Collection and Liquefaction of Guinea Pig Semen.* (24099)

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Investigations of the physiology of reproduction in the male guinea pig and rat have been hampered by lack of methods for semen collection and for "vaginal plug" liquefaction. Consequently, work with these animals has been limited to study of histological and biochemical changes in testes and accessory glands. The 2-fold purpose of this study was to develop a routine method for collection of semen specimens at regular intervals from the guinea pig and to find a simple procedure for plug liquefaction, so that changes in semen characteristics might be used in investigations of this type. Use of electroejaculation for collection of bull and ram semen is now common practice and has been adequately reviewed(1). Dalziel and Phillips(2) developed a technic for electroejaculation of chinchillas and guinea pigs but did not report on the material collected. This paper describes the apparatus and methods used for regular collection of guinea pig semen by electroejaculation and use of proteolytic enzymes in liquefaction of resultant semen coagulum.

Methods. Eighteen mature albino guinea pigs were used to collect 110 semen specimens by electroejaculation during 4 month period. The hair was clipped from lumbar region and electrode jelly applied to clipped area. The guinea pig was strapped on its back to animal board through which there was inserted a round copper disk (diameter 1.75 cm) used as lumbar electrode. The anal electrode was a smooth brass tube (diameter 0.4 cm; length 8.0 cm) lubricated with K-Y jelly and inserted 4-6 cm into the rectum. A simple and inexpensive electronic setup was designed to deliver an intermittent square wave, 25 volt rms. at 1000 cycles, with automatic 3 second "on period" and 12 second "off period" (Fig. 1). The mean number of shocks required for ejaculation was 5 (range 3-14 shocks). The semen specimen was collected in a recalibrated 5 ml graduate in 1 ml of phosphate buffer after White(3), containing appropriate concentration of the enzyme under study; semen volume was read to nearest 0.1 ml. Motilities were estimated under low power of microscope in a slide warmer at 39°C. There was no apparent ill effect of biweekly collection, although as many as 8 specimens were collected from each of 5 animals during the experimental period. While muscle contraction and squealing were noted in the 3 second "on period" during the early part of the study, the effect of conditioning became apparent towards the latter part of the study when there were little or no visible or audible signs of distress during the "on period."

Results. Preliminary trials indicated that semen coagulated almost instantaneously upon ejaculation and in some cases it was ejacu-

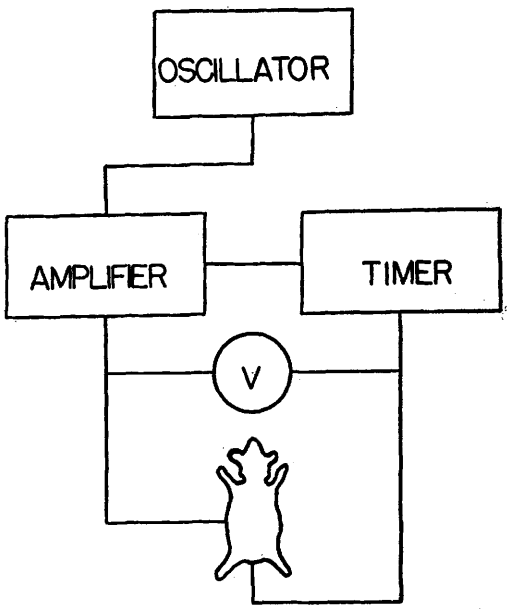

FIG. 1. The oscillator produces a 2.5 volt, 1000 cycle, square wave from the 110 volt, 60 cycle AC line and output at the amplifier is set at 25 volts. The cam operated timer is designed to deliver a 3 sec. "on period" and a 12 sec. "off period."

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lated in plug form, which indicated that it had coagulated in the urethra. This semen coagulum did not spontaneously liquefy even after 6 hours incubation at 39°C. When the coagulum was collected in Ringer's solution or in phosphate buffer, there was no indication of liquefaction at any time during 6 hours at 39°C.

A series of commercially available enzymes were then tried for their liquefying activity in this system.† Since Bunge and Sherman (4) had shown that alpha-amylase would liquefy coagulated human semen specimens, it was tried in Ringer's solution and in phosphate buffer, at concentrations ranging up to 10%. Partial but incomplete liquefaction was noted in 8 specimens with some cells free of the coagulum and feebly motile. Varidase‡ dissolved in phosphate (17,000 units Streptokinase and 10,000 units Streptodornase/ml) was tried with 9 specimens and in every case there was complete liquefaction of the coagulum and vigorous sperm motility. Within minutes, however, recoagulation was noted on the slide and in the collection graduate and, in 5 to 10 minutes (at 39°C), all specimens were completely recoagulated. This phenomenon of recoagulation, as observed under the microscope at 39°C, was made up of several stages; 1. completely liquefied semen was free of coagulum and the cells moved freely and vigorously; 2. small centers of coagulation appeared uniformly throughout the field and the forward motion of the sperm was markedly decreased, although their tails continued beating rapidly; 3. centers of coagulation enlarged to occupy most of the field and there was no forward motion although many tails continued to beat feebly; 4. the coagulum occupied the entire field enmeshing the sperm and no tail motion could be observed. When an additional ml of Varidase was added and stirred into the coagulum, reliquefaction took place and the sperm regained vigorous motility. This was followed by complete recoagulation within 5 to 10 minutes on the pattern described.

