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Percutaneous Absorption of Organic Solvents during Intermittent Exposure in Guinea Pigs

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Skin absorption under intermittent exposure of guinea pigs to n-butanol, toluene, 1,1,1-trichloroethane was studied. Groups of guinea pigs were exposed to test organic solvents for 1 min at 30-min intervals during 4 h, in all 8 exposures. Skin absorption of solvent was assessed by following the concentration of solvent in the blood. This intermittent exposure was compared to continuous exposure over 4 h. Absorption of toluene and 1,1,1-trichloroethane was low, but a considerable amount of butanol was absorbed through the skin on intermittent exposure. A typical serrated absorption profile was seen for butanol that was less pronounced for toluene and 1,1,1-trichloroethane. The absorption of butanol was highest at the end of the exposure period. The differences in absorption profiles may be due to the differences in vapour pressure in the solvents in association with the animal method used. The amount absorbed varied inversely with vapour pressure. Hair stubble may act as a trap for solvents with low vapour pressure. Adequate ventilation reduces unoccluded skin absorption of volatile organic solvents.
Key words: n-butanol; toluene; 1,1,1-trichloroethane; skin exposure; in vivo; animal model.

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Organic solvents are extensively used in many industrial applications. They are used as degreasers, cleaning agents, solvents for plastics and lacquers and in paints. That percutaneous absorption of organic solvents occurs is well known and has been quantified for several solvents. Whether *in vitro* or *in vivo*, experimental studies are generally done under continuous exposure conditions, resulting in steady-state absorption situations (1–9). Continuous exposure provides data for calculation of the pertinent parameters for risk assessment, absorption rates and permeability coefficients – Kp. (10–12). However, continuous exposure under steady-state conditions to solvents is usually not the prevailing situation in industry. Frequent exposures of shorter duration are more common when handling soiled goods or cleaning machinery or manufactured goods, and an experimental situation with intermittent exposure is thus more true to the real situation (1).

The aim of the present study was to investigate the percutaneous absorption of three solvents with various physico-chemical properties – n-butanol, toluene and 1,1,1-trichloroethane – during brief intermittent skin exposures using an animal model.

MATERIAL AND METHODS

Exposure, sampling and analysis of solvents were performed according to a previously published technique (7, 8), described briefly below.

Female guinea pigs, average weight of 600 g, were anaesthetized with pentobarbital sodium (Vitrum, Stockholm). The hair on the back was carefully cut with electric clippers, leaving 0.1 mm stubble, and a glass ring (20 mm diameter) was glued to the skin with a cyanoacrylate glue (Varibond, Thordon Industries, Sollentuna, Sweden). A polyethylene catheter (o.d. 0.97 mm) was inserted into the carotid artery, and 0.5 ml heparinized saline was administered. Blood was subsequently withdrawn for individual standard and blank. Five animals were used in each experiment.

The solvents studied were toluene, n-butanol (Merck Ag, Darmstadt, Germany) and 1,1,1-trichloroethane (Fluka Ag, Buchs, Switzerland), all of analytical quality and used as delivered. The neat solvents were administered into the depot formed by the glass ring, which was covered with a glass lid held in place with a rubber band around the animal and the board to which it was fixed.

The solvent was left on the skin for *1 min* and then removed with a pipette. The skin was then gently wiped with a cotton pad and left to air-dry. Solvent exposure was repeated every 30 min. Each animal was exposed totally for 8 × 1 min. During the experiment the animals were provided with fresh air to breathe by being placed on a ventilated bench.

Blood samples (0.5 ml) were taken every 10 min throughout the

Table I. *Concentration of toluene in blood in guinea pigs (mean±sd) during intermittent and continuous exposure*

S = significant difference, $p < 0.05$, continuous exposure from ref 7.

