ENVIRONMENTAL FACTORS (RH, T) AND THE STRATUM CORNEUM BARRIER FUNCTION TO OCCUPATIONAL CHEMICALS

JOHN HATZIS

Department of Skin and Venereal Diseases, Medical School, University of Ioannina, Greece

SUMMARY

Skin permeation for the occupational chemicals mainly depends on the Stratum Corneum (SC), which behaves as a barrier against the entrance of any substance applied on it. The hydration state of the SC plays a crucial role for its barrier function and the permeation process, especially for the water-soluble substances. In this work the relationship of the environmental Relative Humidity (RH) and Temperature (T) with the Stratum Corneum Hydration state, was studied and measured *in vitro*. We found that the SC hydration state is closely related with the environmental values of RH and T. This means that an increase of RH and T, in the working environment, can promote the toxicity hazards from occupational chemicals.

1. INTRODUCTION

The Stratum Corneum (SC) of the skin consists of the essential barrier between the internal and external environment. This dead layer also controls the permeation of the topically applied drugs or the occupational chemical substances.

Stratum Corneum properties and its protective function can be modified by various environmental factors. Hydration plays a crucial role for SC barrier function and permeation process.

In this work, the relationship between the environmental Relative Humidity (RH) and Temperature (T) with the SC hydration state was studied and measured *in vivo*.

2. MATERIAL

Stratum Corneum sheets from the heel area of the soles of six normal volunteers, three (3) men and three (3) women, 30-52 years of age, were the tested material. The SC sheets were obtained with the aid of a ceratotome. The heel area was moistened for 5 minutes with a wet gauge before cutting. Each SC tested sheet had the following characteristics: Thickness = 0.3 -0.5 mm, Width=6 mm and Height= 15 mm.

3. METHOD AND METHODOLOGY

The SC hydration state was estimated by measuring the shrinkage/relaxation response of the SC specimen, a property which appeared only by dehydration/hydration conditions respectively. This method, which was introduced by us previously, gives the possibility to measure with high accuracy the "Hygroscopicity" and the "Water Holding Capacity - WHC" in structures rich in keratin such as: SC specimens, hair, nail and fish scales [1, 2, 3].

The method was applied as follows:

a) Instrument: The shrinkage/relaxation measurements were recorded by the "Cutech Extensometer" (Stiefel Laboratories Ltd, Holtspur Lane, HP 10 OAU, England), which was used as a dynamometer [Figures 1, 2].

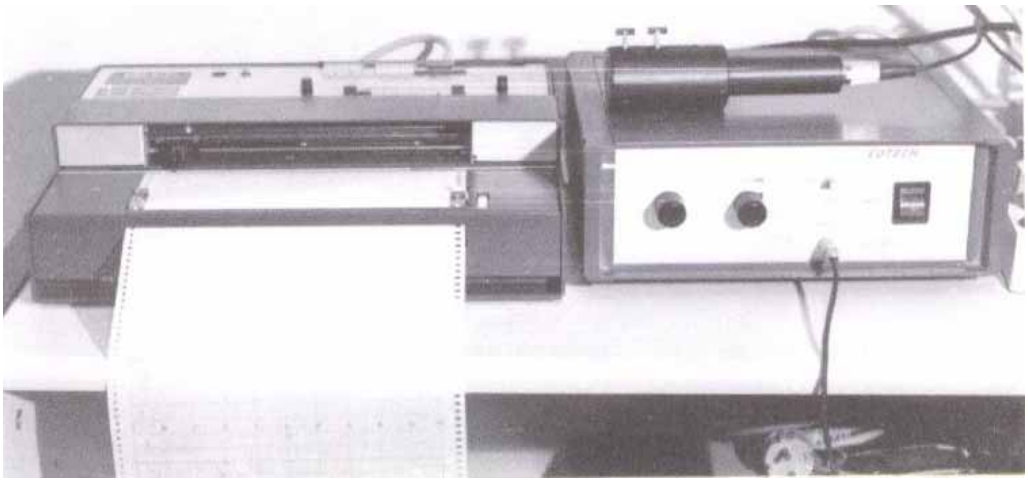

Figure 1: The *in vivo* extensometer, which was used for the *in vitro* shrinkage-relaxation measurements.

