Histological Features of Respiratory Epithelium of Calves Held at Differing Temperature and Humidity

K. W. F. Jericho and S. E. Magwood*

ABSTRACT

The effect of ambient temperature and humidity on the structure of respiratory epithelium of calves was studied. Four calves of each of three experiments were acclimatized to a nonoperational environmental chamber for six days and then exposed to constant extremes of temperature and relative humidity of one of 30°C — 35%, or 27°C — 92%, or 5°C — 92% respectively in this chamber for eight days each. Five calves (3 and 2) were similarly acclimatized then exposed to 1°C — 40%. Nasal swabs were taken from all animals at regular intervals. Swabs of three animals yielded Mycoplasma spp. and one swab yielded the virus of infectious bovine rhinotracheitis. Detailed histological studies of respiratory epithelium of nose, trachea, major bronchus and terminal bronchioli were conducted at four sites. Goblet cells were least in calves held in hot and dry air; calves held in dry air had the least polymorphonuclear cells and the greatest prevalence of hypochromatic cell layers and vacuolation of epithelial cells. Differences between experiments were evident most for sites of trachea and major bronchus.

RÉSUMÉ

Ces expériences visaient à étudier l'effet de la température et de l'humidité ambiantes sur la structure de l'épithélium respiratoire, chez le veau. Au cours de chacune des trois expériences, on acclimata quatre veaux à une chambre pourvue d'un système de climatisation qu'on ne fit pas fonctionner durant les six premiers jours. On les soumit ensuite, durant huit jours, aux combinaisons de température et d'humidité relative suivantes: 30°C — 35%, 27°C — 92% ou 5°C — 92%. On acclimata aussi, de la même façon, un groupe de trois veaux et un autre de deux; on les soumit ensuite à la combinaison suivante: 1°C — 40%. On préleva des écouvillons nasaux, chez tous les sujets, à intervalles réguliers. On isola ainsi Mycoplasma spp., chez trois veaux, et le virus de la rhinotrachéite infectieuse bovine, chez un seul veau. On effectua aussi une étude histologique détaillée de l'épithélium respiratoire du nez, de la trachée, des grosses bronches et des bronchioles terminales, à quatre endroits de ces différentes structures. Les cellules à gobelet se révélèrent les moins nombreuses, chez les veaux soumis à l'air chaud et sec; ceux qu'on avait soumis à l'air sec recelaient le moins de neutrophiles, le plus de couches de cellules épithéliales hyperchromatiques et vacuolaires. Les différences histologiques engendrées par les trois expériences affectaient surtout la trachée et les grosses bronches.

INTRODUCTION

The influence of climate on the incidence of respiratory disease of pigs and calves is well recognized. The temperature and humidity of air, important elements of climate, are precisely regulated on inspiration by the upper respiratory tract (9, 24, 25). Effects of extremes of climate, such as chilling of body surfaces of human subjects, included reduced temperature of the surface of the nasal mucosa with marked reflex vaso-constriction and diminution of blood supply (11, 22). The effect of the temperature and humidity of inspired air on the morphology of respiratory epithelium is not known. It was therefore thought worth-

*Animal Pathology Division, Health of Animals Branch, Agriculture Canada, Animal Diseases Research Institute (Western), P.O. Box 640, Lethbridge, Alberta T1J 3Z4.

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while to elucidate the influence of extremes of temperature and humidity for eight days on the histological features of respiratory epithelium of calves. A study of this type, within narrower limits, was done by Done (12) using pigs kept at either 50°F or 75°F and 60% or 80% relative humidity for up to 28 days. Differences in histological features attributable to these conditions were not observed. Similarly, rats exposed to normal, hot-moist, hot-dry or dry conditions were without increase of goblet cells in any group (15).

The second objective of this study was to gain a better understanding of the structure of respiratory epithelium of calves. The only other report on the structure of healthy respiratory epithelium of calves deals with the nasal mucosa (23). Furthermore this study was undertaken to provide control material for future experiments under similar conditions which would include exposure to viruses and bacteria in aerosol.

A search for certain common respiratory pathogens was included in this work to indicate whether their presence may have influenced the morphology of the respiratory epithelium, and because low humidity conditions have been shown to influence the nasal flora of mice (20).

MATERIALS AND METHODS

Environmental Chamber

The chamber[1] of patterned aluminium measures 2.45 x 2.75 x 4 m. The range of temperature of which the chamber is capable is –25° to 30°C and relative humidity 20% to 90%. Fresh air, after passing a charcoal filter, entered the chamber at a set rate of 4.5 m^3 per minute, which minimized the accumulation of noxious odours. Calves were kept individually in metal crates[2] 125 x 90 x 50 cm. Each crate had a feed trough and a water bucket attached to it. The animals were chained by the neck enabling them to drink, feed and lie down *ad lib.* Two crates were joined by a common side. Two pairs of such crates were in use. Direct contact was possible for calves of each pair, but not between pairs.

