Biorheology, 1977, Vol. 14, pp. 277–292. Pergamon Press. Printed in Great Britain

INVESTIGATION OF THE RHEOLOGICAL PROPERTIES OF HUMAN SEMEN

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(*Received in revised form* 29 *July* 1977)

Abstract—The results of an investigation of the previously undetermined rheological properties of human semen using a modified, multiple-point capillary viscometer are presented.

The design of a viscometer, specifically constructed to give accurate, instantaneous pressure gradient and material flow rate records of biological viscoelastic fluids whose rheological properties are possibly changing with time is given. Using this device, measurements are made on human semen immediately following ejaculation.

An analytical scheme for the data reduction, suitable for non-linear viscoelastic fluids of the Maxwell-type, is offered. An expression is developed for a non-linear Maxwell-type viscoelastic fluid flow in a circular tube, relating the material's elastic properties to the distance of recoil and the pressure gradient. In the case of a power-law elastic behavior this relation couples the wall shear stress with the recoil distance through an apparent shear modulus. Previously established procedures for the viscous response analysis are utilized and an approximate non-dimensional parameter is introduced allowing one to ascertain the relative contributions of the elastic and viscous components to the rate of flow.

Results show the elastic and viscous properties of human semen to be functions of time following ejaculation and frequency of ejaculation. The elastic component is found to have a linear response over the shear stress range investigated, whereas the viscous component is found to exhibit a power-law behavior. The final equilibrium state is characterized by Newtonian behavior, with mean absolute viscosity of 3.37 centipoise. Finally, similarity among all cases examined is found for each material property through consideration of a nondimensional time, t^*, determined from semen liquefaction time and time post ejaculation.

INTRODUCTION

Currently, a plethora of both sophisticated and simple devices with accompanying analytical techniques is available for the rheological determination of biological material properties (e.g. see Van Wazer *et al.*[1]; Dinsdale and Moore[2]; Philippoff *et al.*[3]). For the determination of the rheological properties of a biological viscoelastic fluid whose behavior changes rapidly with time, the capillary viscometer offers some distinct advantages. In addition to its ease of operation, low cost, and simplified test geometry, it provides the capability of performing elastic as well as viscometric measurements on relatively small sample volumes within a short period of time with minimum handling.

The capillary viscometer has been used successfully in numerous previous investigations of biological fluids (e.g. see Glover[4]; Scott Blair and Glover[5]; Philippoff *et al.*[3]; Biondi[6]; Han and Barnett[7]). All these former capillary viscometer designs are of limited use with a strongly time-dependent viscoelastic liquid. Their limitations arise from the fact that even for a simple‡ rheological characterization of a non-Newtonian fluid by means of a capillary tube, a multitude of pressure gradient vs flow rate data points have to be obtained. When the properties of the fluid vary appreciably over a time scale, T, this set of experiments has to be completed in a time much less than T. In addition, in order to investigate the temperal variation, the entire set of experiments has to be repeated several times at intervals of the order of T until final

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‡A non-Newtonian fluid cannot be fully characterized by capillary tube experiments alone. In the following we limit our attention to those fluids for which the form of the functional dependence of the shear stress on the rate of shear strain is the only rheological information desired.

equilibrium has been reached. It is apparent, therefore, that an instrument which can obtain a multitude of data repeatedly over relatively short time is required.

This communication introduces a modified, multiple-point capillary viscometer, specifically designed for the rapid and accurate acquisition of pressure gradient and material flow rate records required in rheological studies of time-dependent viscoelastic fluids. A companion general analytical scheme is presented whereby both the elastic and viscous properties of a viscoelastic fluid of the Maxwell-type can be ascertained simply from the chronology of meniscal progression in a capillary tube. Special consideration is given to the power-law behavior, which adequately characterizes a variety of biological fluids. Further, the capillary viscometer and analytical scheme are used in the determination of the time-dependent rheological properties of human semen.

ANALYTICAL CONSIDERATIONS

In order to develop an analytical scheme to determine viscoelastic properties an assumption on the type of constitutive behavior must be made. Results of previous investigators of biological viscoelastic substances have shown that many can be considered to be "fluids" of a Maxwell-type or "series" viscoelastic nature. In particular, lung mucus, saliva, post nasal mucus, and egg white (Philippoff *et al.*[3]), intestinal contents (Patel *et al.*[8]), and axoplasm (Biondi[6]) have been shown to possess viscous component behavior which is adequately described by a power-law representation. The elastic element of lung mucus, saliva, post nasal mucus, and egg white, which acts in a "series" manner with the viscous element, exhibits either a linear or power-law behavior in the normal physiological range of stress and mostly non-linear behavior at higher stresses (Philippoff *et al.*[3]).

In essence then, it is asserted that the viscoelastic behavior of many biological liquids, in shear flow within the physiological range, can be adequately represented by a rheological model consisting of single power-law elastic and viscous elements in series, hereby denoted as a power-law Maxwell-type model. This simplified approach is certainly restrictive in view of the existence of more sophisticated and rational rheological constitutive theories. Yet, we feel that the adoption of relatively simple rheological models is appropriate when one is dealing with biological materials which exhibit a certain degree of inherent variability and inhomogeneity within the volume of the specimen required for a rheological measurement. In such cases, the nonuniformities present make the rheological classification of the material, along any but the most simple model suggested by the experiment, all but impossible. These considerations led us to the adoption of a power-law Maxwell-type model. Such a model, in addition to the above-mentioned substances, was also adequate in characterizing the gross viscoelastic properties of human semen.

In a less restrictive sense, an analytical approach can be developed to determine the rheological properties of a Maxwell-type viscoleastic fluid without assuming power-law behavior. In the following, this more generalized approach is presented first. Equations are developed relating the experimentally measured quantities of recoil distance and rate of meniscal advance at prescribed shear stresses to the fluid's elastic and viscous properties.

(*a*) *Elastic component analysis.* Let the stress field in the viscoelastic fluid of the general Maxwell-type flowing through a capillary tube be suddenly released. The elastic strain will then be recovered and the material will recoil. From measurements of the recoverable shear strain at a particular point and shear stress at release at the same point, the elastic properties of the material can be determined.

With respect to a cylindrical coordinate system with its z-axis coinciding with the axis of the capillary tube, the recoverable shear strain γ is given by

$$\gamma(\tau) = \gamma_{rz} = \frac{d\xi}{dr}, \tag{1}$$

where ξ is the elastic displacement in the z direction, τ is the shear stress τ_{rz} at release and r is the radial coordinate. The shear stress at release is equal to

$$\tau(r) = -\frac{\Delta P}{L}\frac{r}{2}, \tag{2}$$

where, neglecting end effects, ΔP is the pressure difference at the ends of the column of fluid of length L. The recoil volume V can be related to the shear strain γ as follows.

