AN INFECTIOUS CUTANEOUS FIBROMA OF THE VIRGINIA WHITE-TAILED DEER (ODOCOILEUS VIRGINIANUS)*

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In September of 1952 an adult female Virginia white-tailed deer (*Odocoileus virginianus*) was brought to the laboratory for examination. She had been shot by a game warden in Somerset County, New Jersey, because she had been observed to have numerous masses scattered over her body, as shown in Fig. 1. These were especially plentiful over her head and neck and those about her eyes and over her eyelids were so large as to effectively deprive her of vision. Examination of this animal revealed that the masses were rounded tumor-like growths of the skin ranging from pea to grapefruit in size. They were freely movable over the underlying structures and the larger ones were pendulous and pedunculated. They were very firm and their cut surfaces were white, hard, and almost cartilaginous in consistency. Some of the growths had a deeply pigmented rind of what appeared to be thickened epithelium. Nothing of significance was evident on examination of the viscera at autopsy and especially were no lesions suggestive of metastases found.

Since encountering this first case, 11 other deer, shot in New Jersey and affected to varying degrees, have been examined at autopsy. Two of these animals have shown almost as extensive involvement as did the original deer, while the remaining nine were less severely affected. In some of these milder cases, only one or a few small tumors were to be observed.

The tumor-like condition that we have encountered in New Jersey deer is believed to be the same as that reported in recent years in deer in Wisconsin (1), Virginia (2), California (3), and Vermont (4), and variously identified as "papillomas," "fibromas," "wart-like structures," and "fibrosarcomas." It is the purpose of the present paper to describe the pathology of the New Jersey deer tumor, to report on its transmissibility to normal deer under experimental conditions, and to give evidence, supporting that presented in an earlier paper (5), that the causative agent is a virus.

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Materials and Methods

Tumors from the 12 naturally affected deer served as the starting material for our investigation. These were fixed in formalin or Zenker's fluid for histological study or stored in sterile glycerol-saline to be used in transmission experiments. In such transmission experiments, the material for inoculation consisted in pieces of the tumor masses that had been stored in 50 per cent glycerol-saline at approximately −20°C. for variable periods of time. In the preparation of each inoculum, several pieces of tissue were washed free of adherent glycerol by rinsing in 0.85 per cent sodium chloride solution and were then ground with sand in a mortar to make 10 per cent suspensions in buffered saline (pH 7.0). The supernatants of such suspensions were used for inoculation without further treatment or they were filtered through paper, Seitz pads, or Berkefeld filters before use.

In addition to rabbits, guinea pigs, sheep, and a calf, we have utilized 10 deer as experimental animals in the present experiments. These were obtained in nature as orphaned fawns and were bottle-fed on cow's milk until weaning time. After this they were maintained on a diet of hay and a grain mixture known as omolene (Ralston Purina Co.). They have been used for experimental transmission attempts when they were as young as 5 weeks or as old as approximately 1 year and age has seemingly played no role in the experimental outcome.

Experimental deer were prepared for inoculation and were inoculated in the following manner: The hair was removed by electric clipper from areas roughly 12 x 12 cm. on one or several portions of the body just prior to inoculation. Each denuded area of skin was then scarified with a curved cutting surgical needle deeply enough to cause oozing of blood-tinged fluid along at least portions of the scarifications. Immediately after this the preparation to be tested for infectivity was applied to the scarified surface from a Luer syringe and was rubbed into the scarifications with a sterile glass rod. The animals were held in restraint for a few minutes after inoculation to permit partial drying of the inoculated areas of skin. So far as possible, areas inaccessible to self-licking were selected for inoculation, although, in some instances, this was not feasible and, in these cases, the animals were observed to lick the scarified areas after release from restraint.

