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VISCOELASTIC PROPERTIES OF CUMULUS OÖPHORUS

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Abstract—An experimental technique suitable for the determination of the rheological properties of minute, biological viscoelastic solid specimens is presented. This technique is employed to ascertain the viscoelastic properties of the rabbit cumulus oöphorus. The experimental results when analyzed using the finite theory of elasticity reveal that the cumulus oöphorus can be adequately characterized as an incompressible elastic solid (retardation time less than 0.5 sec) with a strain energy function of the Mooney type. For small deformations, the elastic modulus was determined to be 1500 dynes/cm^2. It is concluded that for the physiological case of oviductal transport the cumulus should be considered to behave essentially as a perfectly elastic body.

INTRODUCTION

An essential step in the reproductive process of mammals is the transport of the newly ovulated egg contained within a viscoelastic mass, the cumulus, through the ampulla of the oviduct to the site of fertilization. Studies (Blandau and Boling[1]) describing this complex mode of segmental peristaltic transport aided by ciliary movement have established the *sine qua non* role of the presence of cumulus in this process. Thus, it is evident that the viscoelastic properties of the cumulus should be incorporated into any physiologically compatible model descriptive of ampullar ovum transport. Surprisingly however, minimal consideration has been given to describing the viscoelastic nature of the cumulus and none to determining its viscoelastic properties.

Previous works ascribed an elastic nature to the cumulus. Blandau[2] refers to the ovulated cumulus as "highly polymerized and sticky". Accounts from the experimental observations of Blandau and Boling[1] describe that when a thin cumulus streamer extending from a recent ovulatory site in the cat is stretched and released, it "snaps back like a stretched band". These studies also note variations in the degree of polymerization of the cumulus among mammalian species. Further, supravitally stained cumuli can be seen to be transported through the ampulla by muscular contractions, undergoing elongation and compression, yet almost always returning to their original shape (Blandau[3]). Finally, Dickmann[4] notes that a gradual reduction in the degree of elasticity of cumuli obtained from gonadotrophin-injected rabbits occurs in the time following ovulation, implying that aging can be associated with a loss in elasticity.

It was in the light of this lack of and the need for the determination of the cumulus' viscoelastic properties that the following investigation was undertaken.

EXPERIMENTAL PROCEDURE

The experimental scheme consisted of retrieving and testing freshly ovulated cumuli from mated New Zealand white rabbits. The rabbit was chosen as the experimental subject because a plethora of investigations on the reproductive physiology of this species has been carried out and an abundance of physiological information is available.

Does (1–2 yr old), housed in individual cages, were mated with two fertile bucks in succession. Nine to $9\frac{1}{2}$ hours later, the does were killed by cervical dislocation and exsanguination and their ovaries surgically removed and placed in a physiological saline solution (0.85% NaCl) maintained at a constant 37°C.

Procurement of the cumuli proceeded within 10 min post mortem by placing the ovary in a warm physiological saline solution (initially 37°C) in a watch glass under a Wild microscope (6–100×). The cumuli were recovered by picking the tip of an enlarged follicle with a No. 25

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syringe needle and exuding the cumuli out of the follicle by gently rolling the needle body over the surface of the follicle. Once the sample was obtained, the ovary was replaced in the 37°C environment. Then the cumulus' resting shape and cellular configuration were noted.

A series of initial tests was conducted by stretching the specimens, submerged in physiological saline solution, between two pairs of forceps over a range of extension ratios of controlled magnitude (up to 300%) and duration (1–300 sec). When the material was stretched to the predetermined length, a stop watch was started and the duration of constant strain recorded. The sample was then released by opening one pair of forceps and the approximate time required for the sample to return to its final position was noted. Finally, upon return, the cellular configuration was checked under 25× magnification to assure that no gross damage was done to the material. In most cases the material was then elongated to another strain for additional measurements.

The results of these tests revealed in all cases examined that the specimens recoiled to their original length almost instantly (well within 0.5 sec). Thus, for the time scales examined, which include the physiological range (Dunn[5]), it was concluded that the cumulus behaves essentially as a viscoelastic solid with a retardation time much less than 0.5 sec.

