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Virus-Associated Tumours of the Harderian Gland in Laboratory-Reared Voles, *Clethrionomys rutilis* (Pallas)

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VIRUS-ASSOCIATED TUMOURS OF THE HARDERIAN GLAND IN LABORATORY-REARED VOLES, *CLETHRIONOMYS RUTILUS* (PALLAS)

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PLATES CXXXII–CXXXVI

To provide a dependable source of experimental animals better suited than the standard species for investigation of indigenous zoonoses in Alaska, colonies of arvicoline rodents, mainly of the genera *Clethrionomys*, *Dicrostonyx*, *Lemmus* and *Microtus*, have been maintained at the Arctic Health Research Center for more than 20 yr. Neoplasia of various types have been observed in nearly all species represented, but with the exception of gastric papilloma in *Microtus abbreviatus* Miller (Rausch and Rausch, 1968), most have occurred infrequently.

In 1967, we became aware of a disorder in the red-backed voles, *Clethrionomys rutilus* (Pallas), characterised by severe deformation of the zygomata and caused by neoplastic changes in the Harderian glands similar to those described by Maisin (1923) and Tucker and Baker (1967) in laboratory mice, *Mus musculus* Linnaeus. Clinical manifestations of the tumour became evident only in old animals, but study of sections of glands from red-backed voles taken at random from the colony disclosed a high rate of early stage changes. The presence of virus in the glands of the animals has been confirmed by means of electron microscopy. Findings in these voles are described in the present paper.

MATERIALS AND METHODS

The colony of red-backed voles was established in late 1957 from wild stock, *C. rutilus dawsoni* (Merriam), trapped near Anchorage, south-central Alaska. Also kept occasionally and in small numbers were specimens of a well differentiated subspecies, *C. rutilus albiventer* Hall and Gilmore, from St Lawrence Island, Bering Sea. Three subcolonies have been maintained: (*a*) the original colony of *C. r. dawsoni*; (*b*) intergrades of *C. r. dawsoni* and *C. r. albiventer*, now in the F_{12} generation; and (*c*) intergrades of the latter showing a mutation for partial " hairless ".

The rodents were kept in stainless steel cages, $41 \times 33 \times 12$ cm, with covers of stainless steel mesh, wood shavings as litter, and cotton for nesting. Food given red-backed voles consisted of carrot, commercially prepared diet for rats, alfalfa pellets, and sunflower seeds, with water *ad libitum*. Breeding pairs remained together until replaced.

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Rausch & Lindsay in Journal of Pathology (April 1974) 112(4).

Harderian glands and other tissues to be studied by light microscopy were fixed in 10 per cent. buffered formalin solution and embedded in paraffin. Sections, cut at 5 and 7 μ, were stained routinely in haematoxylin-eosin, Mallory's aniline blue-orange G, and by the periodic acid-Schiff reaction. Other stains were used for special purposes. For electron microscopy, the glands were removed immediately from voles killed by cervical dislocation. The tissue was cut into small fragments, fixed in 2 per cent. glutaraldehyde in sodium cacodylate buffer (*p*H 7·1), post-fixed in 1 per cent. osmium tetroxide, embedded in a mixture of Araldite and Epon (Mollenhauer, 1964), and cut on a Porter-Blum MT-2 ultramicrotome with a diamond knife. Thick sections (0·5–1·0 μ) were stained in azure II – methylene blue (Richardson *et al.*, 1960), thin sections in lead citrate and uranyl acetate. The latter were examined and photographed by means of a JEM 6-AS electron microscope. Glands used as controls were obtained from live-trapped red-backed voles.

Skulls of affected voles were cleaned by dermestid larvae.

Results

In the red-backed vole, as well as in members of the other arvicoline genera mentioned above, the Harderian gland is similar in form and relative size to that in the laboratory mouse (Merté, 1958). The gland in the red-backed vole is trilobed, filling the orbit around the periorbita and surrounding the optic nerve and associated structures, with a maximum length of about 6 mm and a weight of about 0·030–0·060 g. The ventral lobe is largest, extending posteriad along the zygoma nearly to the level of the jugal-squamosal junction, its posterior end superficial and covered only by a thin sheet of muscle. The dorsal and median lobes extend posteriad to the level of the posterior margin of the eye and to its anterior curvature, respectively. Inner, convex surfaces of the lobes are smooth; outer surfaces are concave, with numerous projecting lobules. Under low magnification (dissecting microscope), the tubules of the gland are discernible through the thin capsule of connective tissue. The main duct opens on a small papilla near the medial canthus just anterior to the vestigial nictitating membrane. The normal gland is white and lacks the superficial accumulations of pigment typically found in the laboratory mouse.