One has for the recoil volume:

$$V = \int_0^R 2\pi r \xi(r) \mathrm{d}r. \tag{3}$$

Use of integration by parts in conjunction with the requirement that the displacement at the wall be zero and change of variables from r to τ gives finally

$$-\gamma_w = \frac{3V}{\pi R^3} + \tau_w \frac{\partial}{\partial \tau_w}\left(\frac{V}{\pi R^3}\right), \tag{4}$$

with "w" denoting the value of the variable at $r = R$, namely the wall of the tube.

Equation (4) can be made more amenable to experimental analysis by noting that $\tau_w = (\Delta P/L)\,(R/2)$. The resulting equation is:

$$\gamma_w = -\left\{\frac{3}{4} + \frac{1}{4}\frac{\mathrm{d}\log(4V/\pi R^3)}{\mathrm{d}\log(\Delta P R/2L)}\right\}\frac{4V}{\pi R^3}. \tag{5}$$

This expression is the elastic case analog of the Mooney–Rabinowitsch formula. Setting $C = (\mathrm{d}\log(4V/\pi R^3)/\mathrm{d}\log(\Delta P R/2L))$ one can write simply

$$\gamma_w = -\frac{3+C}{4}\left(\frac{4V}{\pi R^3}\right), \tag{6}$$

where C is the slope of a log–log plot of $4V/\pi R^3$ *vs* $\Delta P \cdot R/2L$. For a linear stress–strain relationship C is equal to one. Previous investigators (Han and Barnett[7]) have set the recoverable wall shear strain equal to $4V/\pi R^3$. The present analysis indicates that this is only true for fluids with linear elastic responses. In the general non-linear case one has to use the "correction factor" $(3 + C/4)$ to compute the true recoverable wall shear strain.

It should be noted that this expression was derived with respect to a fully developed configuration away from the two ends of the fluid column. One can, however, measure the recoil volume by noting the length of meniscal recoil, X, upon release. Conservation of mass requires that

$$V = \pi R^2 X, \tag{7}$$

assuming that no meniscal change of shape occured upon stress release. Hence, by measuring the recoil distance as a function of pressure gradient one can use equations (2), (5) and (7) to plot the elastic shear stress as a function of the elastic shear strain.

A specific relation between recoverable shear strain and shear stress can be obtained for a Maxwell-type fluid with power-law elastic component. Here, the stress–strain relationship is written as

$$\lambda(\tau) = \frac{\tau}{\beta}\left|\frac{\tau}{\beta}\right|^{b-1}, \tag{8}$$

with β denoting the equivalent of the shear modulus for the non-linear case. Substitution of equation (8) into equation (4), and integration of the resulting equation leads to:

$$\frac{4V}{\pi R^3} = \frac{1}{\beta_{\mathrm{app}}}\left|\frac{\Delta P \cdot R}{2L}\right| = \frac{1}{\beta_{\mathrm{app}}}\tau_w, \tag{9}$$

where

$$\beta_{\mathrm{app}} = \beta^b \frac{(b+3)}{4}\left|\frac{\Delta P \cdot R}{2L}\right|^{1-b}. \tag{10}$$

Further, comparison of equation (6) with equations (8), (9) and (10) reveals that in the case of a power-law elastic component, the correction factor, C, equals the power b.

Expression (10) introduces the concept of an "apparent" shear modulus, β_{app}. It represents the shear modulus that a viscoelastic fluid of the Maxwell-type with a power-law elastic component would "appear" to possess when uncorrected recoverable shear strain values are used. Equivalently, it is the shear modulus that a fluid with a linear elastic component should possess in order to undergo the same deformation at the wall as a fluid with a power-law elastic component subject to the same pressure gradient. It should be noted as seen from equations (6) and (8) that higher apparent shear modulus values imply shorter elastic recoil distances for a given applied shear stress, and hence less "elasticity."

(*b*) *Viscous component analysis.* The general analysis for viscous fluids in steady state pipe flow already has been established and applied successfully to biological materials. Following a procedure analogous to general elastic component analysis, one can develop an expression for a true rate of shear strain (known as the Mooney–Rabinowitsch formula; see Van Wazer *et al.*[1]):

$$\left(-\frac{du}{dr}\right)_w = \left\{\frac{3}{4} + \frac{1}{4}\frac{d\log(4Q/\pi R^3)}{d\log(\Delta PR/2L)}\right\}\frac{4Q}{\pi R^3}$$

$$= \frac{(3+e)}{4}\left(\frac{4Q}{\pi R^3}\right), \tag{11}$$

where Q is the volumetric flow rate, u the axial velocity, and e the correction constant. Here, e is evaluated as the slope of the log–log plot of $4Q/\pi R^3$ vs $\Delta PR/2L$.

For the case of a power-law viscous component where

$$\frac{du}{dr} = \frac{\tau}{k}\left|\frac{\tau}{k}\right|^{a-1}, \tag{12}$$

the correction constant, e, becomes the viscous component power, "a". Hence one arrives at the concept of an apparent consistency defined by:

$$\frac{4Q}{\pi R^3} = \frac{1}{k_{app}}\left(\frac{\Delta PR}{2L}\right) = \frac{1}{k_{app}}\tau_w. \tag{13}$$

For a power-law fluid the apparent consistency is given by:

$$k_{app} = k^a\frac{(a+3)}{4}\left|\frac{\Delta PR}{2L}\right|^{1-a}. \tag{14}$$

The complete analogy of the elastic and viscous component formulae should be noted.

