part of the same defect, an absence of skin and bone with resulting exposure of the underlying structures. The structures which were susceptible to damage may be considered to be directly exposed to contact with the insoluble compound if one assumes that absence of the skin in turn exposes the mesenchyme of the head from which the membranous frontal bone forms between the tenth and twelfth day. Both the eyelids and the frontal bone are in beginning stages of differentiation during the time of exposure. The mechanism by which the defects occur is not known. It may be due to interference with further development of the structures or to actual destruction of the tissues already developed, possibly through irritation or abrasive action. Whether there is any relationship of etiology between these defects and similar defects which are encountered in the human is not known.

With the increasing interest in screening of drugs for possible teratogenic effects it is necessary to consider carefully the physical characteristics of the drug to be tested as this factor influences the experimental testing system utilized.

Summary. Thalidomide produced encephalocele, eyelid defects and beak defects in a significant percentage of chick embryos inoculated into the amnion with the drug at 3 through 6 days incubation. No defects of this type were observed in control embryos inoculated with saline. However 4 other selected compounds which, like the thalidomide, remained in an undissolved state in the amniotic cavity after inoculation, produced the same types of defects. These compounds were sand, ground glass, colloidal alumina and colloidal attapulgus clay. In addition the last 2 produced severe twisting and distortion of the axis and appendages of the embryos, with increased incidence of ectopic viscera.

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Collection of Monkey Semen by Electroejaculation.* (28242)

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Electroejaculation has been used for semen collection in several species, including the bull, boar, dog, ram, fox, chinchilla and guinea pig (1,2,3). This approach has not, to our knowledge, been successful in the primate, notwithstanding the numerous practical applications of such a method in monkey breeding and reproductive research. Recently, this relatively unexplored area of human physiology has been emphasized and unsuccessful attempts at electroejaculation reported(4). The method reported here provides a simple technique for electroejaculation in the macaque, which is not associated with a generalized convulsive reaction and has yielded almost consistently satisfactory results.

Method. Adult male macaques were restrained either by 3 assistants or by one assistant using a choke chain restraint within the cage. Gentle traction was applied to the glans penis. A strip of aluminum foil 2 cm

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wide was applied about the base of the shaft and held with a clamp electrode. The penis was moistened with saline to facilitate contact with a second electrode which was held with a gloved hand against the ventral aspect of the glans near the frenulum. Intermittent charges of 20 volts were delivered at a frequency of between 10-20 impulses/second, and duration of 25 to 50 milliseconds, using a monophasic alternating current.† If ejaculation did not occur within 1-2 minutes, the voltage was increased stepwise to 40. Ejaculates were weighed. The spermatozoa in the liquefied portion of the ejaculate were counted in a Neubauer hemocytometer. The percentage and quality of motility were established by observation of a hanging drop.

Results. An ejaculate was obtained within 3 minutes from 6 *Macaca mulatta,* one *Macacus arctoides* and one *Macacus nemistrinus.* The method failed in 2 *Macaca mulatta* and one *Macacus arctoides.*

To evaluate the effect of repeated electroejaculation, the method was applied at intervals of 2-3 days to each of 2 animals (*Macaca mulatta*). There was no evidence that the monkey became refractory to stimulation. Rather, he became conditioned to the handling required by the procedure. In 40 tries, 37 specimens were obtained. Each time an ejaculate was not obtained, one was noted in the cage pan, presumably the product of recent masturbation.

The semen is liquid on ejaculation. Coagulation occurs within seconds, with the formation of a white, rubbery coagulum. Liquefaction begins within 5 minutes. Thirty minutes following emission, approximately 30% by weight is liquefied. After this initial interval, liquefaction continues at a slower rate, but is never complete.

The liquefied portion contains active spermatozoa. Among 8 monkeys studied, the sperm density ranged between 93 and 807 million/cc (Table I). The percentage of motile forms ranged between 30 and 98%. Those specimens with a low percentage of motility also displayed poor quality of motility.

† Model S-5 Stimulator, Grass Instrument Co., Quincy, Mass.

TABLE I. Spermogram of Monkey Ejaculates.

