THE PHYSICAL PROPERTIES OF THE PEDAL MUCUS OF THE TERRESTRIAL SLUG, *ARIOLIMAX COLUMBIANUS*

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SUMMARY

The pedal mucus of gastropods functions in locomotion by coupling the movements of the foot to the substratum. The pedal mucus of the terrestrial slug, *Ariolimax columbianus*, is suited to this role by the following unusual physical properties.

1. At small deformations the mucus is a viscoelastic solid with a shear modulus of 100–300 Pa.

2. The mucus shows a sharp yield point at a strain of 5–6, the yield stress increasing with increasing strain rate.

3. At strains greater than 6 the mucus is a viscous liquid (η = 30–50 poise).

4. The mucus recovers its solidity if allowed to 'heal' for a period of time, the amount of solidity recovered increasing with increasing time.

INTRODUCTION

The ventral surface of a gastropod's foot is separated from the sub-stratum by a thin layer of mucus. In many cases this mucus layer is left behind as the animal crawls, forming the slime trail that is characteristic of terrestrial snails and slugs. The importance of this pedal mucus in the locomotion and adhesion of gastropods has been known for a considerable time. Barr (1926) reports that slugs deprived of pedal mucus by cauterizing the suprapedal mucus gland can neither crawl nor adhere. Later authors (Lissman, 1945; Jones, 1973, 1975; Miller, 1974) speculate that pedal mucus serves as an adhesive in all gastropods, coupling the force of foot movements to the substratum. However, none of these authors have conducted tests to ascertain the physical properties of pedal mucus, and as a consequence its precise role has remained obscure.

This study reports on the mechanical properties of the pedal mucus of the terrestrial slug, *Ariolimax columbianus*. These properties have been described briefly by Denny (1980*a*) and it will be shown here that *A. columbianus* pedal mucus is an unusual viscoelastic material, the properties of which are ideally suited to adhesive locomotion.

Before describing the properties of this slug's slime it will be useful to briefly review the basic concepts, terminology, and testing procedures used in the study of viscoelastic materials.

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TERMS AND TESTS

If a force is placed on a material the material will deform, and if the material is elastic a given force will result in a unique deformation. This deformation will not change regardless of the time for which the force is applied, and if the force is removed the material will return to its original shape. The properties of elastic solids are quantified by normalizing force and deformation to the size of the sample being tested (see Fig. 1). The deforming force is divided by the area over which the force is applied to give the *stress*, σ. Similarly deformation is divided by sample thickness to give shear strain, γ. The ratio of stress to strain is the material's stiffness, or shear modulus, G:

$$G = \sigma/\gamma. \tag{1}$$

The elasticity shown by soft materials is due to the presence of a crosslinked network of kinetically free, long, polymer chains. When the material is unstressed the chains assume a constantly changing, random configuration. When stressed, the material deforms; the chains pull on each other via the crosslinks, and are stretched to new, more ordered configurations. The change in entropy brought about by this change in configuration can be shown to account for the force required to deform the material. The statistical basis for this form of elasticity has been thoroughly studied and termed the theory of rubber elasticity (Flory, 1953; Treloar, 1970). The details will not be described here, the important point being that any elasticity found in a soft biological material (such as mucus) implies the presence of a crosslinked polymer network.

The primary characteristic of fluids is that they flow. If a force is placed on a fluid the material will deform for as long as the force is applied; the greater the force, the faster the deformation. This property of fluids is quantified as *viscosity*, η, the ratio of stress to strain rate, $\dot{\gamma}$:

$$\eta = \sigma/(d\gamma/dt) = \sigma/\dot{\gamma}. \tag{2}$$

No biological material is purely elastic or liquid, instead these real materials show a combination of these properties. To describe the properties of viscoelastic materials a number of standard techniques have been devised, the objective of which is to separately quantify the viscous and elastic contributions to the overall properties of the material.

(1) *Stress-strain tests*. These tests are conducted essentially as shown in Figs. 1 A, B. A sample suitably held is subjected to an increasing deformation and the force measured at each strain. The deformation may be continued until the material fails, or at some point the deformation can be reversed and force measured as the material is returned to its original dimensions. Fig. 1 E illustrates the property known as hysteresis, the loss of energy accompanying the cyclic loading of a sample. Hysteresis is a result of the viscous nature of a viscoelastic material.

(2) *Stress relaxation tests*. If a material is quickly deformed to a given strain the stress required to maintain this strain can be measured as a function of time. If the material is a pure elastic solid by definition the force will not vary with time. If the material is a pure viscous liquid there is no force since the strain rate is zero. If the material is viscoelastic it shows a stress relaxation curve intermediate between the
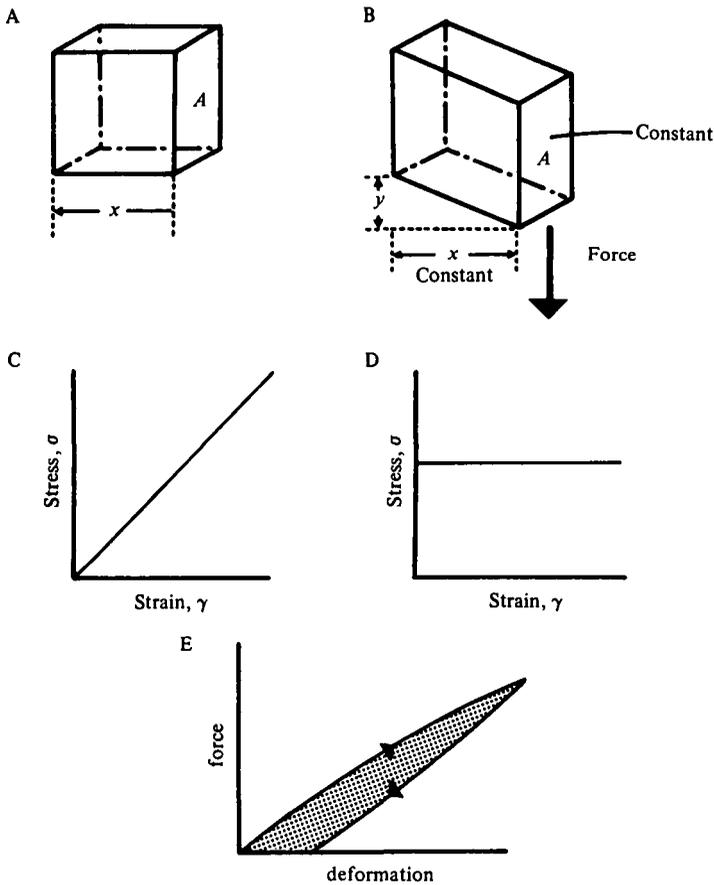
Fig. 1. Elastic solids and viscous liquids. (A) The dimensions of an undeformed sample. (B) The dimensions of a sample deformed in shear. Force is applied over area, A, the *stress* being force/area. Strain is the deformation, y, divided by sample thickness, x. (C) For an elastic solid stress is proportional to strain. (D) For a viscous liquid stress is proportional to strain *rate*, so that at a given strain rate there is a constant stress regardless of strain. (E) Energy is lost in the cyclic loading of a viscoelastic material. The area under a force-deformation curve is a measure of energy. The stippled area is the energy lost in the cycle shown.

solid and liquid curves; the force gradually decays. An ideal viscoelastic material can be characterized by single values for elastic stiffness and viscosity, and in this case stress relaxes exponentially with time. The time needed to decay to $1/e$ (37%) of the initial stress is known as the relaxation time and is a measure of the ratio of viscosity to elasticity. Real viscoelastic materials seldom show a single relaxation time, rather each infinitesimal volume of the material relaxes at a rate determined by the local viscosity and elasticity. There is thus a continuous distribution of relaxation processes occurring as the material as a whole relaxes. The relative number of elements relaxing at a certain rate is quantified as the relaxation spectrum, $H(t)$.

