HLA-A1 and B8, and in one of the studies (7) the A1, B8 haplotype was represented in 8 out of 13 cases with B8. In a study on 13 cases of drug-associated CAH, there was an increase in HLA-A1 and B8 (4). In alcoholic cirrhosis an insignificant excess of HLA-B8 has been reported (5). Other associations of HLA antigens have been found in other types of liver disease. Typing of D-locus antigens in chronic active liver disease has shown a significant increase in DW3 (5) and this association may be of greater importance than the findings within the A and B loci. As mentioned, we have not typed for D locus. Our studies nevertheless support the idea of a genetic predisposition for methotrexate-induced liver cirrhosis and suggest a common factor for this multifactorial condition with the above-mentioned liver disease.

The finding of the A1 and B8 should—just as with other predisposing factors—lead to extra caution in liver control in a psoriatic undergoing methotrexate treatment and, if found prior to treatment, be included in the relative contra-indications to this drug.

REFERENCES

1. Coe, R.: Cirrhosis associated with methotrexate treatment of psoriasis. JAMA *206:* 1515, 1968.
2. Epstein, E. & Craft, S.: Cirrhosis following methotrexate administration for psoriasis. Arch Dermatol *100:* 531, 1969.
3. Kissmeyer-Nielsen, F. & Kjerbye, K.: *In* Histocompatibility Testings (ed. E. Curtoni, R. Mattiuz & R. Togi), pp. 381–383. Munksgaard, Copenhagen, 1967.
4. Lindberg, J., Lindholm, A., Lundin, P. & Iwarsson, S.: Trigger factors and HL-A antigens in chronic active hepatitis. Br Med J *iv:* 77, 1975.
5. Mackay, I.: HLA and liver disease. *In* HLA and Disease (ed. J. Dausset & A. Svejgaard). Munksgaard, Copenhagen, 1977.
6. Nyfors, A.: Liver biopsies from psoriatics related to methotrexate therapy. Acta Pathol Microbiol Scand *85:* 511, 1977.
7. Page, A., Sharp, H., Greenberg, L. & Yunis, E.: Genetic analysis of patients with chronic active hepatitis. J Clin Invest *56:* 530, 1975.
8. Svejgaard, A., Staub Nielsen, L., Svejgaard, E., Kissmeyer-Nielsen, F., Hjortshøj, A. & Zachariae, H.: HL-A in psoriasis vulgaris and in pustular psoriasis—population and family studies. Br J Dermatol *91:* 145, 1974.
9. Weinstein, G., Roenigk, H., Maibach, H., Cosmides, J., Halprin, K., Millard, M., Almeyda, J., Averbach, R., Bergfeld, W., Clyde, D., Dahl, M., Frost, P., Krueger, R., Lee, J., Lundquist, A., Schaefner, P., Scheuer, P. & Zachariae, H.: Psoriasis–liver–methotrexate interactions. Arch Dermatol *108:* 36, 1973.
10. Zachariae, H., Grunnet, E. & Søgaard, H.: Liver biopsy in methotrexate treated psoriatics—a re-evaluation. Acta Dermatovener (Stockholm) *55:* 291, 1975.
11. Zachariae, H., Kragballe, K. & Søgaard, H.: Methotrexate induced liver cirrhosis—studies including serial liver biopsies during continued treatment. Br J Dermatol *101:* in press, 1980.

The Effect of Zinc on the Sebum Secretion Rate

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Abstract. In accordance with a randomized double-blind experimental design, capsules of zinc sulfate, 440 mg total daily dose, and lactose placebo were administered orally to 10 normal Caucasian males for 3 weeks. Sebum secretion rates and serum zinc levels were determined prior to and following treatment. There was a statistically significant change in the before and after mean sebum secretion rates of the zinc group when compared with those of the placebo group ($p<0.028$). The results of this preliminary study indicate that supplemental zinc sulfate may reduce the quantity of skin-surface sebum. Further investigation is warranted.

Key words: Zinc; Sebum Secretion Rate

The essential trace metal zinc has been linked to numerous biological processes in man. It plays a role in at least twenty enzyme systems (7), helps to stabilize macromolecules and biological membranes and influences the phagocytic activity of macrophages (3).

