virus into domestic rabbits (Genus, *Oryctolagus*) results in papillomas of notably aggressive behavior, which frequently become cancerous within a few months.[3] The metastasizing, highly malignant carcinomas that develop then are the outcome of changes in the virus-infected epithelium which are conditional upon various favoring circumstances. The precancerous period of papillomatosis can be much shortened by stimulative procedures, but it has not been done away with entirely by such means, nor has malignancy been induced by introducing extracts of the cancers into normal skin.

The papilloma is formed by multiplication of the epidermal cells with which the virus becomes associated at the time of inoculation, and it is essentially a composite of cell "families," a fact often evident in its aspect. Cancer arises more frequently from some of these families than from others within the same growth. In order to provide a wide range of cell conditions at the time of the initial cell-virus association, as also to give opportunity for any individual differences in the virus entities to assert themselves, advantage has been taken of the tendency of the virus to localize in hyperplastic epidermis.[2] The hyperplasia was secured by tarring the ears of rabbits. After 1½ to 3 months of tarring, when tar papillomas had begun to appear, a large amount of a Berkefeld filtrate, containing active virus, was injected into a leg vein. During the next two weeks—the incubation period of the virus—no significant local changes took place, though some of the growths due to the tarring continued to enlarge slowly, and a few others sometimes appeared. Then in many of the rabbits the growths underwent extraordinary alterations, becoming within a few days discoid, beefy and infiltrative, while many new and similar ones developed. Soon low mounds or ill-defined swellings appeared opposite certain of the tumors, as also elsewhere on the outer side of the ears. The tarring was now discontinued, yet the tumors continued to enlarge; some of the outer swellings ulcerated; the ears became greatly thickened, nodular and distorted, and their hollows filled with coalescing masses of fungoid tissue. These changes usually occupied but a few weeks, and rapidly led to death. Biopsies disclosed the presence at an early period of numerous, discrete, highly anaplastic carcinomas, which frequently had extended through the lacunae in the cartilaginous sheet, causing the ulcerations on the outer side of the ears. Some of the malignant growths developed on the basis of pre-existing tar papillomas but others where none had been visible. In an instance of the latter sort anaplastic cancers 3 mm and 4 mm across developed within 22 days after the virus inoculation. Only where the skin had been tarred did tumors appear.

Most of the nodular or fungous thickening of the ears proved due to growths expressive of the various stages in the transformation of virus-induced papillomas to anaplastic, squamous-cell carcinomas.[3] One could discern, crowded and intermingled in the masses of actively proliferating tissue, benign papillomas, others that were cystic or complicated in pattern and of dubious import, yet others that were frankly invasive and destructive, and carcinomas of all degrees of malignancy. Sections taken early have shown that some of the latter were anaplastic from the beginning.

Many of the benign papillomas could be identified by their slaty hue as due to the action of the virus. Save when they were heavily pigmented, as in these cases, they could not be told from papillomas due to tarring. Indeed all the growths which developed after the virus injection were found to have their counterpart amongst the tumors which develop in rabbits that have been tarred for long periods. Yet the tar tumors can scarcely be caused by the virus now under consideration, or another antigenically related to it, for the blood serum of rabbits carrying tar papillomas does not neutralize the virus *in vitro,* whereas that from animals with virus-induced papillomas usually possesses this power.

In supplementary tests rabbits that had been tarred for many months, with many large and small papillomas in consequence, were injected with the virus intravenously. Again events took the course described. A curious feature was the development of numerous tumors from the hyperplastic epithelial layer covering large, rounded, fleshy, tar papillomas that consisted for the rest of connective tissue. These soon became studded with growing bosses, and were replaced as the new tumors invaded their substance.

The literature on the effects of tarring the ears of rabbits yields no examples of fulminant carcinosis such as are here reported, nor have any been observed in the numerous control animals of the present work.

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[3] *Ibid.,* 62: 523, 1935.

## ISOLATION OF CRYSTALLINE PEPSINOGEN FROM SWINE GASTRIC MUCOSAE AND ITS AUTOCATALYTIC CONVERSION INTO PEPSIN

LANGLEY in 1882[1] observed that slightly alkaline extracts of swine gastric mucosae contained a material which was not pepsin but which could be converted into pepsin upon acidification of the extract.

[1] J. N. Langley, *Jour. Physiol.,* 3: 246, 1882.

This material (pepsinogen or propepsin), which can be converted into pepsin, has been isolated in crystalline form. It is a colorless protein crystallizing in very fine needles from slightly more than 0.4 saturated ammonium sulfate solution at pH 5.2–5.6. Starting with five independent lots of swine gastric mucosae, crystals have been obtained in each instance, and in one case recrystallization was fractionally repeated three times. The optical rotation and specific proteolytic activity[2] were the same for all crystalline preparations.

