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A simple method for testing the effects of reagents on the mechanical properties of sheets of connective tissues

R. D. HARKNESS
Department of Physiology, University College, London, UK

J. A. A. HOOD
School of Dentistry, University of Otago, Dunedin, New Zealand

A simple and rapid method of testing the effects of reagents on a mechanical property of skin or other sheets of connective tissue, by measurements of the force required to punch a hole or drive a conical probe through the tissue, is described. The reagents tested (cysteamine, hydrogen ion concentration, cyanide) had essentially the same effects as in simple tensile tests.

1. Introduction

Experiments on the effects of reagents on a tissue can be used to throw light on the nature of the components that determine the mechanical properties of that tissue [1]. Because of the variation encountered in biological tissue such experiments require a greater number of specimens than do similar tests on non-biological material. It is therefore desirable that any new test method should use specimens requiring minimal preparation and should be quick and easy to conduct.

A major problem in the tensile testing of biological tissue is the difficulty of securely clamping the ends. This problem is avoided if the tissue is cut into rings which can be pulled between hooks or parallel rods, but many tissues cannot be obtained in the form of rings. We report here two simple methods for testing a flat sheet which gave results similar to those obtained previously with tensile tests on rings of flat tail skin [2–4].

2. Experimental procedure

2.1. Mechanical testing

Two fixtures were developed for use with an Instron 1193 mechanical testing machine.

2.2. Punch shear tester

This consisted of a machined block (Fig. 1a) with a lower specimen support plate and an upper guidance plate. Colinear holes 1.5 mm diameter in the long axis of the block penetrated both plates. A 1.5 mm diameter shear pin was driven through it using the cross-head drive of the Instron testing machine. The force required to shear the specimen could be monitored and rates of shearing were varied from 0.5 to 50 mm min^{-1}.

2.3. Cone needle penetrometer

This consisted of a brass base block and a guide (Fig. 1b) each with nine 2.5 mm holes drilled axially through the block and spaced 4 mm apart. Alignment of the holes between the blocks was maintained by a guide pin and two locking screws.

Penetrometer cone needles were made from 2.3 mm diameter HP dental drills which had the cutting heads removed and the necked down shanks (1 mm diameter) ground to points of 15°, 30°, 35°, 40°, or 50°.

Skin specimens, ~ 18 mm square, were placed over the holes on the base block and then the guide block was securely clamped in place using the locking screws. The selected penetrometer cone needle was then placed in one of the holes and driven through the skin specimen at a controlled rate of between 0.5 and 50 mm min^{-1} using the Instron testing machine. The force required for the needle to penetrate the specimen was recorded.

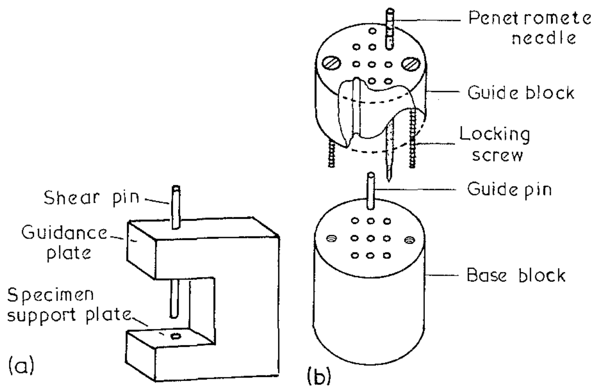

Figure 1 Apparatus used in tests. (a) Punch shear tester. (b) Cone needle penetrometer.

The test assembly allowed for the nine separate penetrations of the clamped skin specimens.

In both the punch and the cone penetrometer tests the skin was penetrated from the outer surface inwards.

2.4. Materials

Skin from the tails of spayed mature albino female rats, weighing about 250 g, was used. In general, tests by the two methods above were done on adjacent areas of skin, a few mm apart, the punch tests being done first.

2.5. Treatment of materials

Animals were killed by stunning and breaking the neck. Tail skin was removed within about 10 min of death, split along its length and put in a sealed container in a deep freeze at -20° C for up to a week before treatment. It was then thawed, washed on the outside with tap water, and the bulk of the epidermis scraped off. Pieces were then cut off and placed in test solutions at 4° C overnight. The following solutions were used.

1. 0.02 M sodium phosphate in 0.13 M sodium chloride (phosphate-buffered saline). This was adjusted to various pH levels by addition of small quantities of aqueous sodium hydroxide or hydrochloric acid.