(3) *Dynamic tests.* As explained above, force for an elastic solid is proportional to the amount of deformation, while for a viscous liquid force is proportional to the rate of deformation. This fact is utilized in dynamic tests to separate the contribution of

elasticity and viscosity to the overall properties of the material. The material is deformed such that

$$\gamma = \sin(\omega t), \tag{3}$$

where ω is the angular velocity (radians/second) and t is time (seconds). By reference to equation 1 the stress for a pure elastic material is;

$$\sigma = G\gamma = G\sin(\omega t) \tag{4}$$

and the stress is in phase with the strain. For a pure viscous liquid

$$\sigma = \eta \, d\gamma/dt, \qquad\qquad \text{(from eqn 2)}$$

therefore

$$\sigma = \eta \, d(\sin(\omega t))/dt = \eta\cos(\omega t) \tag{5}$$

so that stress for a viscous material leads strain by 90°. A viscoelastic material shows a phase shift intermediate between a purely viscous or purely elastic material, i.e. somewhere between 90 and 0°. Thus by measuring the phase shift, δ, between strain and stress a measure of the relative values of elasticity and viscosity can be found. At the same time the ratio of the stress amplitude divided by the strain amplitude yields a stiffness or modulus. This overall modulus (the complex modulus) is denoted G^*. The elastic contribution to G^*, $\cos\delta\, G^* = G'$, is called the storage modulus, and is a measure of the energy stored in each cycle of deformation. Obviously $G' = G^*$ when $\delta = 0°$ and $G' = 0$ when $\delta = 90°$. The viscous contribution is $\sin\delta\, G^* = G''$ or the loss modulus. The loss modulus is often used to calculate a dynamic viscosity, $\eta' = G''/\omega$. The ratio of G'' to G' is $\tan\delta$, a measure at low values that can be related to hysteresis.

These three techniques form the basis for this study of the physical properties of *A. columbianus* pedal mucus. The knowledge gained from these tests can be used to: (1) predict the behaviour of a material in a given situation, and (2) provide some clue to the macromolecular structure of the material.

MATERIALS AND METHODS

Slugs were collected and housed as described by Denny (1980b).

Two types of testing machines were constructed to perform the tests described above, both machines designed to test thin layers of mucus in shear, under conditions as closely as possible approximating those under a crawling slug.

Dynamic testing apparatus

A forced oscillation dynamic testing apparatus was constructed as shown in Figs. 2 and 3. The sample is held between two parallel glass plates, the thickness of the sample being measured and set by a micrometer. The plates are aligned daily while being observed through a dissecting microscope. Typical sample thicknesses are 50 to 150 μm, and sample areas are measured visually by estimating the proportion of the 1 cm^2 glass plate occupied by the sample. One glass plate is coupled to an electromagnetic vibrator and is the instrument for deforming the sample. The actual displacement of this glass plate is measured by a transducer connected to the vibrator
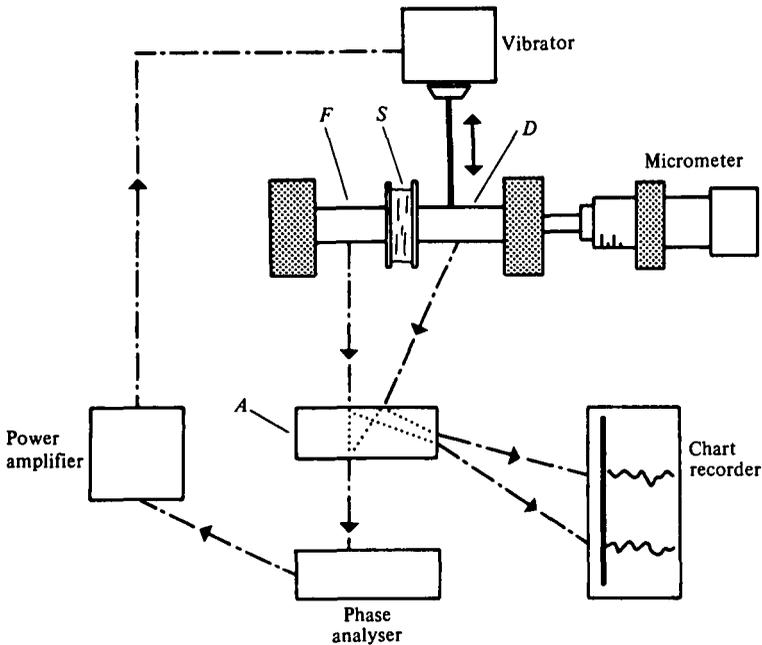
Fig. 2. A schematic diagram of the forced oscillation dynamic testing apparatus used to examine the physical properties of pedal mucus at small strains. *S*, sample; *F*, force transducer; *D*, displacement transducer; *A*, matched carrier amplifiers (SE Laboratories type 4300). The phase analyser is an SE Laboratories type SM 272DP transfer function analyser. The vibrator is a Ling Dynamic Systems model 200.

shaft, and is calibrated by deflecting the measuring beam with a micrometer. Within the range of displacements used in this study the transducer output is linear with displacement. Typical displacements for dynamic tests are 20–50 μm, though displacements up to approximately 400 μm can be used for stress-strain tests.

The second glass plate is supported by a parallel beam transducer which senses the force exerted on the sample during a test. The force transducer is calibrated by hanging accurately known weights from the centre of the glass plate. Forces as small as approximately 10 dynes can be measured accurately and the unloaded resonant frequency of the force transducer is approximately 400 Hz.

During a dynamic test the vibrator is powered by a sinusoidal signal generated by the transfer function analyser. The amplified signals from the force and displacement transducers are in turn compared to this reference signal. For each signal the analyser measures the amplitude of the transducer signal and the phase shift from the reference sine wave. From these data the phase shift of the force signal relative to the displacement signal can be calculated to yield a value for δ. The ratio of the amplitudes of force and displacement signals can be corrected for sample area and thickness to provide a measure of G^*. Samples were tested at frequencies ranging from 0·2 to 100 Hz. Above 100 Hz resonances within the frame supporting the vibrator cause spurious and erratic readings and below 0·2 Hz drift in the semiconductor strain gauges of the force transducer does not allow for accurate measurements.