(*c*) *Component differentiation.* In order to precisely characterize the fluid's elastic and viscous properties one should attempt to differentiate the effect of the elastic and viscous components on the motion of the fluid. Typically, elastic component behavior is best determined from recoil measurements when the viscous component is inactive due to the removal of the stress field. The analysis of viscous component behavior is more involved in that an elastic contribution to the rate of strain will be present whenever the stress field is time-dependent. This is precisely the case in our measuring technique where fluid is drawn in a capillary tube by constant suction pressure. As the fluid advances in the tube, the pressure gradient and, therefore, the stress field diminishes. The temporal variation of the elastic strain gives rise then to a strain rate and a contribution to the flow rate which is not due to the viscous component. Indeed, for the case of a Maxwell-type fluid whose meniscus has advanced a sufficient* distance along the capillary length, the flow rate, Q, is expressed as a function of the shear

*Sufficient implies a large enough L/D ratio such that entrance effects are neglible and the velocity profile is fully developed.

strain rate by:

$$Q(T) = -\int_0^R \frac{\partial u(t)}{\partial r} r^2 \mathrm{d}r, \tag{15}$$

assuming no slippage at the wall. Here, the rate of shear strain is composed of elastic and viscous contributions, namely,

$$\frac{\partial u(t)}{\partial r} = \frac{\partial u(t)}{\partial r}\bigg|_e + \frac{\partial u(t)}{\partial r}\bigg|_v, \tag{16}$$

with the subscripts e and v denoting elastic and viscous component contributions. A non-dimensional number N can be formed as the ratio of the elastic shear strain rate to the viscous shear strain rate by letting

$$N = \frac{(\partial u/\partial r)e}{(\partial u/\partial r)_v}. \tag{17}$$

For the case of a power-law Maxwell-type one can find

$$N = \frac{bk^a}{\beta^b}\left|\frac{\Delta P \cdot r}{2L}\right|^{b-a}\left|\frac{1}{L}\frac{\mathrm{d}L}{\mathrm{d}t}\right|. \tag{18}$$

by noting that

$$\frac{\partial u(t)}{\partial r}\bigg|_e = \frac{\partial}{\partial t}\gamma_e = \frac{b\,\mathrm{d}L}{L\mathrm{d}t}\frac{\Delta P \cdot r}{2L\beta}\left|\frac{\Delta P \cdot r}{2L\beta}\right|^{b-1}, \tag{19}$$

and

$$\frac{\partial u(t)}{\partial r}\bigg|_v = -\frac{\Delta P}{2Lk}\left|\frac{\Delta P \cdot r}{2Lk}\right|^{a-1}. \tag{20}$$

Further simplification can be made by using the substitutions $\lambda_p = bk^a/\beta_b$ and $u_{\mathrm{avg}} = \mathrm{d}L/\mathrm{d}t$, the result being:

$$N = \frac{\lambda_p u_{\mathrm{avg}}}{L}\left|\frac{\Delta P r}{2L}\right|^{b-a}, \tag{21}$$

where λ_p denotes the power-law relaxation parameter.* It is emphasized that N is not constant during the course of an experiment and any statement regarding its magnitude should be made with reference to a particular time.

For the case when both components are linear ($a = b = 1$), equation (21) reduces to:

$$N = \lambda u_{\mathrm{avg}}/L, \tag{22}$$

which is the result found previously for the case of a linear Maxwell fluid (Broer[9]), where N was interpreted as being the ratio of elastic force per unit volume to viscous force per unit volume. Also, in this case, one observes that u_{avg} is proportional to $\Delta P/L$ making N proportional to L^{-2}. Hence, the contribution of the elastic component to the flow rate will diminish rapidly (like $1/L^2$) as the fluid advances in the axial direction. A further interpretation of N as the ratio of the fluid's relaxation time, λ, to the fluid's residence time within the capillary, L/u_{avg}, indicates that N for the linear cases reduces to the Deborah number.

*For the power-law case, λ_p has units of (time). (stress)$^{(a-b)}$.

It is seen from the above that to correctly characterize a viscoelastic fluid's viscous component behavior, measurements must be performed at positions along the capillary where N is sufficiently small ($N \lesssim 0.01$). The determination of N, however, is dependent upon a knowledge of the fluid's elastic and viscous properties. This circuitous problem can be circumvented by conducting viscous component analysis in an iterative manner. Measurements are made over a wide range of flow rates at various meniscal positions and then analyzed via the Mooney–Rabinowitsch formula. The resulting initial viscous property values are used in conjunction with recoil measurement results to obtain the first estimate of the axial positions and flow rates which render N sufficiently small. Then, further viscometric measurements are conducted over the newly specified range, whereby a more precise characterization of viscous component behavior is obtained.

EXPERIMENTAL APPARATUS

The multiple-point capillary viscometer technique entailed the sequential photographing of meniscal position and simultaneous digital representations of suction pressure, temperature, and time as the meniscus advanced along the length of the capillary tube. Specific experimental parameters recorded were the imposed suction pressure, tube diameter, meniscal position, and shape (during advance and recoil), sample temperature, and time. From these quantities all the necessary material properties could be determined.

A schematic of the experimental apparatus is shown in Fig. 1. The apparatus can be divided into the vacuum system, the pressure–time–temperature monitoring system, the photography system and the pressure vent system.

The vacuum system was partially composed of a Duraire pressure vacuum pump, Model No. PB-200, vacuum regulator valve, Ray Model No. 1 pressure snubber, and Klemm charcoal inlet filter connected by means of a 0.25 in (0.64 cm) i.d. rubber hose to the test stand. The vacuum pump was capable of providing a continuous vacuum down to 94 kPa below atmospheric when operated alone. Pressure fluctuations due to the pump's cyclic cylinder operation were eliminated by the pressure snubber. In-line installation of the snubber also reduced the vacuum pressure range that the system could deliver, thus providing a control on the pressure range by insertion of interchangeable pistons (0–69, 0–27, and 0–8 kPa with No. 1, 2, and 3 pistons). Completing the vacuum system were an Asco 3-way solenoid valve and T-junction leading to the pressure transducer and capillary tube mount. Capillary tubes used were Trubore precision glass tubing with internal diameters ranging from 0.203 to 1.016 mm (tolerance ± 0.010 mm) and lengths from 102 to 176 mm.

The suction pressure was recorded using a Validyne multiple range pressure transducer, Model DP15TL, in conjunction with a Carrier demodulator, Model CK10. Diaphragm ranges

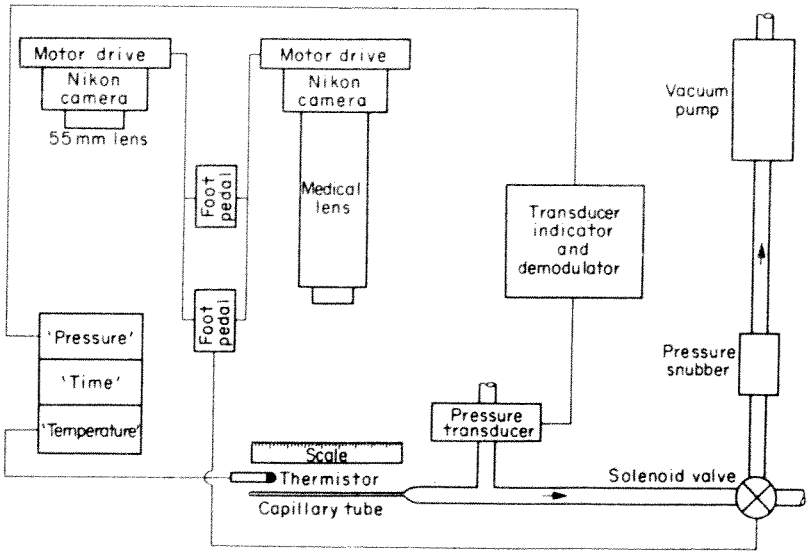

Fig. 1. Schematic of the experimental apparatus.

used during the course of the experiment were 34 and 138 kPa. The digital output of the demodulator was displayed on a DataTech integrating digital multimeter, Model 350 (1 μV resolution). The material temperature was found by positioning a Fenwal UUA32JA uni-curve thermistor (± 0.2°C from 0 to 100°C) adjacent to the capillary tube. The resistance of the thermistor was monitored on a Keithley digital multimeter, Model 160 (0.1 Ω resolution). Time was recorded with a Systron Donner universal countertimer, Model 1034 (1 μsec resolution).