2. Potassium cyanide (AR British Drug Houses) in 1 mM concentration in solution 1, adjusted to various levels of pH.

3. Cysteamine (mercapto-ethylamine, AR, British Durg Houses) 0.1 M in solution 1 at pH 7.0.

In all cases the pH of the solution was measured after the tests. Comparisons of reagents were made in groups of four from the same level in the tail. A ring of 1 cm width (along the tail) was cut and divided into four quarters. One of these was used as a control tested only with phosphate saline at pH 7.4. The other three were treated in different ways and force required to penetrate expressed as a proportion of the force for the control piece of skin. Tests on effects of reagents were done with the punch or 20° cone, both advancing at 5 mm min^{-1}.

2.6. Estimation of collagen content of skin

The collagen content of some of the skin samples was estimated from the hydroxypoline content. Skin was hydrolysed in 6N HCl in an autoclave at a pressure of 9.2 kPa (75 lb in^{-2}) for 5 h and hydroxyproline estimated on an auto-analyser by the method of Grant [5].

3. Results

3.1. Variation in properties of skin with position on tail

A series of tests were done in different positions down the tail. The results are shown in Fig. 2. The force required to penetrate fell with distance from the base of the tail. Subsequent tests where comparisons were being made – for example, between cones of different angle – were done on short lengths of skin (about 15 mm) and so arranged that the average distance down the tail was the same for all the groups being compared. In previous tensile tests no evidence of variation round the tail was found. No evidence of any variation that would affect the results significantly was found in the present tests.

3.2. Effect of rate of movement through skin (cross-head speed)

Variation in the rate of advance of the punch over a hundred-fold range (0.5 to 50 mm min^{-1}) had a comparatively small effect (about 30%) on the force needed to press the punch through the skin

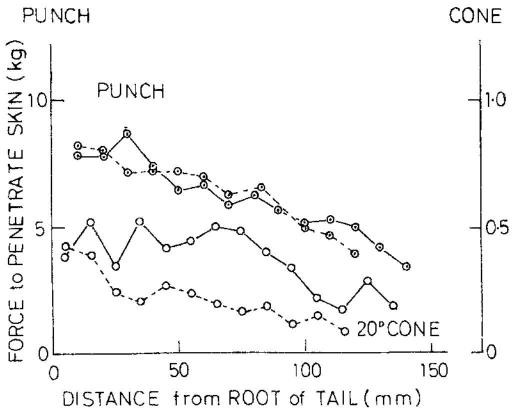

Figure 2 Force to penetrate skin related to distance from root of rat's tail. Relation between position of skin in the tail (abscissa) and the force required to penetrate it (ordinate); ⊙ punch, left-hand scale and circles with dots in them; ○ 20° cone, right-hand scale and empty circles. The complete and interrupted lines show results from two different tails.

(Fig. 3). The force was less with higher speeds. With a cone of 20° over a thousand-fold range of forward movement there was again only about 30% variation in force needed to penetrate the skin, but in this case it rose with rate of movement. Most of the subsequent experiments were done at a rate of 5 mm min^{-1}.

3.3. Effect of different cone angles

The effect of variation in cone angle on force required to penetrate the skin is shown in Fig. 4. Force increased with cone angle approximately linearly. The experiments were arranged so that all the angles in the figure were examined in each inidividual piece of skin. Results were combined by expressing the measurements for individual angles as a proportion of the mean for all angles in that particular test.

3.4. Effect of reagents

The results of tests are shown in Fig. 5. The force needed to penetrate decreased with pH in simple buffered solutions but not in the presence of cyanide. The dotted line gives the relation of tensile strength to pH in rings of rat tail skin, from results previously recorded [4]. Cysteamine reduced the force needed to penetrate. Results for the cone and punch are, in general, very similar. With the punch, the reduction in strength with pH takes place over a range about one unit lower than with the cone. Also cyanide has less effect in increasing tissue resistance in the punch test. The mean value of penetrating force in the range of pH in which it was increased (pH 4 and upwards), as a percentage of the value at pH at 7.4, was 98.5 ± 3.0 (standard error of mean) for the punch, 124.7 ± 5.2 for the 20° cone. The difference in direct comparison of pairs of tests on the same piece of skin was 26.2 ± 4.9, obviously significant.

3.5. Relation between punch and cone tests

There was a general correlation between results, as already noted, but it was not linear (Fig. 6). Increase in the force to penetrate with the cone (abscissa) is not accompanied by a corresponding rise for the punch which tends towards a constant value. In fact, if all the results are combined together, an exponential expression fits well. If P (kg) is the force for the punch and C (kg) for the cone, the expression $P = 8.6 - 8.9.\ 10^{-2.2c}$ gives a close fit to the mean results. For figures bulked (in brackets) predicted mean values for the punch were 2.49 (2.47), 4.38 (4.44), 6.03 (5.97), 7.02 (7.19), 7.72 (7.69), 8.13 (8.12).