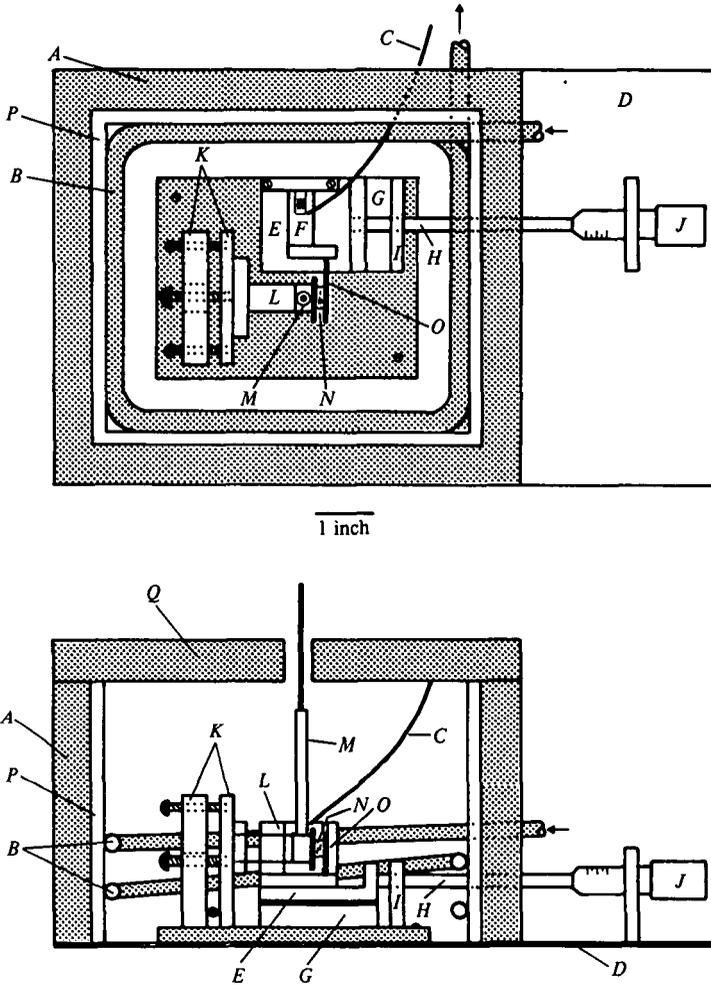Stress-strain tests are performed by powering the vibrator with a triangular wave,

Fig. 3. A construction drawing of the dynamic testing apparatus. A = styrofoam insulation; B, coiled copper tubing carrying water from a controlled temperature bath; C, shielded cable from the force transducer; D, base plate; E, sliding force transducer assembly; F, stainless steel beam (0·008″ thick) with mounted semiconductor strain gauges (BLH type SPB3-20-35); G, glass sliding surface; H, rod connecting the force transducer to the micrometer; I, guide-bearing for micrometer rod; J, micrometer fixed to the base plate; K, alignment system for the displacement apparatus; L, stainless steel beam (0·002″ thick) supporting the displacement glass plate; M, rod connecting the displacement apparatus to the vibrator; N, mucus sample sandwiched between the force and displacement glass plates; O, the force glass plate; P, plexiglass container; Q, styrofoam insulating cover with a gap for the displacement rod.

and the amplified signals from the force and displacement transducers are recorded by a two channel chart recorder. Both the frequency and amplitude of the displacement can be varied within the limits described above.

The testing apparatus is enclosed in an insulated temperature controlled chamber that can be used either as a bath to immerse the sample in a test solution or as a closed chamber to maintain 100% relative humidity during a test. All tests are performed at 22–23 °C with temperature variations during the course of a test less than 0·1 °C.

The usefulness of this apparatus is limited in the two following respects. (1) The semiconductor strain gauges used in the force transducer are exceedingly temperature sensitive. While this poses no problem for tests of short duration such as dynamic or stress-strain tests, tests requiring long term stability (stress relaxation tests) could not be performed. (2) The necessity of maintaining the glass plates parallel and a set distance apart limits the strains that can practically be obtained.

To overcome these limitations the second test apparatus was constructed.

The cone and plate apparatus

The cone and plate machine used for measuring the physical properties of slime at large strains and shear rates and for stress relaxation tests is shown in Fig. 4. In this machine the sample is held between an aluminium plate and a small angle Plexiglass cone. One revolution of the cone produces a uniform strain in the sample of 249 (the geometrical basis for uniform strain in a cone and plate configuration is explained in Fig. 4). The diameter of the sandwiched sample is measured with vernier calipers and the area calculated.

The rotation of the plate is converted to a linear measure by a windlass which supports the core of a linearly variable differential transformer (LVDT), and the amplified signal from this transformer is recorded on one channel of a two channel chart recorder. The LVDT is calibrated by inserting the core with a micrometer, and rotations can be measured to approximately ± 2 degrees.

The cone is supported by a torsion bar. The twisting of this bar, a measure of force, results in the deflexion of a rigid arm which supports the cone of a second LVDT, and the signal from this transformer is recorded on the second channel of the chart recorder. The force transducer is calibrated by turning the transducer on its side so that the LVDT is vertical, accurately known weights then being hung from the core. The force generated during a test is assumed to act at a radius R as explained in Fig. 4. This radius is then compared to the distance from the centre of the cone to the LVDT core to calculate the mechanical advantage and arrive at a final calibration. Forces as small as 100 dynes (acting at a typical effective sample radius of approximately 5 mm) can be accurately measured, and the unloaded resonance of the force transducer is approximately 50 Hz. The electric motor rotating the plate provides strain rates continuously variable above 5/s, and the time required to reach full speed, is about 20 ms.

All tests are performed at room temperature (21–24 °C) with no provision made to regulate the temperature of the sample and apparatus. Temperature variations during tests are less than 0·5 °C. A moist ring of absorbent paper placed as shown in Fig. 4 serves to maintain a high relative humidity around the sample and thereby minimize evaporation.