The photography system included two Nikon F-35 mm single lens reflex cameras driven by Nikon F-36 motor drives, one equiped with a 50 mm *f*/1.4 lens and the other with a Medical-Nikkor auto 200 mm *f*/5.6 lens. The position (marked by a scale graduated in 0.01 in. increments) and shape of the meniscus were photographed with the medical lens equiped camera and the digital representation of the instruments with the 50 mm lens equiped camera. Optimum picture quality was obtained using Kodak Panatomic-X, ASA 32, black and white film and motor drive settings at one frame per sec with 1/125th sec shutter speed and a *f*/1.4 setting for the 50 mm lens and *f*/16 for the medical lens at 2/3 × magnification. Due to the small aperture opening in the medical lens, an external light source was provided by perpendicularly reflecting a General Electric 75 W reflector flood lamp's light off a mirror positioned at 45 degrees from the horizontal immediately below the capillary tube and scale. The 2/3 × magnification provided a subject field of 35 × 53 mm, thereby enabling a sufficient length of the capillary to be photographed. For slower rates of flow this field size was not necessary to obtain numerous data points, so the magnification was increased to 1 × or 1.5 ×, reducing the subject field to as little as 17 × 25 mm. This enabled greater magnification of the meniscus shape. However, magnifications greater than 2/3 × were not essential since magnifications of 23.75 × (53.44 × using 1.5 × on the medical lens) were obtained when used in conjunction with a photographic enlarger. Camera synchronization (to within 0.224 sec) was accomplished by means of a contact closure foot pedal switch connected to each motor drive.

The pressure vent system was composed of a contact closure foot pedal switch joined to the pressure vent circuit, further connected to a solenoid valve and the cameras. It allowed for photographs of meniscal shape to be taken immediately prior to, during, and following recoil. In its normally open position the solenoid valve maintained an open gate between the vacuum pump and capillary tube. Pictures were taken about 10 msec following depression of the foot pedal due to the relay response. Approximately 25 msec later, the solenoid was energized, closing the vacuum pump-capillary tube gate and simultaneously venting the capillary tube to the atmosphere, therby eliciting recoil. Continued depression of the foot pedal enabled a series of pictures to be taken until the substance had fully relaxed.

EXPERIMENTAL PROCEDURE

Prior to experiment, the environmental temperature was adjusted and allowed to come to equilibrium. The specimen was placed in a glass tube (5 cm long of 0.5 or 0.75 cm i.d.) open at both ends. It was positioned such that the sample reached the capillary tube inlet.

Initially, the material was brought into the capillary tube some short distance (~ 2 cm) under minimal pressure gradient (~ 2 kPa). Here the substance was permitted to come to rest. A picture was taken of the resting meniscus. Then, the vacuum pump was reactivated at a desired pressure gradient. By the time the meniscus reached a distance of $L/D \sim 100$*, its advance has become uniform. About 25 pictures were taken either continuously or at intervals as the meniscus travelled further along the tube. Finally, the vacuum was vented and pictures taken until the substance had relaxed fully. This procedure was repeated for a number of trials upon introduction of a new capillary tube for each test.

Experiments were performed on two grades of silicon oil and egg white in order to test the capability and accuracy of the device in measuring both viscous and elastic properties. Silicon oil was chosen because of its Newtonian behavior and minimal viscosity variation with temperature. Commercially available egg whites were selected because of their previously determined characteristics (Philippoff *et al.*[3]).

*Philippoff and Gaskins have established that end effects, and kinetic and elastic energy losses for viscoelastic fluids can be minimized by conducting measurements at $L/D \geq 100$ and recoverable shear strains of order one. See Dunn[13] for further discussion of capillary viscometer errors and corrections.

The beginning tests were conducted with Dow Corning 60,000 centistoke silicon oil over a wall shear rate range from ~ 1 to $\sim 2.5\,\text{sec}^{-1}$. A linear regression analysis applied to the data yielded an absolute viscosity of 53,900 centipoise. This was compared to the manufacturer's value of 57,900 centipoise (corrected to the mean experimental temperature of 28°C), giving a 6.9% error. Further tests were performed on 200 centistoke silicon oil. Results over a wall shear rate range from 21 to $99\,\text{sec}^{-1}$ revealed a 1.8% error when the experimental value of 197 centipoise was compared to the corrected (to 25°C) manufacturer's value of 194 centipoise. The deviations of both experimentally determined absolute viscosities from actual values were checked and found to be within experimental error.

Trials investigating viscoelastic behavior were undertaken using samples of both high and low viscosity portions of egg white (for a description see Philippoff *et al.*[3]). In order to examine elastic behavior, recoil tests were conducted on both portions. The results are presented in Fig. 2. It is noted that for the egg white recoil data presented, no discernible differences in meniscal shape were found before and after stress application, thereby obviating the recoil volume correction factor (Philippoff *et al.*[3]). Examination of the high viscosity sample revealed power-law behavior over the entire range investigated, especially evident at wall shear stresses greater than 500 dynes/cm.^{2} In Fig. 2 both the uncorrected and true recoverable shear strain at the wall are plotted, showing a 6.7% error when corrections are not considered. Further, comparison of the low viscosity sample with the data of Philippoff *et al.*[3] was made. Linear elastic behavior was supported for the low viscosity portion. Variability in elastic shear modulus values was attributed to the different egg white specimens used.

The viscous properties of a low viscosity sample were obtained employing the aforementioned analyses. Results showed the elastic contribution to flow rate to be negligible when compared to the viscous contribution ($N = 10^{-7}$) at the L/D distances examined. A nonlinear regression least-squares power curve fit of the data supported viscous power-law behavior. The resulting values of $k(16.7)$ and "a" ($= 1.59$) were found to be of the same order as those offered previously by other investigators (Philippoff *et al.*[10]). The variability can again be attributed to the difference in commercially available egg white specimens.

