PHYSICAL CHARACTERISTICS OF MOUSE SPERM NUCLEI

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ABSTRACT The nuclei of epididymal sperm, isolated from C57BL/6J and CBA/J inbred mice by their resistance to trypsin digestion, retain the shape differences of the intact sperm head. Various physical characteristics of these nuclei were measured and compared. The measurement of the projected dimensions of nuclei showed that the CBA nuclei are 13.5% longer than C57BL/6 nuclei (8.64 ± 0.02 μm compared with 7.61 ± 0.02 μm), 0.8% narrower (3.51 ± 0.01 vs. 3.54 ± 0.01 μm) with 6.8% more area (22.34 ± 0.10 vs. 20.91 ± 0.09 μm^2). However, the volumes of the nuclei as based on reconstructing calibrated electronmicrographs of serial sections of the nuclei indicated that CBA are about 7% smaller than C57BL/6 nuclei (3.72 ± 0.08 vs. 4.01 ± 0.03 μm^3). The buoyant density of the CBA nuclei is 1.435 ± 0.002 g/cm^3 compared with 1.433 ± 0.002 g/cm^3 for the C57BL/6 nuclei as determined on linear CsCl and Renografin-76 density gradients and confirmed by a technique utilizing physiological tonicities. Therefore, the average mass of the CBA nuclei is less than that of the C57BL/6 nuclei (5.34 ± 0.12 vs. 5.75 ± 0.05 pg). The sedimentation velocities at unit gravity of nuclei from 11 inbred strains differ over a range of more than 6% with CBA nuclei sedimenting about 2.0% more slowly than C57BL/6 nuclei. We show that for these nuclei the sedimentation velocity can be related to their buoyant density, volume and a sedimentation shape factor. Within the errors of our measurements of these various characteristics, it was found that C57BL/6 and CBA nuclei have similar sedimentation shape factors. Therefore, the difference in sedimentation velocity between these nuclei appears to be primarily a result of differences in volume. The possible applications of these techniques to the physical separation of sperm are evaluated in the discussion.

INTRODUCTION

Spermatozoa are highly structured cells with shapes that are characteristic for each individual species (Friend, 1936; Bishop and Walton, 1960). Indeed, there are even significant differences in the shape of sperm amongst inbred strains of mice (Braden, 1959; Sharma, 1960; Beatty and Sharma, 1960; Illisson, 1969; Woolley, 1970). The shape characteristics of sperm seem to be independent of weight and litter size (Beatty and Sharma, 1960; Illisson, 1969), technical and observational factors (Beatty and Napier, 1960), age trends (Beatty and Mukherjee, 1963), as well as maternal environment after fertilization (Pant and Beatty, 1973). Rather the shape seems to be under

strict genetic control (Illisson, 1969; Beatty, 1972) and the prime determinant of sperm structure seems to be the male diploid genotype (Beatty, 1970).

In this report we describe a method of removing and isolating the nuclei of mature mouse sperm of inbred strains known to differ in sperm head shape (Beatty and Sharma, 1960). These nuclei were found to be very rigid structures closely reflecting the sizes and shapes of the sperm heads. We then measured and compared the projected dimensions, volumes, buoyant densities, and sedimentation velocities of these nuclei, especially for the C57BL/6 and CBA inbred strains and showed that a measured difference in the sedimentation rates of these nuclei was most likely due to the difference in their average volumes. Studies such as these afford a better understanding of the physical bases of the genetic differences between sperm of different strains of mice.

Our studies also allow us to better evaluate the ability of various physical techniques to detect very small physical differences. Techniques for the physical separation of whole sperm based on differences in their genetic material have been topics of much interest (Beatty, 1970). Unfortunately, they have met with only limited success. By comparing the physical characteristics of nuclei known to be morphologically different, we have given ourselves a major advantage. In dealing with nuclei we have removed those structural components which could give rise to structural variability unrelated to the genetic material per se. Therefore, those physical techniques which most readily detect the small morphological differences in the sperm nuclei of various strains are likely to be the most applicable to whole sperm separation. These evaluations are further treated in the discussion.

MATERIALS AND METHODS

Mice

Male mice of various inbred strains (see Table II) were obtained from The Jackson Laboratories, Bar Harbor, Maine, at 6–8 wk of age. The animals were housed in wire suspension cages in air-conditioned and light-controlled rooms (16 h day and 8 h night) and allowed food (Purina Chow) and water ad lib.

Preparation of Sperm Nuclei

The suspensions of sperm nuclei were obtained from the spermatozoa in the cauda epididymidis. Two or three mice, 14–18 wk of age, were sacrified by cervical dislocation, their epididymides removed and minced in 2.5 ml of 2 mM $CaCl_2$ (in distilled H_2O); this was followed by moderate pipetting and filtration through an 80 μm stainless steel mesh (Greening Donald Ltd., Ontario). After the addition of 2.5 ml of 0.5% trypsin diluted in water (stock 2.5% trypsin solution in normal saline, Grand Island Biological Co., Grand Island, N.Y.) the contents were incubated at 37°C for 30 min at moderate agitation followed by 1 min maximum agitation on a Vortex Super Mixer (Lawrence Pumps, Inc., Lawrence, Mass.). The concentration of free nuclei in the resulting suspension was in the range of 1.2–1.5 $\times$ 10^7/ml with about an equal concentration of tail fragments. Under these conditions all nuclei were free of their tails and on electron micrographs appeared as homogeneous electron dense particles retaining only some remnants of perinuclear membranes. The acrosome and other sperm structures were removed by this procedure (Meistrich et al., 1975).

