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GEOMETRICAL AND APPLIED OPTICS

Skin Spectrophotometry under the Islet Photothermal Effect on the Epidermal Permeability

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Abstract—The results of experimental investigation of an increase in the epidermal barrier permeability are presented. The results are obtained using the method based on the creation of permeability microzones (islets) during the topical thermal action on stratum corneum. It is shown that the epidermal barrier permeability considerably increases under the island photothermal effect and application of various clearing agents. Detailed spectrophotometric investigations of skin under the conditions of a partial violation of the epidermis barrier functions due to the island effect and introduction of various immersion agents in the skin are presented. The effect of various conditions of the object illumination on the dynamics of spectra is analyzed. A common behavior of the dynamics of the spectra of all kinds during clearing is revealed. Possible mechanisms accompanying the optical clearing process under the island photothermal effect are discussed.

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INTRODUCTION

Spectral and optical measurements hold an important position among modern diagnostic tools of the states of biological systems. Analysis of the spectral characteristics of light transmitted through and/or reflected from a biological tissue can yield diagnostically important information on its composition and structure. However, the majority of biological tissues strongly scatter light and weakly absorb in the visible and near-IR ranges. Such a high scattering restricts the visualization depth and the possibility of spectroscopic study of deep-lying tumors. This considerably reduces the resolving power of optical methods of biomedical diagnostics and prevents the application of the local action upon using phototherapeutic techniques. A sufficiently efficient method to significantly reduce the light scattering is optical immersion, i.e., matching the refractive index of scattering centers (for example, in skin, components of keratinocytes in epidermis, collagen and elastin fibers of dermis) with that of the base (intratissular) substance via introduction of an exogenous biologically compatible chemical agent. As such agents, glucose, glycerol, polyethylene glycol, propylene glycol, radiopaque contrast agents (e.g., sodium amidotrizoate), etc., are applied. The structure and physicochemical organization of many tissues allow their scattering properties to be varied not only by the introduction of immersion liquids, but also by the action of other physical and chemical effects, such as, e.g., compression, extension, dehydration and coagulation of tissues, etc. [1–7].

Recently, much progress has been made in developing techniques for controlling the optical parameters of various biological tissues (sclera, skin, dura mater, bone tissue, etc.). Such techniques are important both for determining the optical characteristics of biological tissues and for developing medical technologies of diagnostics and therapy [1–9]. However, strong scattering of optical radiation by human skin frequently restricts the application of these methods.

At present, the majority of the methods for immersion clearing of biological tissues are based on subcutaneous or intracutaneous administration of an immersion agent in the tissue [7], because the natural barrier function of epidermis (especially, of stratum corneum) considerably retards the diffusion of an immersion agent into the tissue, which makes the surface application of the agent inefficient. Therefore, slow diffusion of immersion liquids and gels through the epidermal barrier complicates the practical application of this method.

In connection with this, we proposed a method for enhancing the diffusion of immersion liquids by increasing the permeability of the epidermal barrier. This method is based on the creation of microzones (islets) of permeability [10] in stratum corneum by subjecting it to the topical thermal action [11–13].

This study presents detailed spectrophotometric investigations of skin in which the barrier functions of the epidermis are partially violated as a result of the thermal island action and introduction of various immersion agents. Based on these investigations, we will show that it is possible to considerably increase the

rate of permeability of immersion substances through an epithelial barrier and to achieve a high efficiency of clearing of tissue. The ultimate goal of these investigations is to increase the optical probing depth of tissues for early detection of malignant tumors and optimal photodestruction of neoplasms.

MATERIALS AND METHODS

All experiments were performed with samples of ex vivo pig skin and ex vivo human skin. Samples of pig skin were taken from the abdominal part of a 1.5-month-old pig. Before each experiment, the skin was stored in physiological solution. Subcutaneous cellular tissue and hair coat were carefully removed immediately before the measurements. The samples of pig skin ex vivo were 1 mm thick.

The samples of human cadaver skin were stored in physiological solution before experiments. For measurements, the samples were cut into 2×2 cm squares, subcutaneous fat was removed, and the epidermis surface was wiped with ethanol to remove possible fatty contaminants. The final sample thickness was about 2 mm.

As immersion liquids, we used a 40% solution of glucose, a 60% solution of glycerol, and a 40% solution of propylene glycol. These substances were chosen due to their biological compatibility and wide use in cosmetology.

