jury." However, in the reports cited, the variety of tissue injuries studied included physical and chemical injuries, but not infectious "injury" *per se,* so they provide no evidence bearing directly on the question of altered resistance to infection in lathyrism. Thus, from the results of the present experiment on the immunologic response, there is no evidence that resistance to infection is altered in lathyrism.

This last observation has led to reconsideration of the original hypothesis, that lathyrism caused decreased resistance to infection. Reexamination of one of the early reports(2) from which this hypothesis was formulated reveals that the experimental and control animals were fed different kinds of peas: lathyrus peas and edible peas. It is possible that the observed differences in the incidence of infection in the 2 groups were related to differences in the nutritional components of the 2 kinds of peas rather than to the presence of the toxic principle in the lathyrus peas. On the other hand, these differences may have been a fortuitous association, for the differences in incidence of infections originally observed in our own laboratory have disappeared. It seems reasonable, therefore, to doubt that resistance to infection is altered in lathyrism, until more evidence is available to support the proposal.

Summary. The immune response to injections of human red blood cells was studied in lathyritic weanling rats by direct agglutination and by electrophoretic analysis of serum globulins. No great differences between the response of the lathyritic and the control rats were found by either study. A minor change in the histology of the spleens of the lathyritic rats is unrelated to the immune response. No evidence is found to support the hypothesis that resistance to infection is altered in lathyrism.

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Production of Tumors in Syrian Hamsters After Oral Administration of Polyoma Virus. (28435)

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Polyoma virus has produced tumors in Syrian hamsters after inoculation by a variety of routes. Subcutaneous inoculation of newborn hamsters resulted in production of sarcomas in the kidneys, heart, liver and subcutaneous tissues(1), while subcutaneous inoculation in young adult hamsters produced only subcutaneous sarcomas(2). Meningeal sarcomas were produced after intracerebral inoculation of the virus in newborn

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hamsters(3) and epithelial lung tumors were produced after intratracheal inoculation in young adult hamsters(4).

Recently, there has been considerable interest in the oncogenic properties in hamsters of SV40, a virus of monkeys(5,6). The simian virus has a number of properties which are similar to those of polyoma virus and both viruses have been classified in the "papova" group (Shope rabbit papilloma, human wart, polyoma, and SV40)(7). Since SV40 was present in some oral poliovirus vaccines and thus was inadvertently fed to large numbers of humans(8), it was of interest to determine the oncogenic potential of "papova" viruses when administered to animals by the oral route. This report describes the results of oral administration of polyoma virus in Syrian hamsters.

Materials and methods. Animals. Thirty male Syrian hamsters, 6 weeks of age, obtained from the Animal Production Unit of National Institutes of Health, were housed in plastic cages, fed Purina laboratory pellets and given water *ad libitum.*

Virus. The strain of polyoma virus used was originally isolated *in vitro* from an extract of a mouse parotid tumor using cultures of milk-adapted P388D1 cells(9). It had been passaged 14 times in milk-adapted P388D1 cells, 5 times in P388D1 cells in 40% pooled human serum and 60% mixture #199, and 2 times in trypsinized primary mouse embryo cultures maintained in Eagle's medium with 5% horse serum. A virus pool prepared from the 2nd passage in mouse embryo cells was used in the present studies. It was titrated in milk-adapted P388D1 cell cultures and contained $10^{8.1}$ $TCID_{50}$ per ml. Another virus pool prepared in a similar manner but not titrated was also used in the experiment.

Control fluid. Discard medium (Eagle's medium with 5% horse serum) from mouse embryo cultures not inoculated with polyoma virus was used as control fluid.

Experimental methods. Virus was administered orally to 20 hamsters by means of a curved pipette with the tip of the pipette placed in the esophagus. The virus pool was diluted 1 to 5 with mixture 199 and each animal was given 0.25 ml twice weekly for 16 months. During the first 5 months of the experiment, the pool of known titer was used, and in the subsequent 9 months the untitered pool was used. Ten animals were given control culture fluid by identical methods.

Results. Fourteen weeks after initiation of the experiment, one of the 20 animals given virus orally was noted to have a subcutaneous tumor measuring 0.5 cm in diameter in the right scapular area. Two weeks later it measured 3.5 cm in greatest diameter. The animal was killed with ether and, at necropsy, the tumor was noted to be well encapsulated and soft. On section, it had a variegated surface with areas of glistening white homogeneous tissue and areas of hemorrhagic necrosis. Microscopically, it was a sarcoma with histologic features similar to those previously described in subcutaneous sarcomas produced in hamsters inoculated subcutaneously with polyoma virus(2). There were no metastases and careful examination of the gastrointestinal tract showed no tumors. Subsequently, 3 other animals in the group fed virus developed subcutaneous sarcomas. Two of these were noted 18 weeks after initiation of the experiment and one was noted after 44 weeks. These animals were killed and necropsied but no evidence of primary tumors arising in the gastrointestinal tract was found.

One of the animals with a subcutaneous tumor had several small white nodules 1-2 mm in diameter in the lungs. Microscopically, these were bronchiolar and alveolar cell tumors similar to those previously described after intratracheal inoculation of polyoma virus in hamsters(4). They were probably the result of aspiration of the virus during the oral inoculations.

Thirteen months after initiation of the experiments, 4 animals in the infected group with no evidence of subcutaneous tumors were killed and necropsied. They had no primary gastrointestinal tumors or tumors in other sites. Three animals in the infected group were found dead 15 months after initiation of the experiments, and they were also free of tumors at necropsy. During the 16 month period, 4 animals were found dead

but partially eaten. Although no evidence of tumors was found in these animals, they could not be properly examined.