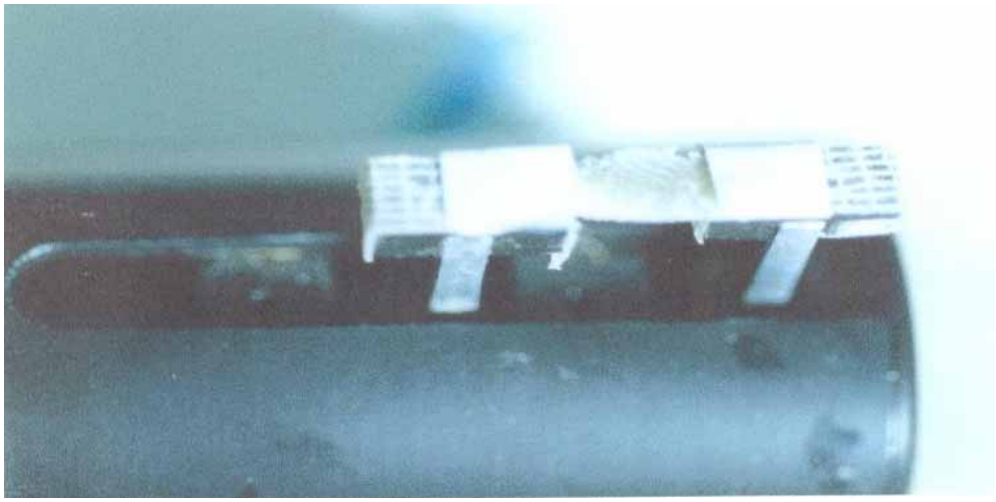

Figure 2: The arms of the extensometer and the tested SC sheet.

The measurements were made (in each specimen) in two different environments of RH, keeping the Temperature constant.

(1) into the laboratory with RH= 52-57% and T= 20-21^{o} C

(2) into a special glass cabinet with a cyclic opening (for inserting the extensometer probe), with RH= 70-75% and T=20-21 ^{o} C. The rise of RH was due to the addition of 2 kg of water into the glass cabinet (Figure 3).

Thereafter, the measurements continued in the two different levels of RH 52-57% and 70-75%, by increasing the Temperature to 30-31^{o} C.

b) Preparation stage: The SC specimen was hydrated with distilled water for 5 minutes. Thereafter, the hydrated SC specimen was stuck onto the extensometer arms (using a cyanoacrylate glue) which had a distance of 10 mm and a width of 6mm [Figure 2]. The dimensions of the tested SC sheet were: Thickness= 0.3 -0.5 mm, Width= 6 mm and Height= 10 mm.