The topical thermal action on the epidermis was obtained by using an island mask with a pattern of centers of a light absorbing pigment (the size of the absorbing centers was ~150 μm, and the intercenter spacing was ~500 μm), which was illuminated with a flashlamp (ExteLux, Palomar Medical Technologies, Inc., USA). The size of the island mask 1×2 cm corresponded to the size of the output window of the flashlamp. A microscopic image of the mask is shown in Fig. 1.

The mask was tightly applied to the sample to achieve an intimate contact between the light absorbing centers and the epidermis and was illuminated with light pulses from the flashlamp. The number of pulses was varied to determine the optimal regime of the illumination. After the illumination, the mask was removed; the samples were wiped with ethanol and were placed on a stage with a holder of a receiving fiber of the experimental setup (Fig. 2). The reference samples were illuminated with the same number of pulses without applying the island mask. Then, clearing liquids were applied to the sample under study and spectrophotometric measurements were performed. The transmission spectra were measured every 5 min within 1 h.

Figure 2 shows the schematic of the experimental setup. As a source, we used a halogen lamp equipped with a fiber-optic cord, which yielded a broad uniform beam to illuminate the sample on the stage. The stage had a hole into which a receiving fiber-optic sensor of

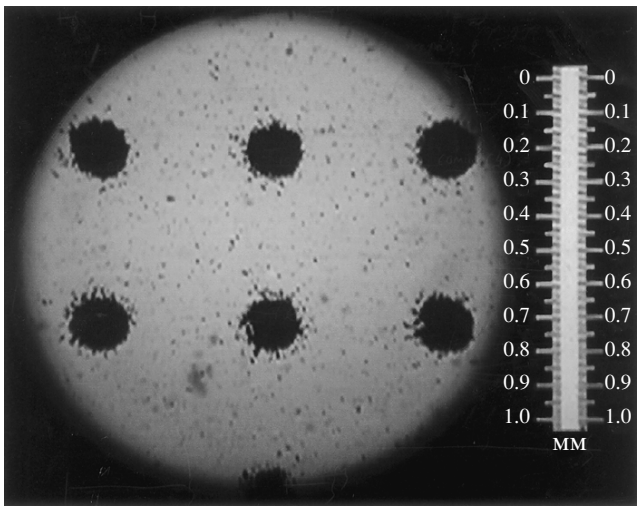

Fig. 1. Image of a matrix for the topical (island) action. The scale is 1 mm.

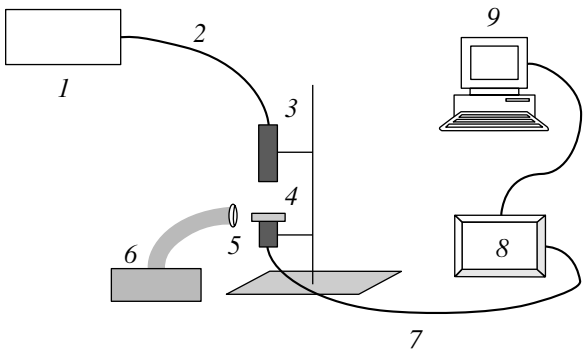

Fig. 2. Schematic of the experimental setup for the topical (island) action: (*1*) radiation source (halogen lamp), (*2*, *3*) optical fiber for the delivery of radiation, (*4*) sample of skin, (*5*, *7*) optical fiber for the collection of radiation, (*6*) air heater, (*8*) Lesa 7med spectrum analyzer, and (*9*) personal computer.

an optical spectrum analyzer (Lesa 7med) was inserted. An air heater positioned to the left of the sample was used to facilitate the penetration of the immersion agent into the bulk of the tissue. The temperature of this heater was monitored by a thermometer placed nearby and was about 50°C. The temperature on the skin surface amounted to approximately 43°C. To prevent drying of the sample, it was persistently wet by the solution of the clearing liquid. The spectral measurements were performed in the range 380–1000 nm.