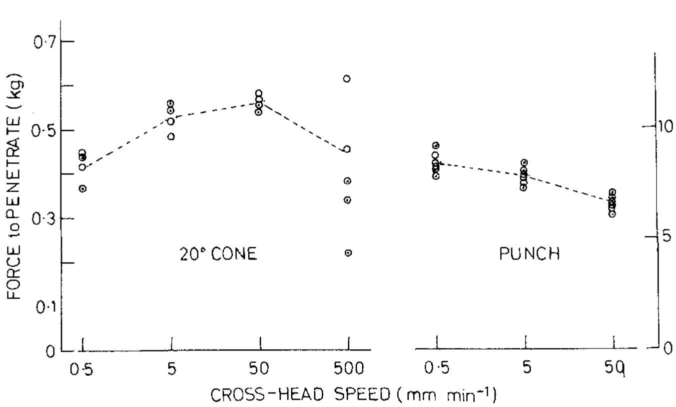

Figure 3 Force to penetrate skin related to rate of penetration. Relation between the rate of forward movement of the penetrating cone or punch (cross-head speed, abscissa) and the force required to penetrate (ordinate). Results were obtained from two tails distinguished by empty circles or circles with dots.

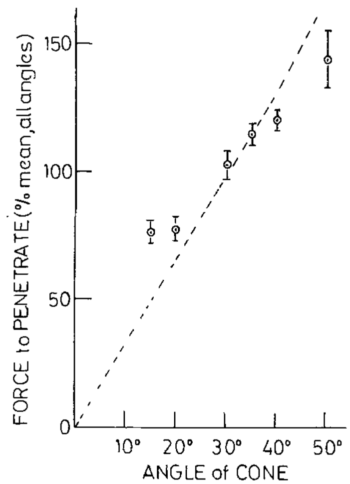

Figure 4 Force to penetrate skin related to angle of cone. Relation between angle of cone (abscissa) and force required to penetrate (ordinate). The points are means of results from six pieces of skin on each of which all angles were tested, 15°, 20° and 30° twice; 35°, 40° and 50° once. The results were combined by expressing those for each test as the mean of all the angles tested. The vertical lines through each point have lengths of twice the standard error of the mean. The dotted line is the relation one might expect if the force in the plane of the skin was proportional inversely to the tangent of half the angle of the cone, which gives the ratio between lateral movement of the surface of the cone and its forward movement. The line has been made arbitrarily to pass through the 30° point.

3.6. Relation between tests and collagen content of skin

Relation between thickness of the skin in terms of collagen (collagen per unit area) for a set of measurements at various distances down the tail is shown in Fig. 7. There is a correlation in both cases but it was closer for the punch (coefficient of correlation 0.96 compared to 0.82).

4. Discussion

4.1. Physical nature of tests

Tests like these are in use in other spheres. Cone tests are used, for example, in defining the properties of soils [6]. But the forces concerned are rather different. In soil, for example, resistance other than from friction is from physical displacement of material by compressive forces. In skin the main resistance apart from friction must be from the tensile properties of the fibrous framework, the material round the circumference of the cone.

The punch test is a standard shear test applied in this case to a rather thin sheet. Calculating from the estimates of collagen per unit area, one arrives at a thickness of the skin in terms of dry collagen, to between a tenth and a quarter of a mm.

4.2. Comparison with simple tensile tests

The evidence about the mechanism of the effects of hydrogen ion concentration and of cysteamine [2, 4, 7, 8] on a simple tensile test on rat tail skin is that they are the result of changes in labile intermolecular cross links in collagen (Schiff-base links [9, 10]). In the present tests, measurements on untreated skin correlated with collagen content, and effects of treatments were essentially the same as found in previous tensile tests. We can conclude, then, that the component of skin that determines the results of these tests is again collagen. The mechanism of the effect of cyanide

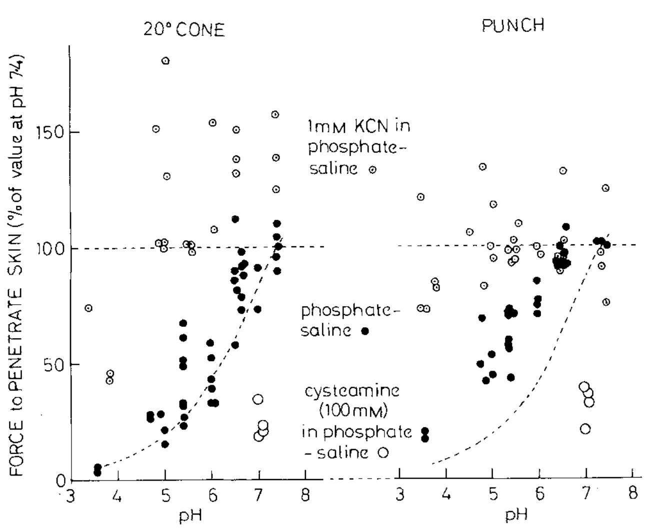

Figure 5 Effect of various treatments on the force required for penetration of rat tail skin. The ordinate is the force required to penetrate the skin expressed as a percentage of the value for a standard control treated with phosphate saline at pH 7.4. The abscissa is the pH of the treating solution. Symbols: • phosphate buffered saline; ⊙ 1 mM KCN in phosphate buffered saline; ○ 0.1 M cysteamine in phosphate buffered saline. The dotted curve shows the effect of phosphate buffered saline in tensile tests on rings of rat tail skin in earlier work [4].