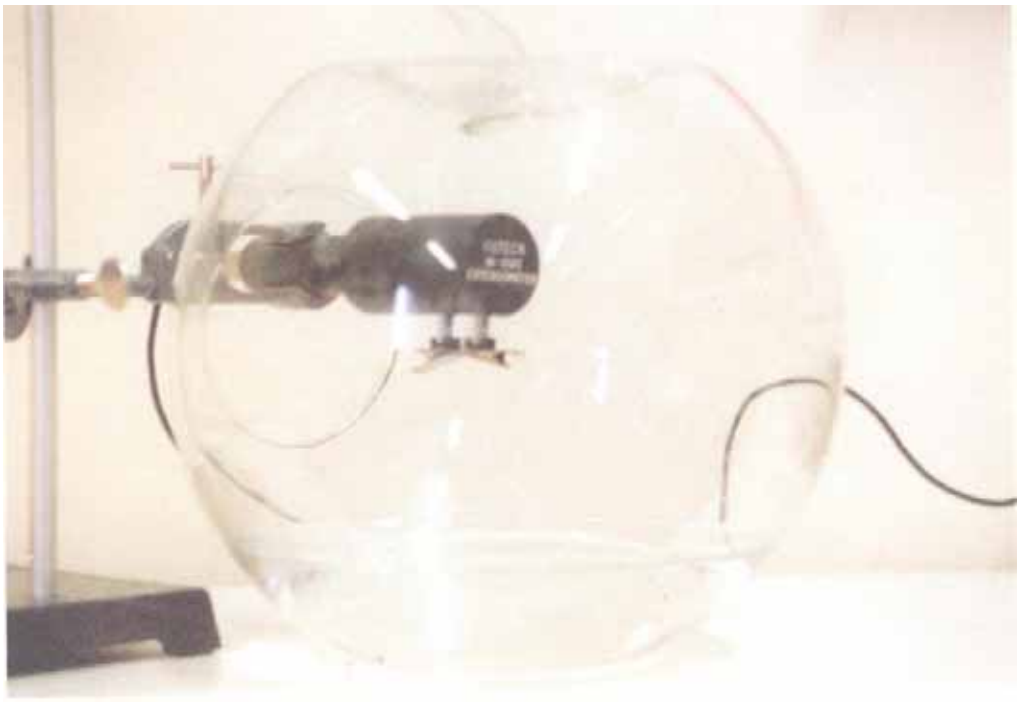

Figure 3: The glass cabinet. The RH was 70% adding 2 kg of water into the bottom.

c) Recording stage:

First record in the laboratory (RH = 52-57%, T= 20-21^{o} C): after the above "preparation stage" we started to record continuously any force applied by the SC specimen on the extensometer arms. At the beginning, no force was applied from the hydrated artificially SC specimen (isoelectric line). However, after a few seconds, as the specimen started to dehydrated into the Laboratory Environment, a "Shrinkage Force" was recorded which gradually reached in a Maximum Level (Maximum Shrinkage Force - MSF) [Figure 4].

Second record in the glass cabinet (RH= 70-75%, T = 20-21^{o} C): While the SC specimen was in the above maximum shrinkage level, the instrument probe inserted into the glass cabinet for two minutes where the RH was 70-75% (higher 15-20% that the environmental) [Figure 3]. This had as result a fast abolishment of shrinkage force (in two minutes) meaning that the SC specimen has the property and ability to be hydrated by the environmental moist [Figure 4].

Third record again in the laboratory (RH= 52-57%, T= 20-21^{o} C): then the instrument probe was put again back into the laboratory environment with a lower RH, measuring again the dehydration by the shrinkage curve [Figure 4].

Fourth record of Reproducibility: Thereafter, the above measurements were repeated in 3 consecutive times in order to evaluate the reproducibility and the direct relationship of the SC shrinkage/relaxation phenomena with the two different levels of environmental RH and under constant Temperature= 20-21^{o} C.

Fifth record**:** the Shrinkage-Relaxation line was recorded in the same SC specimen in two different levels of RH (52-57% and 70-75%) and in a higher level of Temperature = 30-31^{o} C.