DISCUSSION

To estimate the clearing rate and to compare such rates in the case of different conditions of the thermal action on skin and using different clearing agents, we

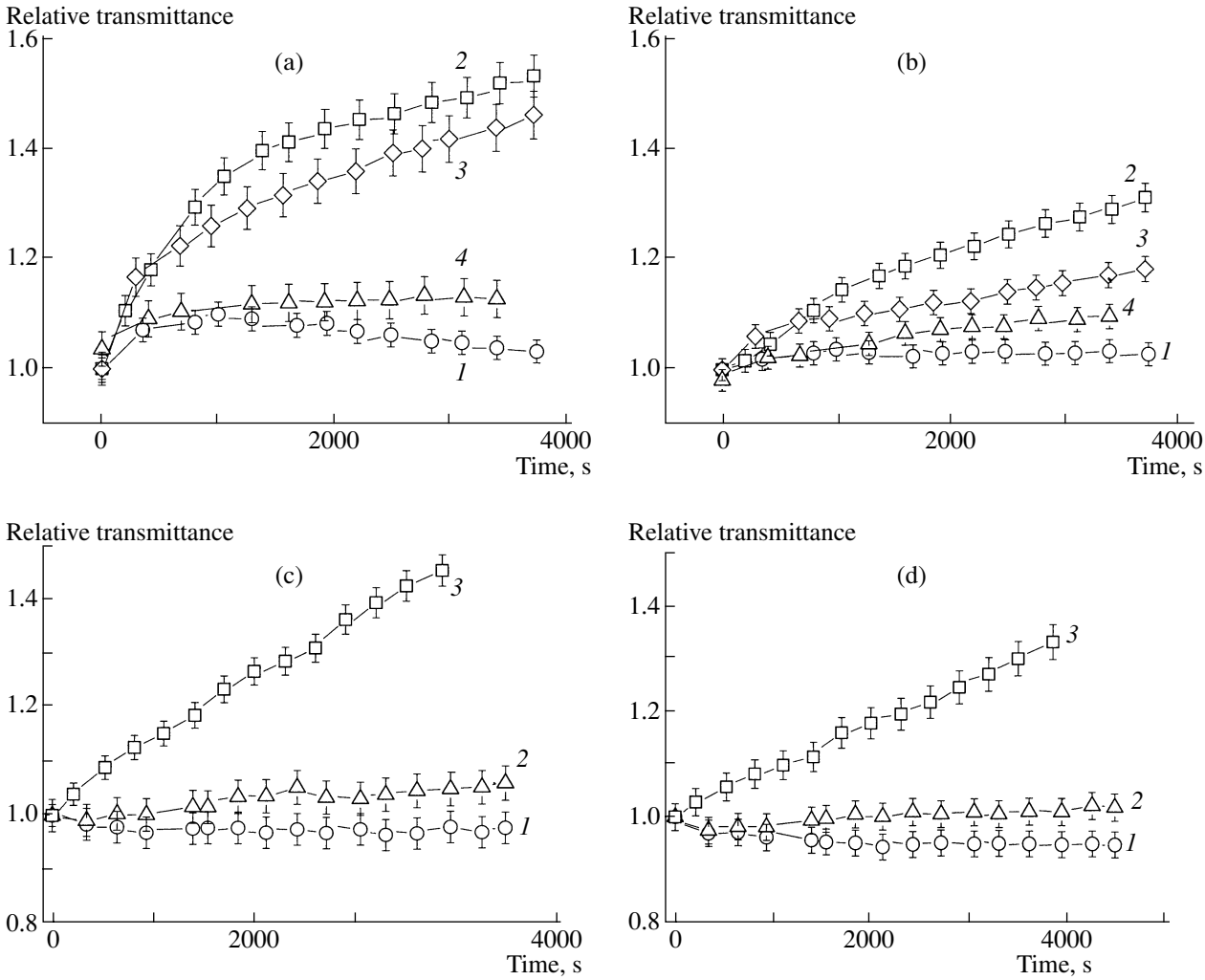

Fig. 3. Time dependence of the transmittance of pig skin illuminated by a narrow quasi-collimated pulsed beam (the pulse duration, 20 ms, and the energy density, 27 J/cm^2) at (a, c) 470 and (b, d) 650 nm. Curves in (a, b): (*1*) reference sample, (*2*) sample illuminated by one lamp pulse with applying the mask and by six pulses without the mask, (*3*) sample illuminated by one lamp pulse with applying the mask and by two pulses without the mask, and (*4*) sample illuminated by seven pulses. Curves in (c, d): (*1*) reference sample, (*2*) sample illuminated by four pulses, and (*3*) sample illuminated by two lamp pulses with applying the mask and by two pulses without the mask. The sample thickness is 1 mm, and the clearing agent is a 40% solution of glucose.

calculated the quantity that we term the relative transmittance,

$$T_{\text{rel}}(\lambda) = I_t(\lambda)/I_0(\lambda),$$

where $I_0(\lambda)$ is the intensity of light incident on the sample, while $I_t(\lambda)$ is the intensity measured at elapsed time t.