Animals

All calves came from one herd of 65 beef cows at the A.D.R.I. (W) and were used during two summer seasons. It is a semi-closed herd, that is, only three bulls have been introduced to it in the last seven years. In the summer the herd grazes on 1,000 hectares of natural prairie grassland without shelter from winds or sun. In the winter the herd is fed hay, salt and mineral supplement and allowed protection from wind. Clinical respiratory disease has never been observed within the herd. Calves, two females and two males, were allotted to crates alternating in order of sex. The age in days of calves ranged from 92 to 152 and the weight in kg ranged from 104 to 129. Water and good quality alfalfa hay was provided *ad lib.* Livers of four calves tested all had rich reserves of vitamin A.

Experimental Design

A total of five experiments were conducted: A, B, C, D and E. Experiment B had three animals and experiment C, which repeated the conditions of experiment B, had two animals. The remainder of the experiments had four animals. Calves were designated by the letter of the experiment and a number, e.g. A1 or B3. The climatic conditions for each experiment are given in Table I and in code form in Table II. Unexposed calves were not included in this study because the extent of histological variation induced by frequent daily extremes of climate, typical of this region, was not known.

The door of the chamber was left open for the first six days and temperature and relative humidity control was not attempted. This is referred to as the acclimatization period. Vapor pressures were determined (Table I) because evaporation of water vapor from a wet surface depends on the difference in the vapor pressure of the air and the vapor pressure at the surface; vapor pressures at various locations of respiratory tracts of calves are not known. On

[1]Controlled Environments Ltd., 661 Madison Street, Winnipeg, Manitoba.

[2]Automatic Equipment Manufacturing Company, Pender, Nebraska.

the seventh day, the door of the chamber was shut and extremes of temperature and relative humidity produced (Table I). The designated settings for each experiment were reached in three steps over a period of ten to 12 hours. Settings for experiments with low temperature exposure required periodic lowering by 1°C to maintain stressful shivering state of the animals. The climatic conditions were maintained continuously for eight days for all experiments.

Aerosol Exposure

On the second day of climatic stress, all calves were exposed individually for five minutes to an aerosol of medium (90% Eagles minimum essential medium (MEM), 10% fetal calf serum (FCS)-inactivated, 0.2% penicillin-streptomycin and 0.1% mycostatin) which was used in later experiments to suspend a culture of the virus of infectious bovine rhinotracheitis (IBR). The aerosol was produced by a Collison atomizer of a modified Henderson apparatus[3] (13) and carried at a flow rate of 155 liters of air per minute to an animal exposure device (6). The atomizer produced particles with mass median diameters of two to three microns (19). The capability of this apparatus to deposit particles from suspension in all parts of the respiratory tract of cattle has been reported previously (14).

Other Technical Procedures

The following activities were performed in the early morning work period: cleaning feces from chamber, recording of rectal temperatures and rates of respiration, water consumption measures, humidity and temperature verified by sling psychrometer and maximum-minimum thermometers. Feed was weighed and dispensed and rectal temperatures were also taken in the late afternoon. Nasal swabs and blood samples, for serum and plasma (heparinized), were taken up to three times from each animal during the acclimatization period and during the periods of climatic control. Nasal swabs were always taken from the left nostril and were of two types. The standard Swube[4] applicators inserted about 5 cm within the nostril were used for virological sampling, and a deep-nasal swab assembly as used by Magwood (18) was used for bacteriological studies.

Histological Studies

On the eighth day of climatic stress (seventh day of experiment A) all animals were weighed and then killed by pithing with a hand gun. As soon as possible the entire respiratory tract was exposed and removed and the lower trachea and right lung fixed by submerging and inflating the lung with 10% buffered formol saline. The following tissues were placed in the same fixative: mucosae of the posterior aspect of the right ventral turbinate and of the right major bronchus 3 cm past the bifuction of the trachea. Selected pieces of tissue were trimmed, embedded in paraffin and two sections five microns thick were cut from each block and stained either by haematoxylin-eosin (H&E) or by periodic-acid Schiff (PAS). Detailed histological study was confined to respiratory epithelium of nose, trachea, major bronchi and of bronchi and terminal bronchioli of pulmonary parenchyma. These lung tissues were from the ventral third of the apical and cardiac lobes, 5 cm from the tip of the diaphragmatic lobe and 5 cm ventral to the latter site. Every effort was made to take tissue samples for histological study from equal locations in all calves. Epithelium of sections stained with H&E was studied for vacuolation, hyper- and hypochromatic cell layers, and infiltration by polymorphonuclear cells. In sections stained with PAS the distribution of goblet cells and other mucopolysaccharide material was studied. The attempt to count goblet cells per histological slide was abandoned because of differences in size of cells and their unequal distribution in histological sections of nose. Instead, the prevalence of goblet cells, polymorphonuclear cells and vacuolation of epithelium was evaluated at each of ten locations as follows: 1 = absent, 2 = few, 3 = mild, 4 = moderate, 5 = many. The broad categories were considered adequate for differences observed. For each experiment, these estimates of each calf were averaged for each

[3]O'Connell, D. C. and G. J. Leahy, Microbiology Section, Defence Research Establishment, Suffield, Ralston, Alberta.