Radioactively labeled sperm nuclei were prepared as follows: 29 days after C57BL/6 mice received intraperitoneal injections of ^{3}H-TdR at doses of 1 μCi/g body wt (14–19.4 Ci/mM, Amersham/Searle Corp., Arlington Heights, Ill.), the epididymides were removed and the sperm gently dislodged in a solution of 1% glucose, 10% dimethyl sulfoxide in physiological phosphate-buffered saline (PBS), filtered through an 80 μm stainless steel mesh and slowly cooled to 4°C. 2-ml aliquots (about 10^7 sperm each) were sealed in freezing vials, further cooled to near 0°C and finally submerged in liquid nitrogen. To recover the sperm nuclei, the vials were warmed to 24°C, the sperm pelleted at 1,000 g for 20 min, resuspended in 0.25% crude trypsin in 2 mM aqueous $CaCl_2$ and treated as were the unlabeled sperm.

Measurement of Projected Length, Width, and Area

After the trypsin treatment the nuclei were pelleted at 1,000 g at 4°C for 20 min, resuspended in 10 ml distilled H_2O and respun. The pellet was then resuspended in about 2 ml of 0.5% aqueous Eosin Y (Fisher Scientific Co., Pittsburgh, Pa.) and after 30 min, air drying smears were prepared and later coverslips added with Permount mounting media (Fisher). The projections of 160 red-stained nuclei were obtained from 10 individual preparations of the C57BL/6 and CBA strains, using a Visopan Projecting Microscope (C. Reichert, Vienna, Austria) at a final magnification of about 7,400. Silhouettes of the individual nuclei were traced and measured for length and width as shown in Fig. 2. The areas were calculated from the weights of individual silhouettes.

Volume Measurements

The volumes of individual C57BL/6 and CBA nuclei were determined by serial electron microscopy. After the nuclei were prepared in trypsin as described above, they were washed in sodium phosphate buffer, fixed in 2% glutaraldehyde for 2 h, and again washed for 1 h in phosphate buffer. Following a second fixation in 2% osmium tetroxide for 1 h, the nuclei were rinsed briefly in distilled water and placed in 4% agar before dehydration with ethanol and propylene oxide. The agar blocks were embedded in 1:1 mixtures of propylene oxide and epon for about 16 h, then transferred to fresh epon and cured for 48 h. Serial sections were cut on a Porter-Blum MTH ultramicrotome with a DuPont diamond knife (Sorvall Operations, DuPont Company, Newtown, Conn.) and collected on single hole Formvar-coated copper grids (Moens 1970). These sections were stained with uranyl acetate and lead citrate, and then observed and photographed with a Philips EM200 electron microscope (Philips Electronic Instruments, Inc., Mt. Vernon, N.Y.). The individual areas of a complete series of cross sections of each nucleus (between 50 and 80 sections for each nucleus) were determined and summed for five nuclei from each strain. Then the grids containing the sections were reimbedded in epon and sectioned at right angles to determine the average thickness of the sections. The volume of an individual nucleus was calculated as the product of the total area of a complete series of cross sections and the average thickness of those sections.

Buoyant Densities

The buoyant densities of the nuclei were determined on linear density gradients. To minimize possible adhesions between nuclei and tail fragments in the gradients, only suspensions of nuclei virtually free of tail fragments were used. To obtain such suspensions, 2.0×10^7 nuclei with tail fragments were suspended in 30 ml of 75% aqueous Renografin-76 (stock solutions consist of 67% meglumine diatrizoate and 9% sodium diatrizoate at physiological pH, and a density of 1.41 g/cm^3, as supplied by Squibb & Sons Ltd., Montreal, Canada). This suspension of density 1.30 g/cm^3 was vigorously agitated for 2 min and layered over 5 ml of Renografin

solution (density = 1.31 g/cm^3) in a 40 ml glass conical centrifugation tube and centrifuged at 1,500 g for 2 h. The pellet was resuspended in 5 ml of 2 mM aqueous $CaCl_2$ with a recovery of about 1.5×10^7 nuclei with less than 1% tail fragments. About 10^7 of these nuclei were then thoroughly dispersed in two 6-ml solutions of 35% and 45% CsCl (wt/wt) of density 1.35 and 1.50 g/cm^3, respectively. These suspensions were then used to generate the linear gradient with a system similar to that of Loir and Wyrobek (1972). The gradients were generated in 13 ml cellulose nitrate, Beckman SW-41 centrifugation tubes (Beckman Instruments, Inc., Fullerton, Calif.) and centrifuged at 8,000 g for 12 h at 24°C. Similar methods were used to generate linear gradients of Renografin-76. However, to attain the required range of densities with Renografin, it was first necessary to concentrate the stock solution to a density of about 1.50 g/cm^3 by evaporation with continuous stirring at 60°C. The density was monitored by refractometry. The two solutions, 1.41 and 1.50 g/cm^3 then provided the range for the linear gradient as described above. About 20, 0.4 ml fractions were collected from each gradient with a Hoefer Fractionator (Hoefer Scientific Instruments, San Francisco, Calif.) and Isco fraction collector at a constant flow rate. For each fraction the concentration of nuclei was determined by hemocytometer and the density of the solution by refractometry, as calibrated against solutions of known density.

