tities of insulin in microdissected islet tissue. The relative simplicity of this method suggests that it will have wide application.

Summary. Tracer amounts of I^{131}-insulin are precipitated by specific antibodies. The change in percent precipitated, as increasing amounts of unlabeled insulin are added, forms the basis of this assay. The method is sensitive to less than one microunit.

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Received February 12, 1962. P.S.E.B.M., 1962, v110.

Comparison of Protein and Zinc Electrophoretic Patterns of Lobes of Rat Prostate.* (27412)

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The presence of large amounts of zinc in prostate has intrigued many investigators since Bertrand and Vladesco's(1) report of its occurrence in the human gland. Others have since established that zinc occurs in high concentrations in rabbit prostate(2) and in dorsolateral prostate of rat(3). The rat lends itself well for study of the prostate since its lobes, ventral and dorsolateral, are distinctly separated. The dorsolateral lobe can, in turn, be separated by gross dissection into its dorsal tip, an area which contains only acini of holocrine type, and its lateral tip, an area which is comprised exclusively of acini of apocrine type(4,5). Histochemical(4,6,7,8), autoradiographic(9,10) and Zn^{65} distribution (4,5) studies in rats have established that the large amounts of zinc are localized predominantly in lateral acini of the dorsolateral prostate. This report presents results of studies on electrophoretic separation of various zinc components found in prostatic lobe secretions and in blood serum. Parallel studies were made of protein migratory patterns in prostatic secretions and serum.

Materials and methods. 1. *Experimental animals.* Previous reports indicate that administered Zn^{65} concentrates in various lobes of rat prostate(4,5,11) parallelling the distribution of natural zinc in various parts of the gland(3,6). To facilitate identification of zinc in prostatic secretions and in serum, Zn^{65}† was administered to rats used in this study; and the presence of zinc on electrophoretic paper strips was thereby determined by radioactive measurement. Mature male Wistar rats, 16-20 weeks of age (350-400 g), were used in these experiments. For studies on lateral prostatic lobe secretion, rats were injected with 0.04 μc/g of Zn^{65} intracardially under ether anesthesia. Twenty-four hours later, when Zn^{65} concentration in lateral prostatic lobe was maximal(4,11), rats were sacrificed by chloroform and exsanguination. Lateral tips of dorsolateral prostate were dissected, as described previously(5).

Since Zn^{65} concentration in dorsal and ven-

* Research supported in part by grants from U. S. Atomic Energy Commission and U. S. Public Health Service, Nat. Cancer Inst.

† Zn^{65} was purchased from Union Carbide Nuclear Co. as $Zn^{65}Cl_2$ in HCl solution with a specific activity of approximately 1000 mc/g. The solution was diluted with physiological saline containing sufficient NaOH to neutralize some of the acid present compatible with solubility. The dilution was made so that the administered dose was contained in less than 0.25 ml.

tral prostatic lobes and in serum is considerably less than that attained in lateral prostatic lobe(4,5,10,11), a larger dose of Zn^{65} (0.12 μc/g) was administered to rats used for study of these specimens. In view of the fact that distribution of Zn^{65} in dorsal(4) and ventral(11) prostatic lobes and in serum (11,12) is maximal at early times following intracardiac injection, samples of these specimens were taken approximately 2 hours after injection of the radioisotope. At that time, rats were anesthetized with ether, and a 3-ml sample of blood was withdrawn by intracardiac puncture and subsequently allowed to clot. The rats were then sacrificed by chloroform and exsanguination, ventral lobes were removed and dorsal tips of dorsolateral prostate were dissected according to technics reported previously(5).

