sheep pituitary tissue obtained from extracts made at 70°C with water, saturated solutions of NaCl and KCl, and with 0.88 M sucrose contained the major part of the gonadotrophic activity. Fractions obtained from extracts made with saturated solutions of barium, calcium and magnesium chlorides, and 6.6 M urea at 70°C were much less active. Dialyzed soluble fractions obtained from saturated NaCl extracts made at 80°C contained little activity and those from extracts made at 90°C were essentially inactive. Extraction at 70°C with the saturated solutions and fractionation of the dialyzed extracts did not separate FSH from LH. The activity of the gonadotrophin was augmented when administered in solutions which were 0.5 saturated with NaCl and KCl.

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Naturally Occurring Fibromas of Grey Squirrels Related to Shope's Rabbit Fibroma. (20099)

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Six grey squirrels (*Sciurus carolinensis*) collected within a year in Maryland counties adjacent to the District of Columbia were covered with multiple tumors which in gross appearance, histologic structure, and on immunological grounds show close relationship to the fibromas originally described by Shope (1) in cottontail rabbits. Afflicted squirrels, which weighed around 300 g or less, appeared to be juveniles. The squirrel fibromas measured from a few mm to 2.5 cm in diameter and varied in number from 5 on one squirrel to between 50 and 100 on others. They were located indiscriminately on all parts of the animals from eyelids to tail and toes. A squirrel with naturally occurring tumors is shown in Fig. 1. As discussed below, woodchucks (*Marmota monax*) were experimentally infected.

Materials and methods. Fibromas when harvested for passage were minced with scissors and ground with sand before being made into 10% emulsions with meat infusion broth. Due to the presence of skin and hair follicles, especially in woodchuck and squirrel biopsies, such emulsions were often contaminated as originally prepared. Addition of penicillin and streptomycin, followed by centrifugation for ½ hour, however, appeared to eliminate contaminants as a factor of importance. In addition animals inoculated with woodchuck or squirrel preparations were given 150,000 units of penicillin in oil. Some of the woodchuck fibroma emulsions were free from contaminants when tested in whole meat broth and on blood agar slants. In *neutralization* tests, the OA strain of Shope rabbit fibroma* was diluted with meat infusion broth, so that after addition to equal volumes of undiluted sera, the final virus dilutions were 10^{-2}, 10^{-3}, and 10^{-4}. Such mixtures in amounts of 0.5

* Kindly supplied by Dr. David R. Ginder, Emory University, Atlanta, Ga.

FIG. 1. Naturally occurring fibromas on grey squirrel.

ml were inoculated intracutaneously in domestic rabbits after incubating not longer than ½ hour at room temperature. All sera were inactivated by heating at 56°C for ½ hour. Each neutralization test included a control consisting of a titration of OA virus run in the presence of normal rabbit serum, fibromas constantly appearing at 10^{-4} and less frequently at a dilution of 10^{-5}. In other neutralization tests, fibroma virus of squirrel origin was used in final dilutions of 1:20 or 1:40, as titers above 10^{-2} have not been obtained so far with these preparations. Results of neutralization tests were read 5 days after inoculation.

Pathology. Histologic sections of tumors recovered from grey squirrels are similar to sections of rabbit fibromas studied in this laboratory and previously described by Shope (1), Andrewes(2), Ahlstrom(3), and Hurst (4). The epidermal covering of the tumors reveals slight hyperkeratosis and occasionally crusting. Focal elongation of the rete pegs is distinct in many sections. Moderate numbers of epidermal cells reveal cytoplasmic vacuolation as well as large cytoplasmic acidophilic and fuchsinophilic inclusions (Fig. 2). They stain with thionine but are not metachromatic. In addition they are unaffected by ribonuclease digestion (crystalline ribonuclease in pH 6 phosphate buffer at 37°C for one hour) and are periodic acid Schiff negative. These inclusions are also noted in islands of epidermis isolated in the upper corium as well as in squamous cells of outer layers of the hair roots. In the corium, and often extending into subcutaneous tissue, circumscribed but non-encapsulated nodules comprised of fibrocytes are present. The fibroma cells are associated with moderate quantities of reticulum and collagen but scanty fibroglia. Cell nuclei are round to oval and frequently vesicular. A rare mitosis is evident. Cell cytoplasms are usually abundant. Intracytoplasmic inclusions (Fig. 3) ranging in size from that of a micrococcus to that of an erythrocyte are observed in fibroma cells after staining sections by the Heidenhain modification of the Mallory collagen method

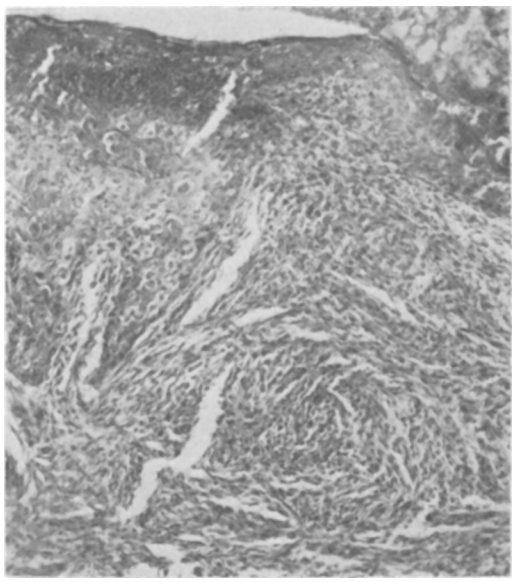

FIG. 2. Photomicrograph demonstrating intracytoplasmic inclusions in epidermal cells of elongated rete pegs at upper left, as well as portion of tumor. (H & E × 95.)