The buoyant density of the nuclei was also determined by differential sedimentation. By measuring the median sedimentation velocities (s_1 and s_2) in two different solutions (1 and 2) of known density (ρ_1 and ρ_2) and viscosity (η_1 and η_2), the buoyant density (ρ) of the nuclei can be calculated by the relationship,

$$\rho = (\rho_2\eta_1 s_1 - \rho_1\eta_2 s_2)/(\eta_1 s_1 - \eta_2 s_2).$$

For this purpose, C57BL/6 nuclei virtually free of tail fragments were layered over both 1 to 3% and 8 to 10% Ficoll (Pharmacia, Uppsala, Sweden) in PBS gradients of measured density (by weight per volume) and viscosity (by Ostwald viscosimeter). The values ρ_1, η_1, ρ_2, η_2 are the average solution densities and viscosities encountered by the nuclei during sedimentation. The median sedimentation rates in these solutions were determined for 24 h of sedimentation at 4.7 ± 0.1°C (see Sedimentation Velocity below). The major assumption underlying this method of density determination is that the volumes and sedimentation shape factors of the nuclei in the two solutions are the same. Since differences in osmotic pressures might effect such changes, it should be noted that the 1% and 10% Ficoll solutions differ by only about 8 mosmol.

Sedimentation Velocity (Staput Method)

Sperm nuclei from various strains of mice were cosedimented at unit gravity with ^{3}H-TdR labeled C57BL/6 sperm nuclei in a cylindrical sedimentation chamber (Miller and Phillips, 1969). The initial layer over the linear 2–4% bovine serum albumin (BSA) in PBS gradient contained 4×10^6 labeled C57BL/6 nuclei mixed with 3×10^7 unlabeled nuclei from the various strains in 5 ml of 1% BSA. The total volume of this gradient was approximately 600 ml with a cylindrical diameter of 12.0 cm and height of about 5 cm. The nuclei were allowed to sediment for 24 h at 4.7°C. About 100 5.8-ml fractions collected by a Gilson collector were scored for the number of sperm nuclei with a Spencer-Neubauer hemocytometer by phase contrast microscopy. The fractions were then individually filtered onto Whatman GF/C filters, washed with 20 ml of PBS, 20 ml of cold 5% aqueous trichloroacetic acid, and 10 ml of 95% ethanol. The filters were dried, submerged in scintillation fluid (2 kg toluene and 14 g Omnifluor, New England Nuclear, Boston, Mass.) and the radioactivity measured in a Unilux II-A liquid scintillation counter (Nuclear Chicago, Des Plaines, Ill.).

The histograms of the hemocytometer and scintillation counts vs. fraction number were analyzed to determine the median fraction numbers and full width at half-maximum height,

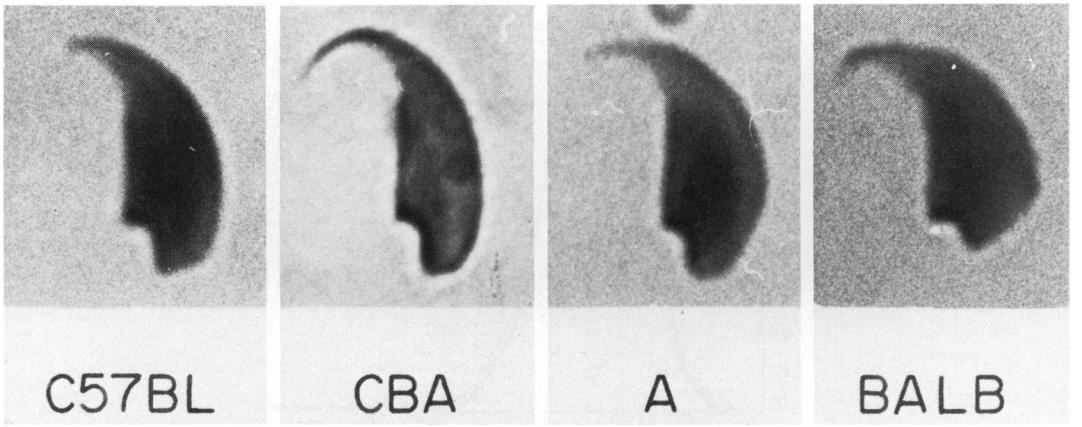

FIGURE 1 Lightmicrographs of stained sperm nuclei from four inbred strains of mice. × 4,000.

the latter used as a measure of the dispersion. To facilitate interpretation all histograms in this article are shown as series of lines connecting the average value of each fraction.

