Made in United States of America
Reprinted from INTERNATIONAL JOURNAL OF FERTILITY
Vol. 22, 1977, No. 4

Variation in Human Semen Viscoelastic Properties with Respect to Time Post Ejaculation and Frequency of Ejaculation

PATRICK F. DUNN* and BASIL F. PICOLOGLOU**

**Department of Physiology, Duke University Medical Center, Durham, NC 27710† and the **Department of Mechanical Engineering and Materials Science, Rice University, Houston, TX 77001*

ABSTRACT

Dunn, Patrick F. and Picologlou, Basil F. (Department of Physiology, Duke University Medical Center, Durham, NC 27710 and Department of Mechanical Engineering and Materials Science, Rice University, Houston, TX 77001). *Variation in human semen viscoelastic properties with respect to time post ejaculation and frequency of ejaculation.*

Int J Fertil **22**: 217–224, 1977.

The present study concerns the rheological characterization of the coagulation-liquefaction process of human semen. Results obtained using a multiple point capillary viscometer reveal marked variations in the elasticity and viscosity of an individual's semen with time immediately following ejaculation and frequency of ejaculation. Similarity among all cases examined for each material property is revealed by relating times post ejaculation to semen liquefaction time, thereby coupling liquefaction time with specific material property values. Further, the final state of liquefied semen is found to be characterized by Newtonian behavior (mean absolute viscosity = 3.37 centipoise). The semen's liquefaction time and ejaculate volume are determined to be functions of the frequency of ejaculation. Steady state ejaculate volume is found to decrease linearly with increasing ejaculation frequency, thereby providing a measure of glandular secretory rate. When collectively considered, these findings provide possible means for monitoring an individual's glandular behavior over an extended period of time and comparing such behavior to established standards.

INTRODUCTION

The *sine qua non* role of semen in mammals as a milieu for spermatozoa during the initial post ejaculation period has been elucidated in recent years. Experiments have established that this admixture of glandular secretions with spermatozoa may serve functionally during the immediate moments following ejaculation in its coagulated form as a mechanism for sperm transport *en masse* (1, 2, 3). In addition, semen is found to possess buffers (4) which protect the freshly ejaculated spermatozoa from the acidic vaginal environment (5) that immobilizes spermatozoa (6), and to contain nutrients which provide the energy required for the sperm to remain motile (7). Other studies demonstrate that several proteolytic enzymes in human seminal plasma facilitate spermatozoan migration by hydrolyzing the cervical mucus and thereby decreasing its viscosity (8, 9).

Further attention has also been focused on the coagulation—liquefaction phenomena of human semen and its relation to fertility and to an abnormality in or the dysfunction of a contributing secretory gland. Investigations note a possible correlation between the degree of coagulation—liquefaction and fertility (10). Tests suggest a direct proportionality between the absolute viscosity of semen and spermatozoa count for subfertile cases (11). Moreover, studies report the lack of the highly viscous coagulate state of semen as a manifestation of congenital bilateral absence of the vas deferens (12), increased semen viscosity as a characteristic of prostate dysfunction (13), and abnormal variations in the degree of coagulation —liquefaction of an individual's semen as a possible portent of prostate carcinoma (14).

† Present address: Engineering Division, Argonne National Laboratory, Argonne, IL 60439.

Clearly then, an analysis of the rheological properties of human semen during the process of coagulation—liquefaction would contribute substantially, not only in coupling specific material property values at times post ejaculation with the status of underlying enzymatic reactions but also in establishing a novel diagnostic tool for evaluating certain glandular pathologies. It was in this light that the present investigation was undertaken.

MATERIAL AND METHODS

Analysis was performed on the semen obtained from a healthy subject (age 26) over a period of eight months, whose semen exhibited a "normal" (13) coagulation immediately following ejaculation and complete liquefaction within 20 minutes thereafter.

Possible influencing environmental factors were controlled since the semen composition of an individual can be "influenced by factors such as light, temperature, season, state of nutrition, etc." (5). Experiments were conducted in a 4m × 6m × 6m room maintained at 36°C (±1°C) with lighting provided by overhead fluorescent lamps. A diet meeting all Recommended Dietary Allowances was administered under the supervision of a Registered Dietitian one month prior to and during experiment.

