VISCOUS FLOW IN COMPRESSED HUMAN AND RAT SKIN*

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When pressure is applied to skin, the tissue is compressed *i.e.* it becomes thinner. This is a commonplace event which can be seen if a fingernail is pressed into the skin over a knuckle. It has been very little studied; the few observations which are available (1-3) are only semi-quantitative. We have therefore performed a short series of experiments in which skin was compressed by a known force acting over a known area, and the time-course of the resultant deformation measured.

EXPERIMENTAL

(a) Preparation of Animals and Subjects

The backs of albino rats of weight 200–250 gm were depilated with barium sulfide. 18–24 hours later each animal was anesthetised with dial and urethane and a fold of the back skin was stretched out and pinned on to a wooden board. Some of the rats were also injected intradermally with hyaluronidase (Hyalase, Benger) in 0.9% NaCl solution containing Evans Blue dye, at several points over the surface of their backs a few hours after depilation, and 15–18 hours before the experiment. In these rats the areas of skin into which the blue dye had spread were chosen for study.

The human subjects were young white male adults. The skin of one leg over the tibia, and the dorsal aspect of one forearm, were dry-shaved immediately before the experiment.

(b) Application of Force and Measurement of Compression

(i) Direct method.—A weighted metal rod, of diameter 4–24 mm and total weight 125–910 gm, was free to slide in a vertical glass tube. The rod was placed on the upper surface of the rat back skinfold. The undersurface of the skinfold rested on a thin metal disc of the same diameter. The vertical position of the top of the sliding rod was registered on a spring-loaded dial micrometer, accurate to 2 μ , and was noted at frequent intervals for 10 min. after the rod had been placed on the skin.

(ii) *Plethysmographic technic.*—This indirect method was suitable for use on skin over a soft backing and was used on rats and men. A methacrylate (Perspex, I.C.I.) annulus, of inside diameter 7 mm and outside diameter 14 mm, was stuck to the skin surface with a synthetic latex, (Evostik; Evode Ltd., England). This annulus formed the wall of a small water-filled plethysmograph, displacements within which were noted by movement of the water along a narrow glass tube.

The plethysmograph moved in a glass slide, so that it was free to move vertical to the skin surface, but not to tip. A 180-2000 gm weight was attached to the plethysmograph and the resulting volume displacement measured for up to 20 min. The compression of the skin (d) was calculated from the equation d = V/A where V =volume displaced and A = area of skin enclosed within the plethysmograph.

(iii) Specimens for histological preparation.— Depilated rat back skin was excised and placed on a piece of cardboard. It was compressed by a 105 gm weight acting over 0.07 cm² (1500 g/cm²) for 20 min., and then covered with Susa's fixative while the weight was still in position. After a further 60 min. the weight was removed; fixation continued for 18 hours.

RESULTS

Direct method.-The weighted rod sank rapidly into the fold of rat skin when first applied, so rapidly that it was difficult to estimate the exact moment at which the rod met the skin. This rapid phase lasted only a few seconds. Thereafter the speed of compression reduced continuously with time, in a nonexponential time-course (Fig. 1). There was a long "tail" of slow compression. In a few experiments which were continued for up to 1 hour the compression was still perceptibly increasing at the end of this time. When the weight was removed, the compression of each side of the skinfold appeared similar, so the compression of rat skin was obtained by dividing the skinfold compression by two (Figs. 1-3, Table I). After the weight was removed the compressed skin slowly resumed its original thickness; the compression remained visible for 2-20 minutes, dependent on the amount of massage the skin received in handling. The maximum compression seen was 0.6 mm (300 μ /skin side), which was obtained with a 910 g force acting on 0.12 cm² for 20 minutes. After this relatively great pressure the compressed area became oedematous.

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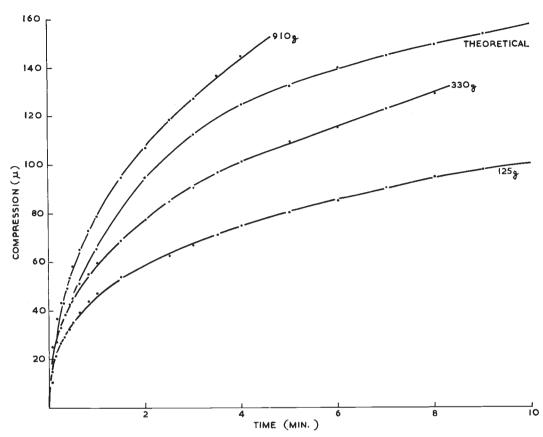


FIG. 1. Compression of skin in a fold of rat back skin, by a weighted rod acting on a 1.1 cm² area. Each experimental line is the mean from 6 experiments. The theoretical line is derived from equation 2 of the mathematical appendix, when $\beta = 1 \text{ min}^{-1}$ and $h_0 = 225 \mu$.