Results of Attempts at Experimental Transmission.—The scratches on the scarified areas of skin healed promptly and nothing was to be seen or palpated at the sites of inoculation until approximately 7 weeks had elapsed. Then, after periods ranging from 46 to 56 days in the 7 out of 10 animals that were positive, the lines along which the needle scratches had been made became pigmented and tiny discrete elevations could be seen and felt along them. These elevations increased slowly in size and by the end of a month after having first become visible were palpable as tiny close set nodules approximately 1 mm. in height and diameter arranged linearly where the scratches had initially been made. The nodules continued to increase and ordinarily after 7 weeks of growth had reached a size of 2 to 3 mm. in height and diameter, as shown in Fig. 2. One deer was sacrificed at this stage for virus and for histological study of its tumors.

The remaining six deer were held under observation and in five of these animals an unexpected happening took place at about 2 months. The tumors which had been growing slowly but steadily up to this time lost their healthy "fleshy" appearance almost overnight, became dry and contracted, and finally sloughed away from the underlying tissues. This regression occurred almost simultaneously in all of the numerous individual small tumors over each inocu-

lated area. In the single animal in which regression did not take place, the tumors continued slowly to enlarge, and finally, 10 months after inoculation, had formed a semiconfluent mass, as shown in Fig. 3, in which individual tumors had reached a size of 3 cm. in height and diameter and had the general appearance of the growths seen in the naturally occurring disease. The animal was sacrificed at this time while its tumors were still healthy in appearance and seemingly actively growing.

It appeared from our transmission experiments that, although most deer were susceptible to the tumor-inducing agent, the tumors themselves exhibited a marked tendency to regress after growing for about 2 months. This would suggest that the deer we have encountered in nature with advanced disease represent but a small proportion of the animals that have undergone infection with the tumor agent. Most naturally infected deer, in all probability, develop tumors that regress before they reach a readily observable size.

Histopathology of the Deer Fibroma.—The tumors produced in experimentally infected deer were identical in histological appearance with those encountered in naturally affected animals and, as shown in Fig. 4, were composed of stellate, angulated, or spindle-shaped cells resembling fibroblasts. These were scattered in an almost regular mosaic throughout a felt work of collagen strands. In individual sections, viewed at low power, as shown in Fig. 5, the cell nuclei seemed to be arranged in a tessellated design of remarkably even distribution, especially in the larger and more long standing tumors. In these older tumors, the cell nuclei were smaller, richer in chromatin, and usually elongated or sausage-shaped in contrast, as shown in Fig. 6, to the large round paler nuclei seen in the cells comprising the mass of younger tumors. In neither old nor young tumors were cells in mitosis to be seen, probably a reflection of the extremely slow rate of growth exhibited by the deer fibroma. The tumors were moderately well vascularized by thin-walled blood vessels and areas of cell necrosis were not seen.

The epithelial layer overlying the tops of tumors was thickened and frequently pigmented (Fig. 7), but that extending down along the sides of the tumor was usually quite thin, as shown in Fig. 8. It seems likely, in view of this arrangement, that the epithelial hyperplasia is actually not a part of the neoplastic process, but rather results from the added friction and abrasion suffered by the protruding surface of the tumor.

The epithelial basement membrane was intact but in some tumors exhibited a remarkable relationship to the collagen structures of the tumor itself. Instead of running in random directions and more or less crisscrossing each other, as they did deep in the tumor substance, the collagen strands at the tumor surface were arranged perpendicularly to the overlying epithelium, as shown in Fig. 9, and extended from the basement membrane paralleling one another deep into the fibroma. This peculiar arrangement of the collagen structures to the overlying epithelial layer was more evident in old than in young fibromas.

Nature of the Agent Causing Fibroma in Deer

Filtration Experiments.—The deer fibroma agent has proven to be filterable. Two out of three attempts to pass the agent through Seitz pads resulted positively, while both of two trials to filter it through Berkefeld N candles were successful. It was apparent from the results that much of the agent was retained by the Seitz pads since, in one instance, a Seitz filtrate failed to cause tumors, while the remaining two Seitz filtrates induced tumors in diminished numbers after incubation periods longer than those observed for the unfiltered fibroma suspensions. On the other hand, the two Berkefeld N filtrates prepared from suspensions of fibroma tissues yielded just as many tumors, without prolongation of the incubation period, as did the unfiltered control suspensions. It would appear from these findings that the deer fibroma agent is readily filterable through Berkefeld N candles, but not so readily filterable through Seitz pads.