[4]Falcon Swube Tube, Fisher Scientific Ltd.

location. The estimates of two bronchi of anterior lobes and two bronchi of diaphragmatic lobes of each calf were averaged for all calves of each experiment. The number of hyper- and hypochromatic cell layers were counted and averaged for each location of each experiment.

Statistical Studies

Although the experiments differed in the number of animals (2 - 5, see Table II), the variables for each location were analyzed statistically as if a completely randomized design was used. The Kruskal-Wallis H Test (26) was used to compare the environments since the data could generally not be regarded as normally distributed with homogeneous variance. The test was carried out only for those variables which showed some response for all environments.

Virological Studies

Nasal swabs taken by Swube applicators, tracheas and pooled tissue of the left anterior and diaphragmatic lobes of the lungs were tested for the presence of the virus of IBR using methods detailed by Darcel and Dorward (10).

Serological Studies

The virus neutralizing titres in sera were determined by a microtechnique for tissue culture employing 100 tissue culture infective doses of IBR virus.

Bacteriological Studies

Deep nasal swabs, tissue of trachea and samples from left anterior and diaphragmatic lobes combined were examined for the presence of *Pasteurella* spp. and *Mycoplasma* spp. Cultural examination for bacteria included the maceration of lung tissue (0.5 gm) in 2 ml of horse serum broth without bacterial or mycotic inhibitors in a Ten Broeck grinder. Mycoplasma media prepared according to the methods of Langford and Dorward (17) and dispensed in 1.8 ml amounts were each inoculated with 0.2 ml of the tissue supernatant. After 48 and 72 hours incubation at 37°C aerobically in the first instance and anaerobically in the last, 0.1 ml was transferred to agar medium for appropriate incubation. All plates were examined at 48 and 96 hours incubation and when growth occurred the cultures were identified using the modified growth inhibition test and the fluorescent antibody technique of Boas and Jasper (8) as modified by Tessler (27) using hyperimmune serum prepared essentially by the method of Morton and Roberts (21).

All lungs were examined for the presence of members of the genus *Pasteurella* by inoculating the bovine blood tryptose agar plate[5] with 0.1 ml of the tissue supernatant and streaking this over the plate. All plates were incubated aerobically at 37°C for a maximum of 96 hours. Each plate was examined at 48, 72 and 96 hours for colonial growth indicative of *Pasteurella* spp. Suspicious colonies were picked and checked by staining and biochemical techniques to determine if they belonged to this genus.

RESULTS

Clinical Observations

Animals of experiments B, C and E were maintained at a mild state of thermal discomfort. These animals would shiver, stand with backs arched and tread in place with their hind legs. This critical temperature was about 3°C higher for conditions of high humidity than for low humidity conditions (Table I). Animals kept at high temperature (experiments A and D) were sluggish and listless.

Averages of two daily recordings of rectal temperatures for all calves of each experiment when plotted were all within the range of 38.5°C - 39.2°C for the periods of acclimatization. Data of experiments B and C were combined as both represented similar climatic conditions and the rectal temperatures were comparable in trend and magnitude. For calves kept at high temperature the average rectal temperatures ranged between 38.8 and 39.4°C for experiment D and between 38.9 and 39.9°C for experiment A. For calves kept at low tem-

[5]Difco Laboratories, Box 1058A, Detroit, Michigan 48232.

TABLE I. Temperature, Relative Humidity and Vapor Pressure During Acclimatization and Climate Control

Experiment	Acclimatization Temperature °C Max	Min	Mean	Humidity % Max	Min	Mean	Pressure mm Hg	Climate Controlled Temperature °C	Humidity %	Vapor Pressure mm Hg
A	30	20.5	25.5	55	37	46.4	10 — 12	30	35	8 — 11
B	24.5	9.5	14	75	65	69.8	8	1	40	2 — 3
C	24.5	18	22	95	59	77	15 — 17	2	40	3 — 4
D	24	16.5	19.9	69	44	55.7	7 — 11	27	92	24
E	24.5	18	21	79	51	66.4	8 — 12	5	92	6

TABLE II. Means of Estimates and Counts and Their H Test Factors of Five Characteristics of Epithelium at Ten Locations for all Experiments, and the Number of Samples for Each Experiment at Each Location