The speed of compression increased when the force was increased (Fig. 1) or if the contact area was reduced (Fig. 2). In order to quantitatively relate the compressive effect to the radius (a) of the contact area the times (t) necessary to reach a given compression were measured for different contact areas. From the ratios of these times $t = ka^{2.45}$.

Pre-treatment of the skin with hyaluronidase also increased its speed of compression (Fig. 3). The time necessary to reach a given compression was reduced by a factor of 3-7(mean = 5.3).

A cross-section cut through compressed rat skin showed that all the layers were reduced in thickness. Most of the compression was in the dermis (Fig. 4). In the compressed dermis the collagen fibers were increased in staining intensity and the spaces between them reduced, and the hair roots were flattened. Adjacent to the compressed area of skin the dermis was expanded, with large spaces between the collagen fibers.

Indirect method.—When the skin plethysmograph was weighted down, there was an immediate displacement of water within it. This displacement was reversible on removal of the weight. It was assumed to be an elastic effect, and was not considered further. After the elastic displacement there was a long-continued water displacement, whose time-course resembled the fall of the weighted rod in the direct method. This displacement remained when the weight was removed.

The calculated compression of a rat skinfold was greater with this technic, for a given pressure and time, than with the direct method (Table I). Hyaluronidase increased the speed of compression, as in the direct method. Human skin was compressed slightly more than rat

120

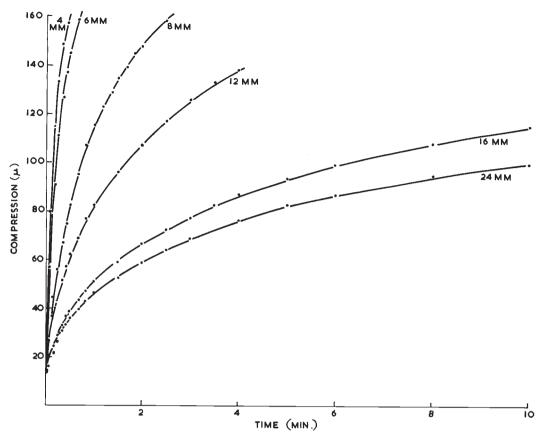


FIG. 2. Compression of rat back skin by a 910 g weight acting on skin areas of the diameters shown. Each line is the mean from 6 experiments.

skin under identical conditions (Table I). The maximum calculated compression of human skin was approx. 0.6 mm (Table I); the values for skin from the tibia region and from the dermal aspect of the forearm were similar to one another. The force necessary to produce this compression (2 kg on 1.2 cm^2) also produced considerable pain, during the compression, and oedema of the area after it was released.

DISCUSSION

The direct method, from which most of our results were obtained, has the great virtue of being easy to interpret: if the rod moves down, the skin has been compressed by that amount. However, it is limited in application, since one must have a firm backing for the skin. The skin plethysmograph, like Schade's elastometer, can be used on a soft backing, since only relative movements between the skin and the plethysmograph are recorded. It is also easy to use. However, it suffers from a theoretical disadvantage: the volume displacement can be caused by two separate effects. The first is the movement of the annulus perpendicular to the skin surface, from which the present results have been calculated. The second is a hypothetical movement of dermal or subcutaneous fluid from under the annulus walls into the central compartment. The comparatively rapid apparent compression of rat skin by the plethysmographic technic may be due to this, or it may be a real difference due to the fact that the annulus walls were thinner than the diameters of the rods usually used (3.5 mm as against 8-16 mm).

The histology of compressed skin shows that most of the compression is in the connective tissue, and primarily in the dermis. Since liquids are virtually incompressible, for the dermis to be compressed liquid must have 122

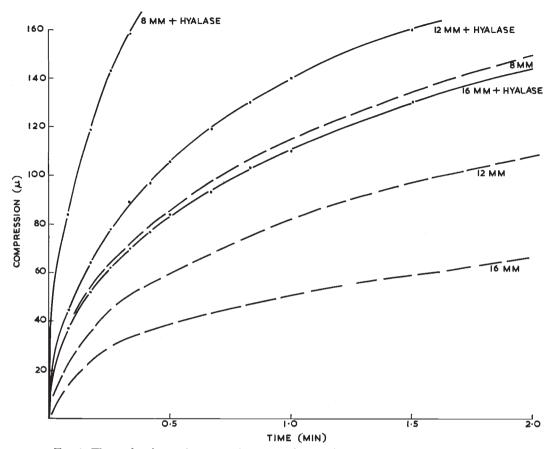


FIG. 3. The early phase of compression of rat back skin by a 910 g weight acting over areas of the diameters shown, before and after the action of hyaluronidase. Each line is the mean from 6 experiments.

