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The Passage of Oxygen through Isolated Sheets of Human Stratum Corneum* (32860)

RAYMOND PENNEYS, WILLIAM FELDER, AND ENNO CHRISTOPHERS
(Introduced by A. M. Kligman)
Vascular Section of the Department of Medicine, and Department of Dermatology, Hospital of the University of Pennsylvania, Philadelphia, Pennsylvania 19104

Since the oxygen content of the skin, and deeper tissues, of the human limb is dependent, in part, upon that of the external environment (1–3), since enough oxygen may come from this route to benefit an ischemic limb, and since the factors that play a role in this process still require further investigation (4–6), we have studied one of these, the passage of oxygen through the stratum corneum. These experiments were prompted by the fact that intact sheets of human stratum corneum can now readily be obtained (7).

We have measured the rate of passage of oxygen when such sheets are placed between air and an essentially oxygen-free liquid because this simulates, to a large extent, those conditions under which the very ischemic limb exists (2).

Method. The method of preparing the sheets of stratum corneum from the abdominal wall of human cadavers, and the chamber used to measure the passage of oxygen through these sheets, have been described elsewhere (7,8).

The phlanges of both the upper and lower sections of the chamber were first thoroughly cleaned and coated with a thin film of Fisher Cello-Seal. A sheet of stratum corneum was placed between the two sections; care was taken to make sure that the central portion (12-mm diameter) of the membrane through which the oxygen would pass was not touched. Then the sections were clamped together; pressure was applied evenly to avoid tearing of the sheet. A magnetic stirring bar was inserted into the lower section of the chamber *via* its sidearm, and nitrogenated (deionized) water was then quickly injected through this sidearm. A Beckman-Clark oxygen electrode (model 160) was then inserted into the water, just long enough to obtain an oxygen tension reading. Finally, the rim of the sidearm, previously coated with Cello-Seal grease, was sealed with aluminum (Al-foil).

The upper section of the chamber then was filled approximately halfway with 4 ml of (deionized) water which had been equilibrated with air; the upper surface of the water was left uncovered, in contact with the outside air. (The volume of the lower section is 10 ml, including the sidearm.)

The chamber was submerged into a water bath of 37°C up to the level of the phlange joint, and the actual experiment was begun by activation of the magnetic stirrer. It was terminated 4 hours later, after which time the Al-foil was removed from the sidearm and another oxygen reading of the water taken. The increase in reading over the original value was calculated, per minute, per micron thickness of the stratum corneum.

Materials of known oxygen permeabilities were similarly studied, as controls, in a number of experiments. These consisted of a material of essentially no permeability (Al-foil), of two very permeable materials (Teflon, 12-μ thick, and cellulose acetate, 22–μ thick), and one less permeable (Kodak Kodar, Mylar type, 19-μ thick).

Results. See Table I. The amount of oxygen that passed through the stratum corneum did

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TABLE I. Passage of Oxygen from Aerated Water into Nitrogenated Water. (Increase, pO_2 mm Hg/min of nitrogenated water/μ of tested material)

	Stratum corneum[a]	Cellulose acetate	Teflon	Aluminum foil[b]	Kodar
No. of experiments	22	21	9	9	6
Average	.008	.007	.014	0	0
Range	.001–.016	.002–.009	.008–.020	0	0
SD	.004	.002	.004	—	—

[a] Thickness of stratum corneum not measured; average thickness of 12 μ (8) used for calculations.

[b] Actual thickness of Al-foil not measured.

not differ significantly from that of cellulose acetate ($p = 0.15$) but was less than that of teflon ($p < 0.005$) and more than that of aluminum foil or Kodar, neither of which showed any permeability to oxygen.

Discussion. Our experiments provide, by simple means, some measurement of the passage of oxygen through the human stratum corneum and show that it is as permeable to oxygen as is cellulose acetate, a fairly "permeable" substance. In making such comparison, however, cognizance must be taken of the variability of our data, illustrated particularly by the wide range of results obtained with the individual sheets of stratum corneum; for one thing, the thickness of each of these sheets was not measured and their intactness was evaluated only by gross examination. Also, biological factors dependent upon the age and previous state of health of the patients from whom the skin specimens were obtained could also have accounted for some of this variability. Other experimental factors, such as the amount of stretch applied to the sheets of stratum corneum, and the other materials studied by us, as they were inserted in the chamber, could also play a role in this variability. More refined methods, similar to those used in standard *in vitro* experiments on membranes (9), probably would reduce this variability.

