presented in Table VI offers another possible way of increasing the sensitivity of tests for relationship of antigens to tumors—both immunization and challenge being inoculated IC.

In those situations where the ability of a given cell suspension to produce tumors is being tested, such as in the *in vitro* transformations by viruses in tissue culture systems, the inoculation into immunologically privileged sites in X-irradiated animals might give positive transplants where conventional routes of inoculation would be negative. The negative results of the attempt to obtain oncogenesis by polyoma virus in adult animals by inoculation into privileged sites are probably explained by the fact that virus particles so inoculated can readily disseminate to other areas, calling forth the immediate usual immunological response(3).

Summary. Two polyoma tumors in mice have been found to have a 1,000-fold increased transplantability if the IC or IT routes of inoculation are used instead of SC. When polyoma virus-immune mice were challenged by the IC or IT routes, the degree of demonstrable resistance was 10^5 and 10^4 minimal transplantable numbers of cells as compared to 10^1 by the SC route. Mice immunized IC or SC with a single dose of X-irradiated tumor cells and later challenged IC showed protection in the IC but not the SC immunized group. The ability of *in vitro* polyoma transformed tissue culture cells to produce tumors on inoculation of isologous adult mice was most markedly enhanced by whole body X-irradiation, but best results were obtained if IC inoculation was made into irradiated animals.

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Characteristics of Rumen Pigment in Lambs Fed Pelleted Rations.* (28304)

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The development of a condition in lambs fed pelleted rations called parakeratosis was described by Jensen *et al.*(1). The affected papillae were dark in color, and, microscopically, the epithelium was thickened. There also were excessive layers of keratinized nucleated cells on the surface. Vidacs *et al.*(2) suggested that the changes in the epithelium were due to chemical factors rather than to the physical characteristics of the ration. The pigment associated with the condition in lambs has not been characterized. The objectives of this work were to study the histological changes in rumen epithelium associated with the feeding of pelleted rations and to characterize the pigment associated with these changes.

Materials and methods. Trial 1. Three lambs were randomly selected from 7 lambs on each of the following treatments for collection of rumen tissue specimens at time of slaughter. The lambs had been on the experimental ration for a 60-day period. The treatments were (1) conventional ration composed of shelled corn, long alfalfa hay and supplement; (2) ration 1, ground and mixed (molasses added); (3) ration 2, pelleted; (4) ration 2 plus minerals and vitamins, pelleted; and (5) ration 2 plus bicarbonate, pelleted. As quickly as possible after slaughter, speci-

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mens of the rumen mucosa were removed, from the anterior ventral sac of the rumen, and placed in cold (4°C) neutral buffered 10% formalin. They were imbedded in paraffin, sectioned at 6 μ, and stained with hematoxylin-eosin stain.

The tissue specimens were embedded in same as described for Trial 1 except that the groups of lambs (7 each) had been on a different series of rations for 69 days. The rations were (1) conventional ration composed of shelled corn, long alfalfa hay and supplement; (2) 80% concentrate (corn cracked) and 20% ground corn cobs; (3) complete ground mixed ration; (4) ration 3, pelleted; (5) ration 3, pelleted (corn ground to consistency of cornmeal); (6) ration 4 plus trace minerals; and (7) ration 4 plus sodium bicarbonate.

The tissue specimens were embedded in paraffin, and serial sections of each specimen were cut at a setting of 6 μ in thickness. Each tissue section was affixed with an albumen adhesive to individual glass microscope slides. Tissue sections from each specimen were stained by the following technics(3): Harris' hematoxylin and cosin; nucleal Feulgen reaction; dinitrofluorobenzene (DNFB) coupled with β-naphthol for the demonstration of sulfhydrol groups; DNFB-β-naphthol for the demonstration of disulfide linkages; ferric-ferricyanide reduction test; periodic acid-Schiff reaction alone and after acetylation (24 hours), acetylation-deacetylation (24 hours each), methylation (24 hours), methylation-demethylation (24 hours each) and bromination (2 and 4 hours); sudan colorants in propylene glycol (24 hours); Gomori's Prussian blue reaction for iron; and the alizarin red S stain for calcium. Unstained tissue sections were deparaffined, mounted in a non-fluorescent medium, and examined by bright field microscopy, polariscopy, and fluorescence microscopy employing exciting light at 365 mμ(3). Deparaffined tissue sections were also microincinerated at 600°C for 30 minutes, mounted dry, and examined by dark-contrast phase contrast and dark field microscopy(3).