TABLE I

Compression of skin

The mean and standard error of each set of observations is given, with the number of experiments in parentheses.

Species and Region	Stress	Time of Application	Compression Produced
	g/cm ²	min	μ
Rat back skin fold (read- ings ÷ 2)	110	8	$95 \pm 6 \ (6)$
	300	8	124 ± 15 (6)
	160 (annulus)	10	250 ± 15 (8)
	7500	20	312 ± 13 (6)
Human skin (all readings from annular stress)			
(a) leg	160	10	$342 \pm 38 (4)$
	1700	15-20	654 ± 79 (4)
(b) forearm	160	10	$303 \pm 28 (6)$
	1700	15-20	$570 \pm 30 (4)$

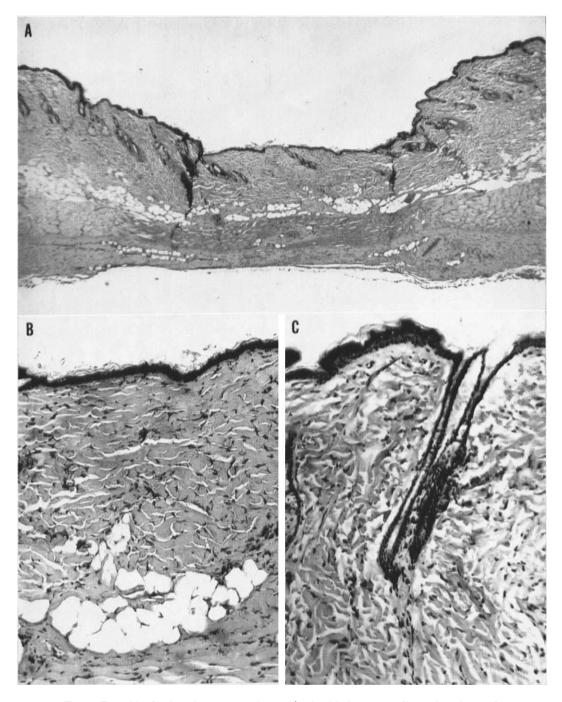


FIG. 4. Rat skin fixed under compression, stained with haematoxylin and eosin. A, low-power view (45 \times) of the compressed skin and adjacent regions. B, the compressed dermis (144 \times); C, uncompressed dermis (144 \times).

flowed sideways out of the compressed area. The dermal fluid, the ground substance, is known to contain enough mucopolysaccharide to make it highly viscous (4, 5). Diffusion through dermis is greatly increased when the polysaccharide is depolymerized by hyaluronidase (6). The present results indicate that the ease of bulk liquid flow is also greatly increased, although the only fact proved is that the injection produced an increase in ease of compression; injection of saline without hyaluronidase was not used as a control experiment, since the saline spread very little throughout the dermis when hyaluronidase was not added, and a very large number of injections would have been necessary.

The flow of ground substance among the collagen fibers may be considered similar to the movement of a viscous liquid in a channel. The viscous resistance to flow, and hence to skin compression, is dependent on the length and width of the resistive channel. Since the effect of a given compressing force was proportional to the radius of the contact area to the power -2.4, the effect of a given pressure (force \div area) was only slightly decreased by increasing the area. Hence it appeared that most of the channels in which the viscous resistance was encountered were less than the radius of the smallest rod used (2 mm) long; within this distance the moving ground substance reached channels wide enough to offer little viscous resistance.

We have considered a model system incorporating these features (cf Mathematical Appendix). In this system a viscous liquid is compressed between parallel plates. The stack radius represents the length of the resistive channels, and the plate separation their width. In such a system the thickness of the "tissue" (h) is given by

$$h = h_0 \frac{1}{1 + \beta t}$$

where β is a constant dependent on the force, viscosity, contact area, interplate spacing, and stack width. This equation represents a timecourse qualitatively similar to that actually found; if $\beta = 1 \text{ min}^{-1}$ and $h_0 = 225 \ \mu$ a good quantitative fit is obtained with the results of the direct method (Fig. 1). It is not possible to obtain a good fit if $h_0 = 300 \ \mu$, the maximum compression actually produced by very high pressure. As this pressure was traumatic, it is possible that it burst cells and thus produced more compression than the smaller pressures would have done.