Exposure time (min)	Intermittent exposure		Continuous exposure		
	Blood concentration Mean ±sd (μM)	n	Blood concentration Mean ±sd (μM)	n	*t*-test
0	0.00	5	0.00	29	S
10	2.30±1.79	5	10.21±5.02	29	S
20	1.54±1.74	5	12.99±5.78	29	S
30	1.17±1.41	5	14.49±6.66	29	S
40	3.51±2.06	5	14.59±7.13	29	S
50	3.28±2.73	5	14.66±7.77	29	S
60	2.26±1.97	5	14.41±8.54	29	S
70	3.81±1.86	5	14.22±8.48	29	S
80	2.37±1.23	5	13.61±7.89	29	S
90	1.51±0.69	5	13.41±7.72	29	S
100	2.98±1.10	5			
105			12.51±7.54	29	
110	2.00±0.89	5			
120	1.40±0.58	5	12.35±7.01	29	S
130	2.53±1.34	5			
140	1.59±0.76	5	12.10±6.37	29	S
150	1.32±0.63	5			
160	2.34±0.94	5	11.01±6.43	29	S
170	1.32±0.65	5			
180	2.34±1.32	5	10.99±6.31	29	S
190	2.06±0.58	5			
200	2.34±1.54	5			
210	1.01±0.36	5	10.04±5.84	29	S
220	1.86±0.47	5			
230	1.30±0.36	5			
240	0.99±0.40	5	9.37±4.62	29	S

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Table II. *Concentration of 1,1,1-trichloroethane in blood in guinea pigs (mean ± sd) during intermittent and continuous exposure*

S = significant difference, $p < 0.05$, continuous exposure from ref 7.

Exposure time (min)	Intermittent exposure		Continuous exposure		
	Blood concentration Mean ±sd (μM)	n	Blood concentration Mean ±sd (μM)	n	*t*-test
0	0.00	5	0.00	19	
10	0.62±0.34	5	6.07±3.33	19	S
20	0.32±0.13	5	9.06±4.74	19	S
30	0.20±0.09	5	10.30±6.24	19	S
40	0.67±0.27	5	10.11±5.75	19	S
50	0.38±0.11	5	9.19±3.92	19	S
60	0.38±0.29	5	8.77±3.76	19	S
70	0.67±0.27	5	8.49±3.69	19	S
80	0.46±0.07	5	8.15±3.56	19	S
90	0.28±0.02	5	7.54±3.40	19	S
100	0.30±0.58	5			
105			7.10±3.10	19	
110	0.48±0.13	5			
120	0.49±0.27	5	6.75±3.10	19	S
130	0.58±0.16	5			
140	0.46±0.16	5	6.50±2.88	19	S
150	0.33±0.11	5			
160	0.78±0.78	5	6.12±2.29	19	S
170	0.43±0.18	5			
180	0.29±0.07	5	5.62±2.29	19	S
190	0.59±0.40	5			
200	0.42±0.20	5			
210	0.30±0.13	5	5.27±2.19	19	S
220	0.67±0.45	5			
230	0.43±0.20	5			
240	0.32±0.16	5	4.97±1.69	19	S

whole experiment and were put into glass sample vials, which were sealed with aluminium foil and a rubber membrane. After each sample, the blood volume was restored by injecting 0.5 ml heparinized saline solution. The solvent concentration in the blood was analysed with a gas chromatograph using a head space technique. A sample, 0.5 ml, of the head space volume in the sample vial was injected into the gas chromatograph. A Varian 3700 equipped with a flame ionization detector was used. N_2, maintained at 30 ml/min, was used as carrier gas and 20% methyl silicone SE-30 on Chromosorb (80/100 mesh) as stationary phase in a stainless steel column. Injector temperature was kept between 110–150°, column between 90–130° and detector 120–190° depending on which solvent was analyzed (8). Groups of 5 animals were exposed for each solvent.

Continuously exposed animals from previous investigations were used as controls (toluene $n = 29$, 1,1,1-trichloroehane $n = 19$, n-butanol $n = 16$) (7). Mean blood concentration of solvent in blood samples from coinciding sample times was analysed using the *t*-test for statistical difference between the continuously and the intermittently exposed animals. As control for butanol at 190 min and 220 min intermittent exposure, the values at 180 and 210 min were used. *P*-values ≤ 0.05 were considered significant. As the number in the exposed group and the control group was different, a test for equal variances was performed prior to the *t*-test.