2. *Electrophoretic studies.* Samples of lateral, dorsal and ventral prostatic lobes that were removed for analysis were placed in chilled 3-ml centrifuge tubes. Tissues were pressed with glass stirring rods to extrude as much secretion as possible. Prostatic secretion samples and blood specimens were then centrifuged in the cold (4°C) at 6400 rpm for 20 minutes. The supernatant from each sample was poured off and recentrifuged as before for 20 minutes. Samples of this final supernatant of each specimen were applied to paper strips in a Spinco Model R Electrophoretic Cell. Each specimen was run in duplicate, one strip subsequently prepared for protein analysis, the other for zinc (Zn^{65}) determination. For protein analysis, application of one 10-μl sample per strip was sufficient for all specimens studied. For Zn^{65} determination, application of one 10-μl quantity of lateral prostatic lobe secretion per strip gave sufficient radioactivity for counting. For the other specimens, *i.e.*, dorsal and ventral prostatic lobe secretions and serum, in which Zn^{65} levels were lower, a total of 100 μl of each specimen had to be applied to strips to obtain sufficient counts. In these cases 10 serial applications of 10 μl each were applied to the paper strips. The strips were run for 18 hours at 3.0 milliamperes, using a Veronal buffer system of pH 8.6 (0.075 ionic strength). Immediately after the electrophoretic run, the strips were dried for 30 minutes in an oven at 130°C. Subsequently, one of the strips on each specimen was processed for protein analysis, the other for zinc (Zn^{65}) determination.

3. *Protein analysis.* Paper strips were stained for protein by the Spinco bromphenol blue technic. Stained strips were cut in 0.5 cm-wide segments along their 30 cm length, yielding a total of sixty 0.5 cm-wide segments per strip. For the bromphenol blue protein determination, each of these 60 segments was placed in a colorimeter tube. The dye was eluted from each paper segment by addition of 5 ml of 0.5% aq. sodium carbonate. The de-colorized paper segment was removed, and optical density of the resulting bromphenol blue solution was determined at 590 millimicrons, using a Bausch and Lomb Spectronic "20". Subsequently, the optical density reading of dye eluted from each 0.5 cm-wide segment was plotted on the ordinate *vs* the cm-length measurement of the strip on the abscissa. The resultant curve was identical with that obtained from an analytrol scan of the entire strip.

4. *Zinc analysis.* The approximate location of zinc on the strip could be revealed by the presence of a pink stain after dipping the strips in 0.1% dithizone (in acetone or chloroform solution). However, due to the deep background coloration of the strip, this method was not suitable for quantitative determination of zinc; and, instead, radioactive measurements of Zn^{65} were used to reveal the presence of zinc.

Electrophoretic strips were measured and cut into 0.5 cm-wide segments, as were those used for protein analysis. At the beginning of these studies, the only type of counting equipment available with an automatic changing device was a gas flow detector for beta radiation. Each 0.5 cm-wide paper segment was secured flat to a planchet by 2 tiny slivers of scotch tape, one at each end of the segment. Later, automatic sample changing equipment became available for a well-type scintillation detector for gamma radiation. By this means, the 0.5 cm-wide segments could be placed in counting tubes without any regard to geometry. When the counts per

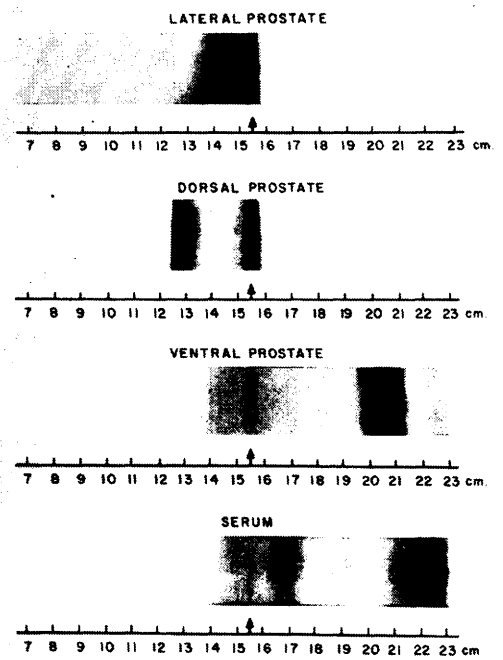

FIG. 1. Paper electrophoretic patterns of proteins (bromphenol blue) of secretions of various lobes of rat prostate and of serum. Arrow indicates point of application of sample. Anode toward the right.

minute obtained from each 0.5 cm-wide segment were plotted on the ordinate *vs* the cm-length measurement of the strip on the abscissa, identical curves were obtained with the 2 types of counting equipment. The curve obtained from an automatic scan of the entire strip (using either gas flow or scintillation detectors) was also similar to the data obtained by counting measured segments. Radiation levels on the strip, however, were far too low to obtain an accurate curve by scanning methods.