Histologic examination revealed a thin capsule of areolar connective tissue, extensions of which divided the lobes incompletely into lobules and were continuous with the intralobular connective tissue surrounding the secretory tubules. The branched tubules composing the gland had a simple columnar epithelium, with the nucleus, containing one or two nucleoli, at the base of each cell (fig. 1). With either the light or electron microscope, two types of epithelial cells were noted. In paraffin sections, the larger nuclei, less dense chromatin, more obvious nucleoli, and lightly staining, vacuolated cytoplasm distinguished one type from the second, in which nuclei were about one-third smaller and the relatively eosinophilic cytoplasm contained fewer vacuoles. In electron micrographs, the two types were differentiated by the arrangement and quantity of endoplasmic reticulum. The ducts were not readily distinguishable in the glands of red-backed voles, since their diameter and epithelium were similar to those of the secretory tubules. The tubules did not contain pigmented material such as is characteristically found in those of glands from laboratory mice.

Rausch & Lindsay in Journal of Pathology (April 1974) 112(4).

PATHOLOGIC FINDINGS

Enlargement of the Harderian gland usually was not evident clinically in voles less than a year old. However, irregularity in size of tubules and abnormalities such as formation of cysts were often discernible in glands from younger animals. The presence of a white, rather viscous exudate, a suspension of desquamated cells and cellular debris in which leucocytes were rarely seen, in the eyes of such animals appeared to be pathognomonic of pre-neoplastic changes. Virus-particles were seen in electron micrographs of desquamated cells within the lumina of tubules (fig. 2).

The first clinical sign of changes in the glands was a slight broadening of the head when both were affected, or an obvious asymmetry when the condition was unilateral. Thereafter, the glands usually enlarged rapidly, producing marked distortion of the head within about a month. Increasing intraorbital pressure caused the zygomata to be displaced laterad, often to an extreme degree (fig. 3).

Examination of the cleaned skulls from six voles with advanced disease (cf. fig. 3) revealed in the orbital walls extensive lesions produced by invasive growth of the tumour and by pressure. Localised osteoporosis and destruction of bone were characteristic. Perforation of the posterior wall of the orbit had exposed the brain in two specimens; in others, openings extended into the nasal cavity or through the orbital floor.

The tumours invaded natural cavities and foramina by extension. Most severely affected was the sphenorbital fissure, which in *Clethrionomys* is situated bilaterally at the middle of the orbital wall, just dorsal to the margin of the palatine process of the maxilla, and is relatively large, about 2·5 mm wide by 2 mm in height in lateral view. Through it pass the optic, abducent, trochlear and oculomotor nerves, the maxillary division of the trigeminal, and the four rectus muscles of the eye. In the six animals, this fissure was markedly enlarged. In one with a unilateral tumour, the orbital wall had been destroyed through formation of an opening about $4{\cdot}8 \times 4{\cdot}5$ mm, which communicated with the cranial cavity posteriorly and with the posterior nares medioventrally. Anteriorly, another perforation through the vertical portion of the frontal bone had exposed the turbinates, and extension of the tumour through the infra-orbital canal had caused erosion of alveolar bone at the proximal end of the right incisor. Destruction of bone of the palatine process of the maxilla had resulted in exposure of the roots of the first and second molars. Two other skulls exhibited similar destruction of alveolar bone, with exposure of the molar roots.

In another specimen, a layer of porous bone extended mediad across the orbit from the ventral margin of the maxillary portion of the zygoma, conforming to the rounded, ventral surface of the enlarged gland. Within the orbit, corresponding to the location of the eye, an irregular mass of porous bone projected dorsad above the orbital margins. Ventrally, the alveoli of the first and second molars were involved, with displacement of the second molar dorsad.

Rausch & Lindsay in Journal of Pathology (April 1974) 112(4).

In the living animals, the relatively rapid development of the tumours was reflected in the deformation of the zygomata. Although the eyes were sometimes displaced mediodorsad by the enlarging glands, exophthalmos usually was not produced. However, this condition was severe in two specimens of *C. r. albiventer*, in which the tumours slowly attained large size and in which the zygomata had not been affected. The sequence of changes in the eyes, often leading to blindness, has not been defined. In one case, an animal that died 68 days after the tumour became clinically evident, destruction of an eye

TABLE

Weights and condition of Harderian glands in red-backed voles

Age of vole	Origin	Weights of Harderian glands (g)		Diagnosis and remarks
<1 yr	Wild, Fairbanks	0·031	0·029	Normal
<1 yr	Wild, Fairbanks	0·040	0·043	Normal
<1 yr	Wild, Fairbanks	0·051	...	Normal
<1 yr	Wild, Fairbanks	0·059	0·057	Normal
<1 yr	Wild, Fairbanks	0·057	0·063	Normal
13·2 mth	Colony	0·040	0·040	Normal
13·2 mth	Colony	0·032	0·035	Premalignant
13·2 mth	Colony	0·023	0·028	Premalignant
13·2 mth	Colony	0·038	0·033	Premalignant
13·4 mth	Colony	0·085	0·092	Malignant
17·2 mth	Colony	0·280	0·119	Premalignant
20·5 mth	Colony	0·024	0·024	Probably malignant Also had subcutaneous mixed basal cell-squamous cell carcinoma with metastases in liver
Captive 21 mth	Wild, St Lawrence Island	0·131	0·124	Malignant
Captive 25·8 mth	Wild, St Lawrence Island	0·477	0·328	Malignant

with necrosis of the adjacent glandular tissue had occurred. The range in size of Harderian glands in normal and diseased voles is indicated by weights given in the table.

