Water-Holding Lipid and Water Transmission Through Homeothermic and Poikilothermic Skins[1] (35427)

CARL JELENKO, III AND JACK M. GINSBURG
Departments of Surgery and Physiology, Medical College of Georgia, Augusta, Georgia 30902

Thermal regulation in homeotherms is, in large measure, accomplished by evaporating water transmitted through the skin. Impairment of thermoregulation proportional to the extent of the burn is observed in burned mammals (1, 2). Earlier, we isolated a hexane-soluble lipid which is a major regulator of passive water holding by mammalian skin (3). Burned human and rabbit skin contained from 0.25 to 30% of the normal amount of water-holding lipid and transmitted up to 4 times more water than intact skin (4). The observation of impaired thermoregulatory function in the postburn surface led us to investigate the relation between skin lipid content and water transmissivity of intact homeotherm (mammals) and poikilothermic (fish and frog) skins and mammalian eschar.

Materials and Methods. Intact skin was obtained from freshly sacrificed New Zealand Albino Rabbits, marine oyster-toad fish (*Opsanus tau*) and grass frogs (*Rana pipiens*). Rabbits were sacrificed by intravenous pentobarbital (100 mg/kg). Fish and frogs were pithed prior to excision of skin samples. Human skin samples were obtained during skin grafting procedures and were stored in isotonic saline at 4° until studied. Analyses were usually initiated within 30 min of excision of human skin and within 10 min of excision of nonhuman skin. Experimental burns were inflicted by applying an 11-cm^2 Pyrex disc heated to 730° to the clipped surface of pentobarbital-anesthetized rabbits for 15 sec. The eschars were excised at 15 min and at 24 and 48 hr postburn. Human eschar was obtained at the time of operative excision. Particular care was taken to exclude subcutaneous fat during the excision of skin and eschar. This was easily accomplished in the case of nonhuman skin, but complete elimination of subcutaneous and rete peg fat was not possible during preparation of human skin and eschar.

The skin samples were placed in a 3.69-cm^2 diffusion chamber so that they separated a water vapor interface from a stream of dry air. The measurement chamber had a radius of 0.925 cm and was equipped with a peripheral air inlet. The central outlet chamber depth was 0.200 cm. The effluent air stream was analyzed for water content using a modified gas chromotograph calibrated with water to a demonstrated accuracy of 98.37% (5). Measurements were made on each sample at six air flows which ranged from 55.02 to 173.00 ml/min corresponding to linear velocities of 4.96 to 15.59 cm/sec, respectively (7). Temperature was regulated at 26.24 ± 0.12° and air pressure at 1.0359 atm.

All samples were analyzed for water-holding lipid in a 1-hr hexane extract of desiccated specimen material. The extract was evaporated to dryness with nitrogen; reconstituted to 2 ml with hexane; and chromatographed by the method of Haahti (7) using an 8-ft Diatoport-S column at isothermal conditions (150°).

Results. Water transmission through fish and frog skin (Fig. 1, G and H) approximated free evaporation (free diffusion) of water, (Fig. 1, I). This represented approximately 6 times more water than was transmitted by rabbit skin (Fig. 1, B) and 20 times more than was transmitted by human skin (Fig. 1, A). Intact poikilothermic surfaces transmitted approximately 1.6 times more water than rabbit eschar (Fig. 1, E and F) and approximately 3 times more water than human eschar, (Fig. 1, C and D).

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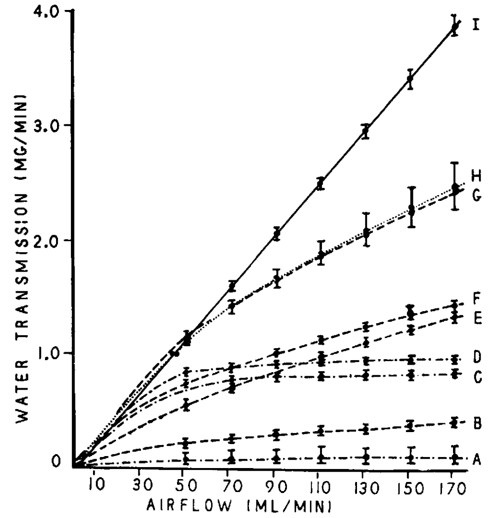

FIG. 1. Comparison of the free diffusion of water with measured water transmissions of fish, frog, human, and rabbit skins are various air flow challenges: Each point is the mean ± SEM (A) Intact human skin (*N* = 124); (B) intact rabbit skin (*N* = 23); (C) mature human eschar (*N* = 63); (D) acute human eschar (*N* = 60); (E) mature rabbit eschar (*N* = 150); (F) acute rabbit eschar (*N* = 72); (G) Marine oyster-toad fish skin (*N* = 10); (H) frog skin (*N* = 6); (I) open water (*N* = 508).

Rabbit eschars transmitted approximately 3 times more water than intact rabbit skin (Fig. 1, compare E and F to B); and human eschars transmitted approximately 9 times more water than intact human skin, (Fig. 1, compare C and D to A).

No water-holding lipid was detected in intact fish or frog skins. We experienced marked difficulty in eliminating subcutaneous and rete peg fat from samples of human skin and eschar, and although water-holding lipid was detected as a chromatographic peak at 4.3 min postinjection, quantification of the material could not be accurately performed in these experiments. Samples of rabbit skin and eschar were both readily obtainable free of subcutaneous fat and the lipid content in this species was accurately measured (Table I). Intact rabbit skin contained 0.01722 ± 0.0028 g of water-holding lipid/100 g of wet weight of skin. Acute rabbit eschar contained 0.00526 ± 0.00026 g% of the material.