RESULTS

Intermittent exposure (8 × 1 min) to solvents in guinea pigs gave detectable amounts of solvent in the blood (Tables I–III). The blood concentrations differed markedly from those following continuous exposure during the same period, 240 min (8). The highest blood concentrations were seen for butanol, followed by toluene and 1,1,1-trichloroethane (Figs. 1–3). The blood concentrations fluctuated during the exposure period, increasing in the sample taken immediately after the exposure and then decreasing again. This variation was most pronounced for butanol, less for toluene and barely detectable for 1,1,1-trichloroethane. The samples taken 10 min after each exposure were generally higher than the following ones within each exposure period. For butanol there was a steady increase in amplitude of the blood concentrations during the consecutive exposures (Fig. 3). This was not seen for the other two solvents studied (Figs. 1, 2). For toluene and 1,1,1-trichloroethane, all samples in the intermittently exposed animals were statistically different from those taken at the same time interval in the continuously exposed animals. For butanol, all coinciding samples except those at 70 and 80 min were significantly lower than the continuously exposed controls. The samples at 190 and 220 min were tested statistically against the immediately preceding values from continuous exposure, as no values at those sample times for in-

Table III. *Concentration of n-butanol in blood in guinea pigs (mean ± sd) during intermittent and continuous exposure*

S = significant difference, NS = no significant difference, $p < 0.05$, continuous exposure from ref 7.

Exposure time (min)	Intermittent exposure		Continuous exposure		
	Blood concentration Mean ±sd (μM)	n	Blood concentration Mean ±sd (μM)	n	*t*-test
0	0.00	5	0.00	16	
10	2.13±1.77	5	6.11±8.47	16	NS
20	1.15±0.74	5	4.98±0.36	16	S
30	0.82±1.12	5	7.35±5.07	16	S
40	2.50±1.45	5	11.14±7.88	16	S
50	0.96±0.27	5	8.38±4.75	16	S
60	0.58±0.54	5	8.64±4.96	16	S
70	5.62±4.45	5	10.51±6.64	16	NS
80	2.00±1.45	5	14.88±17.00	16	NS
90	0.59±0.72	5	12.59±7.39	16	S
100	6.20±5.23	5			
105			12.86±6.58	16	
110	0.15±0.92	5			
120	0.19±3.13	5	16.03±9.71	16	S
130	6.85±6.24	5			
140	2.43±2.91	5	15.66±2.39	16	S
150	1.43±1.45	5			
160	7.93±7.58	5	16.95±8.15	16	S
170	3.24±3.71	5			
180	0.81±5.37	5	15.95±8.64	16	S
190	10.26±14.00	5			NS[1]
200	4.35±6.57	5			
210	6.85±12.81	5	22.11±12.74	16	S
220	12.43±14.96	5			NS[2]
230	6.64±8.68	5			
240	2.29±2.53	5	25.63±16.84	16	S

[1] 190 min intermittent exp tested against 180 min continuous exp
[2] 220 min intermittent exp tested against 210 min continuous exp