Microscopically, the first evidence of changes in the gland was an apparent focal hyalinisation of the tubular epithelium. Although cells of the two types described were affected, changes were more prominent in the eosinophilic cells, in which the cytoplasm exhibited progressively fewer secretion vacuoles, with irregularity of size in those remaining. The diameter of the tubules was not significantly greater, but that of their lumina increased, apparently as a result of degeneration and sloughing of the apical cytoplasm, the tubules becoming lined by low cuboidal rather than columnar epithelium. In early stages also, the nuclei became enlarged, and marginal accumulation of chromatin rendered the multiple nucleoli more obvious. Electron micrographs showed entire, relatively intact cells as well as cellular debris in the lumina.

Adenoma. With hyalinisation, shrinkage of the epithelium, and increased diameter of the tubules, the glands acquired a somewhat cystic appearance.

Rausch & Lindsay in Journal of Pathology (April 1974) 112(4).

The apical degeneration and desquamation of cells perhaps led to occlusion of the ducts, with consequent accumulation of fluids, distension of tubules, and pressure atrophy. However, few of the enlarged glands showed indications of such a process; rather, formation of cysts appeared to be a result of the tubules' becoming confluent (fig. 4). In the adenomas, the hyalinised cuboidal epithelium assumed a columnar or stratified form, with a concomitant two- to three-fold increase in the diameter of the tubules. The large, cystic cavities frequently contained projecting fronds of epithelium (fig. 5). We consider the papillary cystadenoma to be the last stage of adenoma, or possibly the earliest stage of adenocarcinoma. In every instance, the adenomas exhibited varying degrees of anaplasia, with considerable cytologic similarity to adenocarcinoma; histologically, none could be diagnosed as a well differentiated, benign adenoma. For these reasons, they were regarded as being premalignant lesions.

Adenocarcinoma. Three variants could usually be distinguished in the structure of tumours of longer standing. The first type was characterised by cystic areas, the irregular cavities of which were filled with haemorrhagic debris or appeared empty. Such spaces were lined with either columnar or stratified epithelium, usually with projecting fronds (fig. 6). Little of the connective tissue of the stroma was discernible, although in some areas fibrosis imparted a scirrhous character to the tumour. Focal necrosis, often with leucocytic infiltration, was sometimes observed. Closely packed cells with smaller, deeply staining nuclei and scanty cytoplasm were typical of the second form (fig. 7). Little trace of the tubular structure of the gland remained. Oncogenesis evidently involved exuberant proliferation of epithelial fronds that fused and obliterated the cystic cavities. Traces of the original fronds extending from the supporting connective tissue could occasionally be discerned. The third type was characterised by branching and anastomosing plates of cells in progressive disarray (fig. 8), within which hypertrophic tubules lacking lumina could sometimes be distinguished. The cells had larger, lightly staining nuclei and abundant cytoplasm. Tumour giant cells and numerous mitotic figures were present in such solid or consolidating areas.

The malignant character of these tumours was also apparent from their local invasiveness and the occurrence of metastases in other organs. The persistence of areas of hyalinised epithelium in premalignant adenomas, the characteristic anaplasia of the adenomas, and the cytologic similarities between cells of the premalignant adenomas and the adenocarcinomas all indicate that the changes described represented successive stages in the oncogenic process.

Metastases. Secondary foci of the tumour of the Harderian gland were identified in the lungs of two animals (fig. 9); however, only those lungs with macroscopic lesions were preserved. Tumour cells in sections of such well differentiated metastases showed a tendency to organise and form tubules, but no secretory vacuoles could be identified. Poorly differentiated tumours of uncertain origin were found in other organs of four voles that also had neoplastic changes in the Harderian glands. In several animals with clinically normal glands, tumours in other organs included hepatoma, basal cell carcinoma, melanoma, leiomyosarcoma and seminoma.

Rausch & Lindsay in Journal of Pathology (April 1974) 112(4).

Virus. Viral particles were identified in electron micrographs of Harderian glands of all six laboratory-reared voles from which sections were prepared. Five animals, 3–8 mth old, were clinically normal; in the sixth, 18 mth old, both glands were enlarged. The particles, spherical and about 32 mμ in diameter, formed small, intracytoplasmic aggregations, with no visible evidence of a surrounding membrane (fig. 10), in both types of epithelial cells, but not in those of connective tissue. None was found in the glands of two wild voles from which electron micrographs were prepared. Studies to characterise the virus are in progress.