Next a series of simple extension experiments was conducted in order to measure the cumulus' elastic properties. The minute sample size of the cumulus (~0.01 cm^3) and its low elastic modulus made it impossible to employ standard methods of holding the sample and measuring the small applied tensile forces. For this reason a novel technique was devised whereby the sample was held by the action of surface tension, the tensile forces were measured by means of an analytical balance and the resulting deformations were deduced from photographic records.

Specifically, two to four drops of physiological saline solution were placed on the weighing pan of a Mettler analytical balance, Type S6. Then the cumulus, its dimensions having been measured under 25× magnification, was held from one end with a pair of fine forceps and was lowered onto the pan of the balance until it touched the solution surface. The surface tension of the solution then retained the other end while the forceps were retracted away from the pan by means of a Narishige micromanipulator until the cumulus reverted to its original length. Following the establishment of equilibrium, a mass measurement from the balance and a 1 sec exposure photograph using a Nikon F camera with a Medical-Nikkor Auto 200 mm, f 5.6 lens (2/3 × magnification) were taken. Subsequently, the material was elongated to various other predetermined lengths and the procedure was repeated. Force measurements were accurately made by noting the decrease in mass reading registered by the balance (resolution 5 μg). Axial and transverse extension ratios were determined from the photographs and axial extensions approximately confirmed from the micromanipulator readings. Figure 1 illustrates the extension of a typical cumulus specimen, while Fig. 2 displays one test series.

Further, the possibility of time-dependent behavior was explored by varying the order of the extension ratios investigated. Here, two patterns were followed. In pattern one, measurements were recorded for a series of extension ratios increasing up to the maximum value and then decreasing back to the initial configuration. In pattern two, measurements were noted for an alternating series of large-small-large-etc. extension ratios. In both cases the results showed that the same stresses were found at the same extension ratio, thereby eliminating the possibility of time-dependent behavior. Furthermore, identical stresses found for the same initial and final extension used in pattern one assured that no measurable alteration in experimental conditions, e.g. evaporation, occurred during testing.

Finally, the duration of constant extension ratio was increased sometimes, in order to examine the possibility of stress relaxation. In cases with as long as 300 sec constant extension ratio duration no change in the balance reading occurred, thus implying that the specimen behaves as a perfectly elastic body in the range of extension ratio and time intervals examined.

RESULTS

The reduction of the data involved the determination of the cumulus resting length and width and elongated length and width from 35 mm photographic negatives enlarged 23.75 times and the tensile force from the change in mass reading from the balance. Since the extension ratios imposed varied over a range from 15 to 400%, the data were analyzed using the finite theory of elasticity (for example, see Green and Adkins[6], Eringen[7].) Use of this theory is particularly