Results. In the tissue sections examined, discrete granules of brown pigment were observed paranuclearly within scattered cells of the deeper layers of the rumen epithelium. Similar pigment was observed within vacuoles in the more superficial epithelial cells, usually distributed within a lighter colored homogeneous matrix. Such globules of pigment, surrounded by a homogeneous matrix (pigment complex), were also observed to be scattered along the lumen surface of the epithelium or free within the lumen contents adjacent to the epithelial surface. The morphologic similarity to the pigment of the normal human jejunal mucosa, as described by Astaldi and Storaselli(4), was quite striking. Tissue sections of the rumen mucosa from lambs fed the conventional ration did not contain pronounced deposits of pigment. One lamb had a mild degree of pigmentation of the stratum corneum. The cells in the stratum granulosum from one lamb had vacuoles, and the nuclei were degenerating. The stratum germinativum appeared normal. The lambs fed the ground basal ration had mild pigmentation occurring in the stratum corneum, and the cells in the stratum granulosum were necrotic, with large vacuoles and pyknotic nuclei. The stratum germinativum was normal. The lambs fed the pelleted basal rations and fortified pelleted ration had lesions of the rumen epithelium that were similar to the lambs fed the basal ground ration. The lambs fed the basal pelleted ration plus bicarbonate had pigmented cells in the stratum corneum of the rumen epithelium, and marked ballooning of the cells occurred in the stratum corneum and granulosum. The histologic observations of the tissue specimens from the lambs fed the various rations employed in this study are summarized in Table I.

In Table II the results are given of the various histochemical procedures employed in an effort to characterize the pigment in the rumen epithelium of lambs fed several rations. In general the results were the same for the various ration treatments. Pigmentation occurred in the epithelium of all lambs; however, the feeding of processed rations tended to accentuate the condition. This pigmented condition has previously been reported by Jensen *et al.*(1). It would appear from the study reported herein that pigmented rumen

TABLE I. Characteristics of Epithelium Lining the Anterior Ventral Sac of the Rumen of Lambs Fed Various Rations.

Lamb No.	Ration	Rumen epithelium: Stratum corneum	Rumen epithelium: Stratum granulosum	Rumen epithelium: Stratum germinativum
674	Conventional*	No pigment	Vacuoles in cytoplasm; degenerating nuclei	Normal
702	"	Flattened	Mild ballooning of cells	"
897	"	Lack of pronounced pigment	Similar to 674	"
643	Ground basal	Thin, keratinized, flattened	Neurotic cells, large vacuoles, nuclei pyknotic	"
671	"	Slight pigmentation	Similar to 643	"
898	"	Mild pigmentation keratinized	" " "	"
650	Pelleted basal	Dark pigmented, thicker than 643	" " "	"
885	"	Light pigmentation, ballooning	Vacuolation, ballooning	"
660	Fortified pelleted ration†	Very thick	Similar to 643	"
681	*Idem*	Definite pigmentation, ballooning of cells	" " "	"
890	"	Dark pigmented cells, ballooning	Pigmented	Pigmented
765	Pelleted ration + bicarbonate‡	Pigmented, thickened layer, ballooning, vacuolation	Degenerative changes	Thick layer
774	*Idem*	Flattened layer, pink staining	Mild ballooning of cells	Normal
838	"	Marked ballooning of cells; ingesta adherent to cells	Ballooning of cells; degenerative changes	Lymphatic infiltration

* Conventional ration composed of shelled corn, long alfalfa hay and supplement.
† Ration fortified with vitamins and minerals. ‡ Sodium bicarbonate added to ration at a level of 1½%.

TABLE II. Summary of Pigment Reaction to Specific Staining Procedures.