There are conflicting reports in the literature regarding the value of oral zinc therapy in acne vulgaris. A recent report concluded that zinc "has no early clinical effect on male patients with moderate acne". (6) In contrast, Michaëlsson et al. (5) and Hillström et al. (4) suggest that acne improves significantly following zinc sulfate therapy. Interestingly, Michaëlsson noted that the skin of some patients who received zinc sulfate became dryer and

Table I. *Individual sebum secretion rates (mg/10 cm²/3 h) and serum zinc levels (μg/ml) before and after treatment*

Group	Subject no.	Before				After			
		1st	2nd	3rd	Serum zinc level	1st	2nd	2rd	Serum zinc level
Placebo	1	2.50	2.78	2.76	0.58	2.90	3.09	2.30	0.54
	2	3.21	2.19	2.25	1.40	2.36	2.02	3.01	0.48
	3	1.71	2.39	2.90	0.50	3.33	2.85	2.56	0.69
	4	3.46	2.47	3.49	0.61	3.35	3.01	3.61	0.26
	5	2.36	1.47	1.67	0.50	1.50	2.70	3.07	0.70
Zinc	6	2.56	2.11	2.19	0.35	2.51	1.99	2.36	1.17
	7	3.30	3.89	3.18	0.28	4.05	3.40	3.89	0.47
	8	3.18	1.74	3.91	0.45	2.30	2.47	2.84	1.50
	9	2.43	2.23	1.81	0.52	1.58	1.46	2.14	0.69
	10	1.75	2.05	2.47	0.61	1.92	1.38	1.80	0.79

less oily. This was confirmed by physical examination.

Their observation prompted us to postulate that oral zinc sulfate (Orazinc-Mericon) would decrease skin-surface sebum. The following is a report of a double-blind study designed to determine if supplemental oral zinc sulfate (Orazinc-Mericon) decreases the rate of sebum secretion.

SUBJECTS AND METHODS

Ten healthy Caucasian male subjects ranging in age from 27 to 34 years (mean 29.5) were accepted into this study with their informed consent. All 10 were free of facial acne, seborrhea, and seborrheic dermatitis. No topical medicament, tetracycline, or any other antibiotic had been used during the previous 3 months. The study was conducted during April through June 1978.

Treatment with zinc or placebo was administered in accordance with a randomized double-blind experimental design. Zinc sulfate (●razinc-Mericon) was given to 5 subjects as capsules of 220 mg $ZnSO_4 \cdot 7H_2O$ (=50 mg Zn^{2+}). Identically appearing lactose capsules were given as placebo to the remaining 5 subjects. Each subject was instructed to ingest one capsule twice daily immediately after a meal for a period of 3 weeks. Encouragement was given to each subject throughout the study to ensure compliance. No other treatment was permitted during the trial.

The subjects were instructed to continue taking the capsules until all determinations of sebum secretion were completed. The sebum secretion rate of each subject was measured on alternate days prior to and following the treatment period, using the method of Strauss & Pochi (8). This technique is based on the principle of absorbing sebum onto thin cigarette papers as soon as it reaches the skin surface. Each subject's forehead was wiped thoroughly with dry gauze and a 6.45 cm² (1 inch²) area was demarcated by adhesive tape. Four ether-washed cigarette papers were mounted over the defined area, covered by gauze squares and held in place by a one-inch wide rubber tourniquet. The papers were left in place for 15 min and then discarded. This was repeated once to insure uniform preparation. Another set of similarly prepared papers were applied and left in place for 3 hours. Following the 3-hour test period, the portion of papers containing the sebum was placed into 20 cc of anhydrous ethyl ether in a desiccated, pre-weighed aluminium vessel and successive washings using aliquots of 10, 10, and 20 cc of anhydrous ethyl ether were performed. After evaporation of the ether, the vessel containing the sebum was desiccated for 24 hours and weighed using a Mettler fine analytical balance.

The resulting data were analysed and tested for significance using the method of analysis of variance.

Serum zinc levels were determined in each subject before and after treatment. Care was taken to minimize specimen contamination. The zinc analyses were performed by Trace Analysis Laboratory, San Diego, Ca., using X-ray fluorescence.