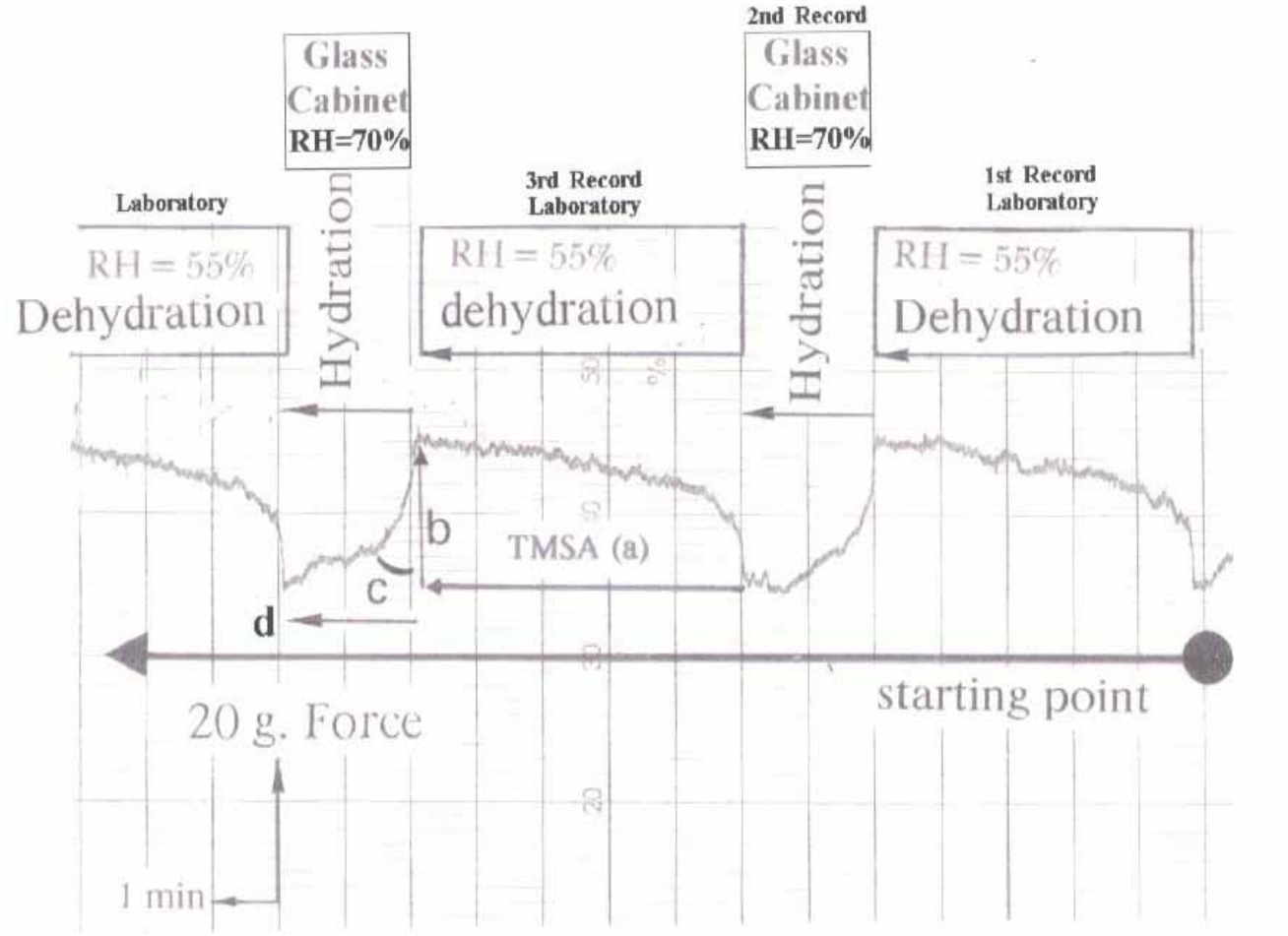

Figure 4: Parameters which were measured in the graph line:
a. TMSA: "Time of Maximum Shrinkage Ability" of SC (in mins)
b. MSF: "Maximum Shrinkage Force" of SC (in gram force)
c. AH: "Angle of Hygroscopicity" (in degrees)
d. TH: "Time of Hygroscopicity" (in mins)

d) Parameters

The parameters used for the evaluation of the Shrinkage-Relaxation curves which were introduced by us [1, 2, 3] are the following [Figure 4):

i) the "Time of Maximum Shrinkage Force-TMSF" (in minutes): represents the needed time in order the SC abolish the sorbed water. Thus, the TMSF consists of a representative value of the SC "Water Holding Capacity-WHC".
ii) The "Maximum Shrinkage Force-MSF" (in grams): is a property of the SC specimen itself and indicates the abolishment grade of its moisture.
iii) The "Angle of Hygroscopicity-AH" (in degrees): indicates oppositely the speed of the SC "Hygroscopicity".
iv) The "Time of Hygroscopicity-TH" (in minutes): measures the duration of hydration progress.
v) SC Dehydration Curve: indicates the speed and the mode of SC dehydration.
vi) SC Hydration curve: indicates the speed and the mode of the SC hydration

4. RESULTS

a. Relative Humidity and SC Hydration

The value of Hygroscopicity of the tested SC specimen from the environment moist was found to be high and similar at both levels of temperature, as the major part of the water is absorbed during the first 5-10 seconds (very small AH) and the "Time of Hygroscopicity" was approximately two minutes [Figures 4, 5, 6].