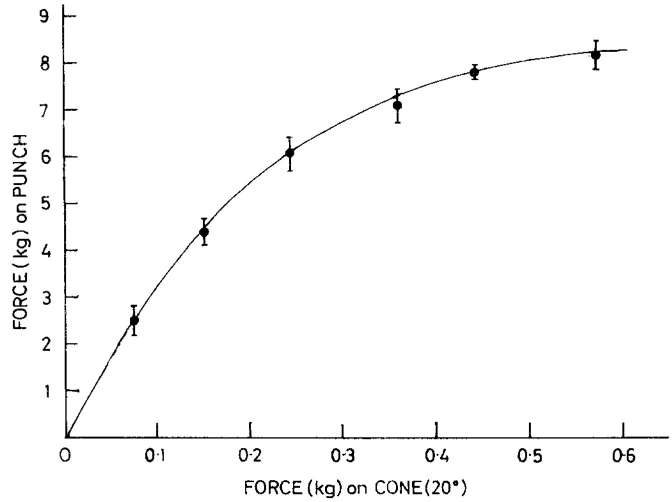

Figure 6 Relation between results of punch and cone tests. The figure shows the relation between punch (ordinate) and 20° cone (abscissa) tests. All the data have been combined together. The vertical lines through the points have lengths of twice the standard error of the mean.

is quite unknown but is again very much the same in these tests as in tensile tests reported previously [3].

4.3. Relation of tests to the biological function of skin and teeth

The mechanical properties of skin are generally regarded as related, functionally, to the role of skin in protecting underlying tissues from mechanical attack. Just what sorts of attack are important in real life is less clear [4]. It is difficult to imagine circumstances in which it would be subjected to forces like those used in simple tensile tests. On the other hand, a relation between the tests described here, particularly the cone test, and situations in real life in which skin is attacked by teeth, horns, claws or plant spines are easy to see. One can see, for example, an obvious analogy between the cone tests and the natural function of canine teeth, used by canivores to puncture skin. It seems, not unexpectedly, from the experiments with cones of different angle that a smaller angle makes penetration easier. Presumably in real life this advantage is offset by increased fragility and the final tooth design gives some compromise between these two factors. Similarly, it would seem that the punch corresponds in its action to incisors or carnassial teeth. In none of these cases does it appear that anything is known in detail about their action on skin.

5. Conclusions

The results of the tests we have described appear to depend upon the collagen in the skin and to be affected by alteration in its properties in the same way as simple tensile tests. When such tests

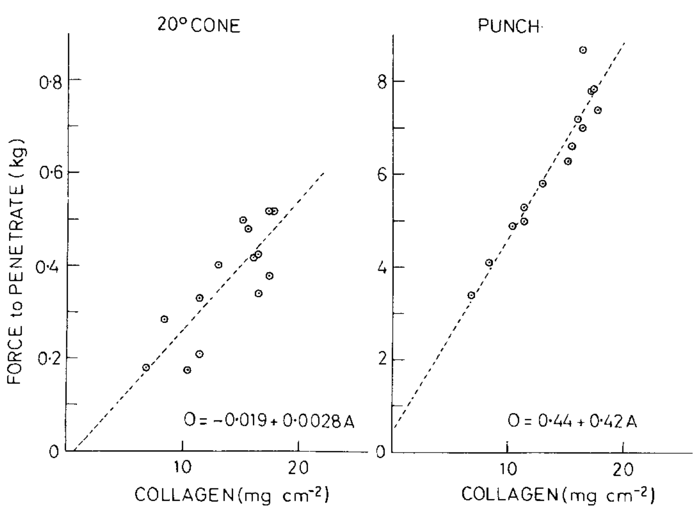

Figure 7 Relation between force required to penetrate the skin and its collagen contents. The figure shows the relation between the collagen content of tail skin (abscissa) at different points down one individual tail and the force required to penetrate (ordinate). The dotted lines are regression lines calculated in the usual way and corresponding to the equations shown: O = ordinate, A = abscissa.

are needed to examine the relation of chemical structure to mechanical properties, these are easy to do and can be applied to a sheet of tissue.

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