Discussion

The number of animals in the colony of *C. rutilus dawsoni* usually has not exceeded 100, and the two subcolonies of intergrades have been maintained at about 50 each. For maximum productivity, breeding pairs reaching an age of 10–12 mth have been replaced by younger animals. A few have been kept longer for special purposes. Tumours of the Harderian gland in the voles do not become clinically evident until the animals are about a year old, the rate thereafter increasing with age. Our practice of removing older individuals from the colony has thus prevented an assessment of the ultimate rate of occurrence of the tumour. At present, the colony of *C. r. dawsoni* consists of 132 animals, with the following composition by age: 1–6 mth, 69; 7–10 mth, 37; 10·5–11·5 mth, 20; 12 or 13 mth, 6. Two, 11·5 and 12-mth-old, have well developed tumours, and the white exudate indicative of preclinical changes in the gland is present in the eyes of a third, age 13 mth. Three of 55 animals in the colony of partially hairless intergrades have clinically obvious tumours.

We have not noted macroscopic evidence of tumours of the Harderian gland in 871 wild red-backed voles, including 63 from St Lawrence Island, that we have captured in snap-traps since Oct. 1950, in Alaska. Before 1957, because early attempts to establish a colony were unsuccessful, 165 red-backed voles were live-trapped and maintained until required for experimental purposes. No clinical sign of the tumour was noted in these. The characteristic exudate was observed in the eyes of laboratory-reared animals soon after the colony was established. Advanced tumours of the gland were found in 12 of 495 voles for which records are complete to Dec. 1971. Several cases have been noted subsequently in animals kept for observation.

The spontaneous occurrence of tumours in the Harderian gland of old laboratory mice, *M. musculus*, has been reported by Maisin (1923) and by Tucker and Baker (1967). In both instances, as in red-backed voles, the tumours described ranged in type from papillary cystadenoma to adenocarcinoma. The condition diagnosed by Maisin as sarcoma appears to have been similar histologically to the poorly differentiated, fibrosed adenocarcinoma found in some of our animals. The tumours studied by Tucker and Baker were slow-growing, poorly differentiated, and regarded as being highly malignant. Exophthalmos was the diagnostic sign, and well differentiated pulmonary metastases were characteristic. Deformation of the zygomata was not reported.

Rausch & Lindsay in Journal of Pathology (April 1974) 112(4).

HARDERIAN GLAND TUMOURS

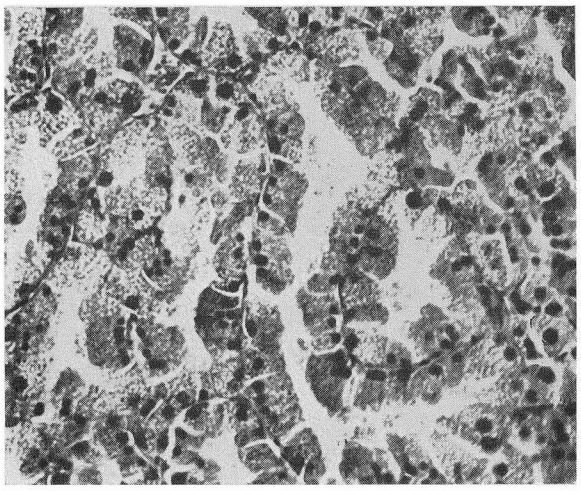

FIG. 1.—Normal structure of Harderian gland from a wild specimen of *Clethrionomys rutilus*. Haematoxylin and eosin (HE). ×225.

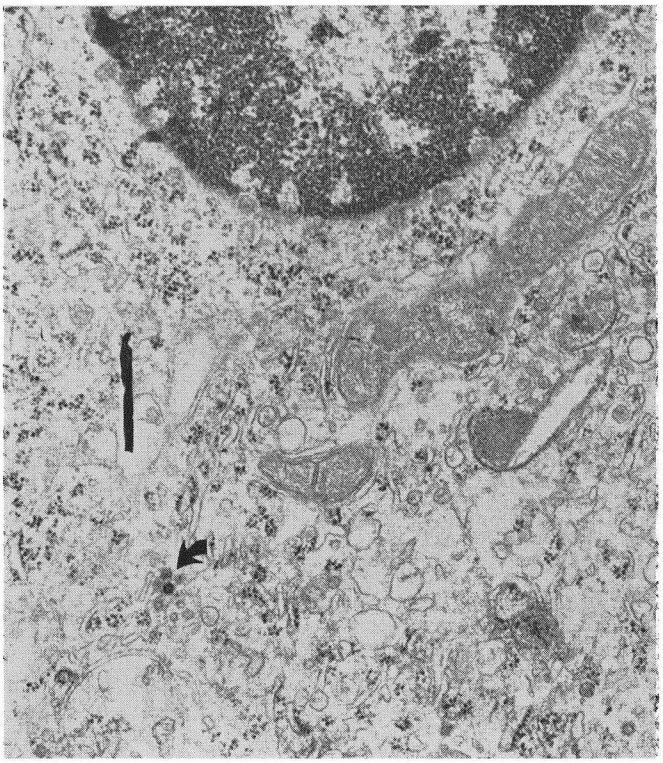

FIG. 2.—Viral particles (arrow) in a desquamated epithelial cell within a tubular lumen of the Harderian gland of a clinically normal red-backed vole. Electron micrograph (EM). ×14,900.