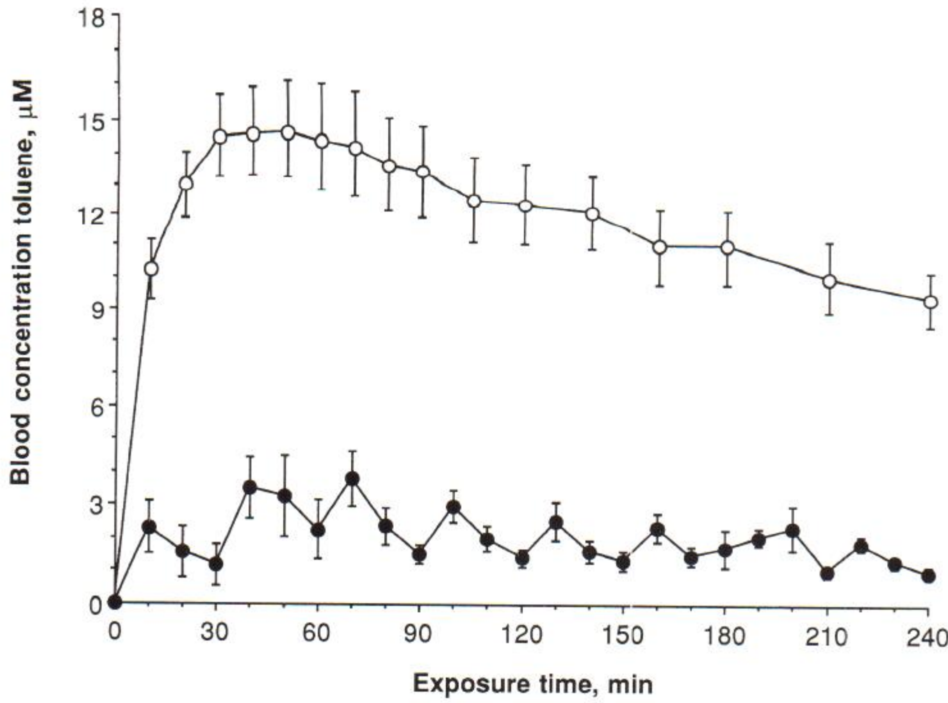

Fig. 1. Percutaneous absorption of toluene in guinea pigs under continuous (–○–) (mean ± SEM, $n = 29$) and intermittent exposure conditions (–●–) (mean ± SEM, $n = 5$). Exposure 1 min each 30 min (Data for continuous exposure from ref 7).

termittent exposure were available. No statistically significant difference was found.

To get an approximation of the total dose absorbed during the exposure, we calculated the area under the time-versus-blood-concentration curve (AUC) with triangulation for each solvent and for the control data, i.e. continuous absorption. The relationship between AUC at continuous exposure and AUC during intermittent exposure versus the vapour pressure was plotted for each solvent. This revealed a non-linear, inverse relationship (Fig. 4) between the solvent vapour pressure and the relative absorbed amount. The solvent with the lowest vapour pressure gave the highest absorbed dose.

DISCUSSION

The three solvents showed markedly different absorption profiles during intermittent exposure. Earlier studies with the same solvents at continuous exposure showed a difference in absorp-

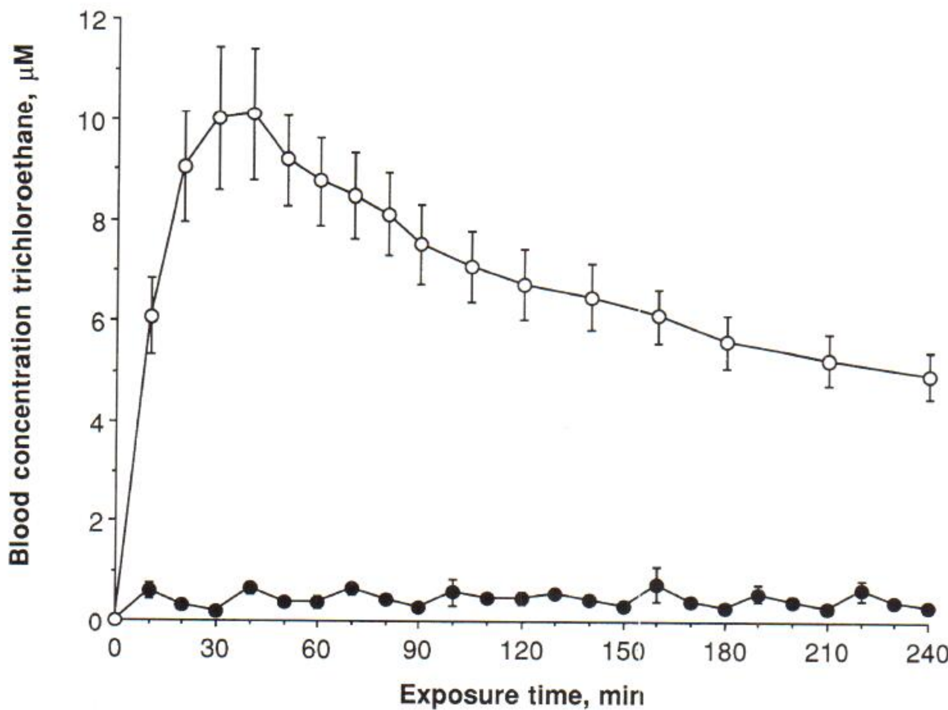

Fig. 2. Percutaneous absorption of 1,1,1-trichloroethane in guinea pigs under continuous (–○–) (mean ± SEM, $n = 19$) and intermittent exposure conditions (–●–) (mean ± SEM, $n = 5$). Exposure 1 min each 30 min (Data for continuous exposure from ref 7).