Hence, it was concluded that the multiple-point, capillary viscometer when used in conjunction with the aforementioned analyses was sufficiently accurate to be used in human semen material property determination.

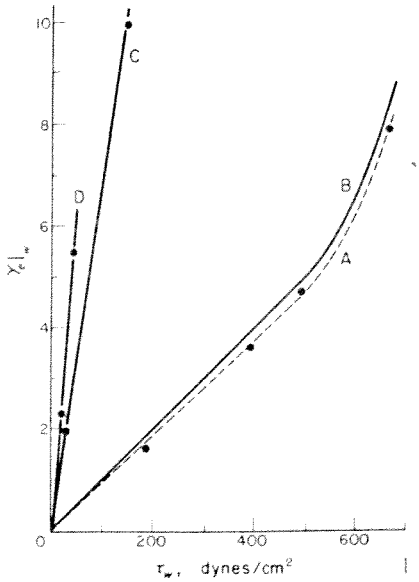

Fig. 2. High and low viscosity portion of egg white: recoverable wall shear strain vs wall shear stress. (A) Uncorrected high viscosity portion; (B) Corrected high viscosity portion; (C) Low viscosity portion; (D) Low viscosity portion of Philippoff *et al.*[3].

RESULTS

The experimental scheme concerned the measurement of elastic and viscous property variations of human semen with respect to time post ejaculation and frequency of ejaculation. This involved the study over a period of eight months of semen from a healthy subject (age 26), who exhibited "normal"* coagulation and liquefaction processes. This "single subject" approach was justified since the inherent physiological variability in semen composition for "normal" single experimental subject (Mann[12]) can be expected to be of the same order as the variance of a "normal" population. The normalcy of the donor subject was confirmed *a posteriori* by our measurements. The mean absolute viscosity of the fully liquefied semen (3.37 centipoise) reported here is in close agreement with the mean value of 1,111 semen samples (3.92 centipoise) obtained by Tjioe and Oentoeng[16] using a Hellige capillary viscometer. In addition, the subject's ejaculate volume dependence on the interval between ejaculations, reported by Dunn and Picologlou[19], agreed well (within 10%) with the findings of Eliasson[15] based on eleven subjects. The specifics of minimizing an individual's variability and thereby establishing a constant sample procurement scheme is discussed elsewhere (Dunn[13]).

The scheme of sample procurement consisted of establishing some initial standard state. This involved performing initial tests only after five days of ejaculatory abstinence, since it has been demonstrated that in the three to seven day period following the last ejaculatory instance the controlling secretory glands maintain a constant stable capacity (Harvey[14]; Eliasson[15]). Additional samples were obtained at four hour intervals. Other initial and continuing tests were conducted after another five day continence period. Following a four month interim, further tests were conducted in order to substantiate the relation between observed liquefaction time and material property variations. In these cases, the initial tests, which proceeded after three days of abstinence, were followed by ones conducted at 6, 12, 24, 48 or 72 hr intervals.

Procedures in addition to those mentioned before were employed with regard to the sampling of semen and measurement of recoil. Following deposition into a 20 ml glass beaker and measurement of ejaculate volume, the sample was inspected to assure that uniform ejaculate mixing had occurred. In few instances when the ejaculate appeared nonhomogeneous, the material was stirred gently by two or three rotations of a fine glass rod. However, selection of a homogeneous portion of the sample was usually accomplished by positioning the small capillary tube opening adjacent to the desired sample portion. During the experiment, the unused portion of semen was stored in the covered beaker and maintained at local test temperature (36 ± 1°C). The sample's time of liquefaction was noted. Finally, to allow for a more accurate determination of elastic properties, additional recoil tests were conducted during a single experimental trial. This was accomplished by performing extra recoil tests in succession after flow rate and initial recoil trials had been undertaken.

(*a*) *Elastic property results.* The initial analysis of elastic component results involved the determination of the shear stress vs shear strain relationship over the shear stress range examined. This was accomplished by plotting the recoverable wall shear strain vs the wall shear stress, as shown in Fig. 3 where the volume of recoil, V, was corrected† due to meniscal shape variation upon recoil. Here, data are presented for an initial sample, No. 5, following a five day abstinence period, and a second sample, No. 6, obtained four hours later. These were typical of the results found for all samples taken.

Examination of Fig. 3 reveals a sparcity of points for a particular test which would aid in the confirmation of linearity. This was because the time interval over which recoil measurements could be made was greater than that during which shear modulus values changed appreciably (especially during the later time periods post ejaculation). However, in some cases, recoil tests were conducted as rapidly as possible over a short period of time (< 45 sec), during which properties did not vary noticeably. Two such cases are presented in Fig. 3, Tests 9–A and 5–D.

*Normal implies coagulation immediately after ejaculation and complete liquefaction within 20 min thereafter (Eliasson[11]).

†Correction factors, which ranged from 0.83 to 1.00, were similar to those found for other viscoelastic biological liquids over the same shear stress range (Philippoff *et al.*[3]).

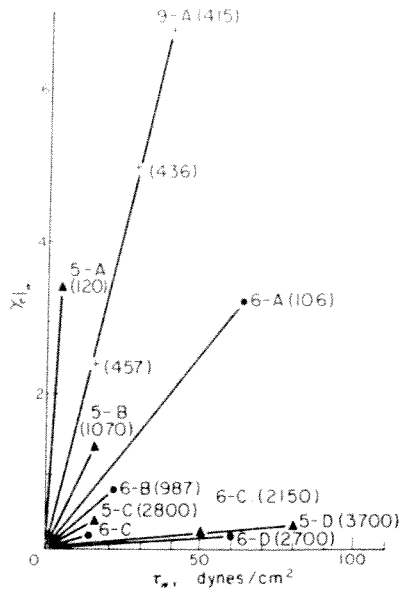

Fig. 3. Human semen: recoverable wall shear strain vs wall shear stress at various times post ejaculation for samples No. 5, 6, and 9 (time post ejaculation in sec given in parentheses).

