experiments were interpreted as examples of methods which failed and were not construed as positive evidence for a non-viral etiology of malignancies in man.

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Chromosome Studies in Certain Subgenera of Spermophilus.* (27649)

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A considerable variation in chromosome number and morphology has been reported among ground squirrels belonging to the subgenera of Spermophilus(1). Furthermore, it was also noted that karyograms of all male specimens of Spermophilus and Ammospermophilus contained a medium size acrocentric Y chromosome(1).

Karyograms of *S. tridecemlineatus* (subgenus Ictidomys) and of *S. beldingi* (subgenus Spermophilus) have not been previously analyzed. It was of interest, therefore, to compare chromosomes of the above species with those described in *S. mexicanus* (Ictidomys) and *S. richardsonii* (*Spermophilus*)(1). Chromosomes of *S. tereticaudus* (subgenus Xerospermophilus) were also analyzed.

The purpose of this investigation was to: 1) evaluate chromosome analysis in comparative taxonomy of closely related species and 2) determine the validity of the medium size acrocentric Y chromosome as a specific characteristic of Spermophilus and Ammospermophilus.

Methods. The following animals were studied: 1) *Spermophilus beldingi beldingi,* 3 males and 2 females. 2) *Spermophilus tridecemlineatus tridecemlineatus,* 3 males and 1 female. 3) *Spermophilus tereticaudus neglectus,* 1 male and 3 females.

Animals were classified as previously described(1). Chromosomes from femoral marrow were analyzed by utilizing a colcemide, hypotonic citrate and acetic orcein squash method(1,2).

Results. Chromosome number. The data are listed in Table I. The modal diploid (2n) chromosome number in *S. beldingi* was 30, in *S. tridecemlineatus* 34 and in *S. tereticaudus* 36.

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TABLE I. Chromosome Counts of Femoral Bone Marrow Cells.

Specimen	Sex	Total cells counted	28	29	30	31	32	33	34	35	36	37
S. beldingi	♂	30	2	1	25	2						
"	♂	10	1		9							
"	♀	60			60							
"	♀	53	1	2	50							
"	♂	23		2	21							
S. tridecemlineatus	♂	65						3	62			
"	♂	30						1	29			
"	♂	30							30			
"	♀	37			1	2	1	4	28	1		
S. tereticaudus	♂	64							1	4	57	2
"	♀	42								3	39	
"	♀	33							1	1	31	
"	♀	43							1	1	41	

Chromosome morphology. Typical karyograms of *S. tridecemlineatus, S. beldingi* and *S. tereticaudus* are illustrated in Fig. 1, 3 and 5, respectively. Karyograms, previously described(1), of *S. mexicanus* and *S. richardsonii* are shown in Fig. 2 and 4 for comparison. It can be seen that the karyogram of *S. tridecemlineatus* (Fig. 1) is distinctly different from that of *S. mexicanus* (Fig. 2) since: 1) more terminal centromeres are present in the 3 largest submetacentric chromosome pairs, and 2) the fourth largest pair of submetacentric chromosomes is smaller and has a more terminal centromere. However, the submetacentric X and acrocentric Y chromosomes of the above two species appear similar (Fig. 1 and 2).

In *S. beldingi* (Fig. 3) the number of chromosomes was less ($2n = 30$ *vs* $2n = 34$) and the Y chromosome was a minute metacentric as compared to the medium acrocentric Y of *S. richardsonii* (Fig. 4). The X chromosome of the above two species was tentatively identified as one of the medium size submetacentrics.

Karyograms of *S. tereticaudus* (Fig. 5) contained only metacentric and submetacentric autosomes. The X chromosome was a medium size submetacentric which was not labeled due to lack of absolute pair identification. All male cells contained a medium size acrocentric Y chromosome similar to those in Fig. 1-4.

Discussion. Chromosome numbers in 5 of 6 subgenera of Spermophilus examined in the present and in a previous study(1) range between 30 and 42. Significant karyotype features in common are few or absent acrocentric autosomes and the presence of a medium size acrocentric Y chromosome in all species examined except *S. beldingi.*

Howell, on the basis of skull characters assigned *S. tridecemlineatus* and *S. mexicanus* to the same group within the subgenus Ictidomys(3). Karyogram analysis in this study supports Howell's conclusion and suggests the above animals are distinct but closely related species.