Rausch & Lindsay in Journal of Pathology (April 1974) 112(4).

HARDERIAN GLAND TUMOURS

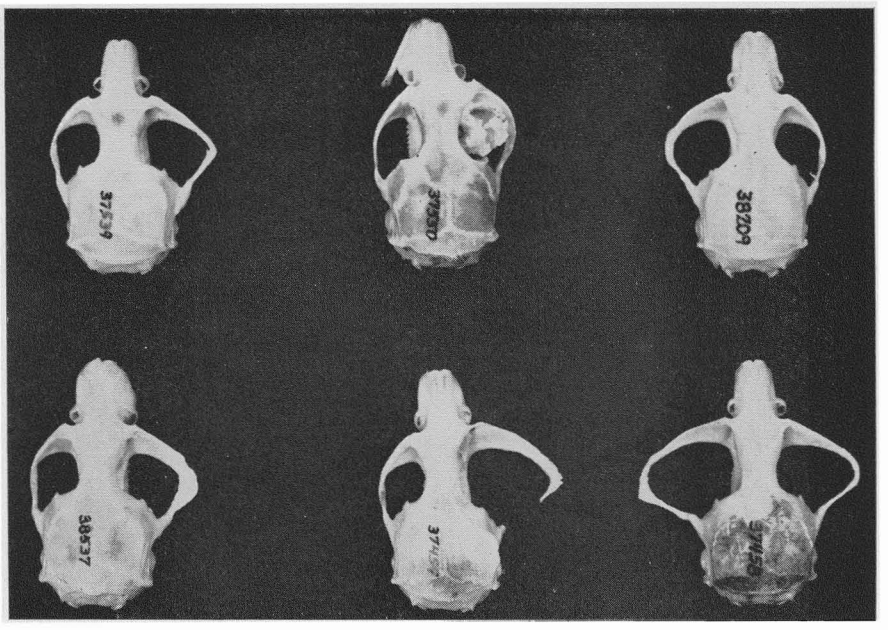

FIG. 3.—Skulls of laboratory-reared red-backed voles showing alterations caused by tumours of the Harderian gland. Ages at death were, left to right from top, 17·2, 19·6, 19·6, 22·6, 22·6 and 30 mth. ×1·8.

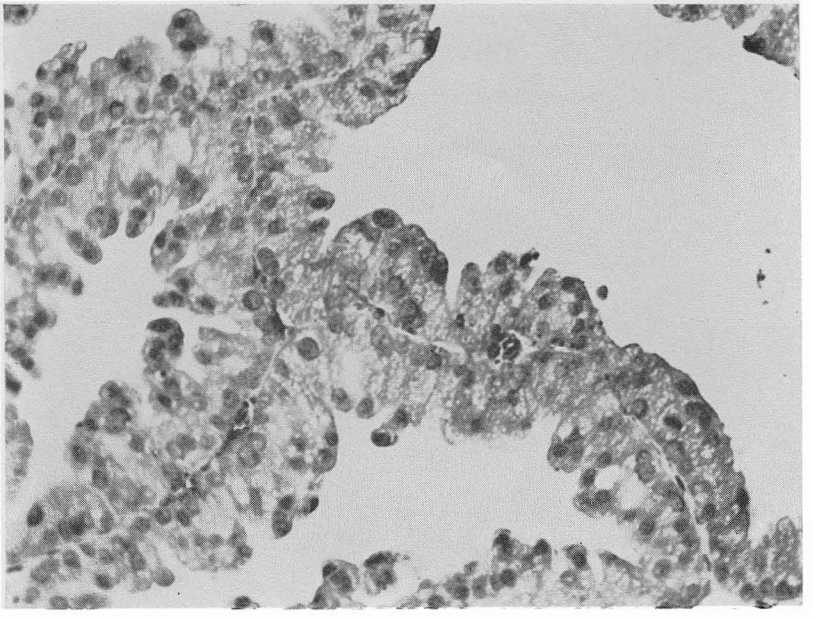

FIG. 4.—Portion of an adenoma showing early formation of cysts. HE. ×225.

Rausch & Lindsay in Journal of Pathology (April 1974) 112(4).