Results. Fig. 1 shows protein patterns of secretions of various components of rat prostate (lateral, dorsal and ventral lobes) and of serum, as revealed by electrophoretic separation on paper. The protein pattern of ventral prostatic lobe secretion shows similarities to that of serum, in that migration is predominantly toward the anode. Whereas the major migratory band of protein in serum consists of albumin (shown in Fig. 1 between 22 and 23 cm), the darkest protein band in ventral prostatic lobe secretion does not migrate quite as far toward the anode as albumin (seen between 20 and 21 cm in Fig. 1). Another smaller fraction, perhaps identical with albumin, is seen in the ventral prostatic pattern.

Dorsal prostatic lobe secretion has 2 major protein components, one migrating slightly toward the cathode (seen at 12.5 cm in Fig. 1), and the other failing to migrate. There are faint migrations toward the anode, with the possibility of a weak band in the area of serum albumin (seen between 22 and 23 cm in Fig. 1). The majority of protein in lateral prostatic lobe secretion also remains at the point of application. Smaller fractions migrate predominantly toward the cathode.

In Fig. 2, the zinc (Zn^{65}) electrophoretic pattern seen in serum shows the presence of at least 4 migratory components. They are probably associated with the albumin fraction, as well as the α-1, α-2 and β-globulins. There appears to be only one zinc fraction in ventral prostatic lobe secretion, possibly associated with its major protein component. There are probably at least 3 zinc fractions in dorsal prostatic lobe secretion; the major one appears to be associated with the non-migratory protein; the lesser fractions migrate toward the anode. The lateral prostatic lobe secretion probably has 4 or 5 zinc components. One of them seems to be associated with the non-migratory protein. The other fractions migrate toward the anode. Of these, the major one has its peak at about 19.5 cm, where there is no definitive protein band demonstrable with bromphenol blue. This, of course, does not rule out that a protein with a very great zinc-binding capacity is involved, one which is not detected as protein in the amounts present. Another possibility exists that there is some other chelate present with which the zinc is combined. This major zinc fraction in lateral prostatic secretion does not apparently represent free zinc, since after electrophoretic application of $Zn^{65}Cl_2$ in comparable amounts in absence of protein Zn^{65} migrated only as far as 18.0 cm.

Discussion. The human prostate is anatomically one organ. It has arbitrarily been given a lobular designation; *i.e.,* an anterior,

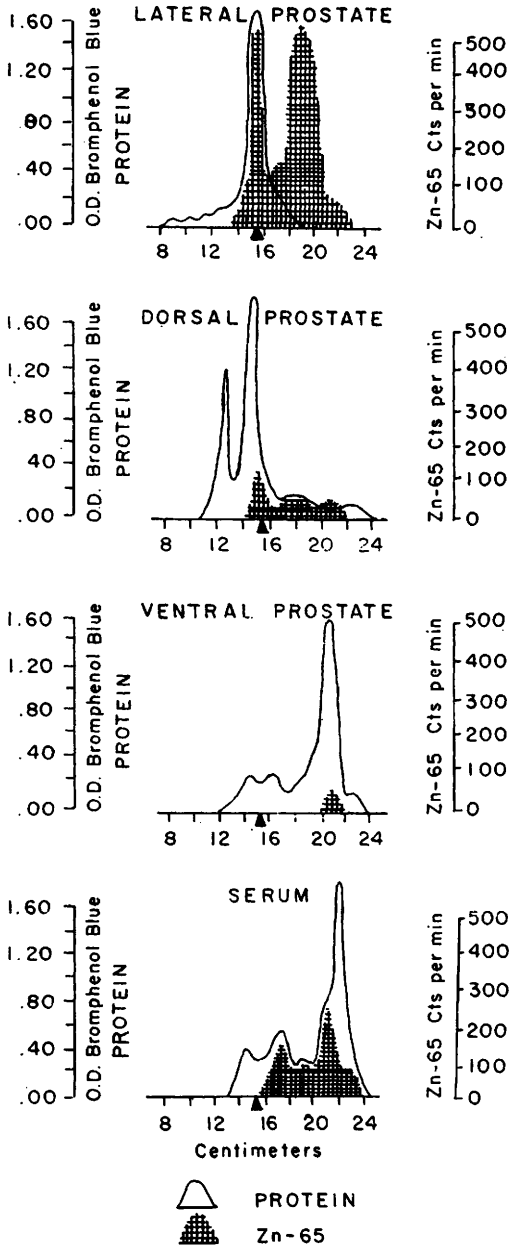

FIG. 2. Paper electrophoretic patterns of protein (bromphenol blue) and zinc (Zn^{65}) complexes of secretions of various lobes of rat prostate and of serum. Arrow indicates point of application of sample. Anode toward the right.