Viscoelastic analysis tests were performed on initial semen samples collected after 5 days of ejaculatory abstinence to allow for the contributing secretory glands to attain a constant stable capacity (15, 16). Successive samples were procurred at 4 hour intervals. Additional test series were conducted following another 5 day continence period. After a 4 month interim, more tests were conducted to reconfirm previous results and to explore possible relations between liquefaction time, ejaculate volume, ejaculation frequency and material property variations. Here, the initial tests, which followed after 3 days abstinence, were followed by tests conducted at 6, 12, 24, 48 and 72 hour intervals.

Measurements of semen viscosity and elasticity were obtained using a multiple point capillary viscometer designed specifically for the determination of the material properties of highly time-dependent viscoelastic liquids. The details of viscometer design and operation along with analytical techniques for property determination are elucidated elsewhere (17).

The basic experimental scheme was as follows: subsequent to sample deposition into a 20 ml glass beaker and measurement of ejaculate volume, a homogeneous portion of the specimen was placed in a glass tube open at both ends. The remaining portion was stored in a covered beaker maintained at 36°C (±1°C) during the course of experiment and the liquefaction time was noted (±15 sec). The sample was then introduced at the capillary tube inlet of the viscometer by application of constant suction. After the fluid had advanced a sufficient distance to achieve steady state, pictures of the meniscus were taken in succession as the fluid continued to advance. Since pressure gradient decreased with increasing length of specimen inside the capillary tube, a large number of pressure gradient versus flow rate data were obtained simply by noting applied suction pressure and meniscal position as a function of time. The fluid's viscous properties were found using known reduction procedures (18). In addition, the suction was periodically and suddenly removed and the meniscal recoil distance was measured. The fluid's elastic properties were then obtained from the resultant stress-strain curve (18).

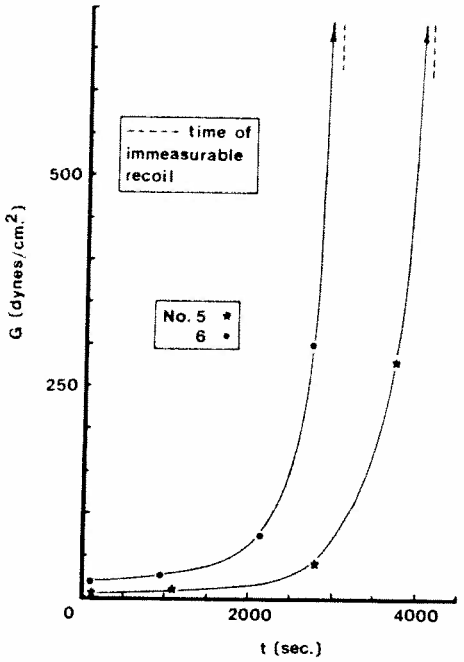

Fig. 1. Shear modulus versus time post ejaculation for initial and second samples.

RESULTS

For all cases examined (n = 9), analysis of the viscoelastic properties [for details, see (17)] revealed semen to be best described within the limits of experimental error as a Maxwell fluid with a linear elastic component characterized by a shear modulus G, and a power-law viscous component characterized by a consistency k and power "a" [shear stress = k (rate of shear

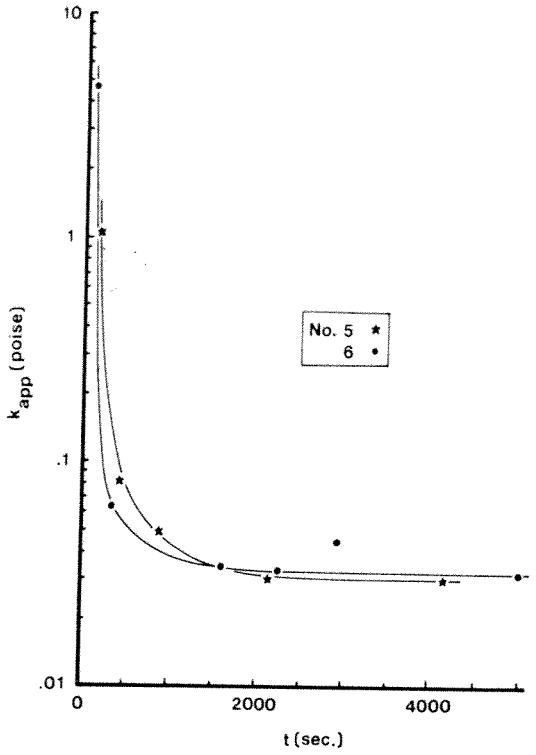

Fig. 2. Semi-log plot of apparent consistency versus time post ejaculation for initial and second samples.

strain)$^{1/a}$]. An example set of results are shown in Figs. 1–3. For means of comparison, the apparent consistency, k_{app}, is presented in lieu of the consistency, k. Here, one observes a substantial variation of all semen viscoelastic properties with respect to time post ejaculation.