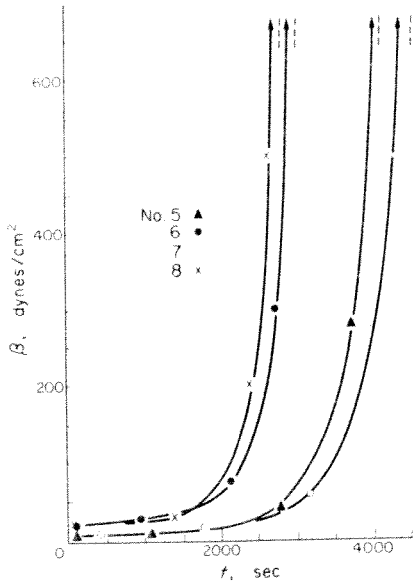

Fig. 4. Human semen: shear modulus vs time post ejaculation for samples Nos. 5–8 (dashed lines denote the times t_∞ that the recoil became immeasurably small).

The behavior is apparently linear. Linearity was also exhibited by other "similar" viscoelastic biological materials previously investigated (Philippoff *et al.*[13]), over the same wall shear stress range.

The shear modulus, β, can be plotted as a function of time post ejaculation (tacitly assuming linearity for the additional tests conducted), as depicted in Fig. 4 for Samples No. 5 and No. 6. This representation allows for comparison of elastic behavior between the initial sample and that of the second sample obtained four hours later. One observes that the shear modulus for the second sample approached large values of β in a shorter time period than the initial sample. Further, recoil measurements showed that at ~ 3000 sec post ejaculation, the recoil distance became immeasureably small, indicating negligible elastic behavior. For the initial sample, however, this did not occur until ~ 4100 sec post ejaculation.

This observation of possible variances between first and second sample shear modulus values at the same time post ejaculation led to performing additional similar tests. Results are depicted in Fig. 4 for another series, initial Sample No. 7 obtained after a five day abstinence period and a second sample, No. 8, obtained four hours later. These results typify those of further tests. Again, differences in shear modulus values at the same time post ejaculation for samples of a particular series were manifested. Here, recoil distances became immeasurably small at ~ 2800 sec post ejaculation for a second sample, No. 8, and at ~ 4500 sec for initial Sample No. 7. Yet, one similarity between all test series was observed. Specifically, the shear modulus values for second samples always approached large values of β in a shorter time period than their initial counterparts. It is noted also that a comparison between, say, the initial tests in Fig. 4 demonstrates a variance in β values for the same time post ejaculation. This was similar to that found in other cases subject to identical experimental conditions.

(*b*) *Viscous property results.* The viscous component analysis for all cases of data examined indicated that the viscous component of post ejaculatory semen is best characterized by a power-law representation (equation (12)). Substitution of the resultant property values for each case into equation (21) to determine N (evaluated at $r = R$) yielded values of N ranging from $\sim 10^{-8}$, for the "most liquefied" test (No. 6–8), up to $\sim 10^{-2}$, for the "most coagulated" test (No. 5–1).

A typical set of stress vs rate of strain data is presented in Fig. 5. For each set of data an averaged least-squares fit was computed, as depicted by the solid line in the figure. The resulting averaged least-squares fits for representative initial and second tests are shown in Fig. 6. For all the curves shown, the maximum deviation from the averaged least-squares fit was less than 15% and the correlation coefficient was greater than 0.97.

One observes from Fig. 6 that if the solid lines representing the "most coagulated" cases (e.g., Nos. 5–1, 6–1, 6–2 and 6–3) were extended to an ordinate axis, they would suggest possible yield stresses be associated with semen in its "most coagulated" state. However, based on comparison with other biological materials investigated (Philippoff *et al.*[3]; Patel *et al.*[8]), this probably is not the case. Instead, at some lower rate of wall shear strain the material most likely assumes another lower value of the viscous component power, "a". Investigation of behavior at these lower rates of wall shear strain was limited by the physical capability of the experimental device.

A more lucid display of viscous property variation with respect to time post ejaculation is offered in Fig. 7 for Samples No. 5 and No. 6. Here, the apparent consistency, k_{app}, as given by equation (14), is presented in lieu of the consistency k to provide for comparison among experiments. Examination of initial and second samples reveals that the apparent consistency for the second sample approached a constant value with respect to time post ejaculation in a shorter time period than the initial sample.

In order to substantiate the noted difference between first and second apparent consistency values for the same time post ejaculation, additional similar tests were conducted. The results of another series, Samples No. 7, No. 8 (obtained four hours after No. 7), and No. 9 (obtained 24 hr after No. 7), which were representative of other tests, are presented in Fig. 8. Again, the

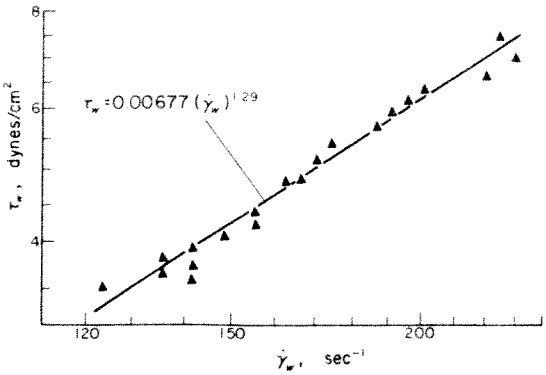

Fig. 5. Human semen: example of log–log plot of wall shear stress vs rate of wall shear strain due to the viscous component (correlation coefficient = 0.99, maximum error = 9%).

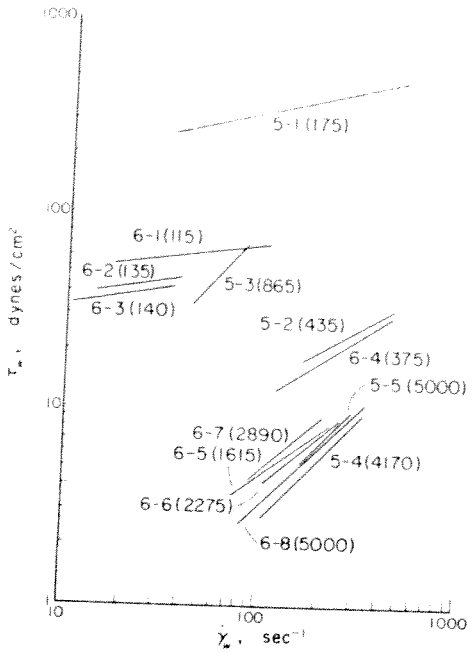

Fig. 6. Human semen: log–log plot of wall shear stress vs rate of wall shear strain at various times post ejaculation for samples No. 5 and 6 (time post ejaculation in seconds given in parentheses).