Storage in Glycerol.—The causative agent of the deer fibroma has proven to be remarkably stable when stored in glycerol-saline at −20°C. Fibromas from naturally occurring cases have been found to contain the agent in apparently fully infective form after periods of storage of 8, 9, and 22 months, and those from experimentally infected deer after periods of 6 and 27 months.

The stability of the fibroma-inducing agent in glycerol and its filterability are properties characteristic of a virus. It is considered, on the basis of these properties, as well as on the basis of our failure to find visible or cultivable microorganisms in the tumors or in infective tumor filtrates, that the causative agent of the deer fibroma is indeed a virus.

Attempts to Transmit the Deer Fibroma Virus to Experimental Animals Other Than Deer.—Deer fibroma virus, known to be infective for experimental deer, has been administered intradermally or applied to the scarified skin of 11 rabbits, two guinea pigs, and two sheep. None of these animals developed tumors or became ill during a period of observation of 3 months or longer.

Lack of Relationship between the Deer Fibroma and the Bovine Papilloma.—Although the growths in bovine papillomatosis are primarily epithelial, they are sometimes accompanied by considerable underlying connective tissue reaction giving them somewhat the appearance of the usual deer fibroma. Furthermore, bovine papillomatosis and the deer fibroma resemble each other in the manner in which the growths of each tend to be distributed over the bodies of their respective hosts. The incubation period of the fibroma of deer is, however, considerably longer than that ordinarily observed for the papilloma of cattle and, furthermore, the deer fibroma usually grows at a considerably slower rate than does the bovine papilloma (6). Nevertheless, because of those features of bovine papillomatosis suggesting a possible similarity to the deer fibroma, it was considered necessary to explore the possibility that the two conditions might actually be etiologically related to one another.

To do this, cross-infectivity tests with the two viruses were conducted in deer and cattle. Deer fibroma virus was applied to the scarified skin and administered intradermally to a Holstein calf, about 2 months old when acquired from a dairy herd free of bovine papillomatosis. The animal was held under observation for 3 months and developed no lesions during this time. In like manner, bovine papilloma virus was applied to the scarified skin and administered intradermally to two deer.[1] The animals were kept under observation for 3 months and neither developed lesions of any sort in the inoculated areas of skin.

These cross-infection experiments indicated that the deer fibroma and the bovine papilloma viruses used in our tests were specific for their homologous species and were, so far as could be told from our findings, not etiologically related to one another. No effort was made to adapt either virus to the heterologous species. However, it is apparent from our results that the bovine papilloma virus is not one that passes readily and with ease from cattle to deer. The findings suggest strongly that the deer fibroma is a specific tumor of deer and not merely a manifestation of infection of deer with bovine papilloma virus.

DISCUSSION

A cutaneous tumor, prevalent in deer in New Jersey, has the general gross and histological appearance of a benign fibroma of remarkably uniform cellular makeup. Individual tumors, of which there may be many scattered over the bodies of affected animals, are firm and white on cut section and are made up of stellate, angulated, and spindle-shaped cells resembling fibroblasts. The overlying epithelium is thickened and may be pigmented. The tumors are benign in the sense that they do not appear to metastasize nor to involve regional lymph nodes. However, their frequent location about the mouths and on the eyelids, and the large size that they may eventually attain, make them deleterious to the continued health and well-being of affected animals.

The causal agent is a virus, readily filterable through Berkefeld N candles, that can be maintained without evident loss of infectivity for periods as long as 2 years in 50 per cent glycerol-saline in the cold. The virus is infective through skin abrasions and induces tumor formation after an incubation period of about 7 weeks.