The "Water Holding Capacity - WHC" of the tested SC specimen, measured by the "Time of Maximum Shrinkage Force - TMSF", was found to be 5-6 minutes at 20-21^{o} C [Figure 5] and much more higher, 7-9 minutes at 30-31^{o} C [Figures 5, 6]. So, the SC can be hydrated from the environmental RH into two minutes (because of SC high Hygroscopicity), whereas the needed time to abolish the absorbed water is 3-4 times greater (because of SC high Water Holding Capacity).

Our results indicate that the SC is able to be hydrated from the water vapor of the environment, because of its high hygroscopicity. Also, alterations in the environmental RH may act directly on the SC changing its hydration level. Thus, the SC hydration state directly depends on the environmental RH.

b. Temperature and SC Hydration

Increasing the environmental temperature from 20-21oC to 30-31^{o} C we had the following results:

a. Increasing the T to 30-31°C during the SC dehydration into the laboratory environment (just after its hydration into the glass cabinet at T= 20-21^{o} C) a rapid abolishment of the sorbed water was observed indicated by a decrease of the TMSF. This decrease represents a reduction of the SC WHC at the moment of entry the SC specimen into a higher environmental Temperature [Figure 5].
b. Thereafter, in a constant increased level of T= 30-31^{o} C in both places of the experiment (Glass cabinet - Laboratory), the WHC of the SC was increased (increased TMSA) in relation to that obtained in a lower temperature [Figure 6]. The increase of MSF and the translocation of the graph curved line to higher values, shows that the SC shrinkage ability is also increased [Figures 5, 6].

5. DISCUSSION

Skin Permeation for topically applied drugs and occupational chemical hazards mainly depends on the Stratum Corneum [4]. The hydration state of the SC plays a crucial role in permeation process especially for the water soluble substances [4, 5, 6, 7, 8, 9]. Thus, any environmental factor, which is able to change the SC hydration state, can act on pharmacokinetics of topically applied drugs or on toxicokinetics of occupational chemical substances.

a. RH and SC Hydration

In this study, it was found that the SC hydration state was increased rapidly after increase of the environmental RH and T [Figures 5, 6]. This SC property has been described previously by various authors using various methods [10, 11, 12, 13, 14]. Singer and Vinson [11] found a logarithmic relationship between SC water contain and RH.

The advantage of this method is that it measures and records continuously: (a) the SC changes related to RH and T and (b) the SC Hygroscopicity and WHC.

