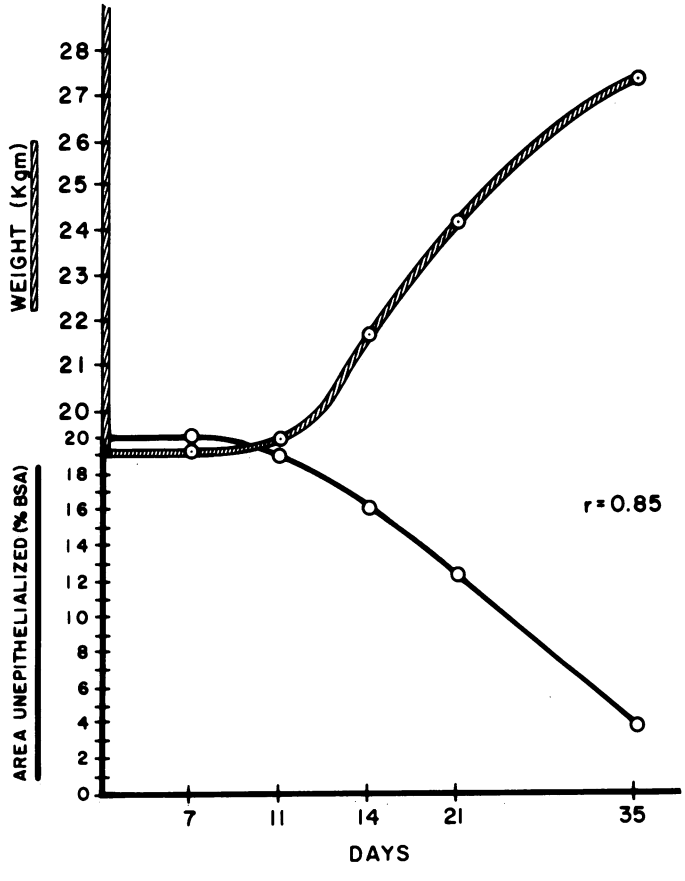

FIG. 1.

TABLE 2. *Lesion Size and Weight Gain Over 35 Days in Burned Swine with 20% Burn, Half of Which Was hĒLate Treated ($\bar{X}$±1SEM)**

	Days 0	7	11	14	21	35
kg Wt	19.05±0.73	19.17±0.86	19.23±0.90	21.63±0.81	23.94±1.29	27.39±0.81
Total Lesion size, T**	20%	20%	19.18±0.50%	15.98±1.25%	12.38±1.78%	2.93±0.73%

*r = 0.85
**T = [20-(0.2xΣ% healed both sides)].

of hĒLate was at—and *only* at—1 hour post-burning. The topical agent was applied at two hours post-burn, and each 24 hours 7 days per week for 21 days. Changes in the wound surface were documented photographically. At death or at 21 days, wounds were debrided and an estimate of healing area was made directly and from the photographs. All animals that died during the period of the study were subjected to necropsy.

Results

Swine

The results of the swine study are summarized in Tables 1 and 2 and Fig. 1. There were no deaths and no manifestations of cutaneous or systemic toxicity in any subject. Those lesions treated with hĒLate demonstrated marked and statistically significantly greater healing over the course of the study than did the non-treated lesions. By day 21, 75% of the treated wounds had healed about 39% of the area burned. At the same time, 58% of the untreated wounds had essentially no manifestion of healing. By the 35th post-burn, post-treatment day, 83% of the treated lesions had healed about 95% of the wound with hair-bearing, non-contracted epithelium. Simultaneously, the untreated areas were severely contracted.

There was little manifestation of healing until the 10-12th day post-burn. Thereafter, healing proceeded in an almost linear fashion (Table 2). Weight gain did not begin to be manifest until the 10-12th day post-burn. Thereafter, it proceeded rapidly (Table 2). There is a significant correlation (r=0.85) between weight gain and total lesion size (Fig. 1).

Rabbits

The results of the rabbit studies are summarized at Tables 3 and 4. In addition, at Table 4 is a X^2 analysis of

TABLE 3. *Healing and Mortality in Rabbits With 20% BSA Burns Treated With hĒLate and Various Other Topical Antibacterial Agents*