RESULTS

Dimensions

A comparison of sperm nuclei from various strains of mice examined by conventional microscopy shows the common features of hooked head and midpiece attachment site but there are some apparent differences between the strains (Fig. 1). Comparisons of measurements of various dimensions of the C57BL/6 and the CBA show that these differences are real (Fig. 2). The CBA nuclei are 13.5% longer than the C57BL/6

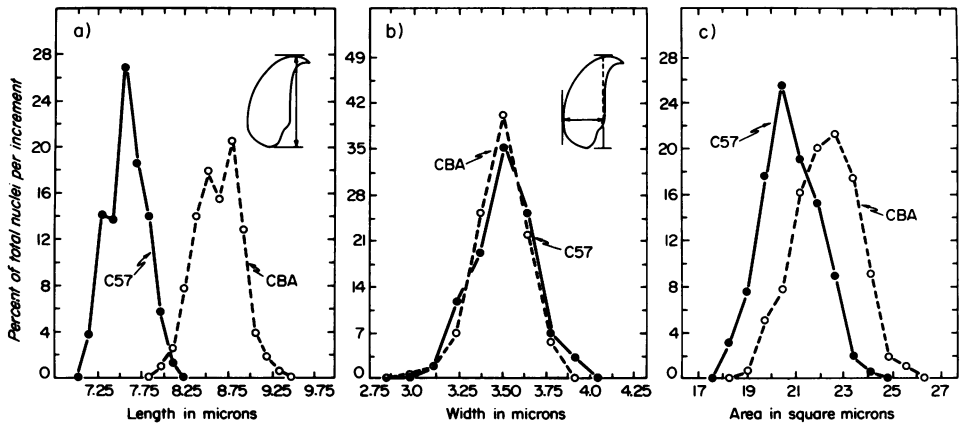

FIGURE 2 Comparison of the projected lengths (*a*), widths (*b*), and areas (*c*) of 160 C57BL/6 or C57 (—●—●—) and CBA sperm nuclei (---o---o---). For each length measurement, the straight portion of the nucleus just above the midpiece attachment site (as best seen in Fig. 1) was aligned with the arrow as shown in the schematic of Fig. 2*a*.

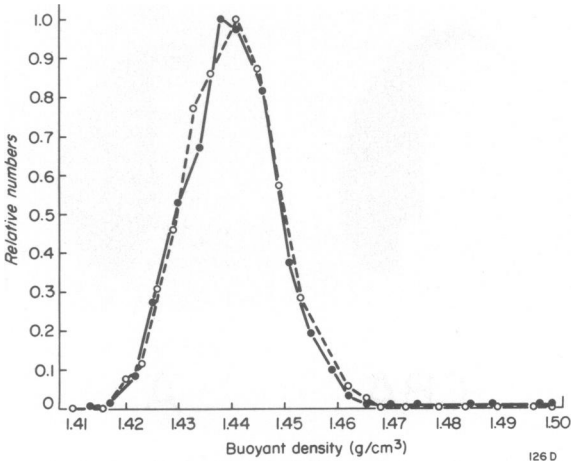

FIGURE 3 Buoyant density distribution of C57BL/6 (—●—●—) and CBA sperm nuclei (---o---o---) in a linear Renografin gradient.

nuclei (the mean and standard error of the mean are 8.64 ± 0.02 μm compared with 7.61 ± 0.02 μm). They have a similar width (3.51 ± 0.01 μm compared with 3.54 ± 0.01 μm), but a 6.8% larger area (22.34 ± 0.1 μm^2 compared with 20.91 ± 0.09 μm^2).

Volume

In the preparation of sperm nuclei used above, the nuclei were lying on their sides flat on the glass. The thickness cannot be measured by conventional optics. Therefore, to determine the volume, serial sections of five nuclei from CBA and C57BL/6 mice were prepared and the volumes calculated as described in Materials and Methods. The average volume for the CBA nucleus was about 7% less than that of the C57BL/6. The mean and standard error of the CBA nuclear volume was 3.72 ± 0.08 μm^3 (3.50, 3.53, 3.78, 3.87, 3.91). The C57BL/6 nuclei had a mean volume of 4.01 ± 0.03 μm^3 (3.90, 3.97, 4.00, 4.07, 4.10).

Density

The buoyant density of sperm nuclei was first measured with linear gradients of CsCl and of Renografin. Typical isopycnic distributions of CBA and C57BL/6 nuclei in Renografin are shown in Fig. 3, illustrating the similarity of the buoyant densities of the two types of nuclei. Similar distributions were obtained in CsCl. The averages of median densities and standard errors (Table I) for CBA and C57BL/6 nuclei were 1.435 ± 0.002 and 1.433 ± 0.002, respectively.