In a more sophisticated study, the dynamics of the passage of oxygen through sheets of material would have to be considered. We have, for instance, calculated the passage of oxygen per micron of tested material, but this disregards the effect of surface phenomena, which would be of relatively greater importance with thinner material. Also, the size of the molecules of the diffusing substance, the sites of its passage through the membrane, and its solubility in the various membranes and the wettability, conditions of storage, and internal structure of the various membranes are all pertinent.

The simplicity of our method, however, readily permits a variety of investigations. The efficacy of various therapeutic agents upon the oxygen permeability of the stratum corneum could be evaluated and, if found to be beneficial, such agents could then be tried on the intact skin. Since the survival of the ischemic extremity depends largely upon the viability of its skin, which has a relatively low oxygen consumption, the amount and depth of penetration of the externally applied oxygen may not have to be very great. Stratum corneum sheets obtained from the extremities would first have to be studied since ours were taken from the abdominal wall, entirely for practical reasons.

Summary. The rate of passage of oxygen through isolated sheets of human stratum corneum was measured *in vitro* and compared to other materials. There was a significant passage of oxygen through the stratum corneum, comparable to that through cellulose acetate, but less than through teflon, and more than through Kodar or Al-foil. Some limitations and therapeutic applications of these experiments are discussed.

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Relation of Pock Size on Chorioallantoic Membrane to Antigenic Type of Herpesvirus Hominis* (32861)

ANDRE J. NAHMIAS,[1] WALTER R. DOWDLE, ZUHER M. NAIB, ANITA HIGHSMITH, REBECCA W. HARWELL, AND WILLIAM E. JOSEY (Introduced by G. R. Cooper)

Departments of Pediatrics, Preventive Medicine, Pathology and Obstetrics-Gynecology, Emory University School of Medicine and the National Communicable Disease Center, Public Health Service, U.S. Department of Health, Education and Welfare, Atlanta, Georgia 30333

Recently we have observed that strains of herpesvirus hominis (HVH) could be differentiated antigenically into two types. Type 1 was found to be primarily associated with nongenital infection and transmission, whereas type 2 was primarily associated with genital infection and transmission (1). In order to further characterize the two antigenic types, biological markers were sought. Other investigators (2–4) found that the pock size on embryonated egg chorioallantoic membrane (CAM) with certain HVH strains were larger. In addition, various types of histological changes have been observed (5–8) in the CAM after infection with different HVH strains. This report presents studies on the relation of pock size on CAM to antigenic type of 79 strains of HVH which were isolated from genital and nongenital sites, and suggests the use of this technique as a presumptive test for the differentiation of HVH strains. Histological studies of the CAM infected with serotypes 1 and 2 also are included.

Materials and Methods. Virus strains. The source and passage history of the majority of the strains used for this study have been described (1). In addition, 4 strains isolated from eye infections, 2 from infections of newborns and a vaginal isolate from an 8-year-old girl are included. The majority of strains were primary isolates with 1 or 2 tissue culture passages, mainly in primary rabbit kidney cells. Only 2 strains had prior egg passage.

Egg inoculation. Embryonated eggs 10–12 days old were inoculated with 0.1 ml of varying dilutions of virus on the CAM using the false air sac technique (9). At least 2 eggs were inoculated with each virus dilution and the eggs were incubated at 34–35°C for 3–4 days. To avoid the possible effect of population density on pock size, measurements were made, whenever feasible, when the number of pocks was between 20 and 100. The membrane was removed onto a plate containing saline. The morphology of the pocks was examined and the diameter of the pocks was measured with the aid of an ocular micrometer mounted to the eyepiece of a binocular dissection microscope. Strains which did not produce pocks with undiluted virus were tested at least twice.

Criteria for "large" and "small" pocks. Up to 20 pocks were measured for each strain. Virus strains were divided into those producing "small" or "large" pocks on the basis of the following criteria: Small—average diameter of pocks counted was less than 0.5 mm and diameter of any one pock did not exceed 1.0 mm (Fig. 1). Large—Average diameter of pocks counted was greater than 0.5 mm and many pocks exceeded 1 mm in diameter (Fig. 2). Pock size measurements were made on

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