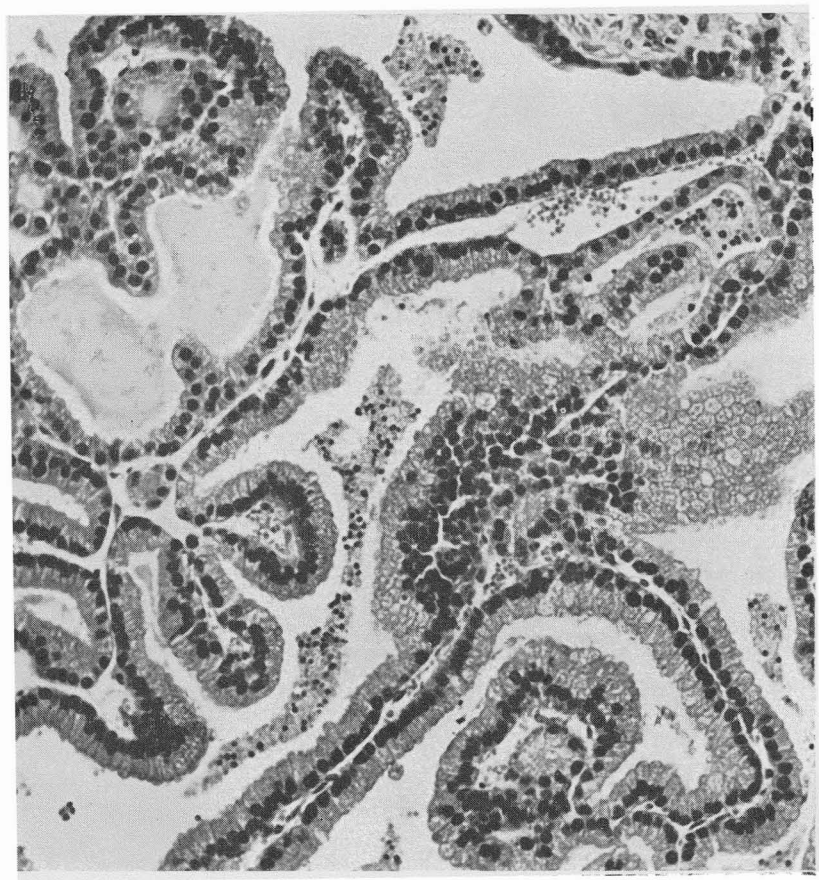

FIG. 5.—Portion of an epithelial frond projecting into a large cyst. HE. ×225.

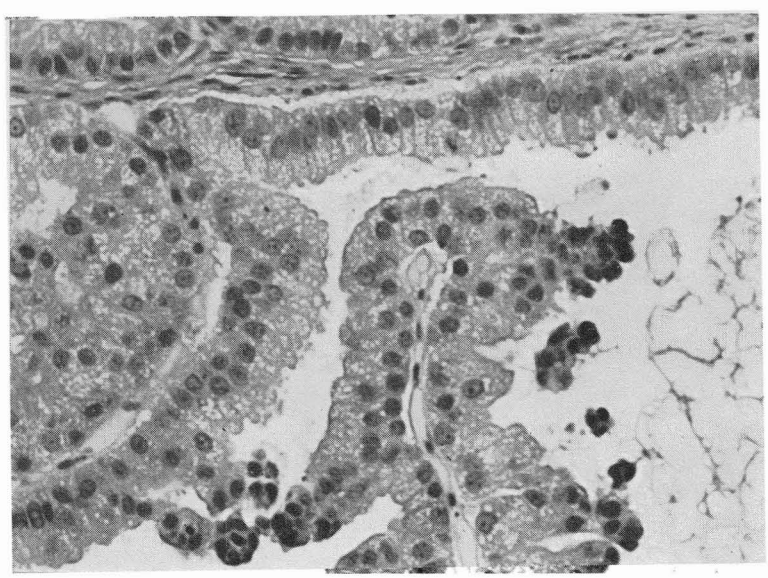

FIG. 6.—Section of an adenocarcinoma showing simple and stratified epithelium, with debris in the cyst-cavity. HE. ×225

Rausch & Lindsay in Journal of Pathology (April 1974) 112(4).

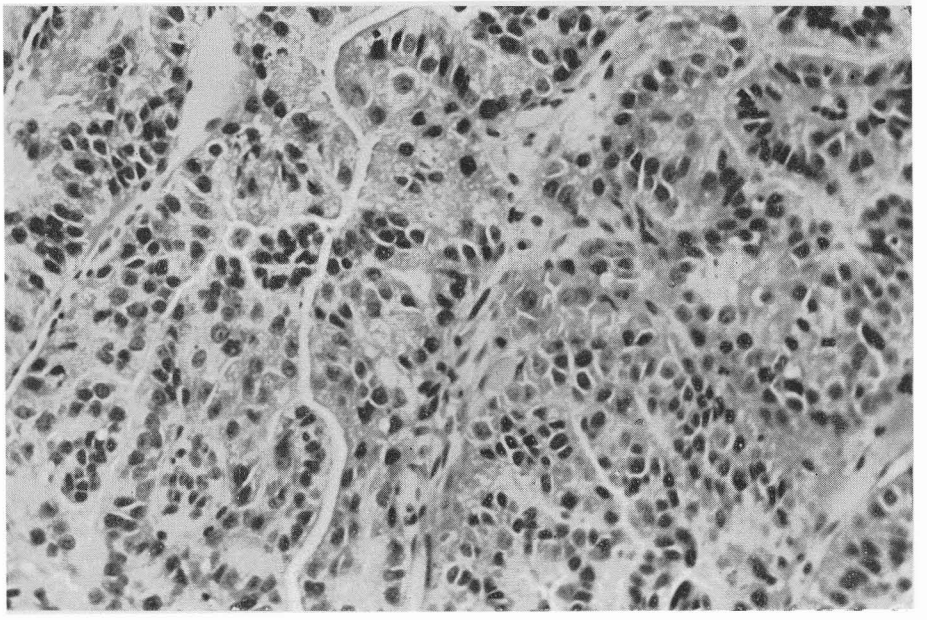

FIG. 7.—Section of an adenocarcinoma showing closely packed cells, with loss of tubular structure of the gland (cf. fig. 8). HE. ×225.