It was possible that the results with CsCl and Renografin were in error because of the high concentration of solutes in the media. A method for measuring density at normal tonicity was therefore employed (see Materials and Methods) which depended on measurements of sedimentation velocity of the sperm nuclei in two Ficoll solutions of slightly different densities and viscosities. This method yielded buoyant densities

TABLE I

MEDIAN BUOYANT DENSITIES OF C57BL/6 AND CBA SPERM NUCLEI

Medium	C57BL/6	CBA
	g/cm^3	g/cm^3
CsCl	1.432	1.435
	1.429	1.432
	1.430	1.430
Renografin-76	1.439	1.440
	1.436	1.440
Average (± SE)	1.433 ± 0.002	1.435 ± 0.002

of 1.41 and 1.45 g/cm^3 for two independent determinations for C57BL/6 nuclei, in close agreement with the measurements obtained with the CsCl and Renografin density gradients.

Sedimentation Velocity

The sedimentation velocity of sperm nuclei was measured at unit gravity in a cylindrical sedimentation chamber (staput). Because the sedimentation velocity was strongly dependent on viscosity which fluctuates with temperature, radioactively labeled C57BL/6 sperm nuclei were cosedimented with the nuclei to be measured, to provide a standard (Fig. 4).

The average and standard error of four measurements of ratios of median sedimentation rate of C57BL/6 compared with the median velocity of the labeled C57BL/6 nuclei was 1.004 ± 0.001 (1.002, 1.002, 1.004, 1.007). The average ratio for CBA nuclei similarly obtained was 0.984 ± 0.004 (0.0971, 0.977, 0.985, 0.988, 0.993, 0.993), suggesting that the CBA nuclei sediment about 2.0% more slowly than the unlabeled

TABLE II

SEDIMENTATION VELOCITY OF SPERM NUCLEI OF VARIOUS STRAINS

Strain	Velocity relative to C57BL/6J nuclei*	
	%	%
A/J	−4.6	−3.9
DBA/2J	−4.6	−3.2
RIII/AnJ	−3.7	−3.1
RF/J	−3.2	−2.4
CBA/J	−2.0 ± 0.4	(6)
C58/J	−1.9	−1.3
SJL/J	−0.6	−0.3
C57BL/6J	0.0 ± 0.1	(4)
SWR/J	+1.3	+1.1
C57BR/cdJ	+1.5	+0.9
BALB/cJ	+2.1	+1.9

*Each percent value represents the result of an independent measurement with the exception of C57BL/6J and CBA/J which are shown as the mean and standard error of the mean for four and six trials, respectively. All values are compared to the median sedimentation velocity of unlabeled C57BL/6J nuclei.

TABLE III

PHYSICAL CHARACTERISTICS OF C57BL/6 AND CBA SPERM NUCLEI

Strain		Length	Width	Area	Volume	Density	Mass ($\times 10^{-2}$ g)	Sedimentation velocity	Relative shape factor*
		μm	μm	μm^2	μm^3	g/cm^3		mm/h	f/f_0
	Trials	160	160	160	5	5	—	21	—
C57BL/6	Mean‡	7.61 ± 0.02	3.54 ± 0.01	20.91 ± 0.09	4.01 ± 0.03	1.433 ± 0.002	5.75 ± 0.04	1.044 ± 0.001	1.75 ± 0.02
	Dispersion§	7.6%	8.2%	12.9%		4.8%		14.9%	
CBA	Mean	8.64 ± 0.02	3.51 ± 0.01	22.34 ± 0.10	3.72 ± 0.08	1.435 ± 0.002	5.34 ± 0.12	1.023 ± 0.004	1.71 ± 0.04
	Dispersion	7.9%	8.3%	15.2%		3.9%		14.6%	
	Percent ‖ differences of means	+13.5%[c]	−0.8%[a]	+6.8%[c]	−7.2%[b]	+0.1%	−7.1%[b]	−2.0%[b]	−2.3%

*The relative shape factor is the sedimentation shape factor of the sperm nuclei divided by the sedimentation shape factor of a sphere.

‡Average of trial results ± standard error of the mean.

§The full width at half-maximum height ×100/median value. For density, the reduced density ($\rho - \rho_{H_2O}$) was used in the denominator.

‖(CBA mean − C57BL/6 mean) ×100/C57BL/6 mean; means significantly different at (*a*) $P < 0.1$, (*b*) $P < 0.01$, and (*c*) $P < 0.001$.

C57BL/6 nuclei. Of nine other strains studied (Table II), the sperm nuclei of the A strain sediment about 4% slower than those of C57BL/6 while BALB nuclei sediment about 2% faster.

Based on the derivation shown in the Appendix the mean sedimentation velocity of sperm nuclei can be related to their mean volume, buoyant density and a sedimentation shape factor. As summarized in Table III, the mean densities and sedimentation shape factors of the C57BL/6 and CBA are not significantly different. This suggests that the significant difference in mean sedimentation rates for the nuclei of the two strains appears to be primarily a result of a difference in mean volumes.

The Dispersion of Sedimentation Velocities

As shown in Fig. 4, the unlabeled CBA nuclei and the ^{3}H-TdR labeled C57BL/6 nuclei have similar dispersion characteristics, as measured by the W of each distribution, i.e., the full width at half-maximum height. To determine whether this dispersion was due to simple particle diffusion or actual physical differences amongst the nuclei, labeled C57BL/6 nuclei were sedimented at unit gravity and their dispersions measured as a function of time. If these dispersions were due to simple particle diffusion during sedimentation then the W of the distribution would be expected to increase as the duration of sedimentation to the $\frac{1}{2}$ power (Tanford, 1961*a*). On the other hand, if these dispersions were due to actual physical differences amongst the nuclei, then the W would be expected to increase linearly with the duration of sedimentation.