The dynamics of the relative transmittance of skin samples at 470 and 650 nm is presented in Figs. 3–7. It is seen from these figures that, upon application of clearing agents (aqueous solutions of glucose, propylene glycol, and glycerol) to the surface of intact epidermis within at least one hour, no changes in the transmittance of samples of pig and human skin are observed. The surface treatment or the sample epidermis by light pulses without applying the island mask made it possible to observe some clearing dynamics (Fig. 3). However, only if the island mask was used, were we able to achieve a considerable increase in the clearing rate (an increase in the intensity of the transmitted light) for all clearing agents used. The measured clearing rates exceeded 3–10 times the rates achieved without applying the mask.

The relative transmittance spectra calculated according to the formula presented for all the three clearing agents and the 1-h time interval are shown in Fig. 6a. It is clearly seen that the best values (the maximal increase in the transmitted light intensity) were obtained for glucose. However, it is hardly possible to compare the action of the clearing agents with respect to this parameter, because, in the case of glucose, the masked sample was illuminated by two light pulses,

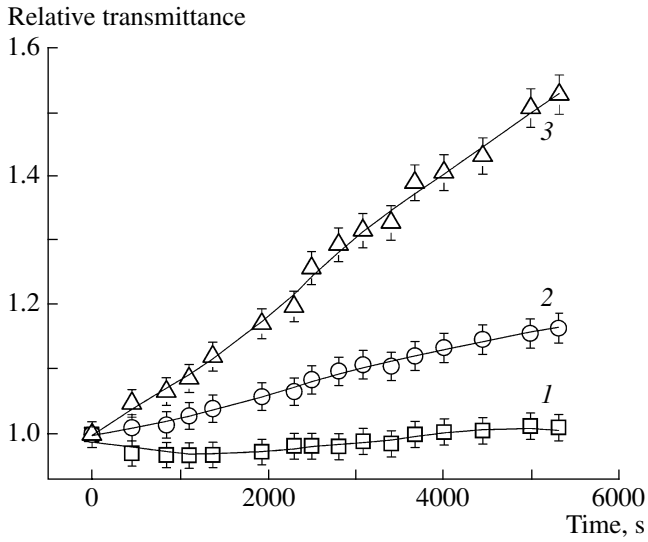

Fig. 4. Time dependence of the transmittance of human skin illuminated by a pulsed beam (the pulse duration, 20 ms, and the energy density, 27 J/cm^2): (*1*) reference sample illuminated by three pulses without applying the mask, (*2*) sample illuminated by one lamp pulse at 470 nm with applying the mask and by two pulses without the mask, and (*3*) sample illuminated by one lamp pulse at 650 nm with applying the mask and by two pulses without the mask. The sample thickness is 2 mm, and the clearing agent is a 40% solution of glucose.

whereas, in the remaining cases, by only one pulse, i.e., the diffusion conditions were different, while the clearing process was not brought to saturation. Nevertheless, the differences in the behavior of the spectral curves attract attention: in general, the slope of the curve for glycerol is greater. As was indicated above upon discussion of the previous series of measurements of the scattering spectra, this can be connected with a higher degree of matching of the refractive indices of scatterers the medium. It is seen that all the spectra presented exhibit the maxima of the α and β bands of hemoglobin and the tendency to a sharp increase upon approach the Soret band in the short-wavelength spectral range. All this allows us to state that the clearing spectra exhibit the manifestation of the absorption of hemoglobin, the main chromophore of the dermis. The clearing (the increase in the transmittance) in the range of the absorption bands is more efficient.

Some experiments revealed a strong dependence of the shape of the relative transmittance spectra on the geometric conditions of the experiment. Figure 7 shows the spectra obtained up illumination of the sample with (*1*) broad and (*2*) narrow light beams, with the detector geometry being the same. If the sample was illuminated by the broad uniform light beam, it was possible to achieve a more efficient clearing in the range of the hemoglobin absorption bands. In the case of the narrow beam, the increase in the transmittance in this range (more exactly, the detected light flux) due to the clearing was less efficient than in the ranges with the absorption was absent.

It is interesting to note that, upon illumination by the broad beam, the contribution of the "positive" effect of the hemoglobin absorption to the clearing efficiency increases as the clearing proceeds. This is clearly seen in the spectrum of clearing of the human skin with glucose (Fig. 6b).