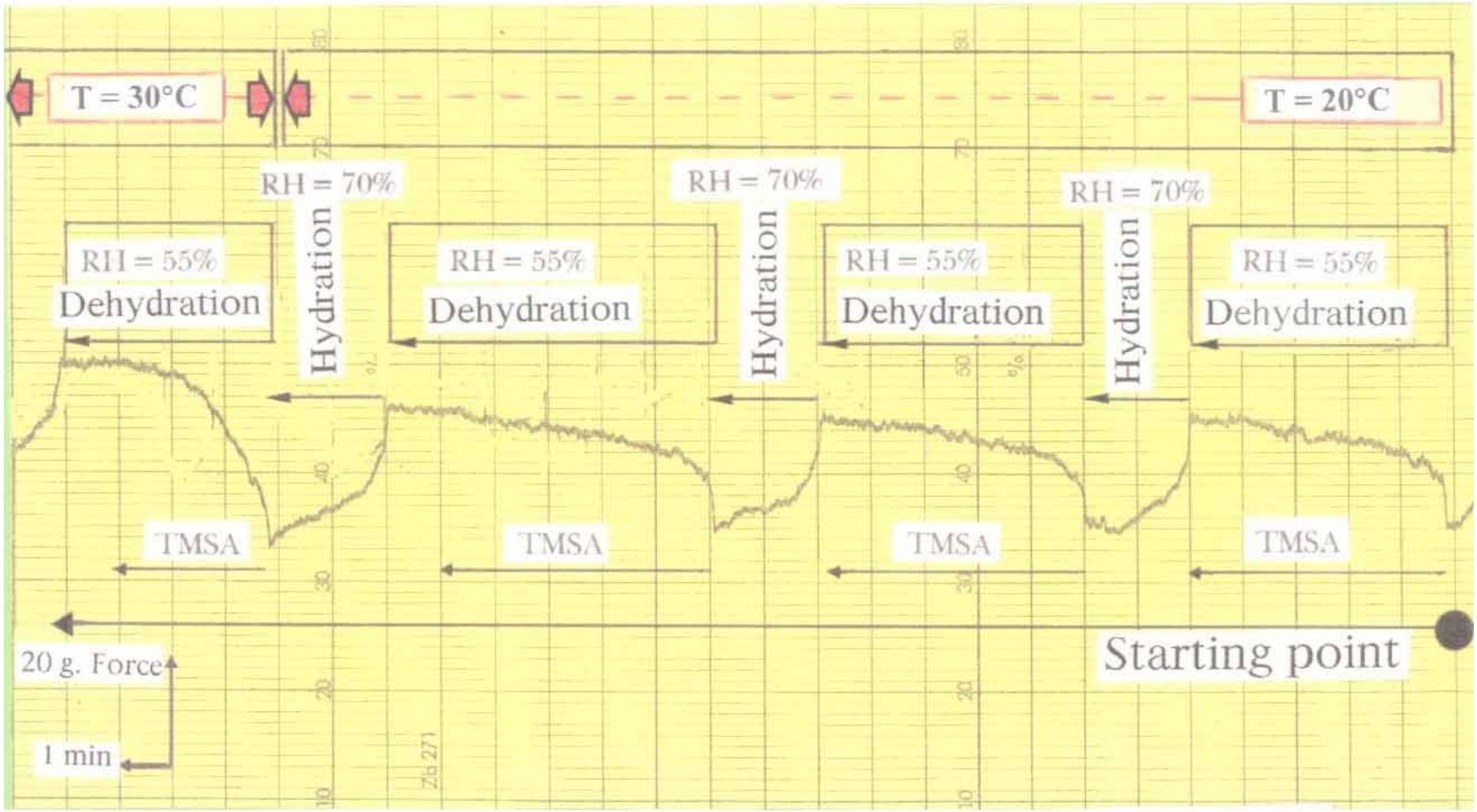

Figure 5: Shrinkage-Relaxation line of SC sheet under hydration and dehydration conditions. During the last dehydration at T=30° C, the TMSA was decreased