Experiment[a]	Location	PAS	Hyperchromatic Cell Layers	Hypochromatic Cell Layers	Vacuolation	Polymorphonuclear Cells	Number of Calves
B + C-T1 H1	Ventral-	3.80	4.40	0.40	1.00	2,20	5[e]
E -T1 H2	Meatus	3.50	6.50	0	1.00	3.50	4
A -T2 H1		2.75	1.50	2.00	2.00	1,00	4
D -T2 H2		4.25	7.75	0	1.75	5.00	4
H Factor[b]		4.62	14.88[c]	—	—	13.38[c]	
B + C-T1 H1	Ventral-	2.00	4.50	0	1.00	1.00	2[f]
E -T1 H2	Turbinate	2.50	6.50	0	3.25	3.25	4
A -T2 H1		—	—	—	—	—	
D -T2 H2		3.00	4.00	0	2.50	3.00	4
H Factor		5.25	7.93[d]	—	3.00	3.75	
B + C-T1 H1	Proximal-	4.40	3.00	0.80	1.00	1.20	5
E -T1 H2	Trachea	4.00	2.00	2.00	1.00	2.75	4
A -T2 H1		3.00	1.00	3.00	1.50	1.25	4
D -T2 H2		3.75	2.75	1.25	1.00	3.25	4
H Factor		9.24[d]	11.55[c]	11.75[c]	—	13.20[c]	
C -T1 H1	Middle-	4.5	3.00	0.80	1.40	1.50	2
E -T1 H2	Trachea	3.85	2.00	2.00	1.00	2.25	4
A -T2 H1		2.25	1.12	3.00	2.50	1.00	4
D -T2 H2		3.85	2.75	1.25	1.00	3.50	4
H Factor		6.06	11.46[c]	12.12[c]	7.89[d]	10.52[c]	
B + C-T1 H1	Distal-	4.00	2.20	1.40	2.00	1.20	5
E -T1 H2	Trachea	4.00	4.00	0	1.00	3.00	4
A -T2 H1		—	—	—	—	—	4
D -T2 H2		4.25	4.25	0	1.00	3.50	4
H Factor		0.80	9.70[c]	—	3.05	9.51[c]	
B + C-T1 H1	Major	3.80	3.00	0	2.40	1.80	5
E -T1 H2	Bronchus	4.00	3.00	0	1.00	1.66	4
A -T2 H1		2.50	1.25	2.50	3.25	1.50	4
D -T2 H2		4.00	3.50	0	1,00	2.25	4
H Factor		5.35	12.71[c]	14.77[c]	7.16	1.43	
B + C-T1 H1	Two Bron-	2.70	2.85	0	2.20	1.50	5
E -T1 H2	chi-Anterior	2.33	2.33	0	1.33	1.00	3
A -T2 H1	Lobes	2.37	2.50	0	3.00	1.00	4
D -T2 H2		2.62	2.06	0	1.00	1.75	4
H Factor		1.44	8.52[d]	—	9.52	—	
B + C-T1 H1	Two Bron-	3.00	2.60	0	2.80	1.00	5
E -T1 H2	chi-Dia-	3.16	2.16	0	1.33	2.00	3
A -T2 H1	phragmatic	2.87	2.37	0	4.37	1.00	4
D -T2 H2	Lobes	3.00	2.50	0	1.25	1.87	4
H Factor		0.67	2.89	—	9.12	3.89	

[a]T1 = low temperature, T2 = high temperature, H1 = low humidity, H2 = high humidity
[b]H statistic for Kruskal-Wallis test
[c]Significant at the 1% level
[d]Significant at the 5% level
[e]Calves of experiment B and C
[f]Experiment C only

peratures average rectal temperatures ranged between 37.8 and 39.2°C for experiment E and between 38.0 and 38.9°C for experiments B and C. A very pronounced circadian rhythm was evident for all days of climatic control of experiments A, B, C and E, but not for the last six days of experiment D. The maximum daily range of average rectal temperatures was 37.8 to 39.2°C on the last day of experiment E. The day following the start of low temperatures animals experienced hyperthermia with a gradual decrease in rectal temperatures as the experiments proceeded. The maximum difference of average rectal temperatures between experiments was 2.1°C.

Averages of two daily recordings of respirations per minute for all calves of each experiment when plotted were all within the range of 40 to 60 for the periods of acclimatization. For periods of climatic control ambient temperature influenced the rate of respiration independently of humidity. For calves kept at high temperatures the average rate of respiration varied randomly and ranged from 60 to 98 for experiment A and 48 to 88 for experiment D. For calves kept at low temperatures a range was all but absent and average respirations were about 22 for experiment E and 18 for experiments B and C. Respirations were shallow for calves at high temperature and deep for calves at low temperature. Abnormal respiratory signs or symptoms were not evident.

Virological Studies

The virus of IBR was isolated from only one nasal swab which was taken from animal E3 on the fifth day of climatic stress. The virus was not cultivated from tracheal tissue of any experiments nor from lung tissue of experiments B, C and D.