RESULTS

The experimental design is outlined in Table I, which also contains the individual replicate serum

Table II. *Summary and statistical analysis of the group mean sebum secretion rates (mg/10 cm²/3 h) and group mean serum zinc levels (μg/ml)*

The change in the Before–After sebum secretion rates of the zinc group is significantly different from that change in the placebo group. $P<0.028$, F-Test for analysis of variance

Group	Before		After	
	Sebum excretion rate	Serum zinc level	Sebum excretion rate	Serum zinc level
Placebo	2.51	0.72	2.78	0.53
Zinc	2.59	0.44	2.40	0.91

secretion rates and serum zinc levels prior to and following treatment.

Table II summarizes the results of the group mean sebum secretion rates and serum zinc levels in order to facilitate comparison. Prior to treatment, the zinc group mean sebum secretion rate was 2.59 mg/10 cm^2/3 h, while the group mean serum zinc level was 0.44 μg/ml. Following zinc supplementation, the group mean sebum secretion rate fell to 2.40 mg/10 cm^2/3 h, while the group mean serum zinc level rose to 0.91 μg/ml. The pre-treatment placebo group mean sebum secretion rate was 2.51 mg/10 cm^2/3 h, with a group mean serum zinc level of 0.72 μg/ml. Following placebo, the group mean sebum secretion rate was 2.78 mg/10 cm^2/3 h and the group mean serum zinc level was 0.53 μg/ml.

The change in the before and after treatment mean sebum secretion rates of the zinc group is significantly different from the before and after change in the mean sebum secretion rates of the placebo group ($p<0.028$*). This corresponds to a significant increase in the mean serum zinc level of the zinc group in comparison with that of the control group ($p<0.024$*).

Side effects were minimal and limited to the zinc group. Three of the 5 subjects who received zinc complained of mild nausea which subsided within 30 min of ingestion of the capsules.

DISCUSSION

The present study is, to our knowledge, the first attempt to assess the effect of supplemental zinc sulfate on the sebum secretion rate in normal individuals. Although the authors of a 1973 investigation (1) concluded that zinc does not affect sebum excretion, they studied only patients with acne. Furthermore, they did not perform a double-blind comparison; rather, all 8 patients received zinc and each served as his own control. Our study, consisting of a double-blind design utilizing a placebo control, suggests that zinc causes a significant diminution in the sebum secretion rate in normal, preclimacteric males.

The rationale behind this undertaking was twofold. As previously mentioned, some of Michaëlsson's patients who received zinc sulfate for a period of several weeks noticed that their skin was less oily than before zinc supplementation. This was verified clinically. Despite conflicting reports regarding the efficacy of zinc sulfate therapy in acne, the possibility that zinc is an effective agent in acne still exists. If this is so, then perhaps the improvement in acne is due to the rectifying of a "disturbed zinc-dependent enzyme system of androgen synthesis" (5) within the sebaceous gland. The dry skin could likewise be accounted for on the basis of enhanced androgen metabolism by a zinc-dependent hydroxysteroid dehydrogenase which has been localized to the junction of the secretory duct and the pilosebaceous follicle (2).

The resultant data indicate that there is an inverse relationship between the group mean serum zinc level and the group mean sebum secretion rate, i.e. the decrease in the zinc group mean sebum secretion rate was associated with an increase in the group mean serum zinc level. Similarly, the decrease in the placebo group mean serum zinc level was coupled with an increment in the group mean sebum secretion rate. The inverse relationship between the sebum secretion rate and the serum zinc level may be attributed to zinc supplementation in the zinc group and nutritional variation of the placebo group.

The results of this study should not be misconstrued. That zinc is an efficacious therapeutic agent for the treatment of acne has yet to be conclusively demonstrated. Our preliminary investigation demonstrates that supplemental zinc sulfate may reduce the output of skin-surface sebum as measured in terms of the sebum secretion rate. The concept that oral zinc supplementation diminishes the sebum secretion rate is a distinct possibility that warrants further investigation employing a larger sample size.

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REFERENCES

1. Burton, J. & Goolamali, S.: Zinc and sebum excretion. Lancet *i* (817): 1448, 1973.
2. Calman, K. C.: Androgens and acne: A new method for the screening of anti-androgens in human skin. Br J Dermatol *82* (Suppl. 6): 26, 1970.
3. Chvapil, M.: New aspects in the biological role of zinc:

* *F*-test for analysis of variance.

