INTRANUCLEAR INCLUSIONS IN THE SALIVARY GLANDS OF MOLES *

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Intranuclear inclusions have been described in the salivary glands of guinea pigs, rats and humans, but only in guinea pigs has it been proved that they are caused by a filterable virus (Cole and Kuttner [1]). We have noted [2] their presence in a fourth species, the common mole. This observation was made in the course of a brief survey, suggested by Dr. E. V. Cowdry, of the distribution of intranuclear inclusions in wild animals in the absence of distinctive clinical symptoms. In this paper we shall describe these inclusions in moles, mention our attempts to transmit a virus and compare the properties of the inclusions in the salivary glands of all four species.

MATERIAL

We list the other animals we examined, as well as the moles, because negative observations may be of some interest to investigators making more complete surveys. They were:

1	Evans king snake (*Lampropeltis calligaster*)
1	Red headed skink (*Eumeces quinquelinaetus*)
1	Electric eel (*Electrophorus electricus*)
1	Bull frog (*Rana catesboana*)
2	Rats (*Mus decumanus*)
2	Mice (*Mus musculus*)
1	Gopher (*Spermophilus franklini*)
14	Moles (*Scalops aquaticus*)
1	Bat (*Myotis subulatus*)
1	Flying squirrel (*Glaucomys volans*)
4	Opossums (*Didelphis virginiana*)
11	Monkeys (*Pithecus rhesus*)
1	Gray cheeked mangabey (*Cercocebus albigena*)
1	Tapir (*Tapirus Indicus*)

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Careful autopsies were made of each animal and specimens were taken from a wide variety of tissues. Zenker and Zenker-formalin fixatives were used in all cases and the sections were colored with hematoxylin and eosin and by the Giemsa stain. Special microchemical tests were applied to the inclusions in the moles and attempts to transmit a virus were made which will be described later.

Observations

Figures 1–6 illustrate the appearance of the intranuclear inclusions in the salivary glands of moles. They were found in all of the 14 moles examined. Careful search in other tissues failed to reveal any. The moles weighed about 150 gm. and were presumably adults. The series did not contain any very young animals. The sections were 5 microns thick. On the average 1.8 inclusions were found per sq. mm. of section. The maximum number was 9 per sq. mm., while in 2 animals the inclusions were very rare, being seen only after intensive study of many serial sections.

The altered cells are usually situated in the terminal tubules of the serous portions of the glands and more rarely in the mucous parts (Fig. 5). None occur in cells that can be definitely classified as duct cells but they are rather more numerous near the ducts. Many appear to be shoved off into the lumens of the tubules (Fig. 6).

An accompanying tissue reaction was noted in 8 animals. It consisted of a slight perivascular and interstitial lymphocytic infiltration. But the extent of the infiltration was not proportional to the number of affected secretory cells. Neither did the location always correspond. In some cases many cells possessing inclusions were observed in the absence of infiltration. Two nematodes were noted. The first, belonging to the genus *Capillaria*, was found in the epithelium of the tongue of 6 moles; and the second, apparently a member of the genus *Porrocaecum*, was observed in the salivary glands of 7 moles. For the identification of both of these we are indebted to Dr. E. W. Price of the Bureau of Animal Industry. There was no sharp correlation between the incidence of the parasites and the inclusions, but it was noted that in several sections the number of inclusions was

definitely higher near the *Porrocaeca*. The parasites were usually well walled off by connective tissue and were frequently accompanied by a mild degree of cellular infiltration, consisting chiefly of lymphocytes. The tissue reaction seemed to bear no constant relation to either the inclusions or parasites. In one mole there was, as might be expected, a heavy accumulation of eosinophile leukocytes near a *Porrocaecum*.

An outstanding feature of the inclusion-containing cells and nuclei was marked hypertrophy. The diameter of the altered cells averaged about 15 microns — approximately 1½ times the diameter of the normal cells. The extent of enlargement for both serous and mucous cells was remarkably uniform, as indicated in Table 1.

