albumin, neither superimposing of the fetal and maternal protein patterns, nor chemical analysis of the 2 bands for polysaccharides reported to contain fetuin(7) were of help in disclosing which of the 2 peaks was fetuin. Both fractions showed a relatively high content of polysaccharides; higher than the corresponding maternal α_1-globulins. Taking an additional reported property of fetuin into account, *e.g.*, the decrease in concentration with the age of fetus(1,8), one can assume that in goats the peak next to the albumin is fetuin. In sheep the conditions are somewhat more complicated, as both the first and especially the second peak decrease with age.

As to the remaining plasma proteins, the α_1-, α_2-, and β-globulins of the goat did not seem to be influenced by the age of the fetus. In the fetal sheep the concentration of α_2-globulins did not show any significant correlation with age; the percentage of the β-globulins tended to increase, but as mentioned before, the number of samples is too small to allow a definite conclusion.

The age-correlated decrease and the relatively high content of polysaccharides in fetuin reminds one of Wharton jelly, a substance of fetal cord rich in mucopolysaccharides(9), which reportedly also decreases in amount with the growing fetus(10). The similar fate and proximity suggest a possible relationship.

Summary. Electrophoretic separation of fetal and maternal plasma proteins in goats and sheep revealed that the fetal plasma lacks γ-globulins and exhibits an additional peak in the area of α_1-globulins. This peak probably corresponds to fetuin. Quantitative conditions of fetal plasma proteins seem to depend largely on age of the fetus. In goats, the percentage of total plasma proteins and of plasma albumin increases; percentage of fetuin decreases with the progressing age of the fetus. The remaining plasma proteins do not seem to be affected. In fetal sheep the percentages of total plasma proteins, plasma albumin and probably of β-globulins increase; levels of fetuin and α_1-globulins decrease with age. The percentage of α_2-globulins remains unaffected.

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Infectious Bovine Rhinotracheitis (IBR) IV. Cytological Changes in Infected Bovine Kidney and HeLa Cultures. (24133)

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Specific intra-nuclear inclusions were observed in routine celloidin preparations of infected HeLa and primary bovine kidney culture monolayers during adaptation to HeLa cells of infectious bovine rhinotracheitis (IBR) virus(1). Since specific localization of increased amounts of desoxyribonucleic acid (DNA)(2) and of other constituents(3) often has resulted from cellular changes produced by infection with other viruses, more detailed cytologic observations of the effects of IBR virus were undertaken. The morphological and chemical effects of IBR virus on cells of an artificial host (HeLa) and of a

natural host such as primary bovine kidney (PBK) were compared. This report presents a comparison of the form and staining properties of HeLa and PBK inclusions following use of Feulgen technic.

Materials and methods. HeLa and PBK cultures were prepared and infected as previously described(1) except that cells were grown on microslips in Leighton tubes. In brief, HeLa cells were grown in Eagle's basal medium(4) with 20% inactivated horse serum. Trypsinized bovine embryo kidney cells, prepared by the method of Dulbecco and Vogt(5), were grown in Earle's basal medium(6) with 20% inactivated cow serum and lactalbumin hydrolysate(7). After monolayers had been established (3-4 days), the growth medium in both HeLa and PBK cultures was replaced by the same control or infected maintenance medium. Control media consisted of supernate from non-infected HeLa cultures diluted (10%) with Ginsberg medium(8). Infectious media consisted of supernate from IBR-infected HeLa cultures diluted (10%) with Ginsberg medium. Examination of both control and infected cultures for signs of cellular degeneration was made daily with a 10x objective. When infected HeLa cultures showed scattered areas of degenerated cells, all cultures were fixed, to insure availability of sufficient cells of both types with inclusions. Waiting for extensive sloughing of HeLa cells would have allowed the PBK cells to degenerate almost completely and to disappear from the glass. The Feulgen technic was based on the method of Gardikas and Israels(9), with substitution of the following wet fix: methyl alcohol 15 ml, distilled water 10 ml, glacial acetic acid 0.25 ml, 40%-formalin 1 ml. For hematoxylin-eosin (H & E) staining, Bouin fixative was used.

Results. H & E staining demonstrated in both types of infected cells a characteristic pattern of nuclear degeneration not seen in control cultures of either type. Inclusions appeared in infected cells before any striking cytoplasmic change could be observed, and resembled in form and general staining properties the H & E-stained primary bovine kidney and human amnion cells reported by Cheatham *et al.*(10). In brief, the normally dispersed, acidophilic, fine nuclear substance condensed and was left surrounded by a fairly uniform clear zone. The nucleoli were reduced in number and displaced toward the periphery of the nucleus. Accompanying margination of the chromatin at the nuclear membrane, resulted in intensified staining with basic dye components, which sharply delineated the nucleus.