As can be seen in Fig. 5, a detailed study of the dispersion of the labeled C57BL/6

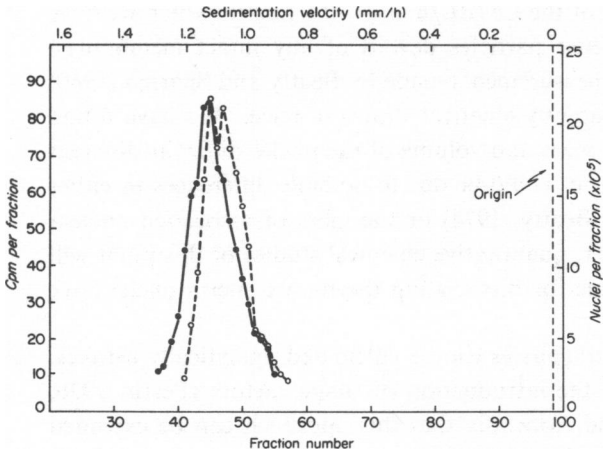

FIGURE 4

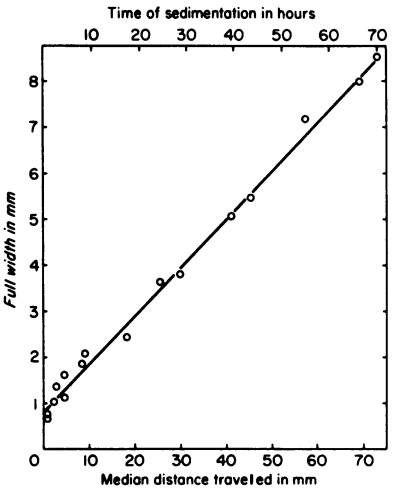

FIGURE 5

FIGURE 4 Cosedimentation of ^{3}H-TdR labeled C57BL/6 nuclei (—●—●—) and unlabeled CBA sperm nuclei (---o---o---) at unit gravity for 24 h at 4.7°C.

FIGURE 5 Dispersion characteristics of C57BL/6 nuclei sedimenting at unit gravity.

nuclei sedimenting at unit gravity for up to 70 h indicates a linear relationship between the median distance traveled (or time) and the W of the distribution. The mean sedimentation velocity of the C57BL/6 nuclei under these conditions was 1.04 mm/h. The points are a very good fit (correlation coefficient = 0.997) to the straight line $W = 0.1049X + 0.8285$, where X is the median distance traveled in millimeters. Since this fit was obtained with only a term linear in X, simple particle diffusion seems to play a very minimal role in the dispersion of the sedimentation rates, especially when observed at 24 h of sedimentation (Fig. 4). This would suggest that the observed dispersion in the sedimentation rates is due to actual physical differences amongst the individual nuclei. Such differences would likely be due to variation in density, volume, and shape factor, as indicated by the dispersions shown in Table III.

DISCUSSION

The nuclei of CBA and C57BL/6 sperm have similar densities but significantly different dimensions, volumes, and sedimentation velocities. The CBA nuclei are 13.5% longer, 0.8% narrower, and have a 6.8% larger projected area. Their volume is, however, 7.2% smaller than that of the C57BL/6 nuclei (Table III). These differences result in a significant difference in the sedimentation velocity of the two nuclei. These measurements raise points concerning the genetic interpretation, the physics of sedimentation, and the possibility of practical applications.

The measurements of the nuclear dimensions confirm and amplify the studies of whole sperm head shape undertaken by other workers (Braden, 1959; Sharma, 1960; Beatty and Sharma, 1960; Illisson, 1969). These groups have shown that sperm morphology of different strains of mice is measurably different and that certain characters have high heritability (Beatty, 1970; Woolley, 1970). It is interesting to note that our measurements of the width and area of the C57BL/6 and CBA nuclei (which we have seen to be homogenous, electron-dense particles devoid of any intact membranes) account for over 92% of the similar measurements made by Beatty and Sharma (1960) on the heads of intact sperm of apparently identical strains of mice. We have found that not only the shape but also the mass and volume of the nuclei differ in different inbred mice (Table III). This difference could be due to possible differences in either the total DNA content (Moore and Beatty, 1974) or the mass of associated nuclear proteins, such as protamine. However, quantitative chemical studies of this point will be difficult until more effective means for dissociating the mouse sperm nuclei have been developed.

