Biomechanical Properties and Microscopic Morphology of Human Stratum corneum Incubated on a Wet Pad in vitro*

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Received October 9, 1972

Summary. Samples of human Stratum corneum (St. c.) collected by the cantharidin method and suspended in a controlled atmosphere usually show a raised extensibility when the relative humidity (RH) is raised, and, consequently, a greater solidity. When the St. c. samples were not suspended but laid on a wet pad, extensibility did not change when RH was raised, but elongation decreased owing to a strong reduction of breaking strength; the St. c. became more fragile. Electron and optical microscopic studies revealed that this latter phenomenon was due to an alteration of the links between cells, i.e. cell interlockings, desmosomes, intercellular cement. In this experiment, St. c. samples were not stiff as when suspended in air, but supple as in vivo; the technics provide a good model for in vivo hyperhydrated St. c.

Recent experiments [3] have shown that extensibility of Stratum corneum (St. c.) in vitro is related to its water content. When St. c. is suspended in air, this water content is related to the relative humidity (RH) of the ambient atmosphere (2.3-4.8). Conversely, its ultimate breaking strength when stretched is in an inverse relation to RH.

* Paper presented at the meeting of the Société Française de Dermatologie, Section Biologique, 8th June 1972.
It must be considered that in these experiments the St. c. was placed in a quite different environment from its natural one which allows its lower face to be continuously supplied with water from the underlying living epidermis. This is probably the reason why it quickly becomes dry and stiff, whereas as it was supple in vivo.

In order to imitate the physiological conditions, we produced an in vitro-device allowing the lower part of the St. c. strip to be continuously supplied with saline, and we had another look into biomechanical properties at various degrees of RH of the atmosphere.

**Methods and Materials**

**Stratum corneum Preparation**

Stratum corneum samples were obtained according to the cantharidin blister procedure of Kligman and Christophers [1] 0.3 cm² 0.5% cantharidine in acetone and were spread on a 16 cm² surface and left to be air dried. 20 min later a wet gauze dressing was put in place and then covered with an occlusive dressing for 18 h. The blister top was excised with scissors and soaked in saline for 1 h so as to be easily flattened onto cellophane; the upper aspect of the St. c. was put onto the cellophane. Strips 3 mm wide and 3 cm long of St. c. on cellophane were cut with a blade and then put on silicon paper; the cellophane was easily detached.

**Incubation**

Saline absorbent cotton wool was put on the bottom of a chamber and covered with a thin sheet of silicone coated paper. The RH of the chamber atmosphere was measured by a hair hygrometer; 98% RH was obtained by complete closing; 33% RH by complete opening; intermediate RH by incomplete closing; this quite simple procedure gave more reliable RH than the use of a silica gel which did not enable us to obtain low RH.

The St. c. strips were maintained for 8 days in the chamber at constant RH and temperature (23—24°C); in this way the strips kept their initial suppleness and transparency.

We could not measure cell hydration by weighing as the results were unreliable: we feel that it was because a small part of the St. c. strip remained attached to the silicone coated paper when it was taken off to be weighed.

**Biomechanical Measurements**

Biomechanical measurements were taken by fixing the 2 ends of the St. c. strips in a Richard textile dynamometer (jaw opening 2.5 cm). Straining was performed at a constant speed and the stress-strain curve recorded on a graphic recorder at the same temperature. This processing lasts less than 3 min and does not allow the sample hydration to be changed enough as to modify the biomechanical properties. All the strips which broke at their edges were rejected.

**Microscopic Study**

St. c. samples were not fixed so as to allow cell shrinking and visualisation of cellular separation due to possible previous alteration of cell links. Then they were dehydrated in more and more concentrated ethanol, as is usual, and included