Growth of experimentally induced fibromas is slow and, in the single animal held under observation for a relatively long period of time, individual tumors had reached a size of only 3 cm. in diameter after 8 months of growth. In most of our experimentally infected deer, progressive growth of the fibromas

[1] We are indebted to Dr. Carl Olson, Jr., for furnishing us with the bovine papilloma virus used and for checking in cattle the infectivity of the preparation which we found to be noninfective in our deer.

continued for only about 2 months and at the end of this time underwent rapid and complete regression. It seems likely, from the high regression rate observed in experimental deer, that, in nature, it is probably only the occasional deer that develops progressive and persisting fibromatosis. In most animals in the wild, infection with the fibroma virus probably constitutes a transient ailment in which the individual tumors achieve, before regression, a size too small to be noticed under the deer's heavy coat of fur.

SUMMARY

A naturally occurring cutaneous fibroma of deer has proven to be experimentally transmissible in deer. The causative agent is a virus that is readily filterable through Berkefeld N candles and that survives in fibroma tissue for at least as long as 27 months in glycerol-saline at −20°C. The experimentally produced deer fibroma has an incubation period of about 7 weeks, a very slow rate of growth, and a high regression rate.

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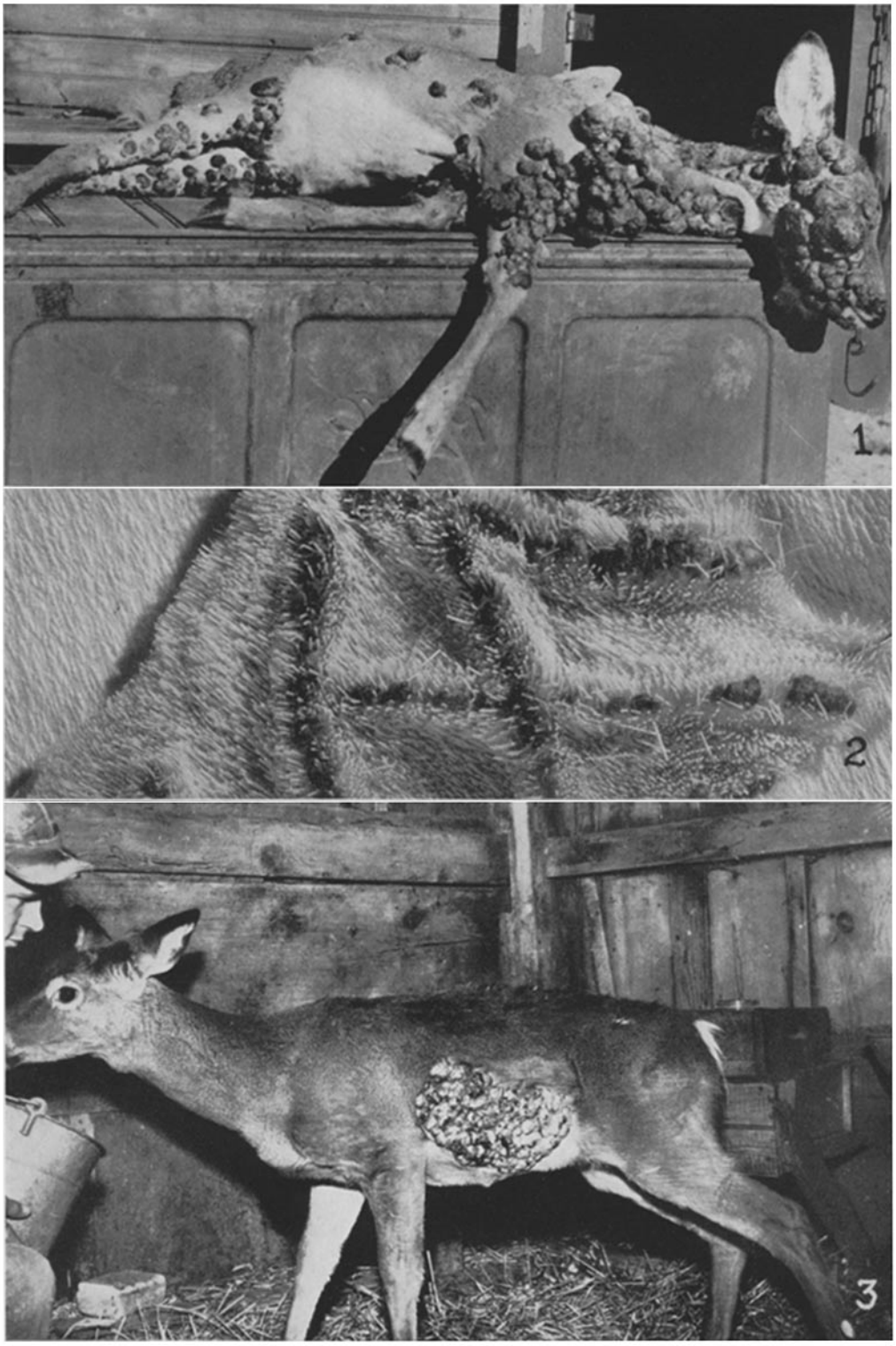
EXPLANATION OF PLATES