Figure 1 illustrates a comparison of shear moduli versus time post ejaculation between an initial sample (No. 5) and a second sample (No. 6) obtained 4 hours later. One observes that the shear modulus for the second sample approached large values of G in a shorter time period than its initial counterpart. For the second sample, recoil distances became immeasurably small at about 3,000 sec, implying negligible elastic behavior, whereas for the initial sample this did not occur until about 4,100 sec. These shear moduli differences between initial and second samples were substantiated in all other cases subject to identical experimental conditions. Specifically, the shear

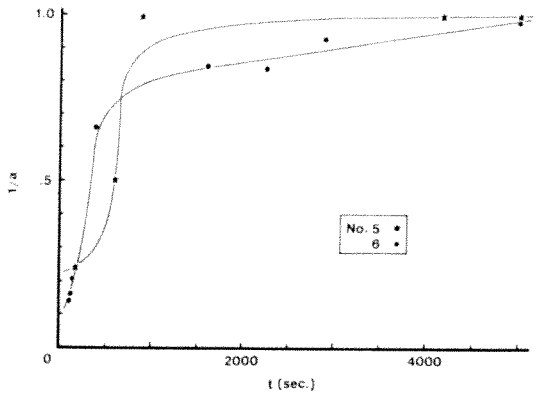

Fig. 3. Inverse of viscous component power versus time post ejaculation for initial and second samples.

moduli values for second samples always approached large values of G in a shorter time period than initial samples. In addition, for all cases examined, an observed decrease in semen liquefaction time from initial to second samples was noted.

Likewise, the apparent consistencies for second samples were found to attain constant values with respect to time post ejaculation in a shorter time period than their initial counterparts, as exhibited by samples Nos. 5 and 6 in Fig. 2. In addition, no characteristic trend of final apparent consistency values with respect to sample order was discerned. An average of final apparent consistency values yielded a mean absolute viscosity of 3.37 centipoise (standard deviation = 0.63 for n = 7) at a mean temperature of 33.2°C (±0.2°C).

Further, as shown in Fig. 3 for sample Nos. 5 and 6, a rapid increase in values of "1/a" occurred, as in all cases examined, at an earlier time post ejaculation for second samples than for initial samples.

In order to investigate a possible similarity among all cases, G, k_{app} and inverse viscous component power, 1/a, were plotted versus a nondimensional time, t^* ($= t/t_\infty$), formed by the ratio of time post ejaculation over t_∞, the time at which all properties attained values consonant with final state Newtonian behavior ($a = 1$, $k_{app} = \text{const.}$, $G \to \infty$). Here, t_∞ was most easily determined for each case as the time

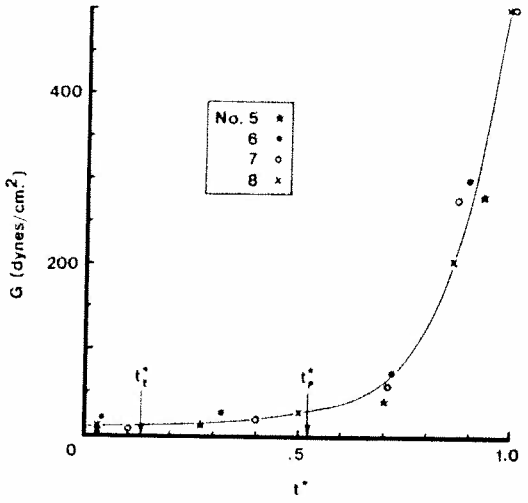

Fig. 4. Shear modulus versus nondimensional time post ejaculation.