S. beldingi and *S. richardsonii* are both included in the same group of subgenus Spermophilus(3). However, this grouping is not supported by the present findings. Chromosome studies, used as a taxonomic character, show significant differences between the above species.

The minute metacentric Y chromosome of *S. beldingi* invalidates a previous suggestion (1) that the larger acrocentric Y of other species of Spermophilus and Ammospermophilus might be a specific characteristic of these genera. The genetic difference between the 2 types of Y chromosomes described above may not. however, be as great as the morphologic difference would indicate. In many animals the Y chromosome is composed primarily of heterochromatin which is said to be relatively inactive genetically(4). Therefore, both types of Y chromosome may contain the same amount of active genetic material and *S. beldingi* may have lost or translocated heterochromatin to alter the karyotype.

It is possible that differences in karyotypes between mammalian species are due to inver-

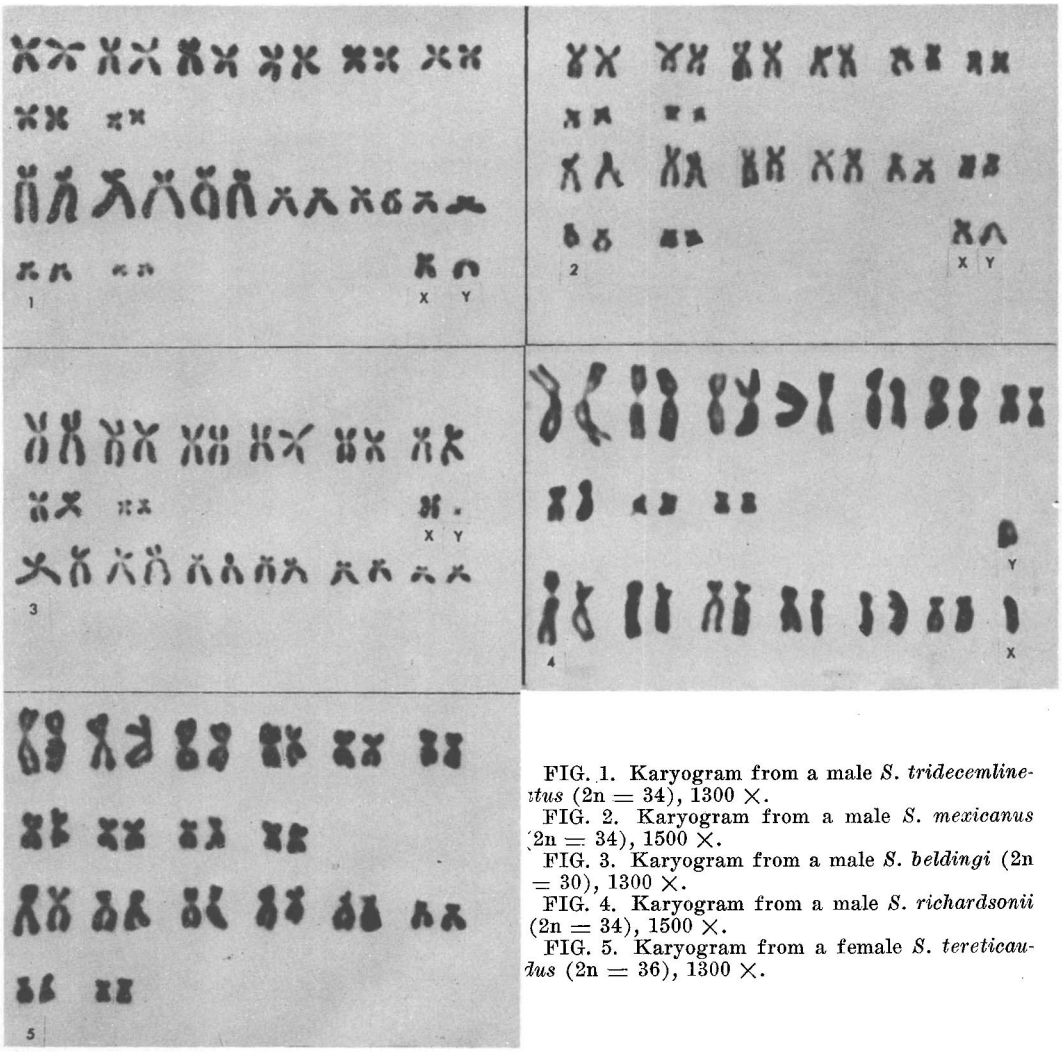

FIG. 1. Karyogram from a male *S. tridecemlineatus* (2n = 34), 1300 ×.
FIG. 2. Karyogram from a male *S. mexicanus* (2n = 34), 1500 ×.
FIG. 3. Karyogram from a male *S. beldingi* (2n = 30), 1300 ×.
FIG. 4. Karyogram from a male *S. richardsonii* (2n = 34), 1500 ×.
FIG. 5. Karyogram from a female *S. tereticaudus* (2n = 36), 1300 ×.

sions, fusions and translocations similar to those noted in Drosophila(5) and other insects(6). Identification of these mechanisms in mammals is difficult due to lack of suitable tissue such as the polytene chromosomes of Drosophila.

The present findings suggest that chromosome analysis is a valuable new character in comparative taxonomy of the genus Spermophilus. However, analysis of mitotic chromosomes alone does not provide definitive information regarding the mechanism of species formation.

Summary. Mitotic chromosomes of 3 species of Spermophilus have been analyzed. The diploid chromosome number was 30 in *S. beldingi beldingi,* 34 in *S. tridecemlineatus tridecemlineatus* and 36 in *S. tereticaudus neglectus.* Karyograms of *S. tridecemlineatus* and *S. beldingi* were compared with karyograms of *S. mexicanus* and *S. richardsonii.* Differences in karyotype were demonstrated and discussed in relation to the classification of the genus Spermophilus. Karyograms of *S. tereticaudus* were different from other species of Spermophilus which supported its subgeneric status. The minute metacentric Y chromosome observed in *S. beldingi* differs from the larger acrocentric Y of other species of Spermophilus. The utilization of chromosome analysis in comparative taxonomy of the genus Spermophilus was demonstrated.