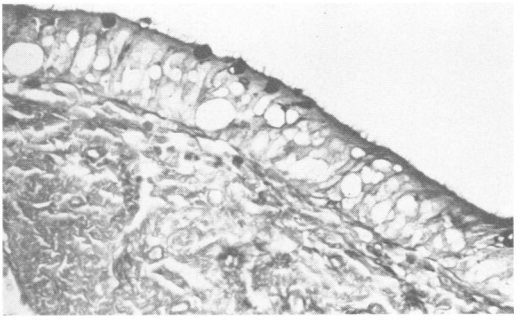

Fig. 1. Major bronchus of animal A3 kept in hot and dry air. Goblet cells are few, small and in superficial position. Vacuolation of epithelium is of variable size but uniform in distribution. PAS. X59.

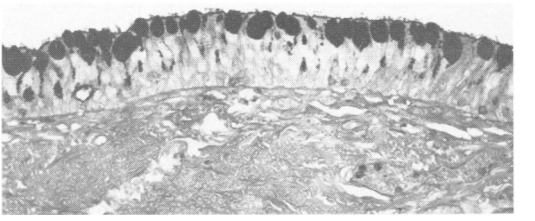

Fig. 2. Lower trachea of animal D3 kept in hot and humid air. Numerous goblet cells uniformly distributed in superficial layer of epithelium. Other mucopolysaccharide material is dotted throughout epithelium. PAS. X48.

Serological Studies

Sera of only four animals, A1, B2, D2 and E1 were negative for antibodies to the virus of IBR throughout the experiments. Animal B2 revealed a positive titre for the period of climatic stress following a serologically negative period during acclimatization. The remainder of the animals had low antibody titres which did not exceed 1/16 and did not change significantly throughout the experiments.

Bacteriological Studies

Pasteurella spp. were not isolated from any deep nasal swabs or lung tissue. For experiments C and E the search for other noteworthy bacteria was included. This yielded nonhemolytic staphylococci from all lung tissue in experiments C and E, and Nocardia-like organisms from all lungs of experiment E. Deep nasal swabs of experiments A, B and E did not yield *Mycoplasma* spp. This microorganism however was cultured from two swabs of animals C2 and D3 before climatic stress and also from swabs of animals D2, D3 and D4 taken on the last day of the experiment. Lung tissue of animal C2 only yielded *Mycoplasma* spp.

Macroscopic Findings

The palatine sinus of animal D2 was filled with cloudy mucoid material. The

only significant lung changes observed were few but obvious fibrinous tags on ventral borders of diaphragmatic lobes of calves kept at low temperature and low humidity in particular, and on some similar lobes of calves of other experiments.

Microscopic Findings

Goblet cells, staining PAS positive, were evident in respiratory epithelium of all locations of all calves. However there were differences in the quantity and distribution of PAS positive material. It was least plentiful in tissue of nose, trachea and major bronchi (Fig. 1) of experiment A. Goblet cells of this experiment were confined to the top one-third of the epithelium. They were small and usually in direct contact with the lumen. In epithelium of calves kept at high humidity goblet cells were plentiful (Fig. 2). At folds of respiratory epithelium goblet cells were generally numerous and not reduced in size even in experiment A (Fig. 3). In all experiments granular PAS positive material was evident in the top two-thirds of respiratory epithelium, but it was least plentiful in experiment A. Comparison of the means for each location of all experiments revealed that goblet cells were fewest for locations of nose, trachea and major bronchus of calves kept at high temperature and low humidity (Table II). Differences were statistically significant at the 5% level for sections of the proximal trachea. Differences in means of goblet cell estimates were negligible for sections taken from the anterior and diaphragmatic lobes.

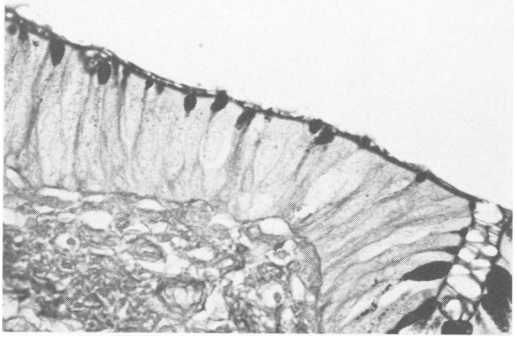

Fig. 3. Bronchus of apical lobe of animal A4 kept in hot and dry air. Goblet cells with PAS positive material are very few, and this material is restricted to the very top of epithelium. At point of folding of epithelium goblet cells are larger and plentiful. Mucopolysaccharides in granular form are evident at site of folding only. PAS. X95.

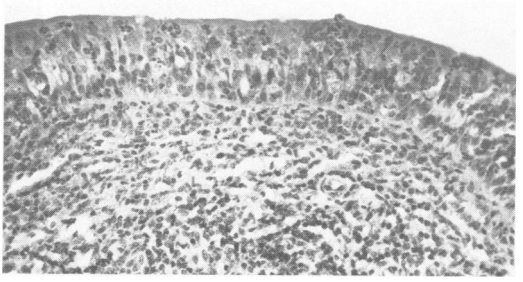

Fig. 4. Right ventral meatus of animal D2 kept in hot and humid air. Epithelium about seven nuclear layers thick is infiltrated by numerous polymorphonuclear cells. The basement membrane is thicker than for bronchi and cilia are not discernible in this instance. Submucosal lymphoreticular tissue is plentiful. H & E. X63.