Modality	No. Subjects	*Healing* greater than control, untreated (observed at 21 days postburn)	Died	% Healing ($\bar{X}$±SEM)
Control (no Rx) daily wash	21	4	6	15.7±13.8
hĒLate (h) alone daily wash	55	38	0	76.4±7.7
Betadine® ointment alone and wash	5	1	5	30
Betadine® + h + wash	6	1	4	45
Garramycin® (Gentamycin) cream alone + wash	6	3	0	24.3±3.5
Garramycin® + h + wash	6	2	0	60.0±25
Neosporin® cream alone + wash	6	2	1	32±8
Neosporin® + h + wash	6	4	0	29.5±6.1
Silvadene®* alone + wash	10	3	4	70.0±10
Silvadene® + h + wash	9	0	3	—
Silver Sulfadiazine** alone + wash	6	3	1	66.7±9.7
Silver Sulfadiazine + h + wash	6	5	0	49.0±3.7
Sulfamylon® + wash	6	0	3	—
Sulfamylon® + h + wash	6	1	5	45

*Patented, Marion Laboratories, Kansas City, Mo.
**Compounded at the Medical College of Georgia for this study only. Base is Unibase.
Betadine ointment®: Purdue-Frederick Co., Norwalk, Conn.
Garramycin cream®: Schering Corp., Kenilworth, N.J.
Neosporin cream®: Burroughs-Wellcome & Co., Research Triangle Park, N.C.
Sulfamylon Ointment®: Winthrop Laboratories, New York, N.Y.

TABLE 4. *Summary of Data in Table 3, and X^2 Analysis of the Summarized Data*

	No. Subjects	Healed	Died
A. No Treatment	21	4	6
B. hELate-treated, no other Rx	55	38	0
C. Topical antibacterial Rx Rx alone (no hELate)	39	12	15
D. hELate + Topical* antibacterial Rx	39	13	13

X^2 analysis (df= 1)

		B	C	D
Healing	A	15.39	0.96	1.37
	B		13.46	11.76
	C			0.06

at df= 1,
p>0.05 0.01
X^2= 3.841 6.635

		B	C	D
Mortality	A	17.06	0.59	0.14
	B		25.17	21.28
	C			0.22

*There is *no* statistical difference in number of subjects healed or in mortality between hELate alone and hELate plus Garramycin cream, Neosporin cream and MCG silver sulfadiazine.

these data. Overall, hELate alone produced highly statistically significant healing and was associated with a highly statistically significantly lower mortality (in fact, no deaths in the hELate-alone group) than any of the other modalities examined. There were no deaths associated with Garramycin® alone or with hELate; Neosporin® cream with hELate; or (MCG) silver sulfadiazine with hELate. Indeed, there is no statistical difference in the number of subjects healed or in the mortality between animals treated with hELate alone and those treated with hELate plus Garramycin®, Neosporin® or (MCG) silver sulfadiazine.

The 24 animals that died during therapy with Betadine ointment®, Silvadene Cream® and Sulfamylon ointment® had severe pneumonitis as the chief terminal event. In addition, the animals that died following treatment with Silvadene or Sulfamylon and hELate demonstrated focal hepatic necrosis and renal cortical (proximal tubular) necrosis.

The subjects treated with hELate plus Betadine ointment, Silvadene, and Sulfamylon appeared ill from about day 3 onward. These animals lost hair from the periorbital areas, lost marked amounts of weight, and were hypersensitive to handling—which apparently produced considerable cutaneous discomfort.

Discussion

It would seem that, under the most adverse conditions and in the most uncontrolled environmental situation, Ethyl Linoleate exerts a salubrious effect on the healing of burn lesions in a skin similar to man's. Even in this highly challenging situation, 75% of the treated lesions in swine healed almost 40% with the re-epithelialization accompanied by some hair re-growth. Simultaneously inflicted, untreated burns on the contralateral sides of these animals showed markedly less propensity to regrowth/epithelium. Sixty-seven per cent of the untreated sides ultimately healed, but in contrast with the treated sides, exhibited marked contracture. In swine, as in our previous studies in rabbits, the Ethyl Linoleate (hELate) mixture was applied in small dose only one time post-burn. The agent was the only material used at any time on the pigs' surfaces.

Interestingly enough, in pigs there was a significant correlation (r=0.85) between weight gain and the area re-epithelized (Table 2). Little or no healing was noted through the 11th post-burn/post-treatment day; and through that time, virtually no weight gain had occurred. Subsequently, asymptotic healing and weight gain was noted. The burns were clearly partial thickness by definition, since they healed. It is interesting to speculate on the relationship between healing rate and rate of weight gain. Previously we have deomonstrated that, in a highly controlled environment in a metabolic chamber, there *is* a significant relationship between evaporative water loss and metabolic heat production in rabbits.[4,5] Perhaps, therefore, it is not unreasonable to postulate that in burned swine the same relationship exists.

Zawacki[6] has shown that in partial thickness lesions in guinea pigs, control of the evaporative water loss by the use of xenograft results in re-epithelialization and in protection from weight loss. The mechanism of action of

xenograft may be to normalize evaporative water loss—and thereby metabolic heat production. It is, therefore, not unreasonable to speculate that Ethyl Linoleate functions in a similar manner with respect to swine and rabbits.