The rate of water transmission in skin and eschar varies inversely with extractable lipid content of the tissue. The coefficient of correlation between measured lipid content and water transmission in the skins and eschar measured at the various air flow challenges ranged from 0.94 to 0.96. Although the absolute magnitude of water transmission depended upon the rate of air flow across the surface, the relative change in water transmissivity of skin with respect to its lipid content

TABLE I. Comparison of Lipid Content to Air Flow Necessary to Yield 1 mg/min Water Transmission for Various Surfaces.

Surface	*N*[a]	Water-holding lipid as (g% wet wt) (mean ± SEM)	Challenge air-flow (ml/min) when water transmission = 1 mg/min (mean ± Syx)[b]
Intact frog skin	6	0.0	39.585 ± 0.106
Intact fish skin	10	0.0	40.475 ± 0.093
Open water	508	0.0	45.347 ± 0.018
Intact rabbit skin	23	0.01722 ± 0.0028	860.150 ± 0.078
Acute rabbit eschar	72	0.00526 ± 0.00026	88.181 ± 0.045
Mature rabbit eschar	150	0.00535 ± 0.00026	113.370 ± 0.501
Intact human skin	124	(2.2738)[c]	115,145.509 ± 0.247
Acute human eschar	60	(0.00537)[c]	235.038 ± 0.223
Mature human eschar	63	(0.01829)[c]	889.280 ± 0.501

[a] *N* = number of samples.

[b] For measured values, coeff. corr. Lipid vs air flow = 0.977.

[c] The difficulties in eliminating subcutaneous and rete peg fat render measured human values inaccurate: Values presented are calculated: (lipid = 1.974×10^{-5} flow + 73.265×10^{-5}). See text for explanation of calculation.

was very similar in the range of air flows used. A decrease in lipid content of 50% resulted in a 2.7-fold increase in water transmission at 50 ml/min air flow and in a 3.2-fold increase at 170 ml/min air flow.

From the relation between water transmission and air flow (Fig. 1) in rabbit skin and eschar, we obtained values for the air flow necessary to produce a 1 mg/min water transmission through skins having lipid content ranging from zero to 0.0172 g%. This air flow value was then plotted against the hexane extractable lipid content of the samples. A least squares regression method was used to fit the curve and the coefficient of correlation was 0.98 (Table I). From this relation the water-holding lipid content of any sample can be predicted if the air flow which causes a 1 mg/min water transmission through the sample is known. The chemical characteristics of the water-holding lipid of rabbit and human skin appears to be highly similar (3) and the above-described relation between air flow at constant water transmission and lipid content may provide an estimate of the water-holding lipid content of human skin and eschar, despite our inability to obtain samples free of subcutaneous and rete peg fat. The air flows required to give a 1 mg/min water transmission through human skin and eschar were obtained from Fig. 1 and we have estimated the water-holding lipid content of acute human eschar, mature human eschar, and intact human skin to be 0.00537, 0.01829, and 2.273 g% wet weight, respectively. This value for water-holding lipid of human skin is 52% less than the value of 4.4 g% obtained by analysis of hexane extractable lipid of human skin known to be contaminated with subcutaneous and rete peg fat (3).

Discussion. Our studies suggest a relationship between thermoregulation, water transmission by the skin, and the content of water-holding lipid in the surface. The effectiveness of thermoregulation by transmission of water vapor through the skin should be to some degree dependent upon the ability of the skin to regulate the passive flux of water through it. The skin of man, rabbit, and rat loses its ability to properly regulate the passive transmission of water following burn injury and the animal tends to become hypothermic (3, 4, 5). In marked contrast to these mammalian skins, the skins of *Opsanus* and *Rana* are remarkably permeable to water vapor. When subjected to air flow challenges up to approximately 5 cm/sec across the surface, their water transmission is virtually identical with free diffusion of water (Fig. 1, compare I to G and H). In the frog, transcutaneous water transmission should depend upon ambient conditions, and the endogenous calories produced by the animal to evaporate this water should be proportional to these conditions. The fish is also subjected to ambient control of thermoregulation while living in his natural aqueous environment. Since the skin is freely permeable to water (Fig. 1, G) which moves along osmotic gradients into a 100% relative humidity milieu at the dew point, no evaporation of water should take place from the surface of the fish. Thus any regulation of temperature should occur from assumption of the temperature of surrounding water and not from endogenous metabolic work. The exceedingly rapid rate of transcutaneous water movement in *Opsanus* skin suggests that this skin is analogous to a heat exchanger and is able to dissipate heat of metabolic origin as rapidly as it is produced by means of the high water flux through the skin.

Postburn water transmission through mammalian eschar increases in amounts proportional to the decrement in water-holding lipid in the surface. Water transmission through mammalian burn approaches the transmissivity of fish and frog skins, which contain no water-holding lipid. Thus, the burned human or rabbit may serve as a model for those mechanisms by which homeothermia is maintained in mammals.

Summary. Water transmission was measured through isolated human, rabbit, marine fish, and frog skins. The water transmission rate of fish and frog skins approximates free evaporation of water, and is 6 to 20 times greater than in mammalian skins. Human and rabbit skins contain a hexane-soluble lipid which appears to be the major regulator of passive water holding. This hexane-extractable lipid was not detected in the fish or frog skin. Burned mammalian skin con-

tained 30% or less of the normal water-holding lipid and transmitted up to 4 times more water than intact skin. Water transmission by the surfaces examined was inversely proportional to the water-holding lipid content of the surface. The postburn decrease of hexane-extractable lipid in burned mammals was associated with production of a surface which closely resembles the skin of poikilotherms in water transmissivity and thermoregulatory capability. The maintenance of homeothermia may therefore be, in part, related to the presence of water-holding lipid in the skin.

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