The value of β (1 min⁻¹) can be produced in the model for a 600 gm force acting over 1.1 cm^2 , as in skin (Fig. 1) if the viscosity of the fluid is 1,000 cpoise and the plates are 4,000 \times as wide as they are apart initially. In terms of skin, the high viscosity needed means that the 0.1–0.4% of mucopolysaccharide which is likely to be present in the ground substance of rat or human skin (4, 7) has to be highly polymerised (8) and to be moving sufficiently slowly for the polymer molecules not to line up and lose their viscous drag (5). The channels have to be approx. $2.000 \times \text{as long}$ as they are wide in uncompressed skin. It is not certain that there are any interfibre channels in normal skin (9), but if there are they might reasonably be supposed to be $0.1-1\mu$ across, in which case they would have to be 0.2-2 mm long before joining together sufficiently to form wide channels of negligible flow resistance.

This model is extremely tentative. Whether it is correct in detail or not, it appears certain that the compression of skin is mainly a property of viscous flow in the dermis.

SUMMARY

1. A force of 100–2000 g acting over 0.1-5 cm² for up to 20 minutes was used to compress rat or human skin.

2. The maximum compression produced was 0.3 mm in rat skin and 0.6 mm in human skin.

3. Histology showed that most of the compression was in the dermis.

4. The time-course of the compression was not exponential; there was a long-continued "tail" of deformation. The compression remained after the force was removed.

5. The speed of compression was increased by increase in the force, decrease in the contact area, or the prior action of hyaluronidase on the dermis.

6. A theory of viscous flow in the dermis is suggested to account for the results.

REFERENCES

- Schade, H.: Die Elastizitätsfunktion des Bindegewebes und die initiale Messung ihrer Störungen. Z. Exp. Path. Therap., 11: 369, 1912.
- 2. Tui, C., Kuo, N. H. and Simuangco, S.: Studies

on scleroderma. J. Invest. Derm., 15: 117, 1949.

- 3. Kirk, E. and Kvorning, S. A.: Quantitative measurements on the elastic properties of the skin and subcutaneous tissue in young
- and old individuals. J. Geront., 4: 273, 1949.
 4. Smith, Q. T.: Body weight, cutaneous collagen and hexosamine of cortisone-treated female rats of various ages. J. Invest. Derm., 42: 353, 1964.
- Jost, A. G. and Stanier, J. E.: The physiological function of hyaluronic acid in synovial fluid. J. Physiol., 119: 244, 1953.
 McLean, D. and Hale, C. W.: Studies on dif-termination.
- fusing factors. Biochem. J., 35: 159, 1941.
- 7. Pearce, R. H. and Watson, É. M.: The mucopolysaccharides of human skin. Canad. J. Res., 27E: 43, 1949.
- 8. Fessler, J. H.: A structural function of mucopolysaccharides in connective tissue. Biochem. J., 76: 124, 1960. 9. Jarrett, A.: The structure of collagen and
- elastic fibres in unprocessed skin. Brit. J. Derm., 70: 343, 1958.
- 10. Barr, G.: A monograph of viscometry. Oxford U.P., 1931.

MATHEMATICAL APPENDIX

The flow of a Newtonian liquid, of viscosity η , between two parallel circular plates of radius a, when the plates are pressed together by a force F, is expressed by:

$$\left(\frac{x_0}{x}\right)^2 = 1 + \frac{4Fx_0^2t}{3\pi\eta a^4}$$

where x = distance between the plates at time t x_0 = distance between the plates at t = 0

This solution is due to Reynolds, and is quoted by Barr (10).

For a stack of such plates, of total height h, the same equation holds:

$$\frac{h_0}{h} = \frac{x_0}{x}$$

Consider a set of n such stacks, close-packed within a circle of radius r. If n is large

$$r^2 \doteq \frac{4na^2}{\pi}$$

If a force F is applied to the set, then F/nbears on each stack ... The equation of motion is:

$$\frac{h_0^2}{h^2} = 1 + \frac{4Fx_0^2t}{3\pi n\eta a^4}
= 1 + \frac{16Fx_0^2t}{3\pi^2 \eta a^2 r^2}$$
(1)

$$\therefore \frac{h}{h_0} = \sqrt{\frac{1}{1+\beta t}}$$
(2)

where

$$\beta = \frac{16Fx_0^2}{3\pi^2 a^2 r^2 \eta} \tag{3}$$

N.B. This treatment assumes that viscous resistance of the fluid is negligible once it has left the stacks *i.e.* that it then passes through wide tubes to the periphery of the major circle.