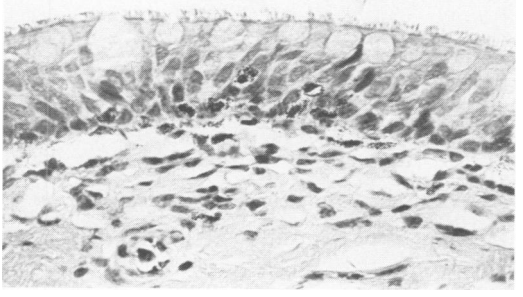

Fig. 5. Major bronchus of animal D3 kept in hot and humid air. Numerous goblet cells are present in the epithelium which has about four layers of cells with intact nuclei. Eosinophils have infiltrated epithelium from the submucosal tissue. H & E. X95.

Epithelium of experiments B, C, D and E was regularly infiltrated by polymorphonuclear cells at all levels of the respiratory tract. This was most prominent for experiment D in which eosinophils predominated often to the exclusion of other inflammatory cells (Figs. 4 and 5). Such infiltrations were least frequent for experiment A. Differences between means of experiments were statistically significant at the 1% level for sections of ventral meatus, and the three locations of trachea (Table II).

Vacuolation of epithelium was evident in all experiments. For locations of middle trachea and distal to it, vacuolation was more plentiful for calves kept at low humidity conditions (Table II). Differences between means of experiments were statistically significant at the 5% level for sections of middle trachea. In epithelium ex-

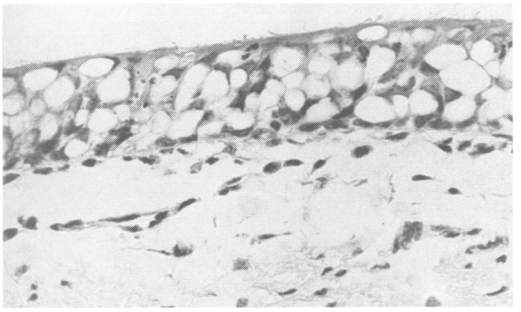

Fig. 6. Bronchus of posterior diaphragmatic lobe of animal A4 kept in hot and dry air. Integrity of epithelium is changed by uniform distribution of vacuoles. Cilia are discernible. H & E. X95.

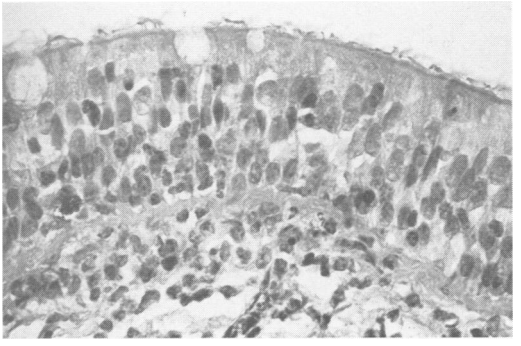

Fig. 7. Right ventral meatus of animal C2 kept in cold and dry air. Epithelium has about seven layers of cells with nuclei stained evenly. Cilia are plentiful and the basement membrane is again much thicker than for bronchi. H & E. X95.

posed to high humidity conditions, vacuolation was infrequent. Vacuoles had no affinity for PAS stain (Fig. 1) and occurred at all strata of epithelium and at all levels of the respiratory tract. Vacuolation was seen generalized (Fig. 6) and localized within bronchi: when localized, it was usually in parts of epithelium which were not folded and appeared immobilized by its firm attachment to its submucosa.

Layers of nuclei of dense chromatic character were of variable number in differing parts of the respiratory tract (Figs. 7 and 8). However, epithelium with hypochromatic nuclear layers was also seen particularly in experiment A. At such sites nuclei with hyperchromatic staining would be reduced to one or two disorderly layers at the basal portion of the epithelium (Figs. 9 and 10). This difference between experiments was significant at the 1% level for sections of ventral meatus, all sites of the trachea and the major bronchus (Table II). Differences in affinity for stain of epithelial cells of air passages of anterior or diaphragmatic lobes were small or absent between experiments.

As a general rule there was agreement in estimates of parameters for any location between animals of any experiment.

The state of cilia varied from intact to disrupted or absent in all experiments at all locations. Intact cilia were also associated with cells with hypochromatic nuclei (Fig. 10). As expected, in terminal bronchioli, cilia were in clusters only.

Basement membranes of sections of nasal mucosa were wider than for mucosa of other sites, where they were reduced to thin lines. In experiments A and B some basement membranes were particularly broad and of amorphous eosinophilic material (Fig. 11).

Metaplastic change of epithelium occurred in two tracheas of experiment A, and was evident as flattened surface lining cells at foci of pathological change (Fig. 12).