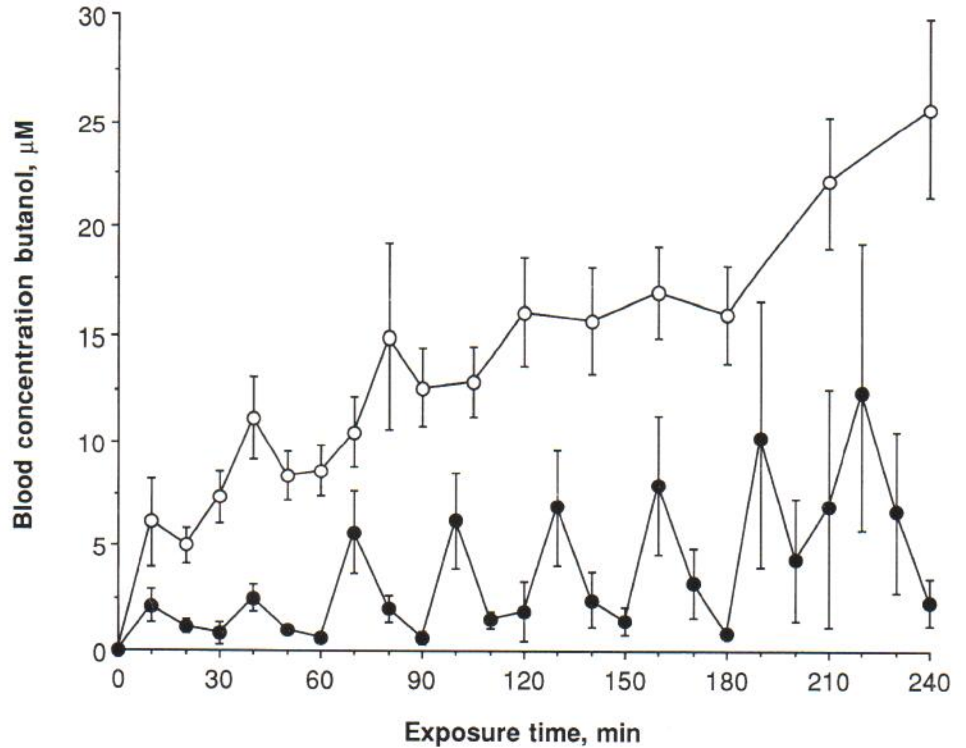

Fig. 3. Percutaneous absorption of n-butanol in guinea pigs under continuous (–○–) (mean ± SEM, $n = 16$) and intermittent exposure conditions (–●–) (mean ± SEM, $n = 5$). Exposure 1 min each 30 min (Data for continuous exposure from ref 7).

tion profiles (7, 8). Toluene and 1,1,1-trichloroethane showed a rapid increase with a peak in skin absorption followed by a steady-state absorption at a lower level, a kinetic behaviour also noted for toluene in rats (9). Butanol on the other hand showed a steady increase in blood concentration during the whole exposure time, establishing steady-state absorption later (7).

The difference in absorption kinetics between butanol, on the one hand, and 1,1,1-trichloroethane and toluene on the other was also seen in the intermittent exposure situation. The peak blood concentration of butanol, just after each exposure, increased as

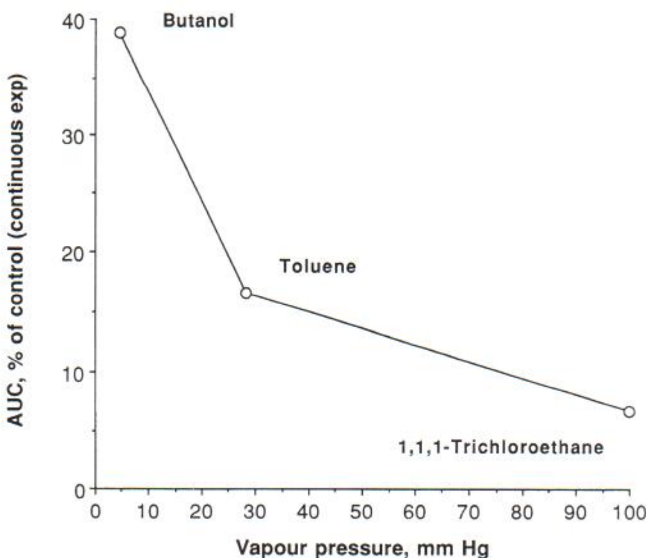

Fig. 4. Relative total percutaneous absorption of three organic solvents in relation to their vapour pressure.

the exposures were repeated, whereas those of toluene and 1,1,1-trichloroethane did not show this increase.