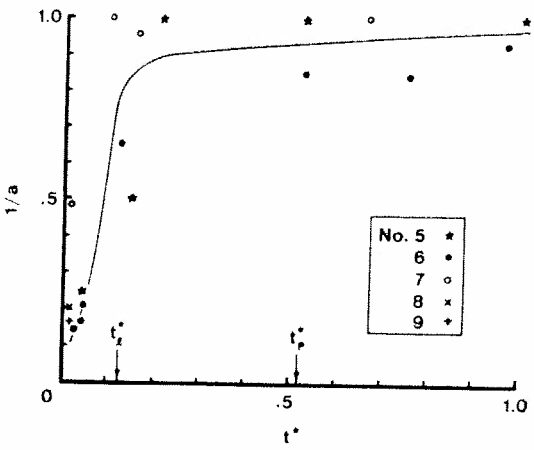

Fig. 6. Inverse of viscous component power versus nondimensional time post ejaculation.

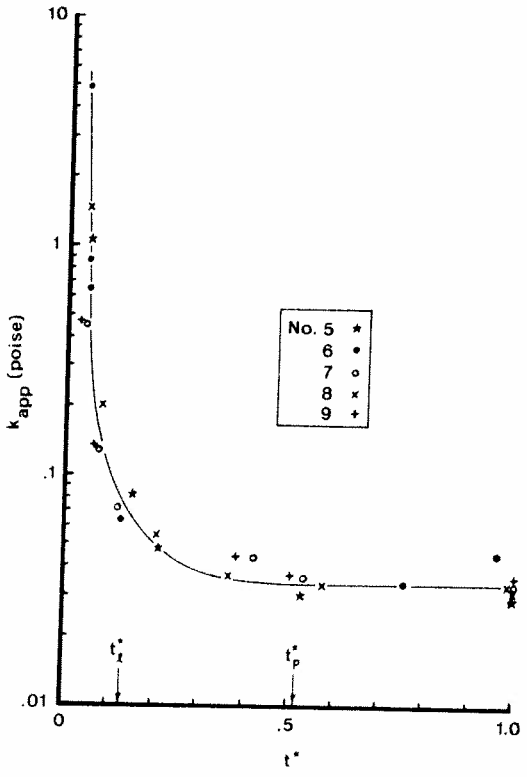

Fig. 5. Apparent consistency versus nondimensional time post ejaculation.

at which the shear modulus reached an experimentally immeasurable value (namely when elastic behavior ceased to exist). The results are presented in Figs. 4, 5 and 6 for initial samples (Nos. 5 and 7), second samples (Nos. 6 and 8) obtained 4 hours later and a third sample (No. 9) obtained 24 hours following the procurement of the initial sample. Similarity was manifested especially in shear modulus and apparent consistency results. Most strikingly, however, was the close (within 4%) agreement of the nondimensional liquefaction time $t_l^* = t_l/t_\infty = 0.13$. Similarity curves for "1/a" did not exhibit such coherence. Here, points outside a single similarity curve yield errors on the order of 10%. It is noted though that this variance was still well within the inherently large experimental error involved in the measurement of "a" (~40%).

To substantiate these findings further and to explore the possible dependence of the liquefaction time on the frequency of ejaculation additional experiments were performed. Following three days of ejaculatory abstinence a number of samples were obtained, for a test series, at fixed time intervals, T, between ejaculations. Different test series were performed for intervals of 4 (two series), 6, 12, 24, 48 and 72 hours. For each series, the liquefaction time, t_l, was nondimensionalized with respect

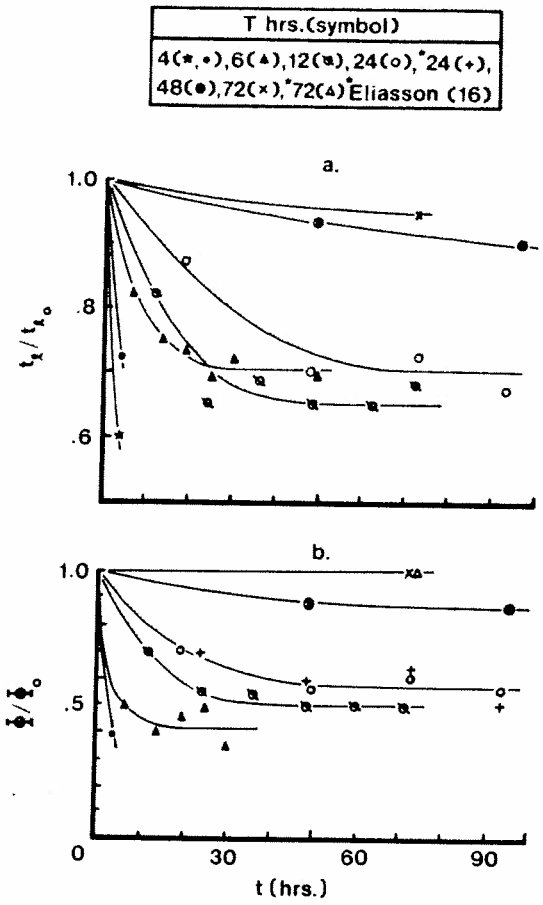

Fig. 7a. Liquefaction time ratio versus time for various ejaculatory time intervals.