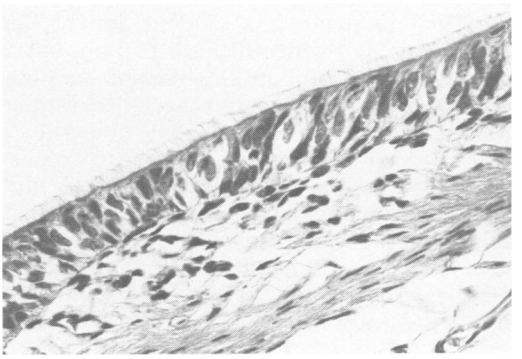

Fig. 8. Bronchus of cardiac lobe of animal D2 kept in hot and humid air has densely staining nuclei of epithelium. Cilia are well preserved. H & E. X95.

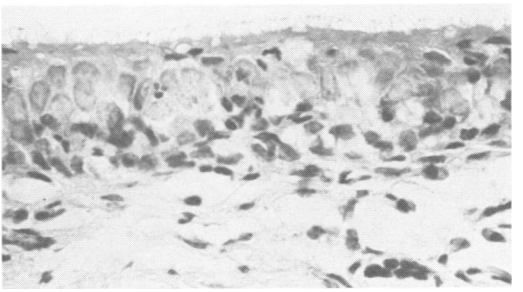

Fig. 9. Middle trachea of animal A2 kept in hot and dry air shows irregular placement of hyperchromatic nuclei and hypochromatic character of top two nuclear layers. Note dense elongated nuclei underneath cilia. Cilia are intact. H & E. X95.

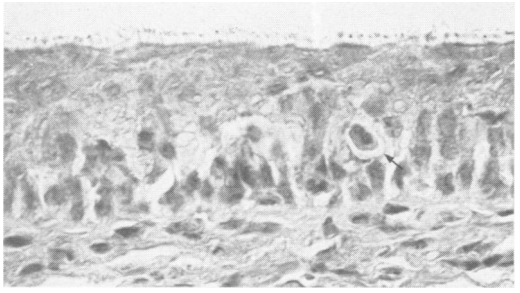

Fig. 10. Upper trachea of animal A3 kept in hot and dry air. Nuclei are only discernible at the basal part of the epithelium. Other nuclei and cytoplasm have formed an amorphous tissue, which is nevertheless supporting a complete complement of cilia. An apoptotic body is surrounded by a halo-like space (arrow). H & E. X95.

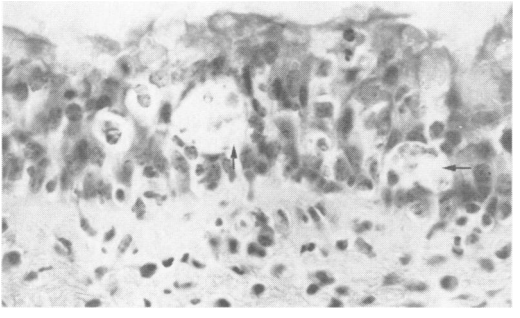

Fig. 11. Right ventral turbinate of animal A1 kept in hot and dry air. This focus of pathological change has a broad amorphous basement membrane. There is degeneration and disorientation of nuclear layers of epithelium. The vacuoles contain inclusion-like material (arrow) with apoptotic bodies nearby. Cilia are not evi-

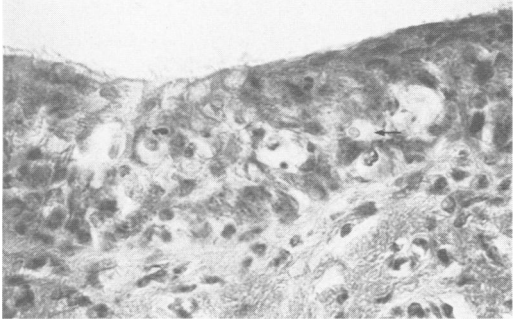

Fig. 12. Upper trachea of animal A1 kept in hot and dry air shows focus of pathological change with vacuolation, degeneration and hypochromatic staining of epithelial cells. One vacuole contains an inclusion-like body (arrow). At the top right, nuclei are elongated and hyperchromatic and cilia are absent. H & E. X95.
dent. H & E. X95.

Focal loss of epithelial integrity occurred in nose, trachea and major bronchi of experiments A and B. Vacuolation, cell infiltration, loss of cilia, epithelial cell degeneration, apoptotic bodies (16) and hypochromatic nuclei were combined in these foci of pathological change (Figs. 11 and 12).

DISCUSSION

The need for physiological adjustments by the calves to the differing climatic conditions was evident from the daily record of rectal temperature and rates of respiration. Evidently the climatic stresses were within the limits of tolerance of the calves. Short-term exposure to higher temperatures and humidity have shown calves capable of responding with much higher rectal temperatures and rates of respiration than required by calves of this study (5, 7). Nevertheless the additional clinical observations of listlessness for high temperatures and shivering for low temperatures indicate that the calves were maintained beyond their zones of thermal comfort.