Collection of pedal mucus

Ariolimax columbianus pedal mucus is collected by allowing the slug to crawl on a glass rod. As the slug attempts to crawl around the rod the rod is rotated forcing the slug to continue crawling lest it lose its footing. In this manner 0·1 to 0·3 ml of pedal mucus can be collected from each slug, an amount sufficient for any of the tests de-
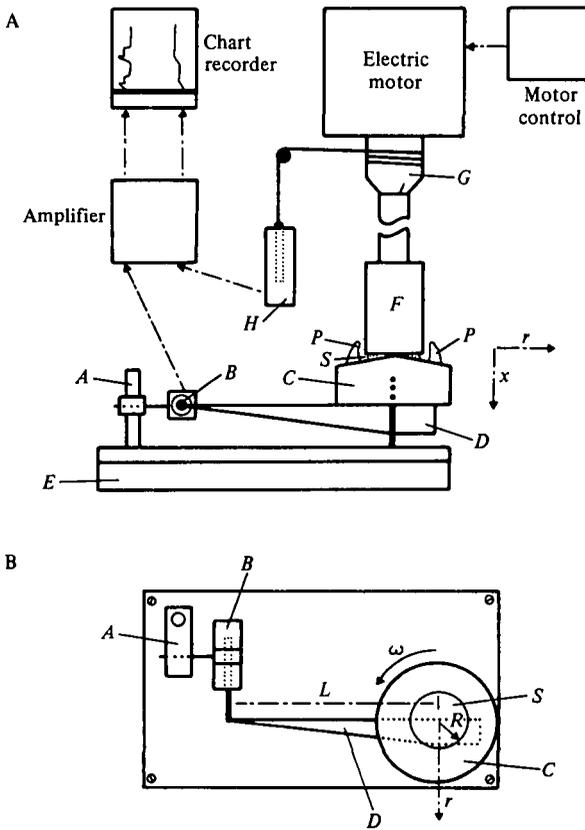
Fig. 4. A combined schematic and construction drawing of the cone and plate apparatus used to examine the physical properties of pedal mucus at large strains. The distance through which a sample is deformed is a function of r, the radius, and ω, the angular velocity and is equal to ωr. The thickness of the sample is again a function of the radius and is equal to mr where m is the slope of the cone. The shear strain thus equals $\omega r/mr = \omega/m$ and is independent of the sample radius. The effective sample radius is the radius of a cylinder coaxial with the cone that contains one half of the sample volume. It is approximately equal to $0.79\ R$. The electric motor is a Cole-Parmer Master Servodyne, and the amplifiers are SE Laboratories type 4300. A, adjustable mounting post for B, a Schaevitz 050 MHR linearly variable differential transformer; C, plexiglass cone supported by an 18 gauge hypodermic needle; D, arm supporting the LVDT core; E, base plate bolted to the horizontally adjustable stage of a milling machine; F, aluminium rod with a polished end acting as the plate; G, chuck attaches the rod to the motor and acts as a capstan; H, Schaevitz 100HR LVDT; L, distance from core to centre of the cone; P, moist paper to slow evaporation from the sample; S, sample. The electric motor is mounted on the vertical adjustable post of a milling machine.

scribed above to be performed on a single sample from one individual. The collection procedure lasts approximately 1 m, and samples are placed in the testing apparatus immediately after collection. No attempt has been made to determine the precise origin of the pedal slime collected, though it is probable that the preponderance is produced by the suprapedal gland. By dusting the dorsal surface of a slug with chalk prior to pedal mucus collection it can be shown that the mucus collected from the foot is not contaminated by mucus from the dorsal epithelium.

It is inevitable that variations be found in the properties of slime, particularly in it hydration when collected. For example, in one set of 18 slime samples collected on a

gle day, the dry weight of the mucus ranged from 2·85% to 4·46% of the wet weight. For certain tests, variation in the hydration of the collected slime appears to cause little variation in the property measured, and for these tests results are averaged and the confidence limits around the mean noted. Other tests are affected by compositional variability to a greater extent. For these tests it often happens that the average of all tests does not closely resemble the measured value for any individual test. In this case the results from the individual sample closest to the average are presented as a 'typical' test and the range of values for all tests is noted.

Physical properties of A. columbianus *mucus at small strains*

Three sorts of tests were conducted to ascertain the properties of *Ariolimax columbianus* pedal mucus at strains less than 5. (1) Stress-strain tests, (2) stress relaxation tests and (3) dynamic tests. The results of these tests will be discussed in turn.

Stress-strain tests

Fig. 5 shows a typical stress-strain curve for mucus deformed in the dynamic testing apparatus to a strain of a few percent at a low shear rate. Under these conditions the slime behaves primarily as an elastic solid, and the stress-strain curve is essentially linear with a shear modulus of approximately 200 Pa. That the mucus is elastic at these strains and shear rates indicates the presence of some crosslinked network structure within the slime, and the viscous nature of the material shows only in the slight hysteresis.

Fig. 6 shows a typical stress-strain curve for mucus at a larger strain and at a shear rate over ten times that of Fig. 5. Under these conditions the viscoelastic nature of the material is evident. Upon loading the mucus shows a G of about 100 Pa but upon unloading it can be seen that a considerable proportion of the energy of deformation has gone to deforming the viscous component of the material, and is non-recoverable. The fact that the material does not return to its original dimensions indicates that whatever network is responsible for the elasticity is either partially broken down at these strains or has rearranged in the course of deformation.

Stress relaxation tests

Fig. 7 shows the averaged results from ten stress relaxation tests carried out in the cone and plate apparatus at a range of strains from 2 to 5. No difference in the time course of relaxation as a function of strain was noted. The relaxation curve cannot be characterized by a single or small number of relaxation times, indicating that there are a variety of different relaxation processes occurring in the material, as is typical for viscoelastic biomaterials.

Within the time course of these experiments (30 min) the relaxing slime does not reach an equilibrium stress, nor does the curve give any hint that an equilibrium would be reached if relaxation for greater periods of time could be measured. In this respect in that it flows, mucus behaves as a fluid. Now it has been shown by stress-strain tests that an elastic network is present during deformation to these strains, at least for short time intervals. Thus it must be hypothesized that this network, while stable over tively short periods of time (seconds), is capable of rearrangement over long periods of time (minutes to hours).
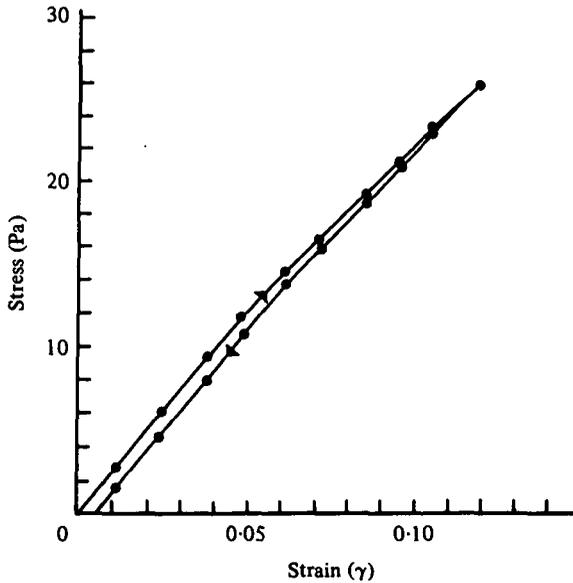
Fig. 5. A stress/shear strain curve for *Ariolimax columbianus* pedal mucus at a small strain. Shear rate = 0·048/s and G = 210 Pa. The hysteresis is 6·9 %.
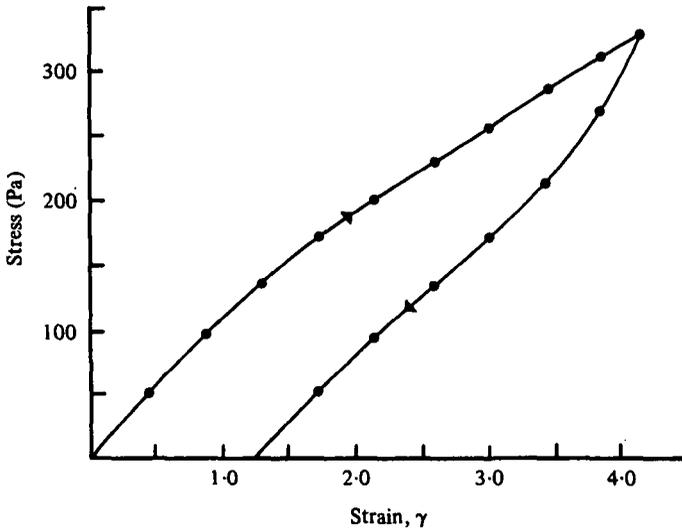

Fig. 6. A stress/shear strain curve for *Ariolimax columbianus* pedal mucus at a moderate strain. Shear rate = 0·56/s and G is approximately 100 Pa. The hysteresis is 44·2 %. Note that the sample does not return to its original dimensions.