Evidence of both increased and unaltered absorption on repeated exposures is found in the literature. Several experiments with repeated skin exposure with drugs reveal an increased percutaneous absorption (13–18). These changes are most pronounced when compounds with known barrier-altering properties (13) or when barrier-altering treatments such as preexposure washing are used (16).

Solvents are known to have a profound action on the barrier properties of the skin. Their lipophilicity results in the extraction of lipid compounds of the skin, i.e. the surface and intercellular lipids. This causes light to severe local reactions – whitening of the skin, dryness, irritation and erythema, as shown in several experimental studies (20–22) as well as clinical experience (23–25). Although a severe barrier-altering effect can be expected from lipid extraction, of two of the three solvents used, due to their relatively high lipophilicity (toluene log $p^{o/w}$ 2.69, 1,1,1-trichloroethane log $p^{o/w}$ 2.49), the only solvent with a substantial increase in absorption during the repeated exposure was butanol, the one with the lowest lipophilicity (log $p_{o/w}$ 0.88).

Additionally, the present study used relatively short time periods between exposures (minutes), compared to the studies with drugs (days), and this may result in a depletion of the intercellular lipids from extraction and lack of time for new synthesis. However, in other experimental systems no effect on absorption at repeated administration has been found (15,17,19). In a study of skin absorption of 1,1,1-trichloroethane, Stewart & Dodd found a great difference in total absorption at continuous exposure, compared to intermittent exposure of the same area for approximately 60 times during 30 min (1).

The differences in absorption of the solvents on repeated

contact may also be attributed to the high vapour pressure of toluene (28.5 mm Hg) and 1,1,1-trichloroethane (120.7 mm Hg), compared to butanol (6.18 mm Hg at 25°C) (26). This results in the evaporation of toluene and 1,1,1-trichloroethane from the exposure site when the short contact with the liquid solvent has ended. The driving force, the concentration gradient across the skin membrane, is therefore reduced in both directions by the combined actions of the evaporation and the transport of penetrating compound by the blood. The importance of evaporative loss in percutaneous exposure has been demonstrated for a number of compounds (27,28). It is shown there that loss of compound due to evaporation ranges between a few per cent and as much as 65% of the applied dose when air flows are passing over the exposed skin and that the compounds with the highest vapour pressure give the largest evaporation loss.

Further, the fine stubble left on the skin from the fur, in our experimental study, may trap small amounts of solvent with a low evaporative loss, resulting in a local high air concentration and thus maintaining a concentration gradient across the skin. This is a disadvantage of the animal model compared to human exposure, as has been pointed out in earlier studies of irritation (21). The influence of evaporation is also demonstrated in Fig. 4. The difference in the total amount of absorbed solvent is thus, in a non-linear fashion, inversely related to the volatility, i.e. the vapour pressure.

The increasing blood concentration of butanol during the later phase of exposure may also be influenced by accumulation and saturation of solvent in the skin during the evaporation period. In addition to this there is a difference in the percutaneous absorption flux and permeability coefficients for the solvents. The absorption flux for butanol has been reported to be 0.65 $\mu mol/cm^2 \times h$ (29), for toluene 2.7 $\mu mol/cm^2 \times h$ (30) and for 1,1,1-trichloroethane 2.76 $\mu mol/cm^2 \times h$ (2). This slower absorption of butanol may result in an accumulation and a formation of a depot of the solvent in the skin. The slower absorption in the skin in both directions after the exposure periods thus gives a saturation effect in the skin, which at the next exposure gives a shorter period for the solvent to reach the blood stream.

The results from intermittent exposure to the three solvents used in the study suggest that a workplace with adequate ventilation is of great importance for reducing not only the respiratory absorption of solvents but also the unoccluded absorption of solvent through the skin after exposure, as the liquid is also lost to the ventilating air stream from the surface of the skin.

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