The absence of *Pasteurella* spp., the low number of other microorganisms and the single isolate of the virus of IBR attests that these common bovine respiratory pathogens did not influence significantly the histological features of the respiratory epithelium studied.

The depletion of mucus of goblet cells of respiratory epithelium of nose, trachea and major bronchi of calves held in hot or dry air suggests a drying effect of these conditions. At sites of folding of epithelium these specialized cells were not reduced in size and number possibly due to reduced ventilation at these sites. Dehydration has been associated with mucus gland depletion. Chicks deprived of water revealed gland depletion by 72 hours (4). However, goblet cell content of terminal bronchioli of calves did not differ between experiments. Air reaching these sites had probably lost its drying power to proximal tissue. Groups of rats exposed to normal, hot-moist, hot-dry or cold environmental conditions for 32 to 34 days have been found without significant difference on goblet cell counts in respiratory epithelium of trachea and lung

(15) although the change in counts for goblet cells was increased for tracheas for hot and dry conditions, and not decreased as in this study. Inter-species differences in physiological parameters of, for example, respiratory rates and volumes may well be of some influence on prevalence of goblet cells when inhaled air is hot, cold, moist or dry.

Hypochromatic nuclear layers were prevalent in calves which were held in hot and dry air and had a similar distribution within the respiratory tract as goblet cells of the same calves. The influence of these air conditions on the staining characteristics of superficial epithelial cells and their cellular detail is without explanation.

Polymorphonuclear cells in epithelium of nose and trachea were numerous in calves kept in humid air and few in calves kept in dry air. This difference was not evident for tissue of major bronchi and bronchioli. Conversely vacuolation was prevalent in tissue of middle trachea and distal to it for calves held in dry air and all but absent for calves held in humid air. These differences are also without explanation.

The influence of temperature and humidity on the functional characteristics of respiratory epithelium of calves is not known. In chickens which were deprived of water for 72 hours movement of mucus flow was decreased in the trachea of intact birds or stopped in the nasal cavities of killed birds (2). Similarly clearance of foreign droplets in trachea of intact birds was related to the temperature and vapor pressure of the inhaled air (3). The effect of humidity on clearance of nasal mucus in humans was investigated by Andersen *et al* (1) who found that neither high or low ambient humidity impairs mucociliary clearance.

The present study has shown that (a) respiratory epithelium of calves has variable histological features at any site and (b) these features appear to be influenced by the temperature and humidity of the inspired air. Further work is needed to confirm results of this study and to learn about the effects of other constant and variable climatic regimes.

The effect, if any, of these histological variations on functional differences of the tissue and in particular to susceptibility to disease are of special interest. For this purpose these experimental conditions were repeated and each calf exposed for five minutes on the second day of climatic stress to an aerosol of the virus of IBR. The results of these experiments will be reported separately.

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BOOK REVIEW

PROCEEDINGS OF THE FIRST VIDO SYMPOSIUM ON NEONATAL DIARRHEA. *Published by Veterinary Infectious Disease Organization, University of Saskatchewan, Saskatoon. 1976. 155 pages. Price $5.00.*

The Veterinary Infectious Disease Organization (VIDO) is described in a foreword to these Proceedings as "an essentially independent research unit..... dedicated to research aimed at controlling indigenous infectious diseases of food-producing animals". The purpose of the Minisymposium was to exchange ideas among workers in the field of neonatal diarrhea and to advise VIDO on a productive applied research path in this area. The symposium was held in May 1976, and VIDO is to be congratulated on publishing the Proceedings in good time, before much of the material becomes out of date. However, this haste may have contributed to the rather poor technical quality of this volume. Thus the arrangement of the tables and figures does not make for easy reference, there are numerous spelling errors, and the reviewer's copy disintegrated after some preliminary thumbing of the pages. The Proceedings contain 16 papers, with a major emphasis on *Escherichia coli*, since seven papers deal with this organism. These range widely in content from the rather basic scientific contribution by Gyles on extrachromosomal virulence-enhancing factors of *E. coli* to those dealing with the use of vaccines in the field, by Myers and Wilson. Relative to *E. coli*, the viruses receive somewhat scant attention, although the bovine rotavirus and coronavirus are dealt with by Mebus, and the same agents receive some attention in papers by Acres and Morin. The porcine enteric viruses are treated even less generously, particularly the porcine rotavirus, the discovery of which was perhaps too recent to be included in the symposium, although it is probably an important pathogen. In addition to the formal papers, the Proceedings contain a transcript of the discussions. Although much of this material appears somewhat banal in print, the exchanges between Drs. Radostits and Wilson on pages 58 and 59 were clearly highly entertaining. Workers in the field of enteric diseases in cattle and swine will wish to be familiar with the content of the Proceedings, and a limited number of copies are still available to interested researchers through VIDO at Saskatoon. — *J. B. Derbyshire.*