To analyze the dynamics of the clearing spectra in more detail, as in the previous series of experiments, we

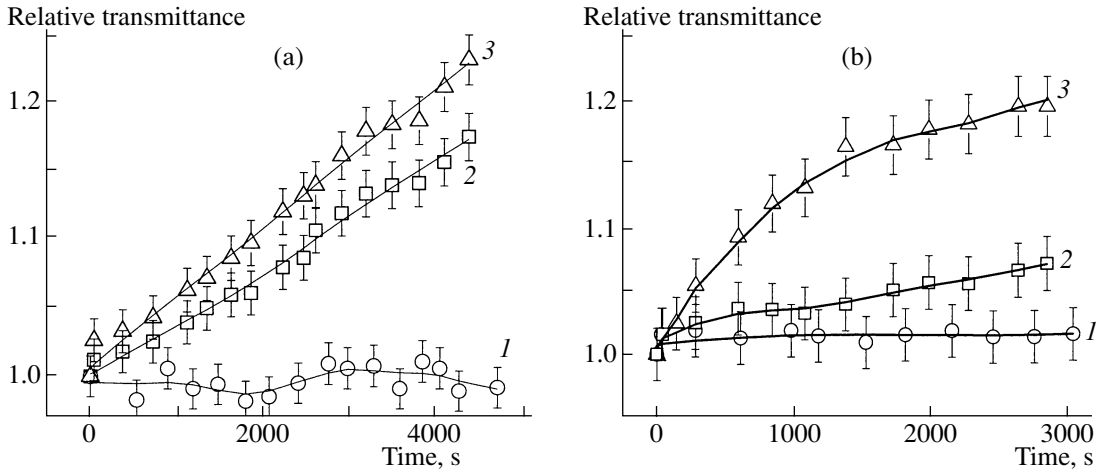

Fig. 5. Time dependence of the transmittance of samples of pig skin (the sample thickness is 1 mm) treated by a 60% solution of (a) propylene glycol and (b) glycerol and illuminated by a pulsed beam (the pulse duration, 20 ms, and the energy density, 27 J/cm^2): (*1*) reference sample illuminated by three lamp pulses without applying the mask, (*2*) sample illuminated by one pulse at 470 nm with applying the mask and by two pulses without the mask, and (*3*) sample illuminated by one pulse at 650 nm with applying the mask and by two pulses without the mask.