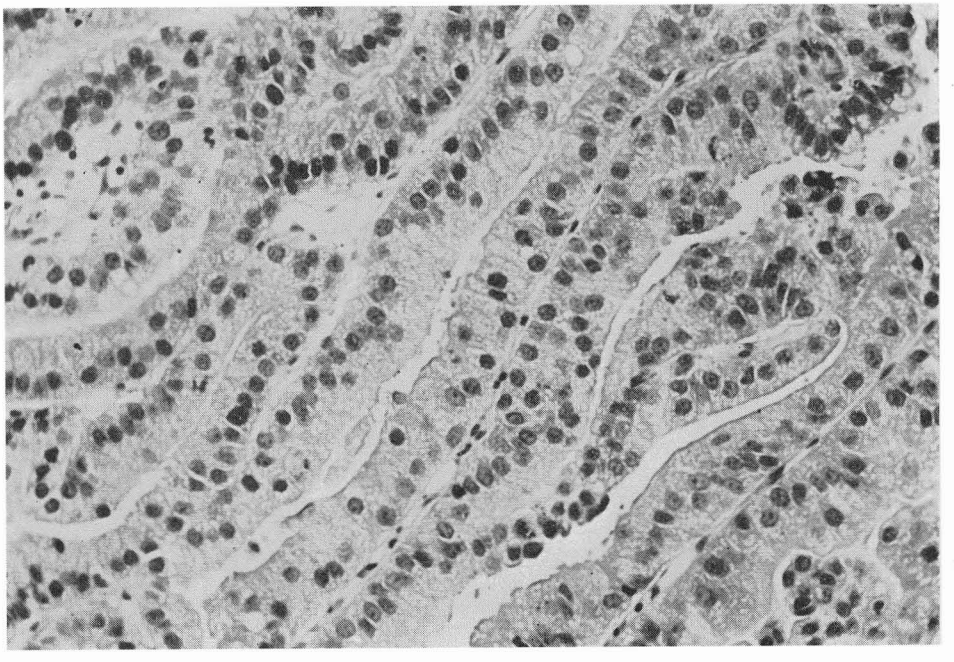

FIG. 8.—Section of an adenocarcinoma showing anastomosing plates of cells. HE. ×225.

Rausch & Lindsay in Journal of Pathology (April 1974) 112(4).

HARDERIAN GLAND TUMOURS

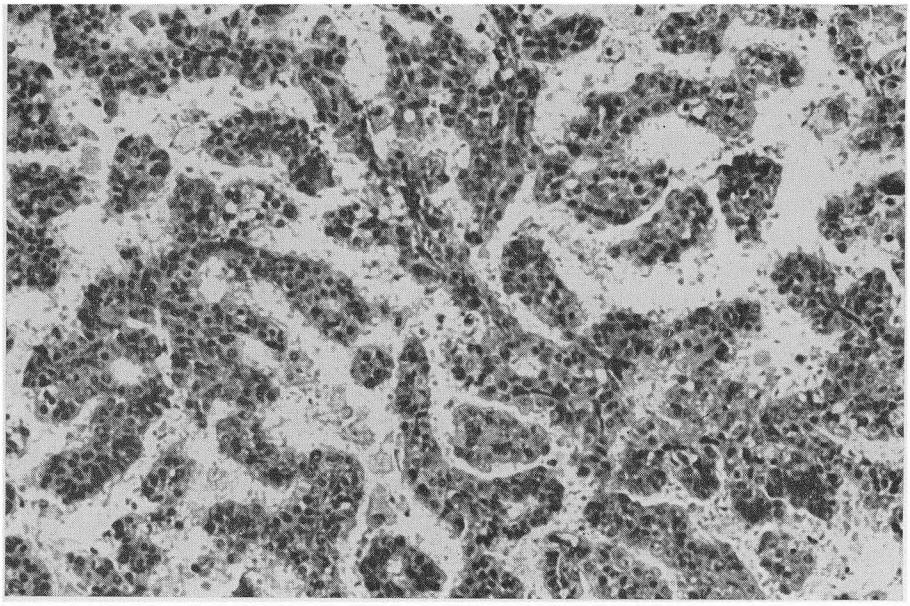

FIG. 9.—Section showing the structure of one of several metastatic nodules in the lung of a vole. HE. ×225.