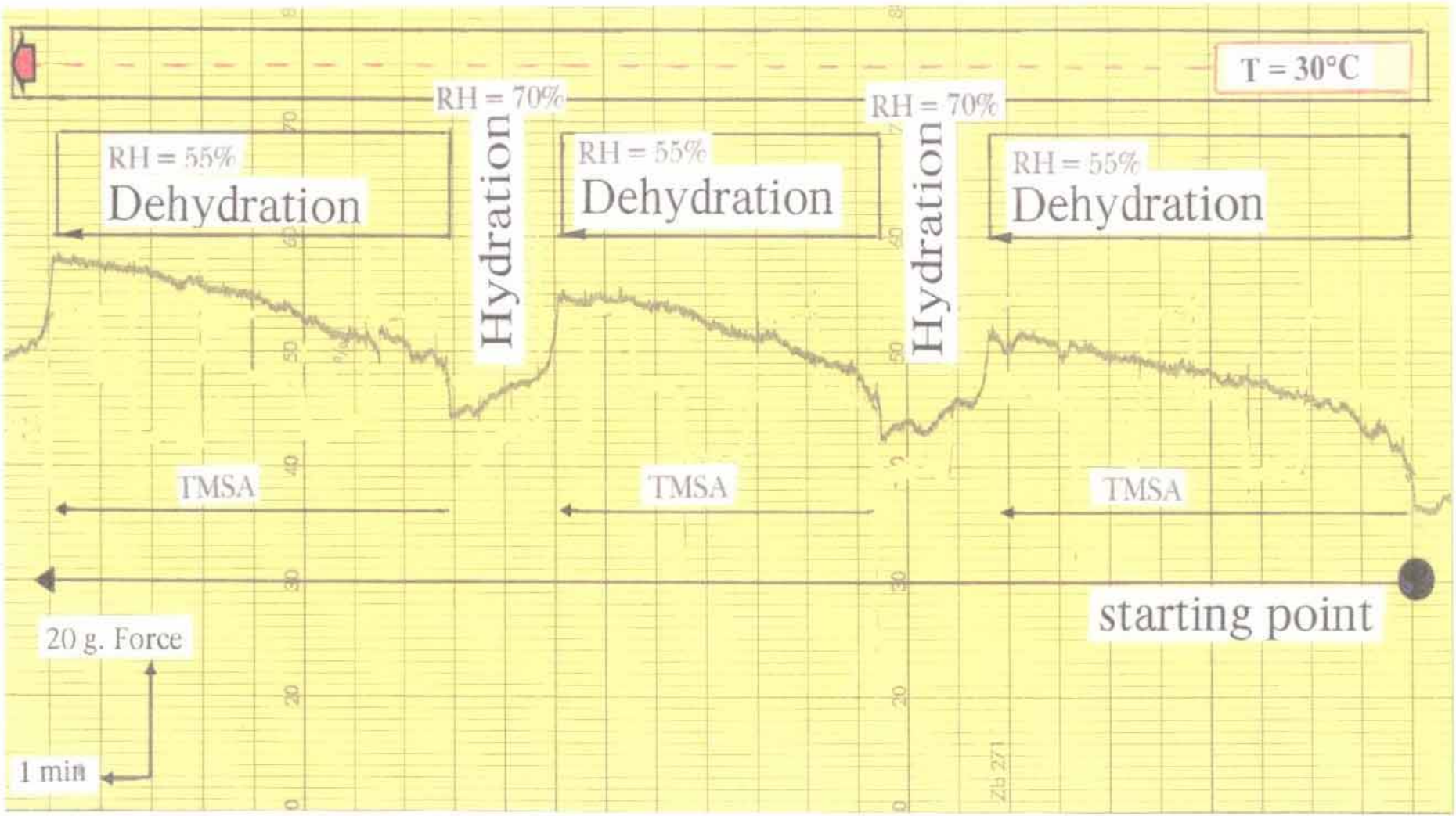

Figure 6: The Shrinkage-Relaxation line in a constant temperature of 30° C

b. T and SC Hydration

Our results indicate that a sudden rise of Temperature from 20^{o} C to 30^{o} C directly increases the SC dehydration speed, whereas the WHC is reduced (the TMSF is reduced) [Figure 5]. Thereafter, as the increased Temperature continues, a new state is established. This state is characterized by an increased Hygroscopicity and WHC of the SC [Figure 6]. The above results indicate that the water mobility (towards into and out of the SC specimen) increases as the environmental Temperature increases. In addition, the SC water increases.

An increase of SC hydration state as a result of T increase has been mentioned by other authors for low and moderate RH values, whereas at 90% RH there were no changes depended from the Temperature [12, 14]. In addition, it has been observed that the water loss from the SC increases at high temperature [10, 15, 16]. An increase of skin temperature *in vivo*, is accompanied by an increase of the TEWL [14]. Our results explain the above data as we observed an increased mobility of the water (into and out of the SC sheet) in a higher temperature.

The increase of SC water loss, water mobility and hydration level during the rise of environmental temperature contributes to the Thermoregulatory function permitting (in a warm environment) a faster water evaporation from the skin surface for a faster lowering of the body temperature.

The skin temperature (or environmental) is considered to increase the permeation of the SC to various substances, by a direct action on this process [4, 9, 17].

The deeper SC layers are much more hydrated, *in vivo*, than the superficial [18] and the outer SC presents a lower Hygroscopicity than the inner SC [1]. Thus, the superficial SC cell layers have the best barrier function.

The environmental RH and T are able to act also on the SC after the application of various emollient substances similarly by increasing the water loss in a low RH or in an increasing Temperature and *vice-versa* [19].

6. CONCLUSIONS

In this study, we estimate the action of the environmental RH and T on SC hydration state and its properties. The conclusions are the following:

1. Our method and methodology, measure and record continuously the SC hydration level in relation to environmental changes of RH and T. Moreover, the “Hygroscopicity” and the “Water Holding Capacity – WHC” of the SC can be estimated.

2. We found that any increase of the environmental RH and T is accompanied by an increase of the SC hydration state. Moreover, T increases the mobility of the water towards into the SC (in high RH) or out of it (in low RH).