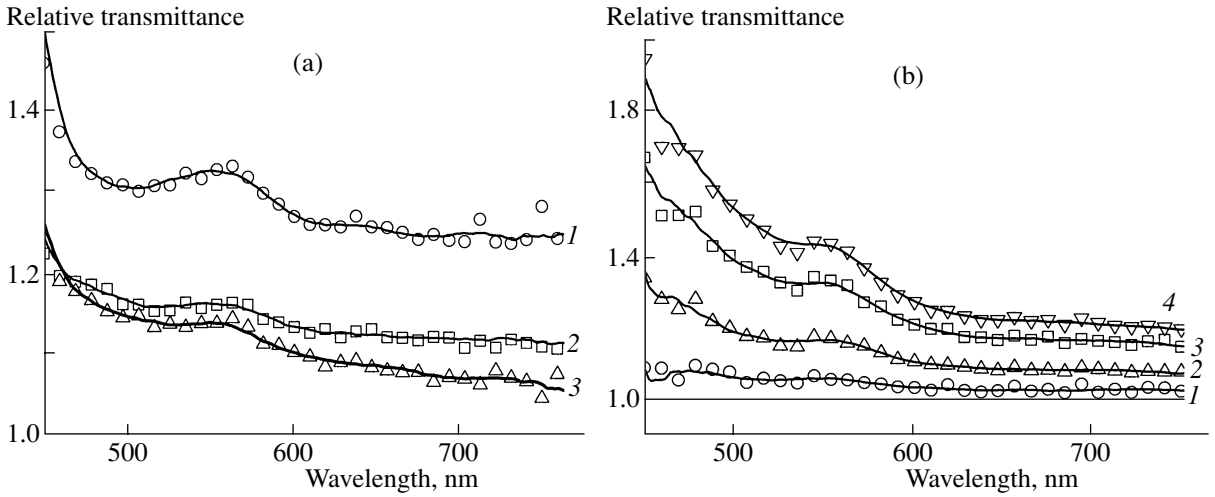

Fig. 6. (a) Relative transmittance spectra of samples of pig skin (the sample thickness is 1 mm) treated by (*1*) a 40% solution of glucose, (*2*) a 60% solution of propylene glycol, and (*3*) a 60% solution of glycerol and measured in the time interval 0–60 min. (b) Relative transmittance spectra of samples of human skin (the sample thickness is 2 mm) treated by a 40% solution of glucose and illuminated by one lamp pulse with applying the mask and by two pulses without the mask. The spectra were measured within (*1*) 15, (*2*) 30, (*3*) 45, and (*4*) 60 min. The energy density is 27 J/cm^2, and the pulse duration is 20 ms.

plotted the relative transmittance spectra for time intervals at the beginning of the clearing process and at the end of observations. For this purpose, we used the relation

$$T_{\text{rel}}(\lambda) = I_{t2}(\lambda)/I_{t1}(\lambda),$$

where $I_{t1}(\lambda)$ and $I_{t2}(\lambda)$ are the intensities measured at the wavelength λ at the elapsed times t_1 and t_2, respectively. In this case, $t_1 < t_2$ because the intensity of the transmitted light increases with clearing.

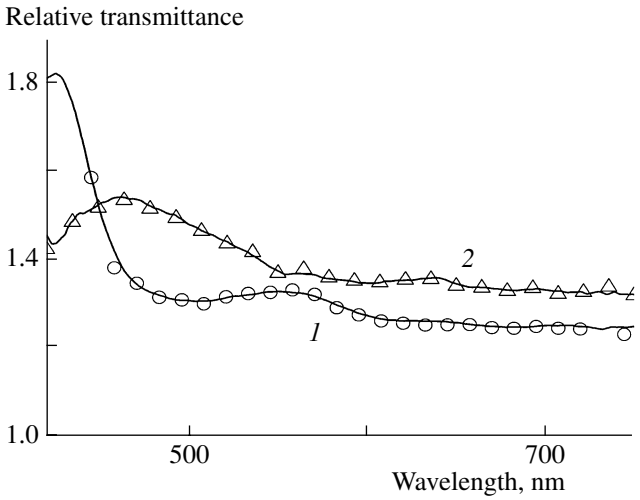

Fig. 7. Relative transmittance spectra of samples of pig skin cleared by a 40% solution of glucose and illuminated by (*1*) a broad beam and (*2*) a narrow quasi-collimated beam. The spectra were measured in the time interval 0–60 min.

Figure 8 presents the spectra of samples of pig skin cleared from the side of the skin epidermis and treated according to the method of creating the pattern of microislets of partial thermal damage of the stratum corneum. Such a "decomposition" of the spectral dynamics in time is shown for the two variants of illumination that were already discussed above. Figure 8a shows the spectra obtained upon illumination by the narrow beam. At the beginning of the process, the clearing prevails in the blue region (an increase in the transmittance, and a decrease in the scattering). At longer times, the spectral curve is smoothed, and, in experiments on measuring the transmittance in this time interval, the rate of clearing in the long-wavelength range is greater.

Analysis of analogous spectra obtained upon illumination by the broad band (Fig. 8a) shows that, at the early stage of clearing, the hemoglobin absorption bands are not observed, whereas, at long clearing times, their "positive" contribution is seen, with the spectral curve tending toward smoothing.

Based on the results obtained, we can propose possible mechanisms accompanying the optical clearing upon island photothermal action. The application of the method proposed results in a local decrease in the thickness of the stratum corneum (the local tissue ablation) in the zones of an increased light absorption (the dark areas of the mask, see Fig. 1). For the illumination regime used (the energy density 27 J/cm^2, the pulse duration 20 ms), an approximate depth of such zones is about 25 μm. In addition, in these islets, the living epidermis under this thermal action becomes more permeable but cells remain living. One can temporarily increase the permeability of a cell without destroying the cell by various physical actions, for example, by