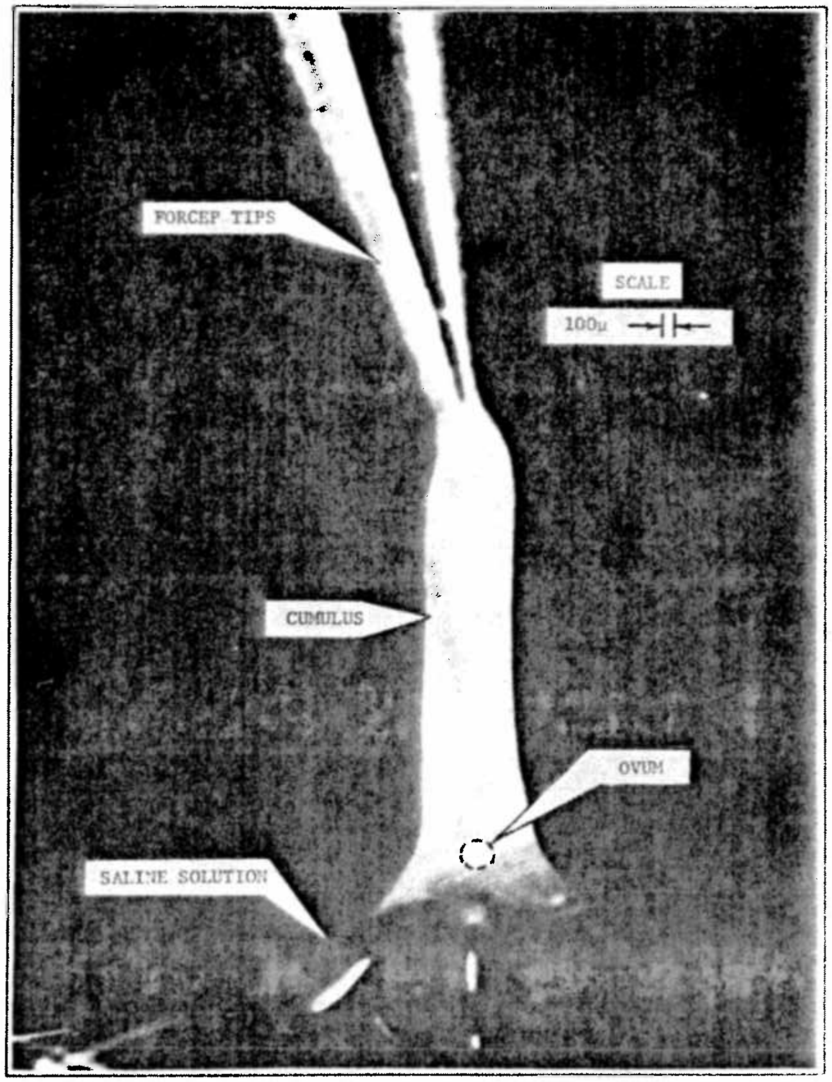

Fig. 1. Elongated cumulus containing ovum.

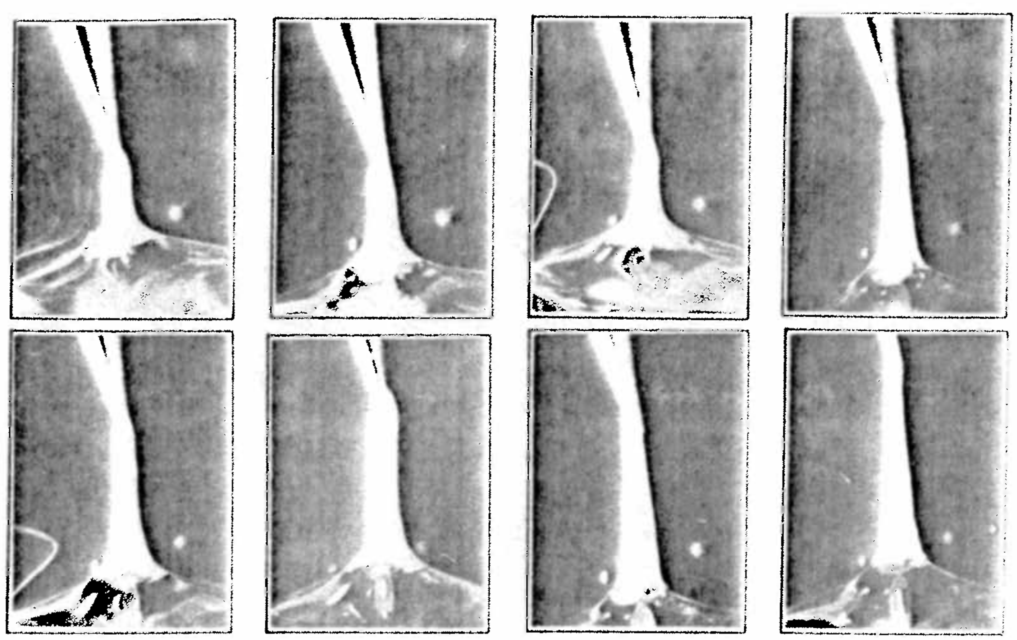

Fig. 2. One typical series of elongation tests (order from left to right, top to bottom).

appropriate for the case of biological viscoelastic solids since most materials of this nature permit large deformations (in some cases up to ~1000% extension ratios) before irreversible effects occur.

For the case of simple elongation of a cylindrical specimen of a homogeneous, isotropic, incompressible,* ideally elastic body, the longitudinal (axial) force, F_L, necessary to maintain equilibrium for the longitudinal extension ratio, $\lambda_L (L_{act}/L_0)$, is given by:

$$F_L = \sigma_{LL} A_{act} = \sigma_{LL} A_0 \lambda_T^2 = \sigma_{LL} A_0 / \lambda_L$$
$$= 2A_0(\lambda_L - 1/\lambda_T^2)\left\{\frac{\partial \Sigma}{\partial I} + \frac{1}{\lambda_L}\frac{\partial \Sigma}{\partial II}\right\} \tag{1}$$