PLATE 51

FIG. 1. Our first naturally affected deer. The animal has myriads of large cutaneous tumor-like masses scattered over its body. These are most numerous over the head, neck, and upper portions of the legs. Photographed by Jasper Sweet.

FIG. 2. Experimentally induced deer fibromas after 7 weeks of growth. The individual tumors are arranged along the lines of scarification and are 2 to 3 mm. in height and diameter. Photographed by Julian Carlile.

FIG. 3. Experimentally infected deer 10 months after inoculation with fibroma virus. The growths, which began as small discrete nodules along the lines of scarification, have enlarged to form a mass of confluent and semiconfluent tumors that individually are about 3 cm. in height and diameter. Photographed by Wendell M. Stanley.

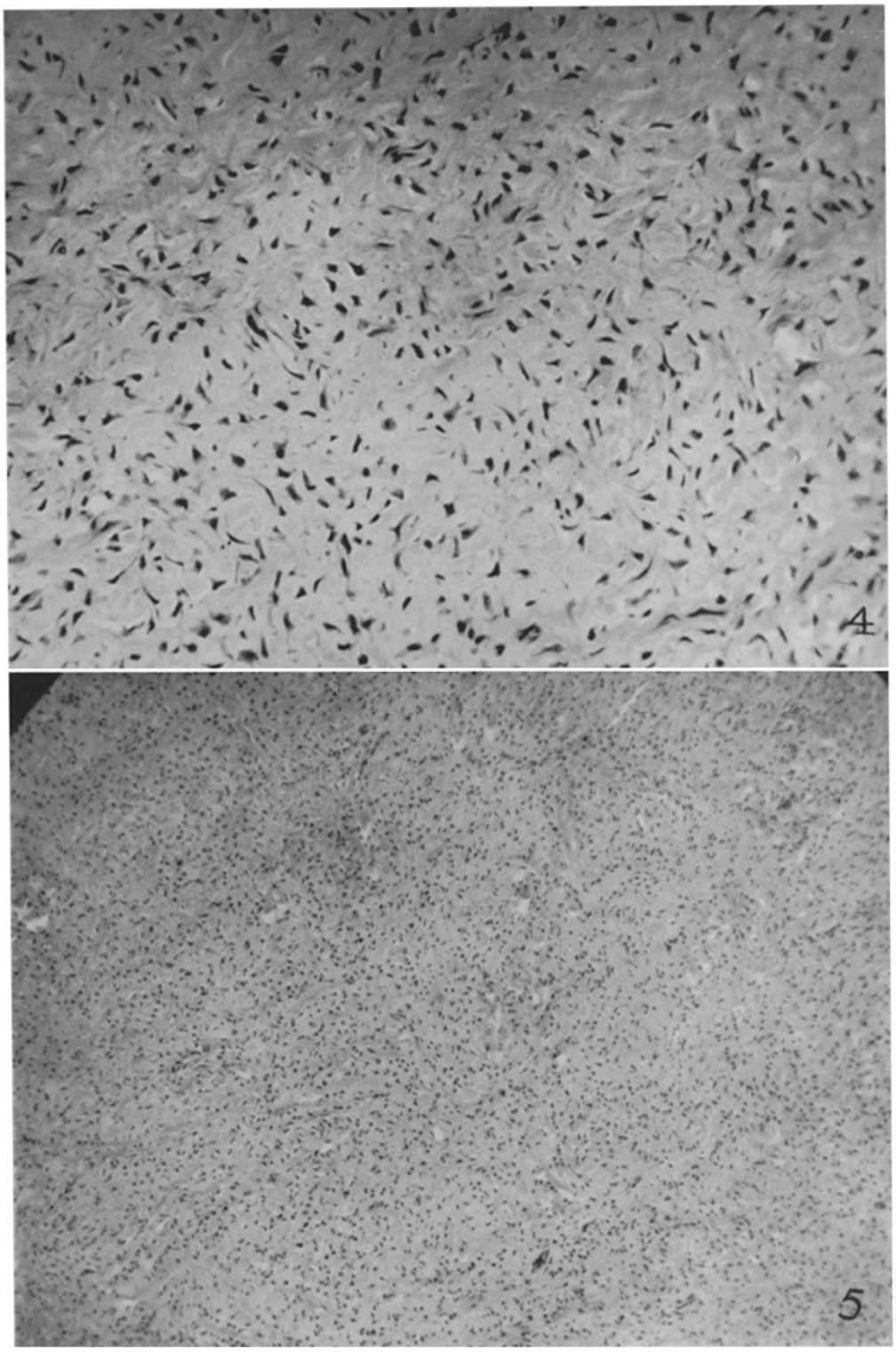


(Shope *et al.*: An infectious fibroma of deer)

PLATE 52

FIG. 4. Section of a naturally occurring deer fibroma. The tumor is composed of stellate, angulated, and spindle-shaped cells arranged in an almost regular mosaic. Hematoxylin-eosin. × 249. Photographed by Julian Carlile.

FIG. 5. Lower power of the above section to show the almost orderly distribution of cells within the mat of collagen strands. Hematoxylin-eosin. × 80. Photographed by Julian Carlile.