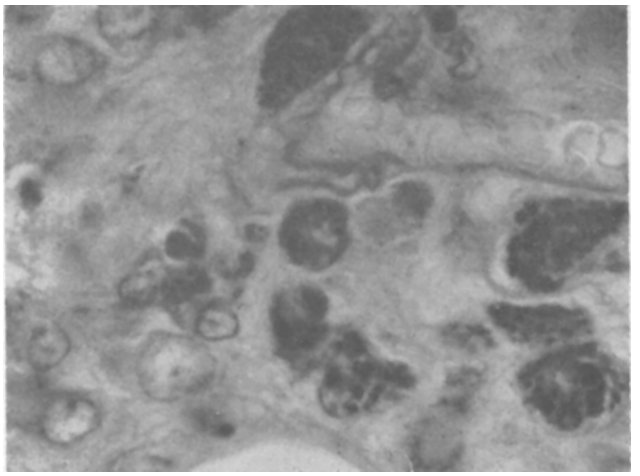

FIG. 3. Photomicrograph demonstrating intracytoplasmic inclusions in "fibroma" cell. (P.A.S. × 950.)

and the periodic acid Schiff procedures. but are not recognized after hematoxylin and eosin staining. These bodies fail to reveal any consistent distribution pattern within fibroma cells and are for the most part homogeneous. In several nodules there is a perivascular infiltrate of lymphocytes, plasma cells and histocytes involving blood vessels of the deep corium. In addition fibroma cells appear to radiate about vessels and to have had their origin from adventitial cells of these structures. Peripheral necrosis is occasionally noted.

Experimental. Transmission of squirrel fibromas was accomplished with difficulty at first. possibly because 5 of the squirrels with the original nodules had been dead for several days when received. Best results were obtained when fibromas from the single squirrel received alive were emulsified in 10% meat infusion broth and passed intracutaneously to a woodchuck. Four woodchuck passages have been carried out. the animals developing thick. plaque-like nodules under the skin which persist for 6 weeks or more. On inoculation into the skin of domestic rabbits. emulsions of woodchuck fibromas have led to the development of small nodules (1.3 cm in diameter) which appeared in 3 days and began to regress in 7 days (Fig. 4). Although growth of these fibromas was unaffected by the presence of normal squirrel or cottontail rabbit serum, their growth was completely or almost completely suppressed when squirrel fibroma-immune squirrel serum or Shope fibroma immune-cottontail serum was mixed in equal amounts with the original inoculum. Two consecutive passages of woodchuck fibromas have been carried out in domestic rabbits. Emulsions of woodchuck fibromas have also led to the formation of nodules on intracutaneous inoculation of grey squirrels. Initial attempts

FIG. 4. Tumors induced in domestic rabbit by intracutaneous inoculation of emulsion of woodchuck fibroma.

to pass the Shope rabbit fibroma to woodchucks and to squirrels have been unsuccessful.

Other experiments have further indicated immunological relationship between squirrel and Shope rabbit fibromas. In current studies 2 domestic rabbits were inoculated with fibroma emulsions, 2 inoculations having been given intracutaneously a week apart. The fibroma-emulsions were from 2 of the original squirrels, each rabbit receiving material from only one squirrel. When bled 10 days after the last inoculation, both rabbits had developed neutralizing antibodies against the OA strain of rabbit fibroma, as indicated by neutralization tests performed in the skin of a fresh rabbit. Preinoculation sera had no neutralizing capacity. Although one of the rabbits died, the second one failed to develop tumors when challenged with virulent OA fibroma virus. Only 2 serum specimens have been obtained from grey squirrels bearing tumors. Both of these sera had a definite capacity to neutralize OA fibroma in dilutions of 10^{-3} and 10^{-4}; whereas sera from 10 normal squirrels showed no neutralizing capacity.

Discussion. Although difficulties in obtaining grey squirrels and woodchucks for laboratory use have somewhat limited the extent of the investigations, our studies demonstrate a close relationship between squirrel and Shope rabbit fibromas. Further studies are in progress to determine how the agent of the squirrel tumor may be transmitted in nature, to define more clearly similarities and differences between it and the Shope fibroma virus, and to investigate the histochemical nature of cellular inclusions encountered.

Summary. Six grey squirrels collected in Maryland were found to have multiple small cutaneous nodules which histologically resembled Shope's rabbit fibroma. The squirrel fibroma was carried through 2 intracutaneous passages in grey squirrels, 4 consecutive passages in woodchucks, and from woodchucks for 2 passages in domestic rabbits. Cross-neutralization tests provided immunological evidence that the squirrel and Shope fibromas are related.

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Effect of Vitamin B_6 Deficiency on Hepatic Transaminase and Cysteine Desulfhydrase Systems. (20100)

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The relationship of vit. B_6 to transamination has been demonstrated in studies on animals(1-3) and microorganisms(4,5) deficient in this vitamin, and also in experiments with isolated enzyme systems(6-11). Schlenk and Snell(1) found that the tissues of vit. B_6-deficient rats exhibited decreased glutamic-aspartic transaminase activity. These findings were confirmed by Ames, Sarma and Elvehjem(2) and similar observations have been made by Shwartzman and Hift in studies on hamsters(3). *In vitro* experiments showed that pyridoxal phosphate activated resolved transaminase preparations(6-11) and recent evidence suggests that pyridoxamine phosphate may also function as a coenzyme for transaminase(12).

Previous communications from this laboratory(13-15) have described an hepatic transaminase system capable of catalyzing the following reaction: L-glutamine + α-keto acid → α-ketoglutaric acid + L-α-amino acid + NH_3. The available evidence indicates that a) a wide variety of α-keto acids may par-