TABLE I

Measurements in Microns of Inclusion-containing Cells in the Salivary Glands of Moles

Cells	Diameter measured	Range	Mean	Median	Mean deviation on mean	Standard deviation
200 inclusion-laden cells in the mixed portion of the gland	Cellular	10.00	15.37	15.06	1.57	2.13
	Nuclear	9.34	10.14	10.67	1.56	1.83
	Inclusion	6.88	6.12	5.69	1.01	1.29
25 inclusion-laden cells in the mucous portion	Cellular	8.13	16.85	16.77	2.03	2.34
	Nuclear	6.25	12.05	12.03	1.75	2.13
	Inclusion	2.50	7.05	6.36	0.81	0.98

The mean is the mathematical average of the measurements obtained by dividing the sum of the diameters by the number. The median is the middle number in the series of numbers arranged in the order of magnitude. The fact that the mean and median are so nearly the same indicates a central tendency. However, one can easily imagine that a distribution in which there is an equal clumping at each end of the range might easily give a median and a mean of almost equal value. To minimize this possibility of error the "mean deviation on the mean" and the "standard deviation" were calculated. In the "mean deviation on the mean" we have a figure showing the average distance of all members of the series from the mean, or average, of the series. This is remarkably small and gives a truer indication of a central tendency. The "standard deviation" is

a measurement which, when marked off on both sides of the mean, will include two-thirds of the cases involved, when the cases have been arranged in the order of magnitude. Interpretation of this central tendency is difficult. It may be that the cellular response, whatever its nature, has reached a kind of end point characterized by relative uniformity in degree of hypertrophy. It is possible also that the early stages of hypertrophy, so rare in our specimens, seem to be lacking because they may be passed through quite rapidly; and that final stages of further hypertrophy or disintegration are absent, because the cells may be cast out through the ducts at this particular stage. Moreover, the infrequency of early stages may indicate that the process is no longer an active one.

The intranuclear inclusions themselves show a parallel uniformity in size. The range from the largest to the smallest was slight. Occasionally they seemed to be paired, or subdivided (Figs. 2 and 4). Their outlines were somewhat irregular. The centers were denser than the periphery. They were definitely basophilic, only rarely showing any tendency towards acidophilia — a property that we do not think can be attributed to unusual fixation or some deviation from standard technique.

The intranuclear inclusions exhibit marked Feulgen and masked iron reactions. Mucicarmine and Millon's reagent gave inconclusive results. Margination of chromatin on the nuclear membrane was not very noticeable. This may be correlated with the retention by the inclusion of basophilic properties (thymonucleic acid and masked iron). The nucleolus was recognizable in some cases either applied to the inclusion or between the latter and the nuclear membrane. A clear thin halo of chromophobic nuclear material is invariably interposed between the inclusion and the nuclear membrane and is very typical.

In the cytoplasm of most cells 10 to 30 small, basophilic inclusions were clearly visible, closely resembling those described in detail in the guinea pig's submaxillary glands by Pearson.[3] They accompanied approximately 40 per cent of the nuclear inclusions and occurred in both serous and mucous types (Figs. 1–5).

Many attempts were made to demonstrate the existence of an active virus in the salivary glands, but saline emulsions injected intracerebrally, subcutaneously and intraglandularly into rats, mice, rabbits and young guinea pigs proved futile (Table II). The animals

that died in a few hours did so as a direct result of injury to the brain when the injection was made and not from the action of a virus. Those that survived until they were sacrificed showed no signs of dis-

TABLE II

Attempts to Pass Virus

Mole No.	Inoculum (saline emulsion)	Recipient animal	Injection	Result
4	Fresh tissue	Rabbit	Intracerebral	Died — 6 hours
4	" "	Young guinea pig	Subcutaneous	Killed — 30 days
5	" "	Rabbit	Intracerebral	Died — 6 hours
5	" "	"	"	Died — 12 hours
5	" "	"	"	Killed — 14 days
5	" "	Young guinea pig	"	Died — 12 hours
4, 5, 6	Glycerin preserved tissue	White mouse	"	Killed — 35 days
4, 5, 6	" " "	" "	"	Died — 3 days
4, 5, 6	" " "	" "	"	Killed — 38 days
4, 5, 6	" " "	White rat	"	39 days
4, 5, 6	" " "	" "	"	39 days
4, 5, 6	" " "	" "	"	39 days
4, 5, 6	" " "	Young guinea pig	"	38 days
4, 5, 6	" " "	" " "	"	38 days
4, 5, 6	" " "	" " "	Submaxillary gland	20 days
12	Fresh tissue	" " "	Intracerebral	Died — 24 hours
12	" "	" " "	"	Died — 12 hours
12	" "	" " "	"	Killed — 62 days

ease. Histological examination of the tissues at the site of injection revealed no significant alterations.

A detailed comparison of these intranuclear inclusions in moles with our own preparations of inclusions in guinea pigs, with speci-

mens showing inclusions in human salivary glands and in rats sent to Dr. E. V. Cowdry by Dr. S. B. Wolbach and by Dr. Juanita Thompson respectively, together with a close study of the literature, brings to light a number of interesting features.