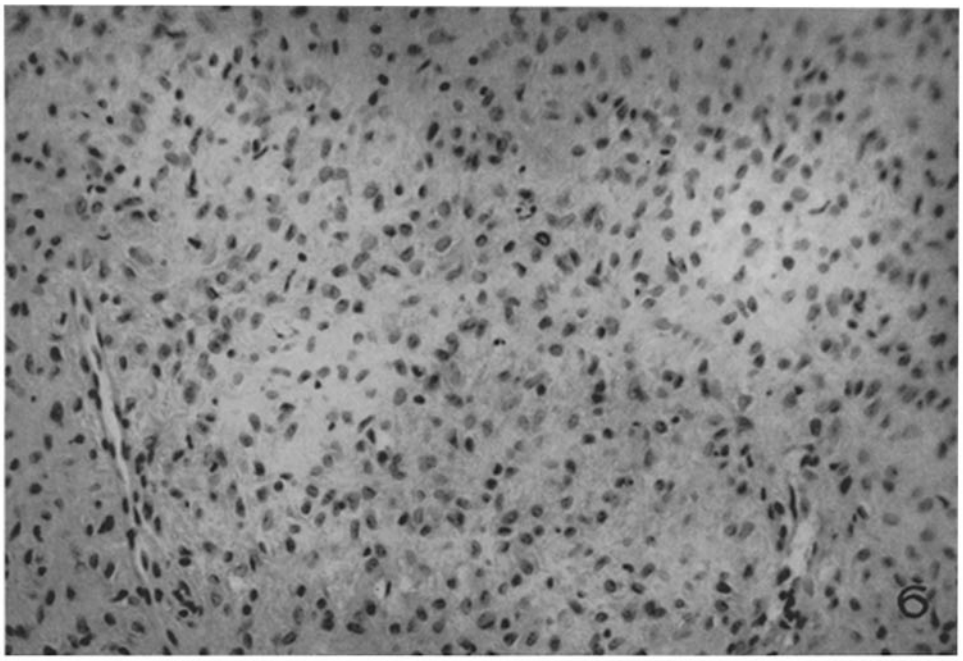


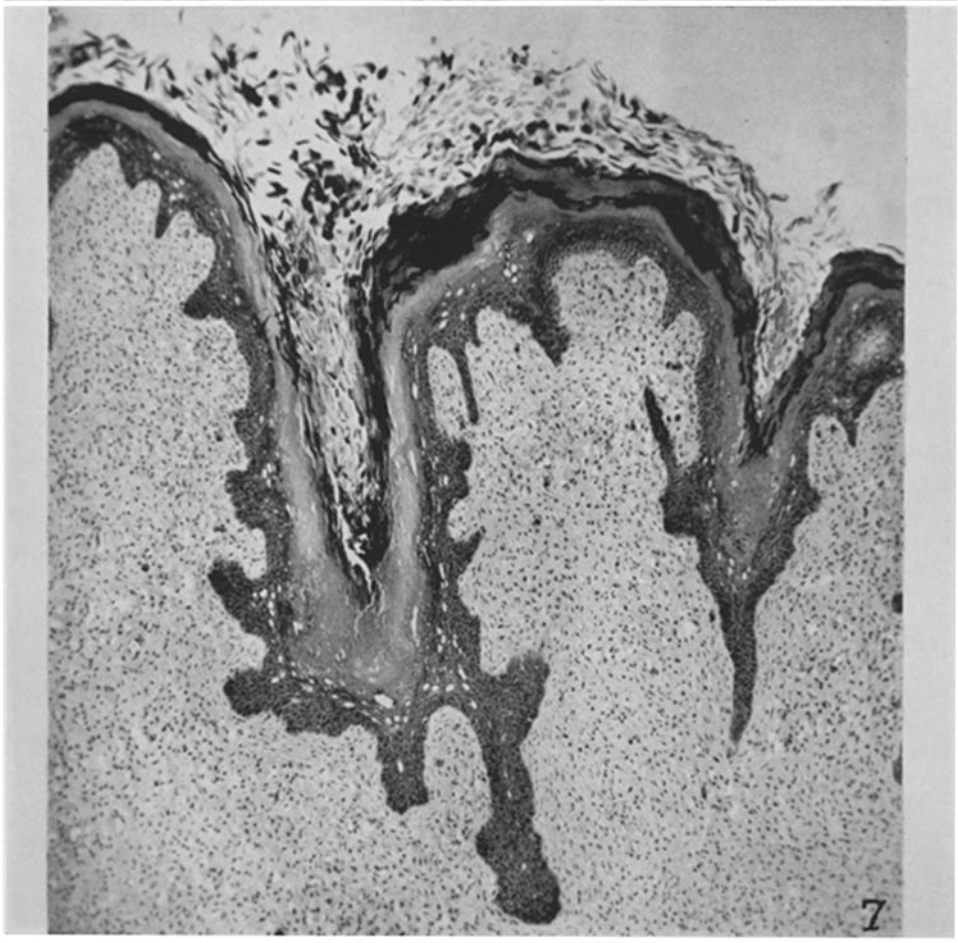
(Shope *et al.*: An infectious fibroma of deer)

PLATE 53

FIG. 6. Section of a 7-week-old experimentally induced deer fibroma. The cells in this young tumor are less angulated than those encountered in older growths and their nuclei are paler and more rounded. Hematoxylin-eosin. × 249. Photographed by Julian Carlile.

FIG. 7. Section of a naturally occurring deer fibroma showing the thickened epithelial layer that characteristically overlies the fibromas. Eosin-methylene blue. × 73. Photographed by Julian Carlile.