Subspecies	Wt of ejaculate (mg)	Count (million /cc)	% of motility	Quality of motility
Mulatta	1182	807	98	4+
"	1291	358	95	4+
"	4985	93	30	1+
"	1283	238	95	4+
"	1550	300	40	2+
"	1649	602	99	4+
Nemistrinus	1273	218	90	1+
Arctoides	0242	264	35	1+

The spermatozoa resembled human forms except that the tail was noticeably longer (Fig. 1). In fixed, stained smears, the length of the tail was approximately 70 μ and that of the head 4 μ, in contrast to values of approximately 50 μ and 5 μ for human spermatozoa.

Discussion. Five of 8 animals whose semen was studied produced specimens which were clearly within the fertile range by human standards. In the remainder, the quality of motility was poor. The quality of motility is the most important single factor in evaluating human male fertility potential(5). The fertility of monkey specimens obtained by electroejaculation remains to be demonstrated by artificial insemination.

Coagulation of monkey semen has been described previously in specimens obtained from the vagina following coitus. The coagulating gland, located by van Wagenen in the cranial lobe of the prostate, is probably responsible for this characteristic(6). Liquefaction of monkey semen is incomplete even after many hours of *in vitro* observation. In

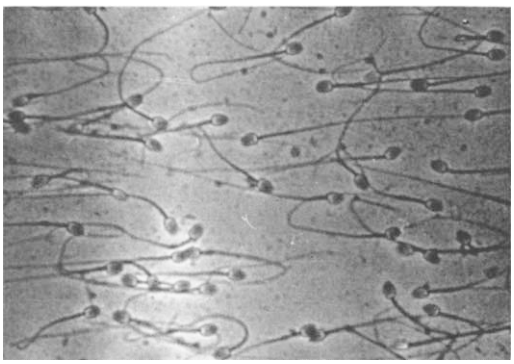

FIG. 1. Fixed, stained smear of liquefied portion of ejaculate *(M. mulatta)* × 80.

this respect, it is similar to guinea pig semen but differs from human semen, which normally liquefies completely within 20 minutes of ejaculation. The coagulum may serve to retain spermatozoa within the vagina post coitum.

Summary. A method for electroejaculation of the macaque was successful in 8 of 11 subjects. Repeated electroejaculation at 2-3 day intervals did not cause a refractory state. Coagulation occurred almost immediately on ejaculation. Liquefaction began shortly thereafter, but was incomplete. Sperm concentration in the liquefied portion of the ejaculate ranged between 93 and 807 million/cc. Tail length was approximately 70 μ, as compared with 50 μ in human material. Electroejaculation is suggested as a practical means of obtaining monkey semen.

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Growth of L Cell Suspensions on a Warburg Apparatus.* (28243)

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Suspension cultures of mammalian cells are frequently agitated by devices which promote a circular motion of the suspension(1,2,3). A simple, inexpensive modification of a Warburg apparatus allowed studies of the growth of L cells when agitated by a reciprocal motion. These are reported here.

Materials and methods. The basal medium was a modification of Waymouth's MB 752/1 (4) with tris(hydroxymethyl)aminomethane (tris) in a final concentration of 14 mg/100 ml substituted for $NaHCO_3$; 0.05% methylcellulose (4000 cps) (Dow Chemical Co., Midland, Mich.), 0.1% Pluronic F68, or 0.1% Pluronic F88 (Wyandotte Chemical Corp., Wyandotte, Mich.)† (all w/v) were usually added as supplements. In serum-free suspensions, 2 of the supplements sometimes were used. Horse serum (10%, v/v), which had been inactivated at 52°C for 1 hour and passed through a sterile Seitz filter, also was added for cultures of cells previously grown in serum-containing media. All cells were sub-lines of NCTC clone 929 (Strain L). Those grown in suspensions with serum had previously been carried on glass in the basal medium with 5% (v/v) horse serum. Those propagated in serum-free suspensions had been cultivated on glass in serum-free basal medium with bicarbonate.

Suspension cultures were grown in 125 ml untreated, screw-capped Erlenmeyer flasks which were held in the Warburg bath (Precision Scientific Co., Chicago, Ill., Cat. No. 6691) by specially designed holders (Fig. 1) attached to manometer supports. The holders with flasks in place could be placed on the bench top without tipping. They may vary in size according to the type of Warburg used but should allow the flasks to be immersed within approximately an inch of the neck while the bath is at the correct water level.‡

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† The assistance of Dr. Trix of Wyandotte Corp. in obtaining the Pluronics is gatefully acknowledged.

‡ The holders also will fit regular Erlenmeyer flaskc and can be made in a variety of sizes and conveniently applied to many operations where shaking in a constant temperature water bath is desired.