No toxicity was noted clinically or at autopsy in the hELate treated swine. Admittedly, a study of more swine is necessary for a full-blown clinical toxicological study, but the implication is great that in this species, as in rabbits, there is not discernible toxicity.

Clearly, in order to undertake a clinical study of the effects of hELate (in terms of reduced calorie demand and, perhaps, burn wound healing,) on the burned human it is insufficient to have shown that hELate used alone is non-toxic. hELate is not an antibacterial substance.[4] Furthermore, the lipid is applied, for therapeutic purposes, only once, and then in small dose. Since infection of the burn wound is an ever-continuing threat until such time as the wound is "closed," it is presumably essential that some acceptable topical antibacterial agent be used concurrently. Our data indicate that three agents are efficacious when used conjointly with hELate and in the manner commonly employed for use of those topical agents. These agents comprise Garramycin® (Gentamycin) cream, Neosporin® cream, and "homemade" silver sulfadiazine in Unibase, USP. There is no statistical difference in the number of burned wounds healed or in the mortality observed in those animals treated with hELate alone and those treated with hELate plus the topical agents cited (Tables 3 and 4).

There are several possible reasons for the ineffectiveness and/or toxicities observed when the hELate-treated subject was concomitantly exposed to Betadine® ointment, Silvadene® or Sulfamylon®. First, it is possible that the lipid obstructed the topical agent's ability to "get at" organisms beneath the surface of the eschar. We have no information that would confirm or deny this possibility. We suspect, by inferred comparison to Garramycin and MCG silver sulfadiazine, that this is not the mechanism: subjects treated with these agents exhibited virtually no toxicity and did, in fact, heal. Since these two agents have some propensity for attacking organisms deep to the surface of the eschar, it is our speculation that the other agents (with the exception of Betadine ointment) had the same opportunity.

It is likely that some interaction occurred between those agents which were ineffective and/or frankly toxic and the hELate itself. The mode of death in those 13 animals that died during exposure to Silvadene and Sulfamylon was grossly similar to the deaths we previously observed in mice challenged with a daily dose of Ethyl Linoleate. There was focal hepatic necrosis and necrosis of the proximal renal tubules in these rabbits, and in addition, there was marked interstitial pneumonitis. The deaths that occurred in animals treated with Betadine ointment appeared to be the result of gross contamination of the burned wound and concomitant sepsis. We therefore speculated that the sulfonamide radical interacted in an untoward manner with one or more of the components of the hELate mixture. It was our thought, originally, that the interaction occurred between the topical agent and the Ethyl Linoleate itself since the modality of death was so parallel to the hydroperoxide/expoxide mortality observed previously[4,5] and described by others.[1,2]

To test the latter hypothesis, and to test the further hypothesis that the responsible toxic inductor was a component of the vehicle of the Sulfamylon and Silvadene agents, we compounded our own silver sulfadiazine by intermixing equimolar quantities of sodium sulfadiazine and silver nitrate. The resultant material was made up into a 1% cream using Unibase USP as the vehicle. This material performed virtually identically to the hELate alone. It produced no deaths—lipid-like, or otherwise—and was associated with healing in 80% of the animals treated.

For a number of reasons, we suspect strongly that the toxic interaction can be traced to one or more materials present in the vehicle into which Sulfamylon and Silvadene are compounded. Studies are underway directed toward defining this hypothesis further. Our current studies indicate, however, that hELate may be used concomitantly with Neosporin® and/or Gentamycin® cream among the commercially-available topical antibacterial agents.

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References

1. Cortesi, R. and Privett, O. S.: Toxicity of the Fatty Ozonides and Peroxides. Lipids, 7:715-721, 1973.
2. Gamage, P. T., Mori, T. and Matsushita, S.: Effects of Linoleic Acid Hydroperoxides and Their Secondary Products on the Growth of *Eschericia Coli*. Agr. Biol. Chem., 35:33-39, 1971.
3. Jelenko, C., III and Wheeler, M. L.: Effects of Antoxidants on the Long-Term Utility of Ethyl Linoleate as a Water-Holding Lipid for Topical Use Postburn. J. Surg. Res., 12:161-163, 1972.
4. Jelenko, C., III, Wheeler, M. L., et al: Topical Lipid Protection of Burned Subjects and Their Wounds. Surgery, 75:892-898, 1974.
5. Jelenko, C., III, Wheeler, M. L., et al.: Studies in Burns; XIII: Effects of a Topical Lipid on Burned Subjects and Their Wounds. Am. Surg., In press.
6. Zawacki, B. E.: The Natural History of Reversible Burn Injury. Surg. Gynecol. Obstet., 139:867-872, 1974.