posterior, medial and 2 lateral lobes, with regard to its relation to the urethra and ejaculatory ducts(13). However, there is no histological demarcation into true lobes. Franks (13) proposes that the human prostate is not truly a uniform gland, as the inner group of glands surrounding the urethra are more sensitive to estrogen and hyperplasia than are the outer circle of glands. Investigators who have conducted zinc(14-16) and acid phosphatase(15) analyses on human prostate have found extreme variations in results even when samples were taken from the same lobe of the prostate. Kerr, Keresteci and Mayoh (16) noted that the anatomical distribution of zinc in human prostate did not follow conventional anatomical planes, but, in general, was greater toward the apex of the gland. In an attempt to explain the inconsistencies of the human prostate, information obtained from studies of rat prostate may be of value. In the rat, the prostatic lobes are discrete and lend themselves to separate investigation. Studies on individual lobes have shown that they differ histologically(4,5,17) and in concentrations of their natural biochemical constituents, particularly fructose(5,18,19), citric acid(18,19), amino acids(20), acid and alkaline phosphatase(6), carbonic anhydrase (2,6) and zinc(3,6). This present paper also emphasizes their marked variation in protein distribution and zinc complexes as revealed by electrophoretic separation on paper.

The question arises as to whether the human prostate is a fusion of the lobes that are found to be more or less discrete in rodents. Thus, even though the human prostate is grossly one gland, it may contain all of the histological, biochemical and functional components found in various prostatic lobes of lower species. This may explain the marked variability of results obtained in studies of human prostate.

Summary. Electrophoretic studies using Zn^{65} indicate the presence of several zinc components in secretions of lobes of rat prostate. The distribution of zinc complexes and the types of protein migratory patterns noted emphasize the marked biochemical variabilities found in the individual lobes, *i.e.*, ventral, dorsal and lateral, of rat prostate. These patterns, in turn, differ from those observed in rat blood serum.

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Received February 13, 1962. P.S.E.B.M., 1962, v110.

Mitotic Chromosomes of Some North American Sciuridae.* (27413)

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A previous study(1) demonstrated that several species of chipmunk (genera Tamias and Eutamias) had a diploid chromosome number of 38. Karyogram analysis permitted classification and identification of most of the chromosome pairs. A close taxonomic relationship between the above genera was demonstrated. These findings suggested that the chipmunk was a suitable experimental animal for cytogenetic studies.

The present study consisted of a survey of mitotic chromosomes in various other genera of the Family Sciuridae. The purpose was to 1) determine taxonomic and genetic relationships within the family and 2) ascertain the suitability of these animals for cytogenetic studies.

Methods. Animals were trapped alive and studied in the laboratory. Identification and classification were made according to Hall and Kelson(2). The following specimens were studied: 1) *Ammospermophilus harrisii harrisii*, 2 males, 2) *Ammospermophilus leucurus leucurus*, 1 male, 3) *Spermophilus richardsonii elegans*, 2 males, 4) *Spermophilus mexicanus parvidens*, 2 males and 1 female, 5) *Spermophilus beecheyi douglasii*, 3 females, 6) *Spermophilus lateralis lateralis*, 1 male and 3 females, 7) *Tamiasciurus hudsonicus fremonti*, 1 male.

Chromosome preparations were made from femoral marrow(1). Animals were first given colcemide intraperitoneally. After 2 hours the marrow was removed, treated with 1% sodium citrate, fixed in 1 part 1 N HCl and 10 parts 2% acetic orcein. The tissue was stained in acetic orcein and squashed between a slide and coverslip. Cells were counted at 1250× and photographed for karyogram analysis. Chromosomes were arranged according to centromere position (median, submedian or terminal) and were paired in order of descending size. Unpaired chromosomes in male karyograms were considered tenta-

* Supported by a grant from The Leukemia Society, Inc.