3. Environment with high RH and T (increasing the SC hydration state and the water mobility), has a positive effect in the permeation process for the topically applied drugs on the skin as well as for the occupational chemicals. In this way, such environmental conditions can promote the effectiveness and the side effects of the topically applied drugs. Moreover, in the working environment such conditions can promote the toxicity hazards from occupational chemicals.

REFERENCES

1. Hatzis J.: Measurement of shrinkage ability of the stratum corneum under dehydration conditions. Skin Research and Technology 1995; 1: 128-136.
2. Hatzis J.: Desquamation mechanism in normal and diseased skin. The 10th International Symposium on Bioengineering and the Skin, 1994, Cincinnati, Ohio, USA. Book of Abstracts, p. 47
3. Hatzis J.: A method measuring the occlusive ability of a nail lacquer preparation. J. Eur. Acad. Dermatol. Venereol (Suppl. 1), 1995; 5: S134-S135.
4. Scheuplein R.J.: Percutaneous Absorption. In: The Physiology and Pathophysiology of the Skin. Vol. 5. Edited by Jarrett A., Academic Press, London, etc., 1978; pp. 1667-1752.
5. Scheuplein R.J. and Ross L.W.: Mechanism of percutaneous absorption. V. Percutaneous absorption of solved deposited solids. J. Invest. Dermatol., 1974; 62: 353.
6. Feldmann R.J. and Maibach H.I.: Penetration of 14C Hydrocortisone through normal skin. The effect of stripping and occlusion. Arch. Dermatol., 1965; 99: 661.
7. Hatzis J.: A contribution in the study of permeability mechanisms of the skin. Medical Publications Paschalides, Athens 1985 (in Greek language).
8. Hatzis J.: Basic Dermatology-Venereology. Medical Publications Litsas, Athens 1994, pp. 794-805 (in Greek language)
9. Schaefer H., Zesch A. and Stuttgen G.: Skin Permeability. Springer-Verlag, Berlin, etc., 1982.
10. Blank I.H.: Factors which influence the water content of the stratum corneum. J. Invest. Dermatol., 1952; 18: 433-440.
11. Singer E.D. and Vinson L.J.: The water binding properties of skin. Proceedings of Scientific Section. Toilet Goods Association, 1966; 46: 29.
12. Spencer T.S., Linamen C.E., Akers W.A. and Jones H.E.: Temperature dependence of water content of stratum corneum. Br. J. Dermatol., 1975; 93: 159-164.
13. Wildnauer R.H., Bothwell J.W. and Douglass A.B.: Stratum corneum biomedical properties. I. Influence of Relative Humidity on normal and extracted human stratum corneum. J. Invest. Dermatol., 1971; 56: 72-78.
14. Grice K.A.: Transepidermal water loss. In: The Physiology and Pathophysiology of the Skin. Edited by Jarrett A., Vol. 6. Academic Press, London, etc., 1980; pp. 2115-2146.
15. Middleton J.D.: The effect of temperature on extensibility of isolated corneum and its relation to skin chapping. Br. J. Dermatol., 1969; 81: 717-721.
16. Middleton J.D. and Allen B.M.: The influence of temperature and humidity on stratum corneum and its relation to skin chapping. J. Soc. Cosmet. Chem., 1973; 24: 239-243.
17. Tregear R.T.: Physical functions of the Skin. Academic Press, London, New York, 1966.
18. Hatzis J. and Marks R.: The relationship between conductance and capacitance measurements and skin surface contour during hydration of the stratum corneum. In: Stratum Corneum. Edited by Marks R. and Plewig G., Springer-Verlag, Berlin, Heidelberg., 1983; pp. 257-261.
19. Strußmann A., Weißen H-J., and Wirtz A.: Water vapour permeability of skin care products in relation to molecular and environmental influences. Intern. J. Cosm. Science., 1993; 15: 227-233.

ABBREVIATIONS

T = Temperature
RH = Relative Humidity
SC = Stratum Corneum
WHC = Water Holding Capacity
TMSA = Time of Maximum Shrinkage Ability
MSF = Maximum Shrinkage Force
AH = Angle of Hygroscopicity
TH = Time of Hygroscopicity