Dynamic tests

The results of the dynamic tests on *Ariolimax columbianus* pedal mucus are summarized in Fig. 8. As mentioned above these tests were carried out at low strains of about 0·2 to 0·5 and frequencies from 0·2 to 100 Hz, and are thus designed to reveal the properties of the elastic network on a time scale of milliseconds to seconds. Under these conditions the material is again shown to behave primarily as an elastic so
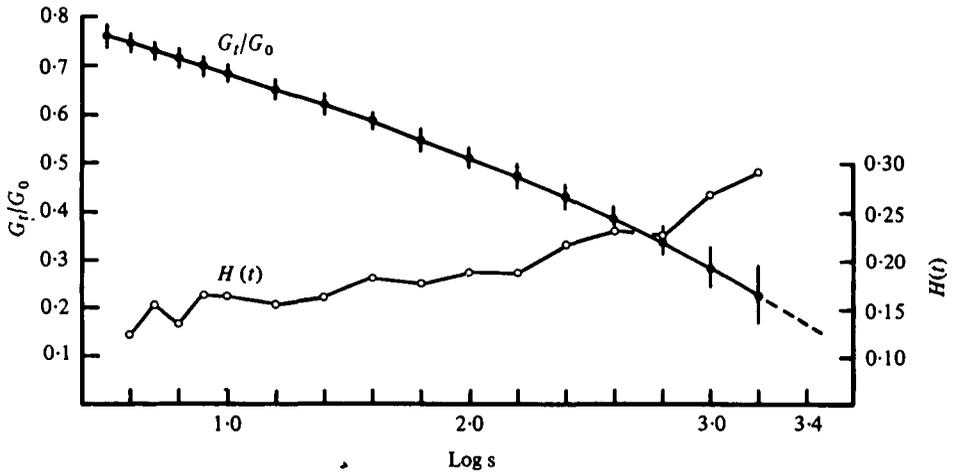
Fig. 7. The stress relaxation characteristics of *Ariolimax columbianus* pedal mucus. The relative shear modulus at a given time (G_t/G_0) and the corresponding relaxation spectrum are shown. The modulus points are averages of ten tests; the bars represent 95 % confidence intervals. The relaxation spectrum is a first estimate calculated from the average relative modulus, $H(t) = d(G_t/G_0)/d(\log{}_t)$.
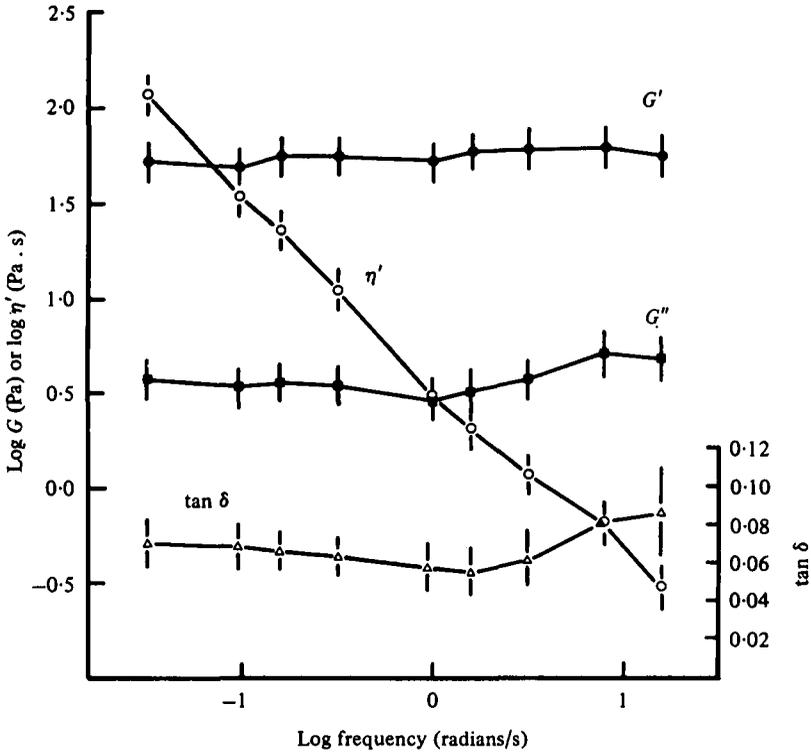

Fig. 8. Dynamic test results. The curves for storage moduls (G'), loss modulus (G''), tan δ, and dynamic viscosity (η') are averages from six samples tested in air saturated with water vapour to reduce evaporation. The bars represent 95 % confidence intervals.

the storage modulus is greater than ten times the loss modulus and the ratio of the two (tan δ) does not vary substantially over the range of frequencies tested. The dynamic viscosity decreases steadily with increasing frequency, as is characteristic of polymeric solids (Nielsen, 1962).

In summary, the tests on *Ariolimax columbianus* pedal mucus at small strains indicate that the material, while viscoelastic, is primarily a solid formed of a crosslinked network, the crosslinks being stable on a time scale of milliseconds to seconds, but unstable at longer periods of time.

Mechanical properties of A. columbianus *pedal mucus at large strains*

While it is useful to know the properties of mucus at small strains, the applicability of this knowledge to the problem of slug locomotion is restricted. For a slug with a step length of 1 mm and a mucus layer thickness of 10 μm, the pedal mucus is exposed to a strain on the order of 100 rather than the 0·1 to 4·0 typical of the tests described above. In order to investigate the properties of *A. columbianus* pedal mucus at larger strains a number of tests were carried out using the cone and plate apparatus.