1. The inclusion incidence of 100 per cent of 14 in moles is to be compared with 12 per cent of 183 in humans (Farber and Wolbach [4]), 84 per cent of 75 in guinea pigs (Cole and Kuttner[1]), and 14 per cent of 70 in rats (Thompson [5]).

2. It is not feasible to compare the number of inclusion-containing cells per unit volume of tissue because the observations on species other than the mole are not quantitative. Probably they are equally numerous in the mole, as in the others.

3. The location of the inclusions in moles is in the serous and mucous gland cells and not in the duct cells, whereas in the others the duct cells are often more frequently affected than the gland cells.

4. In the mole, guinea pig and rat the occurrence of inclusions is apparently limited to the salivary glands, but in humans intranuclear inclusions have been found in other tissues of the same individuals (see Farber and Wolbach [4] and their review of literature).

5. The size of the hypertrophied cells is greatest in humans, about the same in guinea pigs and rats and least in the moles.

6. The morphology of the inclusions is similar in all four. Their outlines are irregular, often their central parts are denser. From the periphery strands of material may extend toward the nuclear membrane. The inclusions are not evenly rounded, spherical droplets of material, nor are they hyaline in appearance like Cowdry's type B inclusions.[6]

7. The inclusions are apparently more basophilic in the moles than in any of the others. They also yield a more marked Feulgen reaction for thymonucleic acid and Bensley-Macallum test for masked iron than Cowdry [7] secured with the guinea pig inclusions. Data on thymonucleic acid and masked iron are not available for the human and rat inclusions.

8. Margination of basophilic chromatin on the nuclear membrane is absent or slight in moles — a circumstance that may be related to the retention of basophilia by the inclusions. This margination is very conspicuous in guinea pigs and comparatively slight in humans and rats.

9. The nucleoli behave in the same way in all four species in so far that they do not contribute noticeably toward the formation of the inclusions.

10. A clear, unstaining halo between the inclusion and the nuclear membrane is most noticeable in the guinea pig, less so in the mole, and least, but to about the same degree, in humans and rats.

11. Similar basophilic bodies occur in the cytoplasm of the inclusion-laden cells in all four species. In the mole, however, they are found on all sides of the nucleus and apparently do not show quite the same tendency to be clumped in the distal cytoplasm, between the nucleus and lumen, as in rats, guinea pigs and humans.

12. No constant accompanying reaction or degeneration of the tissue about the affected cells is found in any of the forms, but, in the case of all of them, mention is made of occasional infiltration by lymphocytes and phagocytic cells.

13. No parasites like those herein reported in the oral epithelium and salivary glands of moles, or of any sort, have been described in humans, guinea pigs or rats.

14. Detection of the inclusions in these four species has been to some extent a matter of chance. In none of them is attention drawn to the inclusions by the exhibition of definite clinical symptoms.

Discussion

The intranuclear inclusions in the salivary glands of moles are sufficiently like those in humans, guinea pigs and rats to suggest a more or less common origin. Only in the guinea pig has a virus been identified as the etiological factor, but the opportunity to demonstrate virus action in the others has not been favorable. It is likely that systematic study of the salivary glands in many species will bring to light further instances of the occurrence of similar inclusions in the absence of distinctive signs of disease. The fact that the intranuclear inclusions in this location are so very large does not of itself indicate that the causative agents if, as in guinea pigs, they turn out to be viruses, are peculiar or very different from those that influence other tissues. When the submaxillary virus of guinea pigs is led to act on the brain it produces inclusions that are not particularly large in cells that are not hypertrophied. Consequently it may be supposed that at least two factors are involved — the nature of the virus and the reactivity of the particular cells.

SUMMARY

All of 14 moles examined show, in the absence of clinical symptoms, intranuclear inclusions in their salivary glands which resemble in many respects those previously reported in humans, guinea pigs and rats.