where A_0 is the sample's initial cross-sectional area, L_0 its initial length, L_{act} its extended length, A_{act} its actual cross sectional area, σ_{LL} the longitudinal stress, Σ the elastic strain function, and I, II the tensor invariants of the spatial strain measures. For the case of the simple elongation at hand

$$I = \lambda_L^2 + 2\lambda_T^2 \tag{2a}$$

and

$$II = 2\lambda_L^2\lambda_T^2 + \lambda_T^4 \tag{2b}$$

where the transverse extension ratio $\lambda_T = d_{act}/d_0$, with d_0 denoting the sample's initial diameter and d_{act} its diameter during elongation.

Characterization of the material is made by determining the functional form of Σ. Equation (1) can be rearranged to express the elastic strain function in terms of the experimental parameters, the result being:

$$\frac{\partial \Sigma}{\partial I} + \frac{1}{\lambda_L}\frac{\partial \Sigma}{\partial II} \equiv Y = \frac{2g\Delta m}{\pi d_0^2}\left(\frac{L_0 L_{act}^2}{L_{act}^3 - L_0^3}\right), \tag{3}$$

with Δm denoting the change in mass and g the acceleration of gravity. The results of a least-squares fit of the data to a straight line are presented in Fig. 3. Here, the functional dependence

$$Y = J_1 + J_2(1/\lambda_L) \tag{4}$$

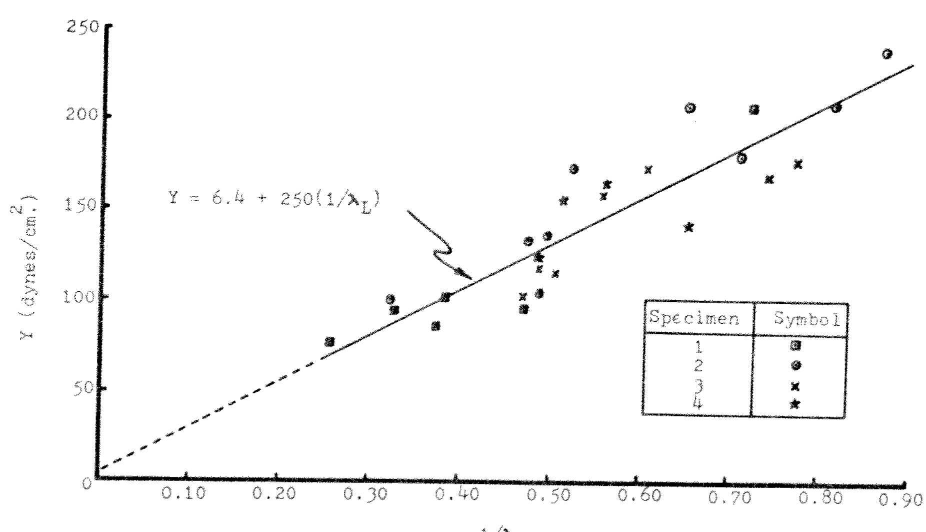

Fig. 3. Simple extension experimental results (Linear regression analysis yields a correlation coefficient = 0.90 and a standard estimate of error = 18.6 dynes/cm²).

*Finite theory advances that if the material is incompressible, $\lambda_L\lambda_T^2 = 1$. From experimental data, the $\lambda_L\lambda_T^2$ mean was found to be equal to 1.02 ($n = 21$, S.D. = 0.16), thereby supporting this assumption of incompressibility.

for a Mooney type material was supported with $J_1 = 6.4$ dynes/cm^2 and $J_2 = 250$ dynes/cm^2. It is noted that this functional dependence is similar to that exhibited by many rubber-like materials (Green and Adkins [6]), where

$$\Sigma = J_1(I-3) + s(II-3)$$
$$= J_1(I-3) + \sum_{i=2}^{\infty} J_i(II-3)^{i-1}, \qquad (5)$$

with J_1 and J_i's denoting constants and where s, a function of $(II-3)$ alone, is represented by a series expansion. In particular, the higher order coefficients of the expansion, i.e. $J_3, J_4, \ldots$ for the experimental case presented herein were found to be of order zero.