Stress-strain tests

The results of shearing pedal mucus to large strains are shown in Figs. 9 and 10. It is evident that these properties are quite different from those at small strains and these differences are best explained by following the time course of a test as shown in Fig. 9. The test is initiated when the motor rotating the plate is switched on. As the plate rotates through the initial few degrees the mucus sample is deformed as in the stress-strain tests described earlier, and stress rises roughly linearly with strain with a modulus of about 100–300 Pa. However, at a strain of 5–6, the mucus abruptly yields and the stress falls. Due to the design of the apparatus the strain at which the mucus yields can only be determined to an accuracy of $\pm 1\cdot4$, however within these limits of accuracy the breaking strain does not vary with the rate at which the sample is deformed. In contrast, the stress at which the sample yields (σ_y) is dependent on the shear rate; the higher the rate the larger the yield stress. A plot of yield stress versus strain rate for a typical sample is shown in Fig. 10. As the mucus is deformed beyond its yield point a new stress level is reached and this level remains constant with further deformation. As shown in the introduction, the maintenance of a constant stress for as constant shear rate is one distinguishing characteristic of a fluid and it thus appears that as the mucus yields its elastic network structure is disrupted to the point where the material behaves as a viscous liquid. If this is so, two other facts should be consequent:

(1) The stress required to deform the liquid should be dependent upon shear rate and this is indeed the case: Samples deformed at higher shear rates show a higher post-yield stress, the flow stress (σ_f). A plot of flow stress versus shear rate for a typical sample is shown in Fig. 10, showing that mucus after it has yielded behaves as a liquid with a viscosity of 30–50 poise (i.e. 3–5000 times more viscous than water). Note that the line of σ_f does not extrapolate to zero, and it is assumed that at shear rates lower than those measured the fluid mucus shows a behaviour as depicted by the dashed line on the graph.
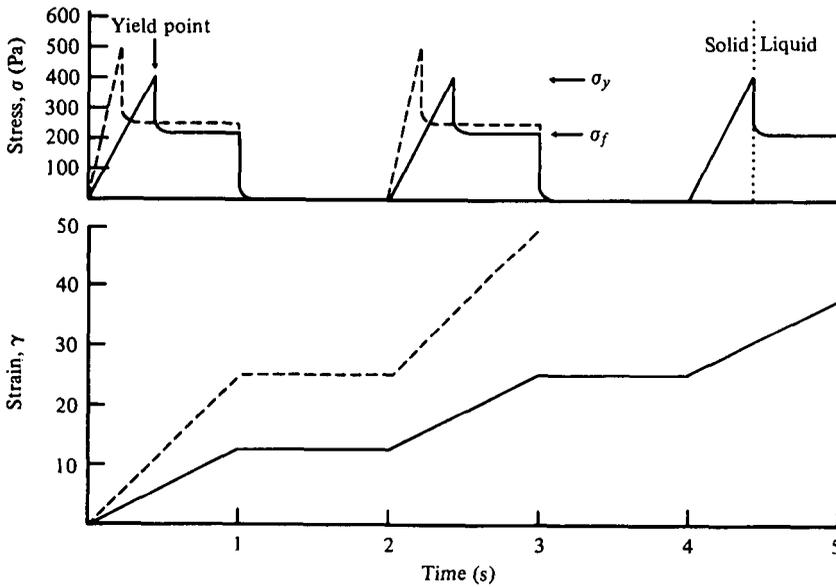
Fig. 9. The characteristics of *Ariolimax columbianus* pedal mucus at large strains. The strain regime and the consequent forces are shown. The mucus yields at a stress $= \sigma_y$ and a strain of approximately 5 to form a viscous liquid. The stress necessary to cause the mucus to flow at the imposed strain rate is the flow stress (σ_f). Yield and flow stresses vary with strain rate. The mucus 'heals' if allowed to rest unstressed.
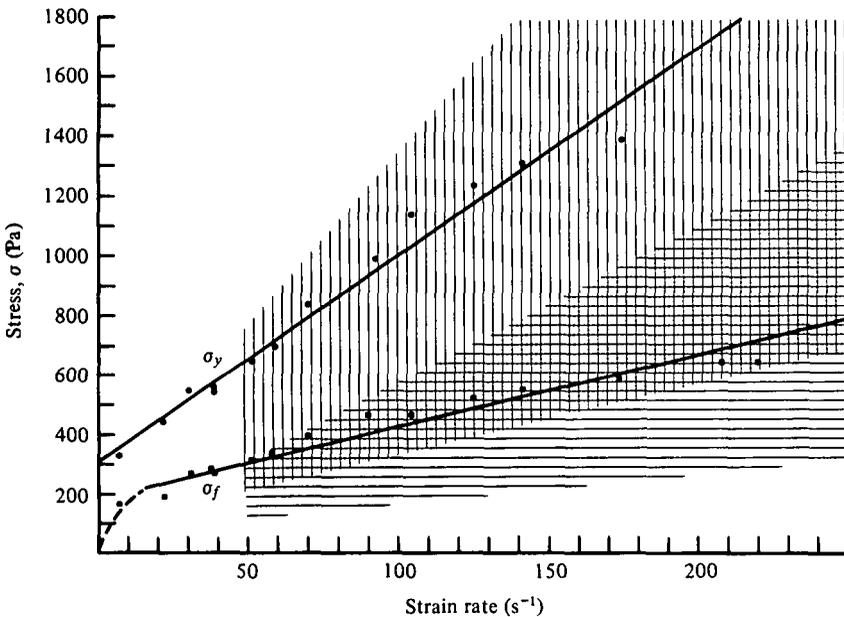
Fig. 10. A plot of yield stress (σ_y) and flow stress (σ_f) for *Ariolimax columbianus* pedal mucus. The data points and regression lines (heavy lines) shown are from a representative sample. The ruled areas show the total range observed in all the samples tested for the strain rates likely to be present under a crawling slug: vertical rulings, yield stress; horizontal rulings, flow stress.
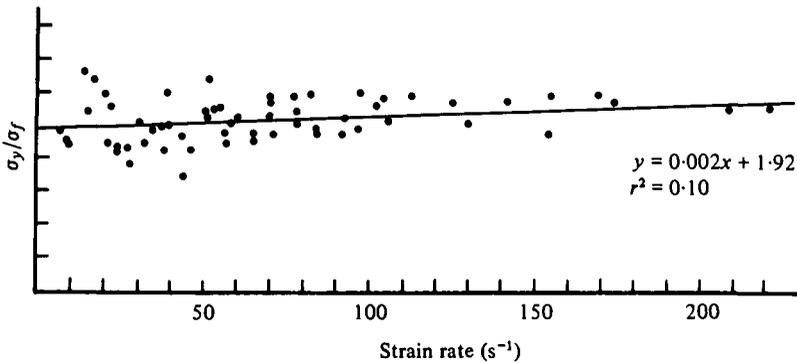
Fig. 11. The ratio of yield stress (σ_y) to flow stress (σ_f) for all samples tested plotted against strain rate. The variability in σ_y and σ_f (shown in Fig. 10) is not reflected in their ratio.

(2) When deformation is halted a solid maintains a positive value of stress; for a liquid the stress immediately decays to zero. As shown in Fig. 9 the highly deformed mucus again behaves as a liquid.

Two physical interpretations are consistent with these results. First, the elastic network may break down throughout the entire sample allowing the sample as a whole to behave as a liquid. Second it is possible that only the network in the portion of the sample adjacent to either the cone or plate is destroyed, forming a thin layer of liquid separating the still solid sample from the cone or plate. In this second case it is essentially the properties of this thin layer that are being tested rather than the sample as a whole. If this is so, and depending on the thickness of the fluid layer, the calculated values of shear rate may be considerably in error. It is impossible from the tests performed in the course of this study to distinguish which of these two possibilities is the correct one. While it would be interesting to be able to resolve this question, the fact that it has not been resolved does not affect the accuracy of the results of these tests in describing the effective properties of slug pedal mucus.