The similarity of PBK and HeLa inclusions demonstrated by H & E staining also was demonstrated by use of Feulgen technic. After fixation, the microslips with control and infected cultures of both cell types were removed from their tubes and transferred to the same carrier, making it possible to subject all culture slips simultaneously to hydrolysis, treatment with Schiff's reagent, and counterstaining with Fast Green. In some preparations of both control and infected PBK and HeLa cultures, the Fast Green counterstain was omitted.

In inclusions of both PBK and HeLa nuclei, the absence of DNA was invariably demonstrated, regardless of staining method used or duration of infectivity. In both PBK (Fig. 2) and HeLa (Fig. 4) cultures, the Feulgen-negative inclusions stained readily with Fast Green. Nucleoli of both cell types stained deep green, in sharp contrast to the less intense green of the condensed inclusion and the nuclear membrane. The marginated chromatin of the latter was strongly Feulgen-positive. A decrease of DNA from the nuclei of both cell types also accompanied proliferation of the HeLa-strain IBR virus. This reduction of Feulgen-positive material was evident even at the periphery of the nucleus where the chromatin had marginated, as evidenced by less intense staining.

Use of the Feulgen technic also disclosed two interesting differences between the HeLa and the PBK cells in control cultures. (A) PBK cytoplasm showed relatively less affinity to Fast Green, taking approximately 10 times as long to stain as HeLa. (B) Without counterstaining, the strikingly greater concentration of Feulgen-positive material observed in the smaller PBK nuclei suggested that they contained relatively more DNA

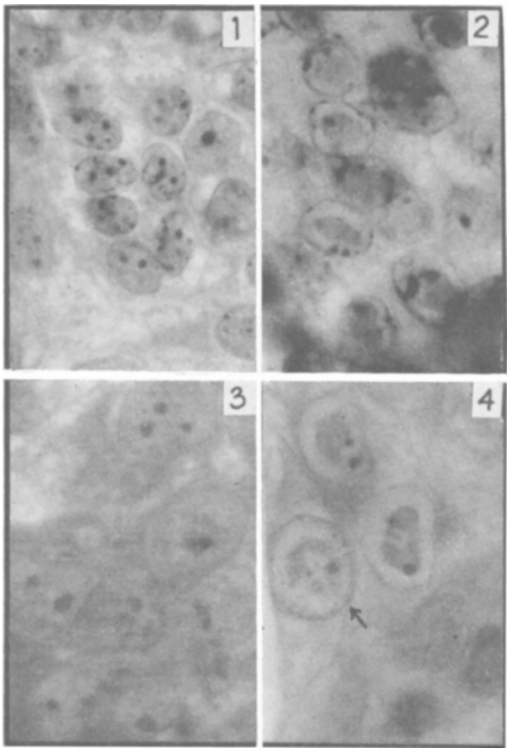

FIG. 1. Uninfected bovine kidney monolayer. Chromatin, acidophilic nuclear substance, and nucleoli are uniformly distributed.

FIG. 2. IBR-infected bovine kidney monolayer with majority of nuclei showing "inclusions."

FIG. 3. Uninfected HeLa monolayer with normal complement of nucleoli, dispersed chromatin and acidophilic element. Note that HeLa nuclei tend to be more obscured by Fast-Green-stained cytoplasm than bovine nuclei in Fig. 1.

FIG. 4. Typical IBR-infected HeLa nuclei, with one (arrow) demonstrating breakdown of the nucleoli into smaller deeply stained (Fast Green) particles.

(All fields are of Feulgen-Fast-Green-stained preparations photographed at ×588.)

than the HeLa nuclei.

Discussion. Although HeLa and PBK cells differed somewhat with respect to size, staining properties and rate of virus-induced degeneration, no difference in morphology or staining properties of the inclusions was demonstrated by infecting the 2 cell types with the same strain of IBR virus in the same maintenance medium. Infection was followed by loss of DNA in both HeLa and PBK cells, and no Feulgen-positive inclusion material could be demonstrated, either during early stages of infection when only a few isolated cells showed inclusions, or in later stages when almost all remaining cells contained well formed inclusions. In this way IBR virus differs from those viruses which cause increased intranuclear DNA inclusion as infection progresses, as is seen in tissues infected with canine hepatitis(11) and in HeLa cells infected with adenovirus(2).

Summary. In both bovine kidney and HeLa cultures, extensive focal nuclear degeneration followed inoculation with a HeLa-adapted IBR virus strain. The pattern of nuclear and cytoplasmic degeneration was similar for the native host (PBK) and the HeLa cell. In cells of both types, nuclear inclusions were Feulgen-negative, and morphologic nuclear changes were accompanied by a reduction of DNA.

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