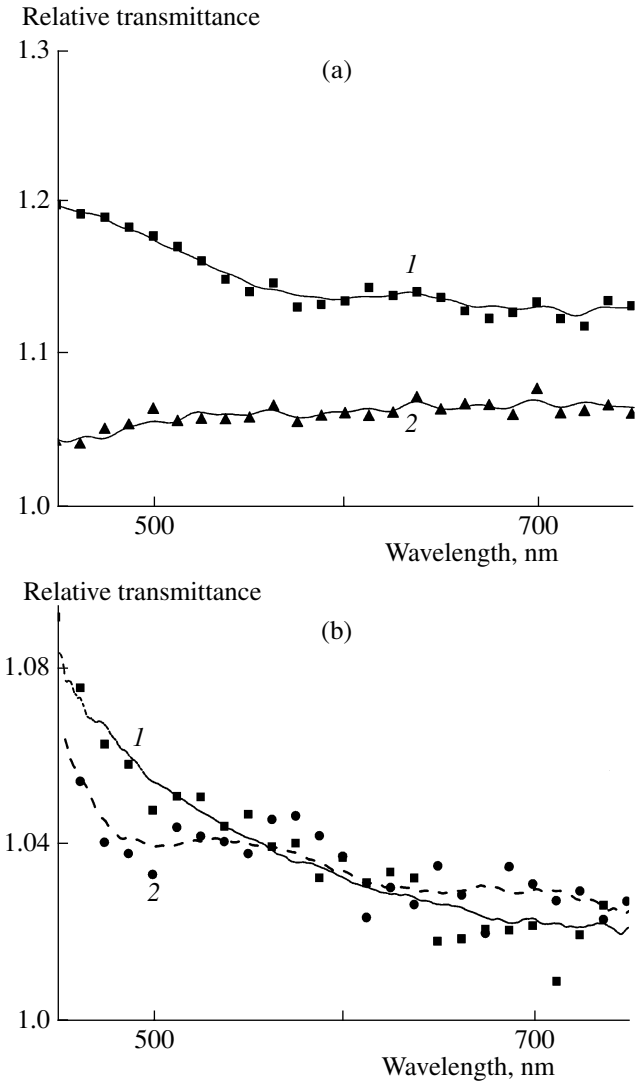

Fig. 8. Relative transmittance (*1*) 750/0 and (*2*) 3000/1500 spectra of samples of pig skin (the sample thickness is 1 mm) treated by (a) a 40% solution of glucose and (b) a 60% solution of propylene glycol and illuminated (a) by one pulse of a narrow quasi-collimated beam with applying the mask and six pulses without the mask and (b) by one pulse of a broad beam with applying the mask and two pulses without the mask. The energy density is 27 J/cm^2, and the pulse duration is 20 ms.

exciting acoustic waves by a pulsed laser (the photomechanical waves) [1]. With this method, it was demonstrated in [14] for the first time that the permeability of the stratum corneum for aminolevulinic acid, which is used in photodynamic therapy of tumors. Comparing the method used in the present study with the photoacoustic action on membranes of living cells of epidermis, it is necessary to compare the pulse durations. In [14], the pulse duration was short, being about 250 ns, whereas, in our study, we used the light from the flashlamp with an energy density of 27 J/cm^2 and considerably longer pulse duration, about 20 ms. The action of such light is equivalent to the action of continuous radiation, which is more likely thermal in character rather than acoustical.

The partial perforation (as a result of the island ablation) of the stratum corneum and the moderate thermal action on cell membranes of the living epidermis (as a result of the increase in their permeability, because phase transitions (between the liquid crystal phase and the liquid phase) occur in lipids at elevated temperatures, which facilitates increasing their permeability) [15], makes it possible to considerably increase the skin permeability for immersion agents and drugs.

CONCLUSIONS

We showed that the pulsed optical action causes a considerable increase in the optical clearing rate for all the immersion liquids used. We tested the method of the creation of the pattern of microislets of a partial thermal damage of stratum corneum and showed that the application of this method makes it possible to increase the permeability of an epithelial barrier and to perform immersion clearing of skin upon surface application of clearing agents. We found that the dynamics of all the spectra in the course of clearing has a common regular feature: at the initial stage, the clearing is more efficient in the short-wavelength spectral range, whereas, at the end of the process, the clearing rate levels off, which is connected with the saturation of the process. We revealed that the spectra obtained under different conditions of the sample illumination have substantially different dynamics. In the case of illumination with a narrow beam, the efficiency of clearing (of increasing the intensity of light transmitted through skin) for wavelengths in the range of the hemoglobin absorption bands is lower compared to neighboring spectral ranges. The method proposed makes it possible to more efficiently control the optical characteristics of skin. It should be expected that this method will make it possible to considerably increase the depth of the optical probing of tissues, improving the visualization of hidden inhomogeneities and neoplasms in surgery and therapy.

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