As shown in Fig. 10 there is considerable range in the values for breaking stress and flow stress in the samples tested. Though the hypothesis was not tested it seems likely that these variations are due to the normal variation in the concentration of glyco-protein present in the mucus, as explained earlier. This variation in the magnitude of yield stress and flow stress does not, however, affect the ratio between the two for any one sample, nor is the ratio dependent on shear rate. Fig. 11 shows a plot of the yield stress/flow stress values for all samples tested. This constant value of yield stress/flow stress (equal to about 2·0) is important for the locomotion of the animal. It has been shown (Denny 1980b) that the ability of the mucus to change from a solid to a liquid is a necessity for adhesive locomotion, and the invariant ratio of solid strength to viscosity described here allows the mucus to function effectively within a wide range of hydrations and shear rates.

Do these tests then imply that beneath a moving slug (where the mucus is periodically deformed to a strain of about 100), all the mucus is in its liquid form? After the rotation of the plate is stopped and the stress has decayed, the sample is allowed to remain unstressed for one second (Fig. 9). This period was chosen as equivalent to th
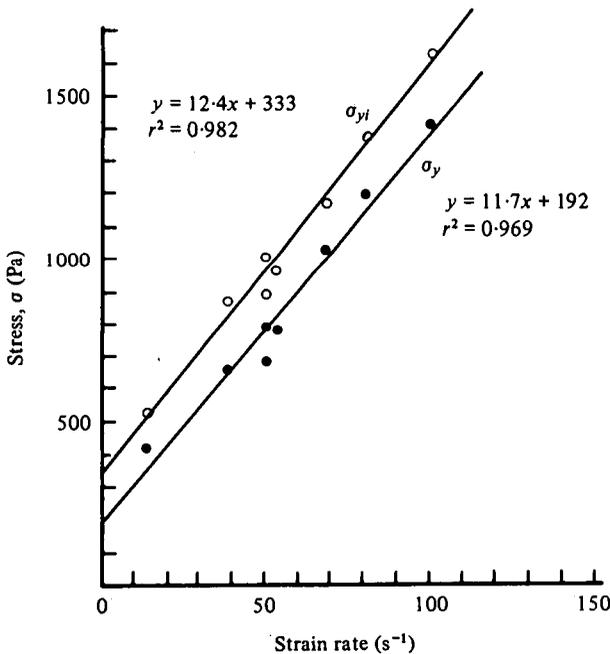
Fig. 12. The initial yield stress (σ_{yi}) after a sample has been left unstressed for a period of time is greater than subsequent yield stresses (σ_y) after only short term 'heal' periods.

time that mucus would be beneath the interwave (and therefore not deformed) under a moving slug. After this time the plate is again rotated. It is found that the mucus has 'healed' and again behaves as a solid, rather than showing the characteristics of a liquid. Stress rises linearly with strain and at a strain of 5–6 the material again yields and so forth. The record of this second period of deformation is identical to the first. This 'yield-heal' cycle can be repeated 20 to 30 times before the mucus begins to show signs of failing to recover its solidity. Thus, the elastic network, which must be broken for the mucus to act as a liquid, reforms quickly. Additional evidence of this process is provided by two further tests:

(1) After a sample had been deformed a number of times at a given shear rate, the sample was allowed to rest for 10–12 s while the controls were set for another set of deformations at a new shear rate. It was noted that the initial deformation in this new series showed a considerably higher yield stress than subsequent deformations. The values of yield stress shown in Fig. 10 are for deformations subsequent to the initial deformation as these are more characteristic of the properties of mucus under a moving slug. Fig. 12 shows values from a typical sample comparing initial (σ_{yi}) and subsequent (σ_y) values for yield stress as a function of shear rate. It is apparent that the elastic network formed when the mucus rests for 10–12 s is stronger than that formed when rest is allowed for only 1 s.

(2) The time course of the healing process was examined through another series of tests using the cone and plate apparatus as shown in Fig. 13. In these tests one complete cycle of deformation is performed on the sample, and the sample is then allowed to 'heal' for varying lengths of time. After healing the sample is again stressed, but
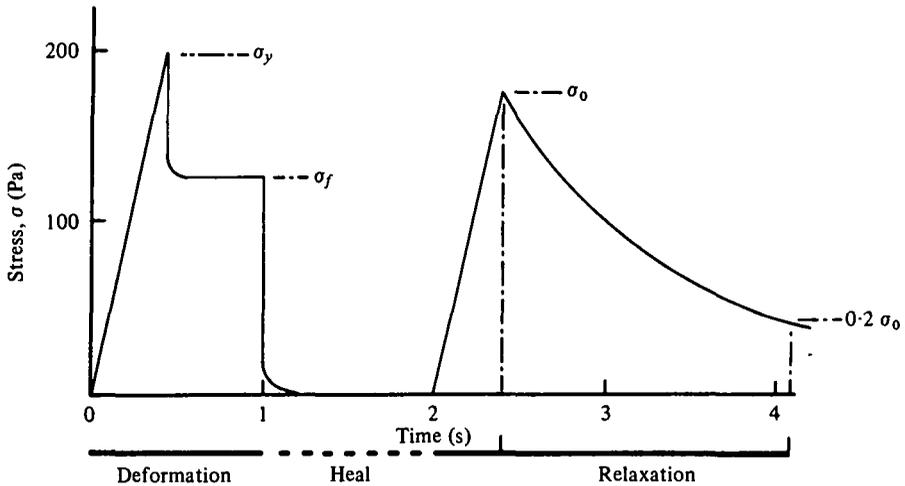
Fig. 13. The testing procedures used to determine the effect of 'healing' time on the recovery of solidity. The mucus is stressed to yielding, and the resulting liquid is allowed to heal for varying lengths of time. The material is then again stressed, but to a stress (σ_0) less than the yield point. The relaxation time (seconds to relax to 0·2 of σ_0) is measured. The longer the relaxation time the more solid the material.

only to a sub-yield level. Rotation of the plate is then halted and a stress relaxation time (τ) is measured. As a matter of convenience, τ was chosen as the time (in seconds) required for the stress to relax to 0·20 of the initial stress. The results of a typical tests are presented in Fig. 14. Relaxation times for any given heal time proved to be quite variable. However, the general trend of increasing τ with increasing heal time is apparent and is taken as evidence that the sample becomes more solid the longer it is allowed to heal. The relaxation time for liquid mucus is too short to be accurately measured by this testing procedure but is certainly less than 0·1 s.

The physical properties of *A. columbianus* pedal mucus can be summarized as follows:

(1) At strains less than 5–6 the mucus behaves as a viscoelastic solid. The shear modulus is on the order of 100 to 300 Pa, increasing with increasing shear rate.

(2) The mucus shows a sharp yield point at a strain of 5–6. Yield stress increases with increasing shear rate.

(3) At strains of greater than 6 the mucus behaves as a fluid with a viscosity of 30–50 poise.

(4) The ratio of yield stress to shear rate for any one sample is about 2·0.

(5) The fluid mucus recovers its elasticity if allowed to heal for a period of time.

(6) The amount of solidity recovered increases with increasing time.