Pepsinogen does not clot milk at pH 5.0 nor liquefy gelatin at pH 4.7, although pepsin has marked activity under these conditions.

Pepsin prepared by the acidification of pepsinogen has been crystallized twice and its crystalline form is indistinguishable by inspection from pepsin crystallized from commercial pepsin by the method of Northrop.[3] The optical rotation and specific proteolytic activity[2] are nearly the same for the two pepsin preparations.

The conversion of pepsinogen into pepsin at pH 4.6 is an autocatalytic reaction similar to the previously described autocatalytic conversion of trypsinogen into trypsin.[4] Since the conversion is caused by the active enzyme and since no linkage but peptide linkages are known to be split by pepsin, it seems probable that the change involves the rupture of a peptide linkage, although little if any non-protein nitrogen is liberated during the conversion.

The procedure used in crystallizing pepsinogen may be summarized as follows:

(1) Minced swine fundus mucosae extracted with 0.45 saturated ammonium sulfate in M/10 sodium bicarbonate; filtered after the addition of 10 per cent. Filter Cel and 5 per cent. Hyflow Super Cel.[5]

(2) Pepsinogen precipitated from 0.7 saturated ammonium sulfate.

(3) Pepsinogen adsorbed from solution at pH 6.0 by cupric hydroxide suspension and eluted in M/10 pH 6.8 phosphate.

(4) Treatment with cupric hydroxide repeated.

(5) Soluble carbohydrate remaining removed by treatment with Filter Cel at pH 7.0.

(6) Pepsinogen crystallized in fine needles over night at 10° C., 0.4–0.45 saturated ammonium sulfate and pH 5.2–5.6 (orange red to methyl red).

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[2] By "specific proteolytic activity" is meant the activity per milligram of protein nitrogen. The activities were determined by the hemoglobin method.

[3] J. H. Northrop, *Jour. Gen. Physiol.*, 13: 739, 1930.

[4] M. Kunitz and J. H. Northrop, SCIENCE, 80: 505, 1934.

[5] Johns-Manville products.

## SELENIUM AND DUCK SICKNESS

SELENIUM in varying concentrations has been known to exist for some time in certain marginal and submarginal areas. It has been found not only in the soil of these areas but also in the vegetation in varying quantities from mere traces to high concentrations.[1] Occurring in grains and grasses in some localities, it has been believed to be a source of chronic and acute disease (formerly known as "alkali disease") among live stock.

Because of the marked similarity of the effects of toxic grains known to contain selenium on experimental animals, as studied by Franke and colleagues,[2] and others,[3] which show symptoms very closely related to those of duck sickness, it is possible to consider selenium as a cause of duck sickness. In general, the range of duck sickness has been confined to regions of alkaline waters of western United States and Canada which are characterized by marshes, mud-flat areas and overflowed lands.[4] Greatest concentrations of selenium have occurred during dry years and in areas identical with those in which great numbers of migratory waterfowl have perished.

Although the evidence for botulinum poisoning as the causative factor[4] is considered valid, it seems desirable in the light of recent work to thoroughly investigate the possibility of another cause, namely, selenium and salts of related metals. Experiments in which tame and decoy ducks were used show that sodium selenite added to drinking water in concentrations of 50 p.p.m. and above, produce lethal results in about ten to twelve hours, depending upon the amount of water consumed. When 20 p.p.m. sodium selenite were added, death usually followed within fifteen to twenty-four hours. Delayed and less severe symptoms resulted when lower concentrations of the toxic element were added to the water.

It is noteworthy that a definite parallelism may exist between selenium poisoning and the syndrome of duck sickness. A difficulty in respiration was first noticed. This was followed by weakness in the legs as a state of unbalance became apparent. The birds showed difficulty in holding their wings in position and, while resting, would support their heads and necks over their backs with bills resting on their breasts. Watery discharges flowed from the eyes and nostrils and in some cases formed encrustations which partly closed the external openings. A characteristic, green, fluid diarrhea was present in all cases. Subnormal body temperatures (100 degrees F.), followed by rapid drop as low as 96 degrees F. just before

[1] H. G. Byers and H. G. Knight, *Ind. and Eng. Chem.*, 27: 902, 1935.

[2] U. S. D. A., Circular 320, August, 1934.

[3] O. A. Beath, J. H. Draize, H. F. Eppson, C. S. Gilbert, and O. C. McCreary, *Jour. Amer. Pharm. Assoc.*, 23: 94, 1934.

[4] U. S. D. A. Bull. 411, May, 1934.