b. Ejaculate volume ratio versus time for various ejaculatory time intervals.

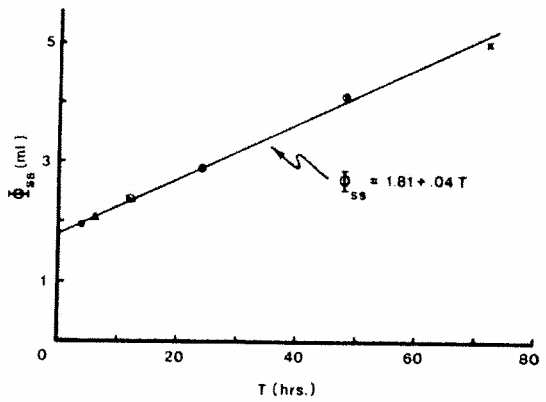

Fig. 8. Steady state ejaculate volume versus ejaculatory time interval (correlation coefficient = 1.00, standard error = 0.03).

to its value for the initial sample t_{t_o}. The results are shown in Fig. 7a. It is evident that for all cases examined the liquefaction time of the second and successive samples is less than its initial counterpart. This supports the findings reported earlier in this communication. Examination of the limiting values of t_t/t_{t_o} reveals that small time intervals (4 hours) between ejaculations yielded a substantial reduction in liquefaction time whereas large time intervals (72 hours) produced no appreciable effect. For intermediate time intervals (6, 12, 24 hours), liquefaction times attained a steady state, with the time required to reach this state being longer for increasing time intervals.

To understand the reasons for the dependence of liquefaction time on the frequency of ejaculation, the ejaculate volume, Φ, nondimensionalized with respect to its initial sample value, Φ_o, was plotted (Fig. 7b). It is seen that for each interval between ejaculations, the ejaculate volume attained a steady state after a certain number of ejaculations. Both the time required to reach steady state and the ejaculate volume were found to increase with increasing T. Also contained in Fig. 7b are the results of Eliasson (16) averaged for 11 "normal" subjects at ejaculatory intervals of 24 and 72 hours. A surprisingly close (within 10%) agreement was revealed.

These findings suggest further consideration of the dependency of the steady state ejaculate volume on T. When our results are plotted in the manner shown in Fig. 8, it is seen that the steady state ejaculate volume, Φ_{ss}, is linearly related (via a least-squares fit of the data) to the interval T by:

$$\Phi_{ss}\,(\mathrm{m}l) = 1.81 + 0.04\mathrm{T}(\mathrm{hr}). \qquad (1)$$

This relation is valid for all time intervals less than or equal to the interval (here, 72 hours) during which a constant stable capacity can be maintained by the contributing secretory glands (15, 16).

The physiological implication of such a relation is that, above some minimum amount

which could always be delivered upon ejaculation (here, 1.81 m*l*) the volume of contributing glandular secretions produced per unit time (here, 0.04 m*l*/hr), remained constant for a "normal" individual over extended periods of time (here, at least 8 months).

DISCUSSION

The results presented herein identify the coagulation-liquefaction of semen to be a highly dynamic process in which viscoelastic material properties are altered one hundred-fold over a period of 15 minutes. To date, there have been no reported studies on the measurement of the viscoelastic properties characteristic of the coagulation-liquefaction process of human semen. At present, the results of this study can only be compared to previous studies concerned with semen analyses performed hours after ejaculation.

The similarity found among all cases for each material property suggests the contraction or expansion of the time scale of the same underlying chemical behavior. For example, assuming observed liquefaction time to approximate the completion of the first lytic reaction of the coagulate, using simple chemical kinetics for a first approximation, one can argue that liquefaction time should be directly proportional to substrate/enzyme concentration ratio and inversely proportional to enzymatic activity (17). Therefore, changes in liquefaction time with ejaculation frequency can be interpreted as manifestations of alterations in substrate/enzyme concentration ratio and/or enzymatic activity of the primary liquefaction reaction.