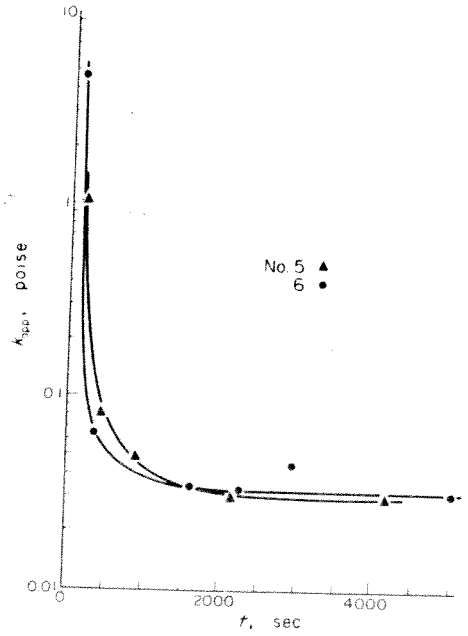

Fig. 7. Human semen: semi-log plot of apparent consistency vs time post ejaculation for samples No. 5 and 6.

same type of variance between initial and second samples was manifested. Yet, an additional third sample (No. 9) attained a constant k_{app} value over an intermediate time period. Comparison between tests performed under identical experimental conditions, e.g. Samples No. 5 and No. 7, yielded dissimilar k_{app} values for the same time post ejaculation. This variance, typical of that found in other cases, is another example of variations under identical experimental conditions (as displayed before in shear modulus behavior).

One further observes in Fig. 7 and 8 the lack of final apparent consistency dependency upon

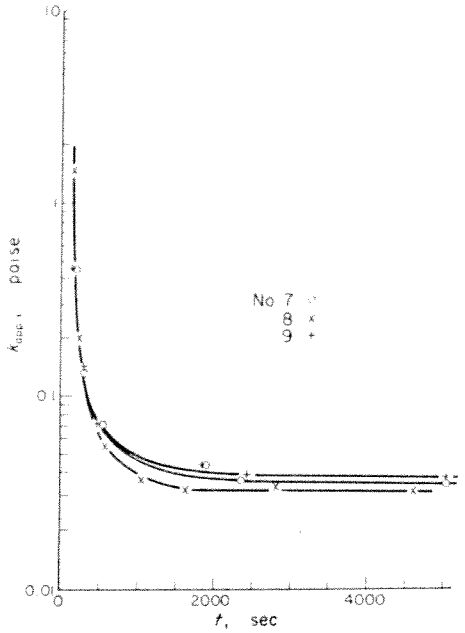

Fig. 8. Human semen: semi-log plot of apparent consistency vs time post ejaculation for samples Nos. 7–9.

sample order. For all cases examined, no characteristic trend of the final k_{app} values with respect to sample order was manifested. Calculations using the final k_{app} values of these experiments revealed a mean absolute viscosity of 3.37 centipoise (S.D. = 0.63 for $n = 7$) at a mean temperature of 33.2°C (±0.2°C). This value compared well with that obtained by Tjioe and Oentoeng[16], using a Hellige capillary viscometer, where the mean viscosity determined from a total of 1111 semen samples examined within the second hour post ejaculation was 3.92 centipoise (range of 1.2–23.3 centipoise).

Further, the viscous component power, "a", was found to be dependent upon the time post ejaculation. The only similarity found between all cases examined was that the rapid decrease in the values of "a" occurred always at an earlier time post ejaculation for second samples than for initial samples. Also, variations in values of "a" for tests performed under identical experimental conditions were found.

DISCUSSION

To quote Scott Blair[20], "Very little work appears to have been done on the 'viscosity' (certainly anomalous) of semen, even in man". Consequently, one cannot fully discuss our findings in relation to previous investigations. In those few instances where information, similar to that reported here, was available the agreement was quite good. In contrast, considerable work has been done on the bull semen by Szumowski[21], Walton[22], Glover and Scott Blair[23], [24], Melrose[25] among others. The majority of that work is directed towards the understanding of the "wave motion" and "rheotaxis" of bull spermatozoa and the relationship between their motility and fertility and is not directly related to the present work. Particularly interesting from our point of view is the work of Glover and Scott Blair[23], [24] which attempts to correlate the sperm motility and viability to semen viscosity. These investigators present in[23] curves exhibiting the temporal variation of the viscosity of bull semen for a variety of shear stresses and they attribute this variation to the activation and death of the sperm. Although it is very tempting to extrapolate these interesting results to account for our measurements, it is nevertheless a hazardous undertaking. A most distinct difference between semen of man and bull is the fact that the latter lacks the coagulation–liquefaction process so prevalent in the former. Indeed, in man this process is mainly responsible for the pronounced temporal variation of the rheological properties of semen. This position is supported by our measurements as we will show shortly. The work of Glover and Blair suggests, however, that part of these changes might be due to the motility of the sperm. This is an interesting premise

and is worthy of further investigation. Unfortunately, we were not equipped to pursue this question.

In order to explain our findings on the basis of the coagulation–liquefaction process, the underlying mechanisms involved were considered.

Currently, this process is believed to involve three distinct, continuous stages (Mann[17]). During the course of ejaculation and immediately thereafter, a coagulate is formed by the action of a gelating enzyme produced in the prostate gland on the fibrinogen-like protein substrate provided by the seminal vesicles. Liquefaction (the rapid dissolution of this coagulate into lysed strands via an initial enzymatic reaction catalyzed by a plasmin-like enzyme of prostatic origin) occurs within twenty minutes following emission (Eliasson[11]). Further dissolution of the lysed strands results via a second enzymatic reaction, leading to the final formation of a water-like solution (completed *ca.* 1 hr post ejaculation; Jacobsson[18]).

In this light, variations in the liquefaction times among samples can be interpreted as variations in the rate of the enzymatic dissolution of the coagulate† . This implies that variations in material property values among samples for a particular time post ejaculation are simply the result of extensions or contractions in time of the same chemical behavior. One is led to conclude that similarity should exist if times post ejaculation are non-dimensionalized with respect to a time representative of a particular chemical event such as liquefaction onset or the completion of the total liquefaction process.

In order to test this assertion, the representations of shear modulus, apparent consistency and inverse viscous component power were replotted with the time post ejaculation nondimensionalized with respect to the time required for completion of the total liquefaction process, t_∞. Here, t_∞ was determined for each case as the time at which elastic behavior became immeasurable. The results are presented in Figs. 9–11. Similarity was manifested especially in shear modulus and apparent consistency results. It is noted, however, that such good similarity is not exhibited in the inverse viscous component power results where deviations as large as 20% occur. This is not alarming since the error in the measurement of "a" is inherently larger than that for the other parameters (see Dunn[13]). Most strikingly, is the close (within 4%) agreement of the observed nondimensional counterpart $t_l^* = (t_l/t_\infty)$ of the liquefaction time $t_l(t_l^* = 0.13)$. A similar agreement was displayed in the nondimensional counterpart $t_p^* = (t_p/t_\infty)$ of the times at which the apparent consistency approached a constant value $t_p(t_p^* = 0.52)$. The immediate and remarkable consequence of this result is that the post ejaculatory chronology of an individual's semen material properties can be determined at any future time following one initial viscometric test solely by noting the time of liquefaction, independently of the frequency of ejaculation.