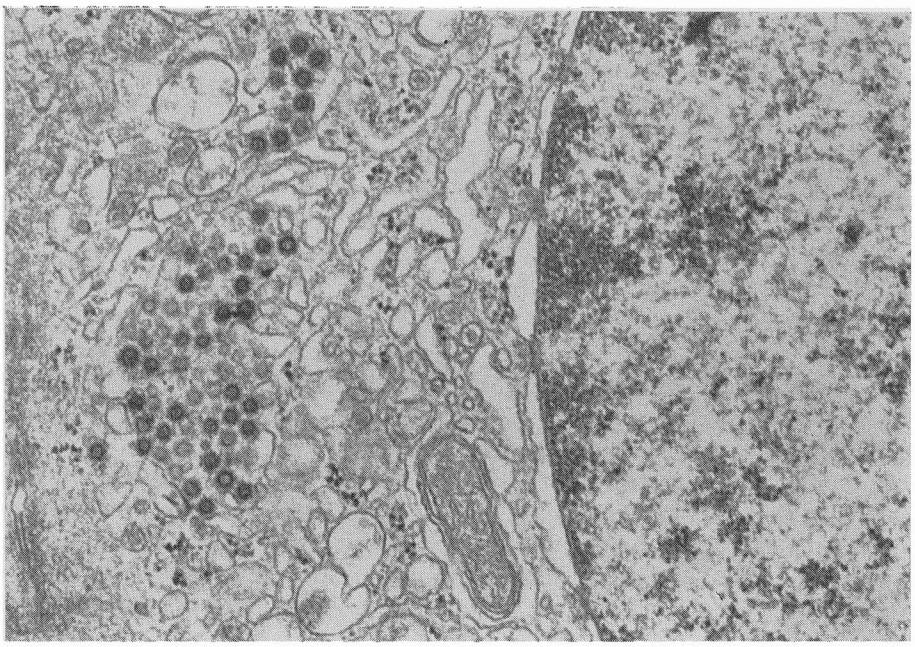

FIG. 10.—Viral particles in cytoplasm of one type of epithelial cell in the Harderian gland of a clinically normal red-backed vole. EM. ×38,200.

Rausch & Lindsay in Journal of Pathology (April 1974) 112(4).

Furth *et al.* (1954) observed adenocarcinoma of the Harderian gland in mice 15–17 mth after their exposure to ionising radiation. The tumours were locally invasive, producing changes in the surrounding soft tissue and in the skull similar to those we observed in red-backed voles.

Viruses evidently have not been previously demonstrated in tumours of the Harderian gland in rodents. The apparent prevalence of viral particles and the high rate of subclinical, pre-neoplastic changes in the glands of our captive voles suggest that the virus has an aetiologic role. Under laboratory conditions, virus present in the secretion of the Harderian glands probably would be transmitted to young animals before they are separated from their parents. Findings in two specimens of *C. rutilus albiventer*, trapped on St Lawrence Island in Mar. and Aug. 1969, and kept for 21 and 26 mth, respectively, suggest that their tumours were caused by a virus acquired in captivity. Both animals, killed when moribund in late May 1971, had large adenocarcinomas bilaterally, with marked exophthalmos. *C. r. albiventer*, formerly considered to be a distinct species, differs cytogenetically from *C. r. dawsoni* in the morphological characteristics of certain chromosomes (V. R. Rausch, unpublished). Tumours enlarged rapidly in intergrades of the two forms.

The numbers of animals in our colonies of *Dicrostonyx*, *Lemmus* and *Microtus*, established during 1951–56 and maintained continually, have considerably exceeded those of the red-backed voles. Before 1968, colonies of *Lemmus* and *Dicrostonyx* were kept in a separate room adjacent to that in which voles (*Clethrionomys* and *Microtus*) were maintained. Subsequently, all species have been kept together, segregated only to the extent that the different colonies occupy different sections of the cage-racks, and the opportunity for transmission of the virus to rodents of other species would seem now to exist. With a single exception, an F_1 hybrid *Dicrostonyx*, abnormalities of the Harderian gland have not been observed in individuals from the other colonies. In the hybrid lemming, the glands were enlarged bilaterally, and in sections we observed changes compatible with the diagnosis of well differentiated adenoma. The virus thus appears to exhibit a high degree of host-specificity.

Summary

Tumours of the Harderian gland occurred frequently in red-backed voles, *Clethrionomys rutilus* (Pallas), in a captive colony maintained since 1957. Although the tumours became obvious only in animals about a year old and older, early stage changes were histologically evident in glands from younger, clinically normal voles. The tumours ranged in type from poorly differentiated adenoma to adenocarcinoma, with invasion of adjacent tissues and characteristic deformation of the zygomata. Electron micrographs disclosed viral particles in the cytoplasm of glands from clinically normal animals as well as in glands showing malignant changes. The tumour was not observed in arvicoline rodents of other species maintained in the same rooms with the red-backed voles.

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