Criteria	Rations: Conventional*	High concentrate	Ground mixed	Pelleted†	Pelleted‡	Pelleted + trace minerals	Pelleted + bicarbonate
No. lambs	3	3	3	3	3	3	3
Gross color§	Flesh with dark tendency	Dark	Dark	Dark	Dark	Dark	Dark
H & E pigment	+	+	+	+	+	+	+
Nucleal Feulgen reaction‖	+	+	+	+	+	+	+
Ferric ferricyanide reduction test	+	+	+	+	+	+	+
Oil red O	Maj —	—	Maj —	Maj —	—, +	Maj —	Maj —
PAS	+	+	+	+	+	+	+
Acetylation PAS	—	—	—	—	—	—	—
Deacetylation PAS	Maj —	Maj —	+, —	—	+, —	+, —	Maj —
Methylation PAS	—	—	—	—	—	—	—
Demethylation PAS	—	—	—	—	—	—	—
Bromination PAS	+	+	+	+	+	+	+
Alizarin Red S	Maj +	Maj —	+, —	+, —	Maj +	Maj +	Maj +
Gomori iron reaction	—	+, —	Maj —	Maj —	Maj +	+, —	Maj —
Sulfhydryl "	—	—	—	—	—	—	—
Disulfide "	—	—	—	—	—	—	—

Maj = Majority.
* Conventional ration was composed of shelled corn, supplement and long alfalfa hay.
† Ration composed of ground shelled corn, ground alfalfa hay, molasses and supplement.
‡ Same ration as in footnote (†) plus trace minerals.
§ In this set of samples the gross color was not as different as had been noticed in previous trials.
‖ The pigment was removed by the mild acid hydrolysis in the Nucleal Feulgen reaction.

epithelium may be found in lambs fed a wide variety of rations.

In all instances in which the pigment was observed, the pigment complex was non-fluorescent at 365 mμ, yet exhibited intrinsic blackish-brown color in unstained sections. It was not birefringent. The complex was PAS positive. It lost its PAS positivity upon acetylation or methylation and regained it on deacetylation or demethylation, respectively. It maintained its PAS positivity after bromination. The nucleal Feulgen reaction was invariably negative because of the removal of the pigment by mild acid (HCl) hydrolysis employed in the technic. The complex gave a positive reaction to the ferric-ferricyanide reduction test and a negative reaction to tests for sulfhydryl and disulfide linkages. The pigment was usually associated with (1) substances that gave a positive Prussian blue reaction, (2) stained red with the alizarin red S stain and (3) gave a reddish or white ash residue at the sites of some deposits after micro-incineration. Occasionally, the more superficial deposits of the pigment were associated with sudanophilic substances.

On the basis of these results, the rumen pigment complex may be characterized as containing vicinal 1,2-glycolic and/or 1,2-aminohydroxy groups. It contains substances which are capable of acting as reduction sites, which evidently are not sulfhydryl groups or disulfide linkages of protein and cannot be attributed to lipids since the pigment is not invariably sudanophilic, but invariably reduces ferric-ferricyanide. The pigment is frequently, though not invariably associated with polymerized or epioxidized unsaturated lipids as shown by the frequent presence of solvent resistant sudanophilic substances. It is usually associated with minerals (iron and/or calcium) and is labile to mild acid hydrolysis.

Discussion. It is possible that the pigment could have been a flavonoid compound, such as quercitrin or tricin. These compounds occur naturally in plants (Redemann, 5). The bioflavonoids are insoluble compounds, and most of them form a dark color with Fe^{+++}. Chelation is a characteristic of the flavonoid compounds, (Martin and Szent-Gyorgi, 6). This is only speculation at this time; however, the flavonoids have been shown to accumulate in certain tissues of the body (Martin and Szent-Gyorgi, 6).

The reasons the pigment tends to be deposited to a greater extent when pelleted rations are fed to lambs are not understood at present. Since the character of rumen fermentation is changed on pelleted rations (Rhodes and Woods, 7), it is possible that this may account for the increased deposition of pigment in the rumen epithelium of lambs fed this form of ration. The condition is a surface phenomenon and would be more subject to the influence of the rumen fluid. This would be in agreement with the suggestion of Vidacs *et al.*(2) that the deposition of pigment is a chemical rather than a physical factor.

Summary. The pigment found in the rumen epithelium from lambs fed various rations was characterized by histological and histochemical examination. The pigment was found to be a surface phenomenon and appeared to be more pronounced when lambs were fed pelleted rations.

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