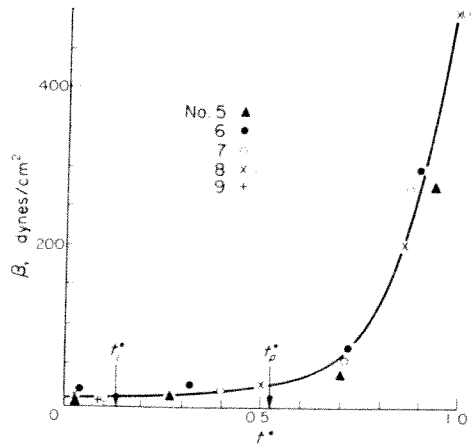

Fig. 9. Human semen: shear modulus vs nondimensional time post ejaculation (t_l^*:nondimensional liquefaction time; t_p^*: nondimensional time when k_{app} became constant).

†See Dunn[13] for a more detailed discussion.

*Liquefaction times were determined by noting when the coagulate was observed to have completely dissolved into a water-like consistency (estimated accuracy ± 15 sec: maximum error = 6%).

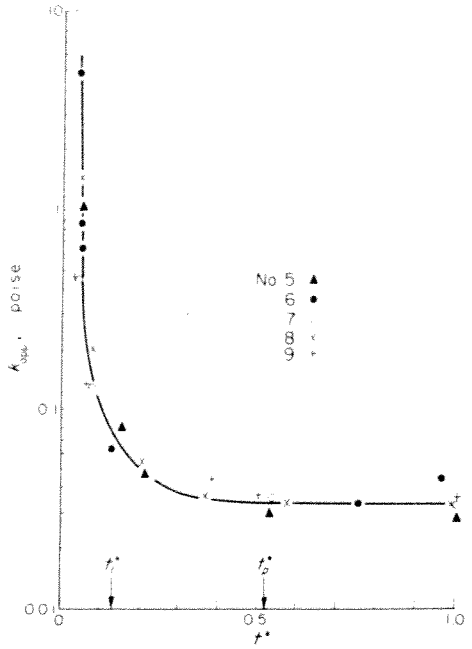

Fig. 10. Human semen: apparent consistency vs nondimensional time post ejaculation (t_l^*: nondimensional liquefaction time; t_p^*: nondimensional time when k_{app} became constant).

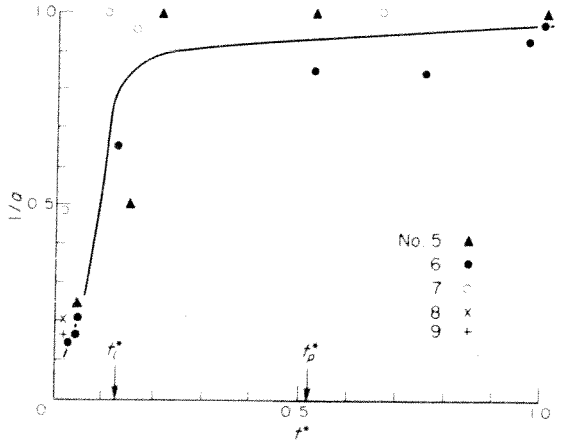

Fig. 11. Human semen: inverse of viscous component power vs nondimensional time post ejaculation (t_l^*: nondimensional liquefaction time); (t_p^*: nondimensional time when k_{app} became constant).

Finally, the above considerations allow one to postulate the following material property representations of the chemical reactions of liquefaction. First, the coagulate state is characterized by the large apparent consistencies (~1–10 poise), small shear moduli (~1–20 dynes/cm^2) and high viscous component powers (1–10 poise), displayed during the initial times post ejaculation corresponding to $t^* \gtrsim 0.05$. Then, liquefaction proceeds to the time at which the semen clot is observed to completely dissolve, at $t^* = 0.13$. At this time (denoted by t_l^* in the figures), the shear modulus departs from a constant value, the apparent consistency approaches a final value, and the inverse viscous component power markedly increases toward a value of 1.0. It is noted, however, that this time probably may not be the exact time of completion of coagulate lysing. Rather, over some short time period thereafter, the remainder of the coagulate, not visible under casual observation, undergoes dissociation. During this period, also, the initial lysed strands are subjected to the further degradation of the second

enzymatic reaction. The time of the probable completion of the first reaction and predominance of the second reaction corresponds to $t^* = 0.52$ (denoted by t_p^* in the figures). After t_p^*, shear modulus values rapidly increase, apparent consistency values become constant and inverse viscous component power values become approximately constant. The second enzymatic reaction reaches completion in a time of the order of 1 hr. This final, steady state is marked by the attainment of Newtonian behavior of the semen, where the shear modulus becomes immeasurably high (i.e. the semen loses its "elasticity"), the apparent consistency maintains a constant value (in this case, 3.37 centipoise) and the viscous component power becomes equal to the value of 1.

Acknowledgement—The authors wish to thank Professor Paul S. Lykoudis, Department of Nuclear Engineering, Purdue University, for his helpful discussion of the semen property results. In addition, Patrick Dunn acknowledges support received via a David Ross Research Grant, Purdue University, during the course of this study and Basil F. Picologlou acknowledges partial support of National Science Foundation Grant ENG75-10522.

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ERRATA

"Investigation of the Rheological Properties of Human Semen," BIORHEOLOGY, Vol. 14, No. 5/6, p. 277-292, by P. F. Dunn and B. F. Picologlou.

1. p. 277, para. 2: Scott Blair should be Scott-Blair
2. p. 277, footnote: Aernautics should be Aeronautics
3. p. 279, Eqn. (8): λ should be γ
4. p. 281, Eqn. (15): Q(T) should be Q(t)
5. p. 281, Eqn. (17): $(\partial u/\partial r)e$ should be $(\partial u/\partial r)_e$
6. p. 281, expression in text between Eqs. (20) and (21): β_b should be β^b
7. p. 281, para. 2: equiped should be equipped
8. p. 284, para. 3: k(16.7) should be k(=16.7)
9. p. 289, para. 3: Scott Blair should be Scott-Blair (occurs three times)
10. p. 292, Ref. 8: B. F. Should be B. F.,
11. p. 292, Ref. 19: In press should be changed to 22, 217 (1977).