While the sedimentation velocity of spheres can be calculated analytically (Stokes, 1851), more complex forms require the introduction of shape factors (Perrin 1936, Herzog et al., 1934). We show in the Appendix that this approach can be extended to any rigid particle of arbitrary shape such as the sperm nucleus. This permits us to compare the sedimentation velocities of the CBA and C57BL/6 sperm nuclei in relation to their buoyant densities, volumes, and sedimentation shape factors. Using the values of Table III, we find relative sedimentation shape factors of about 1.75 as given in the last column of the table. For comparison, oblate ellipsoids of revolution

with axial ratios of about 18 have similar relative shape factors (Tanford 1961*b*). Such a ratio is very similar to one obtained by dividing the geometric mean of the projected length and width of the nuclei (Fig. 2) by a thickness calculated from the data given in Table III. Based on the relationship derived in the Appendix it can also be shown that since the densities and shape factors are similar within the errors of our measurements, the difference in volumes between the C57BL/6 and CBA nuclei appears to be the principle factor contributing to the observed difference in mean sedimentation rates.

Sperm nuclei are remarkably uniform biological particles, but there is a measurable dispersion in each of the parameters. This may be quantitated as the full width at half-maximum height (Table III). In the case of the density, this was about 4%, with the length 8%, and with the sedimentation velocity 15%. The greater dispersion in sedimentation velocity is presumably a consequence of the dependence of this parameter on three other parameters (density, volume, and shape), each of which may show dispersion. As shown, good measures of the dispersion are available only for the density and sedimentation velocity. From the data presented, however, it is possible to make two estimates of the dispersion in volumes, one based on the dispersion amongst the ten nuclei measured for volume, and the second based on an extrapolation of the dispersions in the projected dimensions. These estimates yield values of about 6% and 23%, respectively. Employing a simple model of error propagation Melissinos, 1966), one finds that the measured dispersion in density together with the estimated dispersion in volume account for either 40 or 100% of the observed dispersion in sedimentation rates, depending on which estimate of the volume dispersion is used. It should be noted that for these calculations, no dispersion estimate was made for the shape factor.

It would seem possible that the dispersion in sedimentation rates could be a consequence of the inclusion of the sperm nuclei at different steps of maturation. This is unlikely, however, in view of the fact that the dispersion of labeled nuclei (solid line, Fig. 4) which are presumably in very similar states of maturity is similar to that of the total population of nuclei (dashed line, Fig. 4).

Finally it is interesting to consider the practical possibilities of sperm separation. By exploring differences in sperm nuclei rather than sperm, we have clearly given ourselves an advantage. Because of their physiological heterogeneity, mature intact sperm are likely to show much larger dispersions than seen in the sperm nuclei themselves. For example, when sedimenting intact mature mouse sperm, dispersions of 25–35% were not uncommon (unpublished results), about twice the dispersion found for individual sperm nuclei (Table III). Even when examining only the nuclei, however, it is clear that density separation offers little prospect for separation and sedimentation velocity has only limited usefulness. For instance, under no condition was there evidence of a resolution of sperm with X and Y chromosomes. The sedimentation velocity method can separate grossly abnormal sperm, such as those with diploid nuclei from normal sperm, but those with small differences in structure, such as minor variations in head shape, cosediment with the normal sperm unpublished results). Of all the physical

characteristics investigated in this study, length and area measurements of individual nuclei most readily distinguished between the CBA and C57BL/6 nuclei. Based on these observations future separation studies are likely to depend on the development of techniques utilizing measurements of individual sperm. One such technique may be an individual cell sorting device based on the light scattering properties (Mullaney and Dean, 1970) or perhaps even the differential staining properties of sperm (e.g., Evans, 1972). It is our opinion that sperm shape and perhaps differential staining appear to offer the greatest possibility for separation of sperm cells with different nuclear properties.

APPENDIX

We wish to demonstrate that the sedimentation rate, s, of an object of arbitrary shape sedimenting in its most stable orientation through an incompressible viscous fluid, is given by

$$s = [(\rho - \rho_o) V^{2/3}/\eta f]g, \tag{1}$$

where ρ and ρ_o are the densities of the object and fluid, respectively, V is the volume of the object, g is the acceleration of gravity, η the viscosity of the fluid, and where f is a function only of the shape of the object. The terminal sedimentation rate of the particle is determined by balancing the frictional force, K, resisting the motion with the gravitational and buoyant forces on the object:

$$K = (\rho - \rho_o) V g. \tag{2}$$

Combining Eqs. 1 and 2 yields

$$K = f\eta V^{1/3} s. \tag{3}$$

This is true for a sphere (Stokes' Law), $f = 6\pi(3/4\pi)^{1/3}$, and for a variety of other regular objects (Happel and Brenner, 1965) but we are not aware of any proof of this equation for the general case of any shape. That this is the case will be demonstrated below.