Fibre formation

The properties summarized above were measured under conditions designed to simulate those beneath a crawling slug. However, being lethargic beasts, slugs spend large periods of time simply sitting in one spot. In the cages used to house the slugs for this study the preferred resting position was halfway up the vertical glass walls,
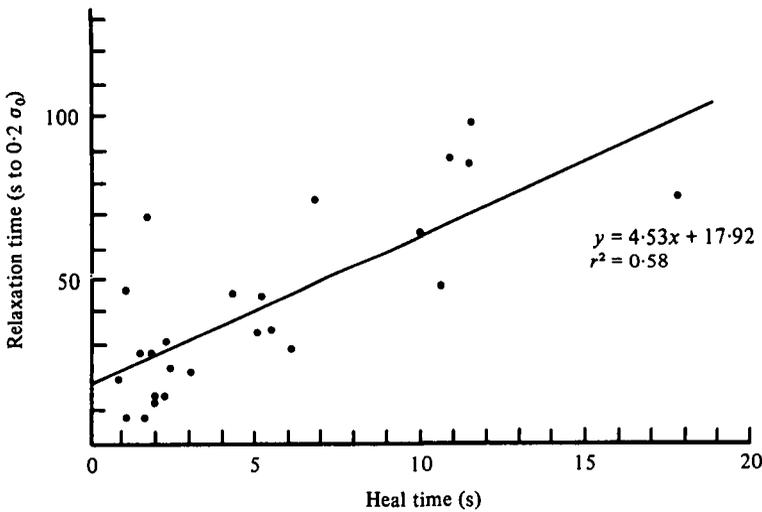
Fig. 14. A representative plot of relaxations time versus heal time for *Ariolimax columbianus* pedal mucus. The range shown in these tests was:

$$\text{low} \quad y = 0 \cdot 11x + 0 \cdot 41 \; r^2 = 0 \cdot 71.$$
$$\text{high} \; y = 5 \cdot 75x + 27 \cdot 59 \; r^2 = 0 \cdot 51.$$

and slugs commonly spent periods of 12–14 h thus attached. Since it has been shown by stress relaxation tests that pedal mucus will flow over long periods of time, why do slugs attached to vertical walls not gradually slide down under the force of gravity? The answer to this question may lie with another property of pedal mucus, its ability to form fibres.

If a slug that has been attached to a vertical wall is gently pried off, a white layer of mucus often remains behind. Upon examination under a polarizing microscope the mucus is found to contain, in addition to the usual debris, a dense feltwork of fine (1 μm), birefringent fibres. It is difficult to trace a single fibre from end to end but they appear to be quite long (up to about 0·5 mm). The fibres do not dissolve if the sample is placed in distilled water. The time course of fibre formation beneath a resting slug has not been studied.

It is possible to induce fibre formation in pedal mucus in the dynamic testing apparatus. Mucus alone (at 100% relative humidity or in distilled water) either does not form fibres of they are formed too slowly to be detected in these tests. If, however the sample is immersed in a salt solution, fibres rapidly form and the formation of fibres is accompanied by a dramatic increase in the shear modulus of the sample. This modulus was measured by stress-strain tests at strains less than 0·10 and shear rates less than 0·10/sec. Fig. 15 shows the results of one series of tests following the time course of this increase in modulus for various solutions. An insufficient number of tests were performed to be able to attribute the different time courses found as being a result of the different salts applied. It can be concluded, however, that while fibre formation is dependent on the presence of salts (no fibres being formed in distilled water) the process is not dependent on either a specific cation or anion, or the valence of either the cation or anion. Again, once fibres are formed they do not dissolve
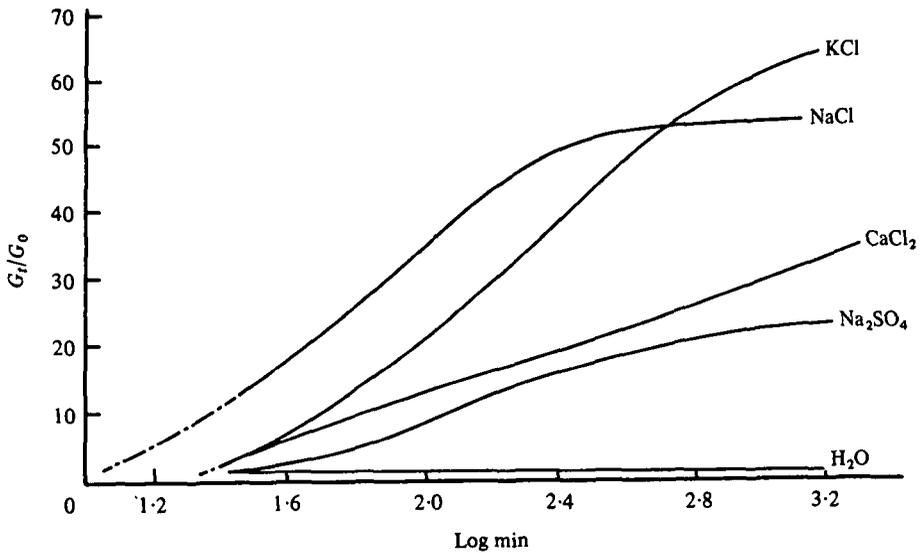
Fig. 15. The time course of the effect of various salts on the relative increase (G_t/G_0) in shear modulus due to fibre formation in *Ariolimax columbianus* pedal mucus. All salts were present as 0·05 M-aqueous solutions.

if the sample is exhaustively dialysed against distilled water and the salts thereby removed.

To accurately predict the behaviour of fibrous mucus under a resting slug it would be necessary to perform 'creep' tests where the sample is subjected to a constant stress and the strain measured as a function of time. Unfortunately machines that allow for creep tests to be performed in shear (as would be necessary for mucus) are difficult to design and construct and no attempt has been made in this study to test the creep characteristics of fibrous and non-fibrous pedal mucus. It is possible however to speculate as to what these properties might be.

It may reasonably be assumed that when fibres form in the pedal mucus under a slug some, but not all, of the glycoprotein chains of the mucus are bound into fibres. The birefringence of the fibres indicates that their molecular structure is ordered, and without exception all other biological birefringent fibres have a modulus considerably higher than that of the randomly arranged mucus network (see Wainwright *et al.* 1976).

Under the microscope the fibres appear to be unconnected. Thus it seems reasonable that this fibrous mucus consists of a low modulus matrix (the mucus elastic network) through which run discontinuous higher modulus fibres. Another class of biological materials with a very similar structure – sea anemone mesoglea – has been studied by several authors, notably Gosline (1971 *a, b*) and Koehl (1977). The relevant findings of these authors are: (1) The modulus of a discontinuous fibre reinforced composite such as mesoglea increases as the proportion of fibres to matrix increases. (2) For a given rate of deformation of the material as a whole the presence of fibres serves to increase the shear rate acting on the viscous matrix component. As a consequence, a fibre reinforced material creeps more slowly than one that is not reinforced. It has been shown that the modulus of fibrous mucus increases as one

would expect if the material were to behave analogously to mesoglea, and it can thus be guessed that the fibrous mucus will creep more slowly than the nonfibrous slime. If this is indeed so, it could explain the ability of slugs to remain attached to vertical walls without slipping.

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