Studies on the composition of human semen (16, 19), however, have reported that the relative concentrations of constituents remain "constant" for an individual, independent of semen volume and frequency of ejaculation. Further, Harvey (20) has ascertained the fibrinolytic activity in human semen by mixing a fixed volume of oxalated blood plasma with varying amounts of semen and has found it to be independent of volume. The above studies have demonstrated also that the relative concentrations and activities are "constant" for and characteristic of a given individual, despite a wide range of distribution in concentrations and activities among individuals (21).

This apparent incongruity is reconciled when one notes that variations of approximately 12% (well within the "constant" state variances of approximately 25% in the cited studies) are sufficient to produce the changes in liquefaction time observed herein. Consider, for example, the limiting case of a decrease in substrate concentration accompanied by an increase in enzyme concentration and activity of 12% from initial to second samples. This would allow for approximately a 30% decrease in liquefaction time from initial to second samples (17). Thus, it appears quite reasonable that the probable chemical basis of this observed decrease in liquefaction time is some combination of a reduction in substrate/enzyme concentration ratio and/or an increase in enzymatic activity. Possibly, a slight difference in the glandular production rates of the prostate to replenish its fibrinolytic enzymes or the seminal vesicles to produce the gelatinous substrate could account for such a decrease.

Further, notwithstanding the complexity of the chemical processes involved in the liquefaction of semen, the following chronology of material property representations of the underlying lytic reactions can be postulated. First, the coagulated state is characterized by large apparent consistencies (~1–10 poise), small shear moduli (~1–20 dynes/cm^2) and high viscous component powers (~4–9) displayed during the initial times post ejaculation corresponding to $t^* \sim< 0.05$. Then, liquefaction proceeds to the time at which the semen clot is observed to dissolve completely, at $t^* = 0.13$. At this time, (denoted by t_l^* in the Figs.), 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. It is noted, however, that this time probably may not be the exact time of fibrinolysis completion. Rather, over some short time period thereafter, the remainder of the fibrin-like clot, not visible under casual observation, undergoes

dissociation. During this period, also, the initial strands of lysed fibrin are subjected to further dissolution by a 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 Figs.). 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 on the order of 1 hour as manifested by the accumulation of nonprotein nitrogen, free amino acids, and at a somewhat later state, free ammonia (22, 23). This final, steady state is marked by the attainment of Newtonian behavior of the semen, where the shear modulus becomes immeasurably high, the apparent consistency maintains a constant value (in this case, 3.37 centipoise) and the viscous component power becomes equal to a value of 1.

Comparisons of the results obtained in the present study with those of other investigators tend to support the "normal" status of the data presented herein. The mean absolute viscosity of 3.37 centipoise compares well with that obtained by Tjioe and Oentoeng (11), using a Hellige capillary viscometer. The mean viscosity determined from a total of 1,111 semen samples examined within the second hour post ejaculation was 3.92 centipoise (range of 1.3–23.3 centipoise). Agreement to within 10% of the ejaculate volume versus T findings with those of Eliasson (16) further tend to support normalcy. A consequence of such close agreement would be the establishment of a "normal" standard to which a subject's glandular secretion rate could be characterized and evaluated.

When considered *in toto*, this study provides unique means to characterize an individual's glandular behavior and his coagulation-liquefaction process. An initial series of one viscoelastic and two ejaculate volume versus frequency tests could be used for characterization of coagulation-liquefaction and glandular production rate. Then, monitoring could be extended over a period of months (in this study, 8 months) simply by measuring ejaculate volume for a prescribed ejaculate frequency at any future instance.

In conclusion, the variations in the viscoelastic property values characteristic of the coagulation-liquefaction process of an individual's semen for various ejaculation frequencies manifest similarity when post ejaculation times are considered in relation to semen liquefaction time. This similarity suggests that each change in liquefaction time with ejaculation frequency can be interpreted as an alteration in substrate/enzyme concentration ratio and/or enzymatic activity. By considering ejaculate volume as a function of ejaculation frequency, an individual's glandular production rate can be identified. This fact, when used in conjunction with the viscoelastic property analysis of the coagulation-liquefaction process, could provide the means to monitor indirectly an individual's semen's material property behavior over an extended period of time. This would be of significance since most clinical semen analyses cannot be readily conducted in the period immediately following ejaculation during which liquefaction occurs.