Consider two rigid objects of similar shape, S and S'; S' is n times S in each linear dimension. If S is described by the equation

$$a(x, y, z) = 0, \tag{4}$$

then S' is described by

$$a'(x, y, z) = a\left(\frac{x}{n}, \frac{y}{n}, \frac{z}{n}\right) = 0. \tag{5}$$

(The prime notation is used to designate properties associated with S'). Furthermore it can be demonstrated that if a vector or scalar field, F', can be related to the field F by the following expression:

$$F'(x, y, z) = F\left(\frac{x}{n}, \frac{y}{n}, \frac{z}{n}\right), \tag{6}$$

then any order spatial derivates of these fields are related by:

$$\frac{\delta^m}{\delta x}[F'(x,y,z)] = \left(\frac{1}{n}\right)^m \frac{\delta^m}{\delta x}\left[F\left(\frac{x}{n},\frac{y}{n},\frac{z}{n}\right)\right]. \tag{7}$$

We shall use the above equations to relate the frictional forces on S and S' by carrying through a process similar to the derivation of Stokes' Law presented by Page (1952). If the velocity field of the fluid flowing relative to S is given by $\mathbf{v}(x,y,z)$, then $\mathbf{v}$ must satisfy the boundary conditions:

$$\mathbf{v} = 0 \quad \text{at the surface of the object,} \tag{8}$$

$$\mathbf{v} = s \quad \text{at } \infty, \tag{9}$$

and the equations of motion:

$$\nabla^2\mathbf{v} = (1/\eta)\nabla p, \tag{10}$$

$$\nabla\cdot\mathbf{v} = 0, \tag{11}$$

where p is the hydrostatic pressure. If ω is defined as the mean angular velocity given by

$$\omega = \tfrac{1}{2}\nabla \times \mathbf{v}. \tag{12}$$

Eq. 10 becomes

$$\nabla^2\omega = 0. \tag{13}$$

The velocity field of fluid relative to S' may be given by

$$\mathbf{v}'(x,y,z) = \mathbf{v}\left(\frac{x}{n},\frac{y}{n},\frac{z}{n}\right). \tag{14}$$

That this is indeed the case is demonstrated by the fact the $\mathbf{v}'$ satisfies Eq. 8 at S′ and 9 at ∞ and since the terms in the equations of motion 11, 12, and 13 are all spatial derivates of $\mathbf{v}$, and $\mathbf{v}(x,y,z)$ is a solution, then using Eq. 7 $\mathbf{v}'(x,y,z)$ must also satisfy the equations of motion.

The pressure field associated with S' can be related to that of S, using Eq. 10. The result is

$$p'(x,y,z) = \frac{1}{n}\, p\left(\frac{x}{n},\frac{y}{n},\frac{z}{n}\right) + \text{const.} \tag{15}$$

The arbitrary constant represents the pressure over the outer boundary of the fluid but may be neglected in the calculation of the frictional forces.

The stress dyadic, $\overline{\overline{\psi}}$, for an incompressible fluid, is defined as

$$\overline{\overline{\psi}} = -p\overline{\overline{I}} + 2\eta\,\overline{\overline{\Phi}}, \tag{16}$$

where $\overline{\overline{I}}$ is the identity dyadic and $\overline{\overline{\Phi}}$ is the rate of strain dyadic. All terms in $\overline{\overline{\Phi}}$ are first spatial derivates of the velocity field. Therefore, by Eq. 7, 15, and 16:

$$\overline{\overline{\Phi}}'(x,y,z) = \frac{1}{n}\,\overline{\overline{\Phi}}\left(\frac{x}{n},\frac{y}{n},\frac{z}{n}\right), \tag{17}$$

$$\overline{\overline{\psi}}'(x,y,z) = \frac{1}{n}\,\overline{\overline{\psi}}\left(\frac{x}{n},\frac{y}{n},\frac{z}{n}\right). \tag{18}$$

To calculate the force on S, the stresses on the surface, given by $\overline{\overline{\psi}}$, are integrated over the entire surface.

$$\mathbf{K} = \int_S \mathbf{e}_n \cdot \overline{\overline{\psi}}\, d\sigma \tag{19}$$

where $\mathbf{e}_n$ is the unit vector perpendicular (outward) to the surface. For the force on S' is given by

$$\mathbf{K}' = \int_{S'} \mathbf{e}'_n \cdot \overline{\overline{\psi}}'(x,y,z)\, d\sigma' = \frac{1}{n}\int_{S'} \mathbf{e}'_n \cdot \overline{\overline{\psi}}\left(\frac{x}{n},\frac{y}{n},\frac{z}{n}\right) d\sigma'. \tag{20}$$

For each point on S', (x,y,z), the equivalent point on S is $(x/n, y/n, z/n)$. For such equivalent points the normal vectors are the same and the differential surface areas are related by $d\sigma' = n^2 d\sigma$. Therefore

$$\mathbf{K}' = \frac{1}{n}\int_S \mathbf{e}_n \cdot \overline{\overline{\psi}}(x,y,z) n^2 d\sigma = n\mathbf{K}. \tag{21}$$

Thus the frictional force for similar objects is proportional to n, the linear dimension, or $V^{1/3}$. Returning to the equations of motion, 8, 9, 11, 12, and 13, it is apparent that the velocity field for a given object is independent of η and proportional to s. By Eq. 10 the pressure field is proportional to both η and s, as is $\overline{\overline{\psi}}$ by Eq. 16. Thus K is proportional to $V^{1/3}\,\eta s$. The only factors which the proportionality factor can be a function of, are shape and orientation. But by a similar analysis of the torque, τ, instead of the force, K, it can be shown that $\tau' = n^2\tau$. Thus the equilibrium orientations ($\tau = \tau' = 0$) of S and S' would be the same. Hence the proportionality factor, f, is solely determined by shape.

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