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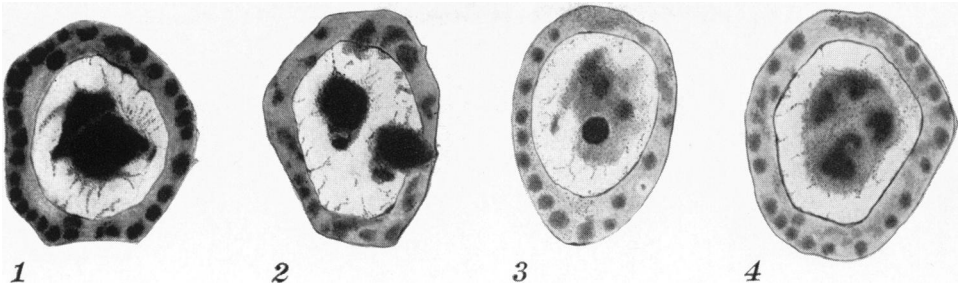
DESCRIPTION OF PLATE

PLATE 144

FIGS. 1 to 4 inclusive. Drawings of affected cells to show detailed structure of intranuclear inclusions and position of cytoplasmic inclusions. Note the paired inclusion in Fig. 2 and the presence of the nucleolus in Fig. 3. × 2300.

FIG. 5. Drawing of mucous acinus to show relative size and position of the affected cell. Note the encroachment upon the lumen. × 2300.

FIG. 6. Drawing of mucous and serous acini showing involved cells in which cytoplasmic inclusions are absent. × 1300.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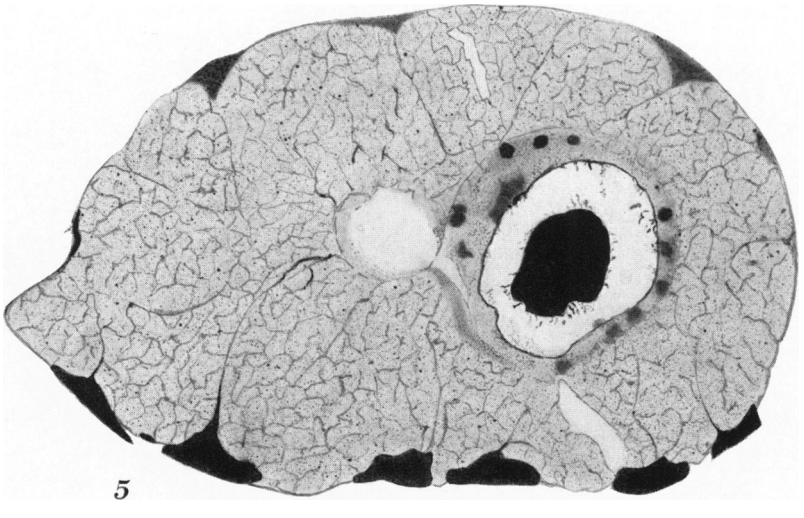
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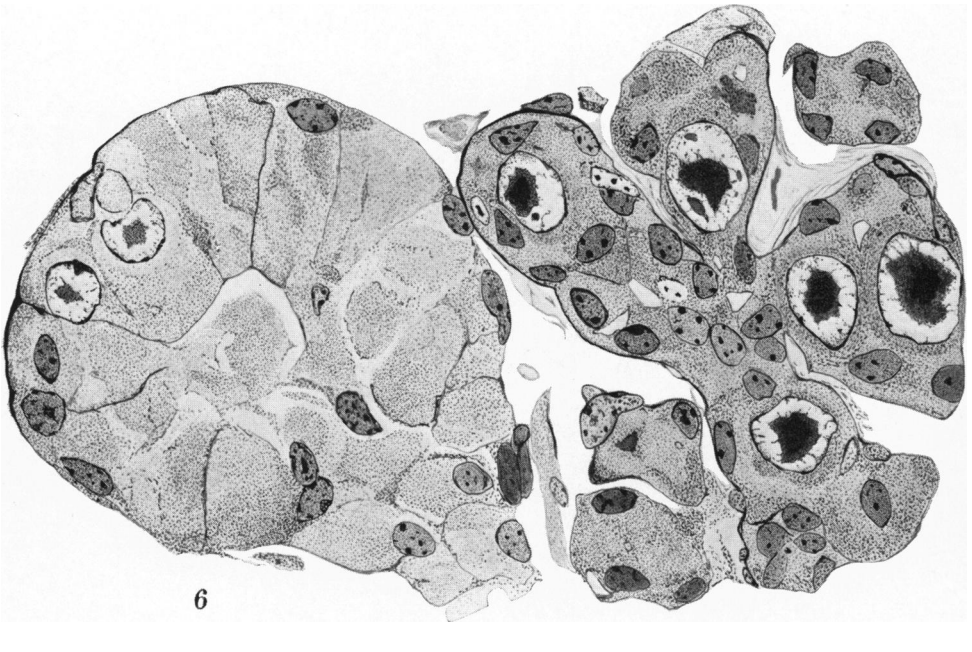
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