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Effect of Diphenylhydantoin Administration upon Concentration of Liver and Aortic Lipids.* (27650)

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Intraperitoneal administration of large amounts of sodium bi-phenyl hydantoinate (Dilantin) to rats has been shown to alter profoundly the chemical composition of the connective tissue (dermis) of these animals (1). Recently(2) it was shown that Dilantin administration resulted in a significant decrease in dermal concentrations of both neutral glycerides and phospholipid. The present study was to explore whether similar effects upon lipid concentration would be observed in other tissues such as liver and particularly the aorta.

Methods. A group of 22 male Wistar rats weighing 300-360 g each were divided into 3 groups: one group of 8 animals was kept as normal controls; another group of 6 animals was subjected to 12 daily intraperitoneal injections of 1 ml of isotonic saline to serve as injected controls; and the remaining 8 animals received 12 daily intraperitoneal injections of 1 ml of saline suspension of 25 mg of Dilantin. Following an overnight fast, all animals were sacrificed by chloroform. These animals had been fed and watered *ad lib.* on Purina laboratory chow. Approximately 2.5 g of liver tissue was removed from each animal and lipids extracted with chloroform-methanol. The thoracic aortas of each rat were removed and cleaned of adherent tissues with the aid of a magnifying glass. These aortas were randomly pooled into 2 equal collections each representing half of the animals in each of the 3 experimental groups. These pools of aortic tissue were similarly extracted with chloroform-methanol and cholesterol, phospholipids and neutral glycerides determined. Methods for lipid extraction and chemical analyses have been described(2). The results were expressed in mg of lipid per g of fresh wet tissue.

Results and discussion. The effect of Dilantin administration and control injection upon mean and standard deviations of the lipid content of liver tissue are compared in Table I with the results obtained from normal intact animals. Although intraperitoneal injections of isotonic saline resulted in a significant increase in the neutral glyceride concentration of the liver tisue, Dilantin administration resulted in a significant decrease of this lipid in the liver in both normal and control animals.

Similar data for lipid concentration of the aorta from normal, control and Dilantin treated animals, presented in Table II, indicates that the amount of neutral glycerides in both pools of aortic tissue from the Dilantin treated animals differed by more than 3 standard deviations (3×2.5) from the mean concentrations of both pools of aorta obtained from normal and control groups of animals.

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