Finally, the material's properties appropriate to small deformation theory can be recovered from the finite theory of elasticity by letting $\lambda_L = 1 + \epsilon_L$ in (1) with $\epsilon_L \ll 1$. Evaluating $\partial\Sigma/\partial I$ and $\partial\Sigma/\partial II$ at this essentially nondeformed state, i.e. at $I = II = 3$, one obtains:

$$\sigma_{LL} = E \cdot \epsilon_L = 6(J_1 + J_2)\epsilon_L. \qquad (6)$$

From the J_1 and J_2 values calculated from experiment, the elastic modulus, E, was determined to be equal to 1500 dynes/cm^2. In addition, and for small deformations only, this value of E and the fact that Poisson's ratio is equal to the limiting value of 0.50 (Timoshenko and Goodier [8]) yields a value for the shear modulus G equal to 500 dynes/cm^2, through use of the equation

$$G = \frac{E}{2(1+\mu)}. \qquad (7)$$

DISCUSSION

In the previous analysis many assumptions were advanced without comment. Material homogeniety was supported by the careful examination of the specimen at 100× magnification under the microscope. Only cumuli whose cellular configuration appeared to be uniform were sampled. In those cumuli containing eggs, testing was performed only when the egg was positioned in the material held by the forceps or in the material held by the solution's surface tension. Isotropy was more difficult to check. Ideally, it could have been tested by sample rotation, yielding different orientations in a series of measurements at the same strain. However, due to the small sample volume and, moreover, difficulty in obtaining uniform surface tension on the wider (more irregular) portion of the sample, this was not possible. Yet, isotropy can be argued by noting that for eight cumuli examined from two does no detectable variation in results occurred among the samples, all tested at different orientations for the same extension ratio.

Also, it is noted that results of simple elongation experiments can only suggest a functional form of Σ (Green and Adkins [6]). The certainty with which the functional form of Σ can be stated increases with the number of different deformation fields under which the material can be tested. Since in the present situation only simple extension experiments could be performed the possibility of slightly different functional dependence of Σ upon I and II still exists. Such possibility also arises from the uncertainty introduced by experimental error, as encountered* especially in testing extremely small specimens of approximately cylindrical shape.

The determined value of $\lambda_L\lambda_T^2$ (=1.02) compares well with the value of 1.00 found for most biological materials (Lighthill [9]), natural rubber and other rubber-like materials (Forsythe [10]). The elastic modulus of the cumulus (1500 dynes/cm^2) however, is relatively low for a material of such low viscous component. Resilin (Weis-Fogh [12]) exhibits similar elastic response but possesses a much higher modulus of elacticity of the order of 10^7 dynes/cm^2. Biological materials with lower elastic moduli (e.g. whole sea urchin eggs, Hiramoto [13], low viscosity portion of egg white, Philippoff [11]) possess much higher viscous components than the cumulus. The predominant but weak elastic behavior of the cumulus is indicative of its coherent yet loosely packed cellular matrix observed under light microscopy (Dickmann [4]). It should be pointed out

*The *maximum* experimental error was computed to be 9% in λ_L and 46% in Y (Dunn [5]).

here that our experiments did not rule out the possibility that a sufficiently viscous element can exist in series with the Kelvin model to provide a relaxation time much higher than 300 sec.

CONCLUSIONS

The main results of the present work are: (1) the development of an experimental technique whereby the material properties of a minute biological viscoelastic solid can be ascertained, and (2) the utilization of this technique in conjunction with the finite theory of elasticity to characterize the elastic strain function of the rabbit cumulus oöphorus, and hence, its material properties for the case of small deformations.

Specifically, the results of initial tests demonstrated the cumulus to be of a perfect elastic nature for the range of extension ratios and stretch durations examined, with a retardation time much less than 0.5 sec. Via simple extension experiments, the elastic strain function was found to be of the same simple linear form as that exhibited by many rubber-like materials (Mooney rubber-like materials). For the case of small deformations, these results allow one to determine the cumulus' modulus of elasticity to be 1500 dynes/cm^2. Since the material was determined to be incompressible, the shear modulus was computed to be 500 dynes/cm^2. Hence, it is concluded that for the physiological case of ovum transport during which reasonably small deformations occur and where the wave transport time ($\sim$1–2 sec) well exceeds the cumulus' retardation time ($\ll$0.5 sec), the cumulus should be modelled essentially as a perfectly elastic solid.

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