Two of the control animals were sacrificed after 9 months of treatment and complete necropsy revealed no evidence of tumor. The remaining 8 animals given control tissue culture fluid orally have remained well throughout the experiment and have no evidence of tumors. They will be observed throughout their lifespan as will the remaining 5 animals in the group given virus orally.

Discussion. Although intracerebral inoculation of polyoma virus resulted in meningeal sarcomas(3) and intratracheal inoculation resulted in lung tumors(4), no gastrointestinal tumors have been produced after repeated oral inoculation of the virus in Syrian hamsters. This would suggest that the gastrointestinal epithelium of the hamster is resistant to the oncogenic activity of polyoma virus; however, it is also possible that the exposure of the epithelium to virus was inadequate, due, perhaps, to the protective effect of the intestinal mucus or to some other local factors.

It is of interest, however, that 4 of the 20 animals given virus orally developed subcutaneous sarcomas similar to those produced by subcutaneous inoculation of the virus. This observation suggests that virus was absorbed from the gastrointestinal tract and reached the susceptible subcutaneous tissue where it exerted its oncogenic activity. It is not known whether the virus could have been absorbed through intact gastrointestinal mucosa; however, the animals may have had mucosal erosions in the gastrointestinal tract at some time during the experiment and virus might have been absorbed through such lesions. Care was taken to avoid traumatizing the esophageal mucosa during the oral inoculations, and no esophageal lesions were noted in the necropsied animals; however, it is possible that minimal injury of the esophagus by the pipette may have produced a portal of entry for the virus.

In view of the relatively low incidence of subcutaneous sarcomas in the inoculated animals, the possibility that the tumors were "spontaneous" must be considered; however, this seems unlikely. Dunham and Herrold studied 360 hamsters from the National Institutes of Health animal production unit and observed only one subcutaneous sarcoma(10). This occurred in an animal 27 months of age whereas the tumors described in our experiment appeared in animals less than one year of age. In our own studies of approximately 200 Syrian hamsters inoculated with human tumor extracts, we have seen no subcutaneous sarcomas in animals less than one year of age.

The fact that oral administration of chemical carcinogens may result in tumor induction at sites other than the gastrointestinal tract is well established(11). A similar phenomenon has been demonstrated with polyoma virus in the hamster, and further studies of oral administration of oncogenic viruses seem indicated.

Summary. Polyoma virus was administered orally to young Syrian hamsters twice weekly for 68 weeks. Four of the 20 animals developed subcutaneous sarcomas 14, 18, 18 and 44 weeks after initiation of the experiment. The tumors were similar to those produced in hamsters by subcutaneous inoculation of the virus. The 4 animals with subcutaneous tumors and 7 animals from the infected groups without subcutaneous tumors have been necropsied, and no evidence of primary tumors of the gastrointestinal tract has been found. No tumors have been observed in the remaining animals in the group fed virus and in 10 control animals given tissue culture fluid free of virus. These findings indicate that although oral administration of an oncogenic virus may not result in primary tumors of the gastrointestinal tract, subcutaneous tumors may be produced.

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Effect of Sodium Diphenylhydantoinate on Oral Mucosa of Rats.* (28436)

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The gingival hyperplasia resulting from dilantin therapy has been reported by various investigators(1-5). Shafer *et al.*(6) observed an increase in the tensile strength of healing skin wounds in dilantin treated rats. They suggested that this may be due to an increase in collagen synthesis accompanying the proliferative response of fibroblasts to dilantin therapy. No effect on the proliferation of epithelial cells could, however, be noted. Houck *et al.*(7) have shown that dilantin administration to rats resulted in accumulation of super-normal amounts of collagen within the dermis. Shapiro(8) reported acanthosis of the epithelium in human gingiva but failed to observe any alteration in keratinization of this tissue. The purpose of this investigation was to demonstrate the effect of systemic administration of sodium dilantin on the oral mucosa of rats.

Materials and methods. Five litters, each of 10 newborn albino rats, were used in this study. Beginning at 3 days of age, 8 rats in each litter were subjected every 3 days to intraperitoneal injections of 0.04 ml suspension of 100 mg/kg body weight of dilantin sodium in isotonic saline. The remaining 2 animals in each group received 0.04 ml of isotonic saline and were used as controls. The experimental rats in each litter received from 1-5 doses of dilantin sodium.† Two dilantin treated animals from each litter were sacrificed by decapitation 6 days after administration of the last dose. The controls were sacrificed at corresponding intervals. The oral mucosa from the cheek region was removed and fixed in 10% alcoholic formalin. The tissues were processed by routine histological procedure and embedded in paraffin. Serial sections were cut at 6 μ thickness and stained with Hematoxylin and Eosin, Masson Trichrome, Periodic Acid Schiff, and Mowry's procedure for acid mucopolysaccharides.

Results. No visible clinical changes were noted in the mucous membrane of the cheek of experimental animals. The initial *histological* changes in the mucosa were observed in animals which had received only one dose of sodium dilantin (Fig. 1-2). An increased mitotic activity in the basal layer of the epithelium was noted. Intercellular edema within the epithelium appeared to have caused prominent spongiosis and acanthosis. There was an apparent disturbance in cornification resulting in parakeratosis. In animals which had received 4 doses of sodium dilantin (Fig. 3 & 4), hyperplasia of epithelium was a common feature. The new cells produced were devoid of nuclear detail. The rete pegs showed elongation and splitting. Occasional intertwining of the rete pegs produced isolated areas of connective tissue stroma among the epithelium. Unusually large numbers of discrete basophilic keratohyaline granules were diffusely distributed throughout the epithelial cell layers. This was always associated with hyperkeratosis. Increased vascularity and scattered areas of chronic inflammation were sometimes observed in the connective tissue. Elevated fibroblastic activity

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† Parke-Davis (Sodium 5,5 diphenylhydantoinate).