A stabilizer of macromolecules and biological membranes. Life Sci *13:* 1041, 1973.
4. Hillström, L., Petterson, L., Hellbe, L. et al.: Comparison of oral treatment with zinc sulfate and placebo in acne vulgaris. Br J Dermatol *97:* 681, 1977.
5. Michaëlsson, G., Juhlin, L. & Vahlquist, A.: Effects of oral zinc and vitamin A in acne. Arch Dermatol *113:* 31, 1977.
6. Orris, L., Shalita, A. R., Sibulkin, D. et al.: Oral zinc therapy of acne absorption and clinical effect. Arch Dermatol *114:* 1018, 1978.
7. Parisi, A. F. & Vallee, B. L.: Zinc metalloenzymes: characteristics and significance in biology and medicine. Am J Clin Nutr *22:* 1222, 1969.
8. Strauss, J. S. & Pochi, P. E.: The quantitative gravimetric determination of sebum production. J Invest Dermatol *36:* 293, 1961.

Table I. *Staining (S) and erythema (Er) by 24-hour exposure of 0.05% dithranol (D), 10-acetyldithranol (2C), 10-propionyldithranol (3C) and 10-butyryldithranol (4C) in 50 psoriasis patients*

Grading 0–3

S	Er	D	2C	3C	4C
0	0			16	44
0	1	3	19	26	6
0	2	23	27	8	
1	1	1			
1	2	23	4		
Total		50	50	50	50

Irritation and Staining by Dithranol (Anthralin) and Related Compounds: II. Structure-Activity Relationships among 10-Meso-Substituted Acyl Analogues

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Abstract. Irritation and staining by dithranol and some 10-meso-substituted acyl analogues was studied with the chamber-testing technique in a series of 50 psoriasis patients. The test concentrations for dithranol, 10-acetyldithranol, 10-propionyldithranol and 10-butyryldithranol were 0.05 and 0.5%, for 10-valeryldithranol 5% and for 10-myristoyldithranol 10%. As a rule, with increasing length of the carbon chain of the 10-acyl substituent (from 2C to 14C) the staining and irritative properties of the molecule decreased.

Key words: Psoriasis; Dithranol; Anthralin; 10-Acyl-1,8-dihydroxy-9-anthrones; Delayed skin irritation; Staining of the skin; Chamber test

In a previous paper (3), skin irritation [1, 2] and staining caused by dithranol and some related anthrones was studied by applying the chamber-testing technique of Pirilä (4). In order to reduce the staining and the irritation either one or both of the hydrogen atoms of the reactive methylene group at the 10-meso-position of dithranol was replaced. When both hydrogen atoms were replaced to form 10,10'-*bis* (formylethyl) dithranol, the new molecule had lost not only the irritative and staining properties of dithranol but also the antipsoriatic activity of the parent compound (cf. 6). When only one of the two hydrogen atoms was replaced with an acetyl group to form 10-acetyldithranol, the new molecule retained most of the properties of dithranol.

In this paper, it is shown that with increasing length of the carbon chain of the 10-acyl substituent the staining and even the irritative activities of the molecule decrease.

MATERIALS AND METHODS

An unselected series of hospitalized psoriasis patients was tested. The test compounds dithranol (D), 10-acetyldithranol (2C), 10-propionyldithranol (3C), 10-butyryldithranol (4C), 10-valeryldithranol (5C) and 10-myristoyldithranol (14C) were pure samples synthesized[2] by Erkki Honkanen, Ph.D. and Aino Pippuri, MSci (Orion Pharmaceutical Co., Helsinki, Finland). The proper concentrations of the test compounds were screened by testing on the back skin of the author. The concentrations selected were 0.05% and 0.5% for D, 2C, 3C and 4C. For 5C and 14C the concentrations were 5% and 10%, respectively. The chamber-test method was applied as described (3). The diameter of the erythema, edema and brownish stain was measured and the visual intensity estimated using a scale graduated from 0 to 3. When staining hampered the estimation of erythema, contact thermography was used.

[1] Irritation is defined as non-immunologic local inflammatory reaction of the skin, characterized by erythema, edema or corrosion, following topical application of a chemical substance (cf. 2).

[2] Patents pending.