ACKNOWLEDGMENT

The authors wish to thank Professor Paul S. Lykoudis, Department of Nuclear Engineering, Purdue University for his helpful discussions of the semen property results. In addition, P. F. Dunn acknowledges support received via a David Ross Research Grant, Purdue University during the course of this study.

REFERENCES

1. Blandau, R. J.: On the factors involved in sperm transport through the cervix uteri of the albino rat. Amer J Anat **77:** 253, 1945.
2. Blandau, R. J.: Gamete transport–comparative aspects. Pages 129–162 *in* E. S. E. Hafez & R. J. Blandau, eds. The mammalian oviduct. Univ. of Chicago Press, Chicago, 1969.
3. Blandau, R. J.: Sperm transport through the mammalian cervix: comparative aspects. Pages 285–304 *in* R. J. Blandau & K. S. Moghissi, eds. The biology of the cervix. Univ. of Chicago Press, Chicago, 1973.
4. Nocenti, M. R.: Reproduction. Pages 992–1028 *in* V. B. Mountcastle, ed. Medical physiology, Vol. I, 12th ed. C. V. Mosby Co., St. Louis, 1968.

5. Mann, T.: The biochemistry of semen. John Wiley & Sons, New York, 1954.
6. Moghissi, K. S.: Sperm migration through the human cervix. Pages 305–327 *in* R. J. Blandau & K. S. Moghissi, eds. The biology of the cervix. Univ. of Chicago Press, Chicago, 1973.
7. Mann, T.: Energy requirements of spermatazoa and the cervical environment. Pages 329–338 *in* R. J. Blandau & K. S. Moghissi, eds. The biology of the cervix. Univ. of Chicago Press, Chicago, 1973.
8. Neuhaus, O. W., and Moghissi, K.: Composition and properties of human cervical mucus. III. A preliminary study of the mucoid component. Fertil Steril **13:** 550, 1962.
9. Moghissi, K., and Syner, F. N.: The effect of seminal protease on sperm migration through the cervical mucus. Int J Fertil **15:** 43, 1970.
10. Sobrero, A. J., and MacLeod, J.: The immediate postcoital test. Fertil Steril **13:** No. 2, 184, 1962.
11. Tjioe, D. Y., and Oentoeng, S.: The viscosity of human semen and the percentage of motile spermatozoa. Fertil Steril **19:** 562, 1968.
12. Amelar, R. D.: Coagulation, liquefaction and viscosity of human semen. J Urol **87:** 187, 1962.
13. Eliasson, R.: Parameters of male fertility. Pages 39–51 *in* E. S. E. Hafez & T. N. Evans, eds. Human reproduction, conception and contraception. Harper & Row, New York, 1973.
14. Mann, T.: The biochemistry of semen and of the male reproductive tract. John Wiley & Sons, New York, 1964.
15. Harvey, C.: Fructose and citric acid in human semen. Proc Soc Study Fertil **3:** 56, 1951.
16. Eliasson, R.: Effect of frequent ejaculations on the composition of human seminal plasma. J Reprod Fertil **9:** 331, 1965.
17. Dunn, P. F.: The rheological characterization and experimental determination of biological material properties. Ph.D. Thesis, Purdue University, 1974.
18. Dunn, P. F., and Picologlou, B. F.: Investigation of the rheological properties of human semen. Biorheol. (submitted, 1977).
19. Eliasson, R.: Biochemical analyses of human semen in the study of the physiology and pathophysiology of the male accessory genital glands. Fertil Steril **19:** 344, 1968.
20. Harvey, C.: Fibrinolysin in human semen. Proc Soc Study Fertil **1:** 11, 1949.
21. MacLeod, J., and Heim, L. M.: Characteristics and variations in semen specimens in 100 normal young men. J Urol **54:** 474, 1945.
22. Jacobsson, L.: Free amino acids in human semen. Acta Phys Scand **20:** 88, 1950.
23. Huggins, C., and Neal, W.: Coagulation and liquefaction of semen: proteolytic enzymes and citrate in prostatic fluid. J Exper Med **76:** 527, 1942.

Submitted for publication August 2, 1976