Use of bromelain, papain, cathepsin, or trypsin in concentrations ranging to 0.1% in phosphate yielded variable results, with partial liquefaction of the coagulum in some specimens and no liquefaction in others.

Chymotrypsin was tried at concentrations ranging from 0.001 - 0.1% in phosphate. At the 0.1% concentration it consistently produced liquefaction and the specimens did not recoagulate during 6 hours of incubation at 39°C. At lower concentrations of chymotrypsin, liquefaction was incomplete and, in a number of cases, recoagulation took place. Of the 78 specimens collected in 0.1% chymotrypsin-phosphate, 69 liquefied rapidly and contained motile cells (mean motility = 51%; range = 10-90%), 4 did not liquefy, 4 liquefied but showed no sperm motility, and 1 specimen was sperm free. The motility of these cells was characterized by curvilinear swimming patterns, irregular forward motility, and "head to head" rouleaux. Mean ejaculate volume was 0.5 ml (range 0.1-1.2 ml).

During the 4 month study, a marked trend was noted for the ejaculated semen to remain liquid and fail to clot, *i.e.* those pigs that had been electroejaculated several times, at biweekly intervals, began to deliver specimens that did not coagulate at ejaculation or even after several hours of incubation. The liquid semen specimens were either completely coagulum free or else had only small, scattered islands of coagulum and the sperm motility was, in all cases, good. The possibility that the failure of the semen to coagulate was due to damage to the accessory glands by repeated electroejaculation was investigated by histological methods. Two "electroejaculate" pigs and 2 "control" pigs (breeders that had never been electroejaculated) were sacrificed and tissue from the coagulating gland, dorsal and lateral prostate, seminal vesicle, testis, and rectum was fixed in Bouin's, sectioned at 4 μ, and stained with hematoxylin and eosin. There was no evidence of gross morphological or histological differences between the "electroejaculate" and the "control" pigs.

† Trypsin, chymotrypsin, alpha-amylase (Nutritional Biochemicals); trypsin, chymotrypsin, alpha-amylase (Bios Labs); bromelain, papain, cathepsin, pepsin (Mann Research Labs).

‡ We are indebted to Dr. J. M. Ruegsegger, American Cyanamid, N. Y. City, for the Varidase.

It might be suggested that the ejaculation of liquid semen after repeated biweekly electroejaculation is the result of some physiological change affecting either vesiculase production by the coagulating gland or else some other factor involved in the clotting mechanism. This might be due either 1) to frequency of ejaculation or 2) to electroejaculation of larger volumes of semen than might be delivered under the normal conditions of coitus, with the result that the ability of the accessory gland system to regenerate some essential part of the clotting mechanism is exceeded. A further investigation of this problem will use the addition of a purified vesiculase to these liquid specimens to determine whether failure to coagulate is due to a lack of vesiculase.

Summary. A method and an apparatus for routine electroejaculation of guinea pigs were developed, the semen coagulum or "vaginal plug" examined, and the coagulation process described. A series of proteolytic enzymes were tried for their liquefying activity and 0.1% chymotrypsin was found to result in consistent liquefaction and good sperm motility. Mean ejaculate volume was 0.5 ml and mean motility at 39°C was 51%. There was no evidence of gross morphological or histological changes in the reproductive system of guinea pigs electroejaculated at biweekly intervals.

I am indebted to Mr. Lloyd Schoenbach for the design and construction of the electroejaculator and to Mrs. Olga Radimska for histological preparations.

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Apparatus for Continuous Intravenous Infusion in Dogs. (24100)

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Several types of apparatus for intravenous infusion of fluids in dogs have been developed, each designed to meet particular needs of different investigations(1,2,3). The main feature of the apparatus described here is a standard assembly consisting of a reservoir bottle, drip device and connecting tubes leading to indwelling intravenous catheter, commonly employed in clinical hospital practice. The polyethylene catheter is introduced into an external jugular vein as described by Zimmermann(4). The bottle is suspended under a swivel, with a flexible support for the connecting tubing arranged so that the dog has almost complete freedom of movement in a spacious cage while fluids are infused during varying periods of time.

Apparatus, Fig. 1: *1.* Pulley roller on ¾ inch round rod, held 4 feet above top of cage, permits backward and forward movements of dog. *2.* Swivel hook hanging below pulley, turns freely through a complete circle. *3.* Square ⅜ inch steel rod, with hook at top, suspended from swivel. *4.* Light coil spring, fastened at its top to the rod by adjustable clamp and at its lower end to a bracket (*5*), which slides up and down on rod. Attachments of spring are adjusted to give tension strong enough to hold the bracket, tubing and harness at a level comfortable for the dog. *5.* Sliding bracket with 2 square holes fitting the steel rod loosely. Excursion of the bracket

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