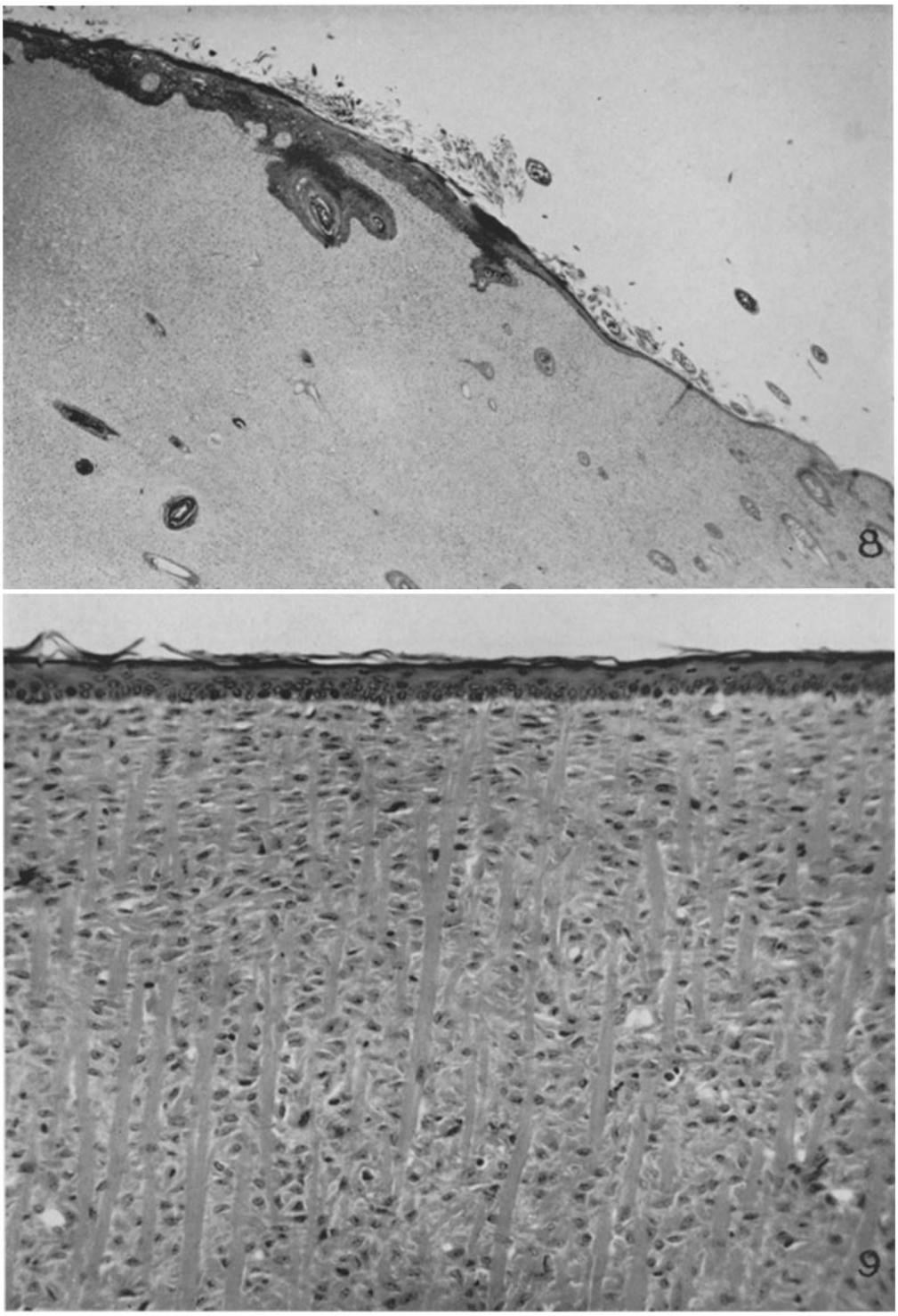


(Shope *et al.*: An infectious fibroma of deer)

PLATE 54

FIG. 8. Section of a naturally occurring deer fibroma. The epithelial layer covering the top of the fibroma is thickened and hyperplastic while that over the sides and at the bases of the tumor is quite thin. Hematoxylin-eosin. × 35. Photographed by Julian Carlile.

FIG. 9. Section of an 8-month-old experimentally induced deer fibroma to show collagen strands arranged perpendicularly to the epithelial layer and paralleling one another deep into the fibroma. Eosin-methylene blue. × 249. Photographed by Julian Carlile.



(Shope *et al.*: An infectious fibroma of deer)