clostridial lecithinases, and perhaps the majority of hemolytic toxins, may depend on their ability to disrupt the delicate lipid-protein relationships within membranes of living mammalian cells. Specific lipoproteins, like the experimentally induced streptolysin O inhibitor in mouse plasma, or the naturally-occurring streptolysin S inhibitor in human and animal sera(11,12), may, by way of similarity to some structural sub-unit of the susceptible membrane, combine with and competitively inactivate such toxins.

Summary. A simple technic is described for separating streptolysin O inhibitor from the plasma of mice made refractory to the lethal effect of streptolysin O. Results of fractionation and subsequent analysis indicate that the bulk of the inhibitory activity of refractory mouse plasma is associated with a lipoprotein of extremely low density (<1.006) containing abundant lipid relative to protein. All classes of serum lipids are represented in the inhibitor-rich fraction; their concentrations, as well as the concentration of protein, are significantly greater than values obtained for comparable fractions from normal mouse plasma. Esterified cholesterol shows the greatest increment and seems most closely to parallel the inhibitory activity. It is suggested that the capacity of the induced lipoprotein to inhibit streptolysin O depends on an abundant content and unique orientation of lipids that are capable of interacting with the toxin.

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Cytochemical Observations on Intranuclear Inclusion of Feline Viral Rhinotracheitis Virus. (28620)

ROBERT A. CRANDELL* AND DAVID F. HERSEY† (Introduced by S. S. Kalter)
Armed Forces Institute of Pathology, Washington, D. C.

The sequence of the development of inclusion bodies in cultures of feline renal cells infected with feline viral rhinotracheitis virus has been reported(1). In hematoxylin and eosin preparations, the earliest discernible change was stippling of the nuclei with basophilic chromatin. Later, these chromatin granules appeared as fine particles dispersed on a light-staining background of nuclear substance. These granules and the nucleoli eventually marginated and the nuclear material contracted, forming an acidophilic inclusion surrounded by a clear halo.

Histochemical methods have been used to demonstrate the presence of desoxyribonucleic acid in animal tissue and cell cultures

* Present address: USAF Epidemiological Laboratory, Lackland Air Force Base, Texas.

† Present address: Science Information Exchange, Washington, D.C.

infected with a number of viruses(2,3,4-6,7). The May-Grunwald-Giemsa stain and the Feulgen technique have been used for demonstration of desoxyribonucleic acid. Acridine orange staining has recently(8,9) been employed for differentiation of desoxyribonucleic acid and ribonucleic acid in cells.

In the present study, these 3 methods were used to observe the cytochemical and the morphologic changes during infection of feline renal cell cultures with feline viral rhinotracheitis virus.

Materials and methods. Cultures of feline renal cells were prepared on 11 × 22 mm coverslips in Leighton tubes by previously described methods(10). The maintenance medium at time of inoculation was 0.5% lactalbumin hydrolysate containing 2% lamb serum. The cell cultures were inoculated with 100 $TCID_{50}$ of virus and incubated at 37°C. At 12, 24, 36, 48, and 72 hours following inoculation, control and infected coverslips were stained with May-Grunwald-Giemsa stain according to Jacobson's method as described by Orr(5). At the same time intervals, duplicate coverslips were stained by Feulgen technique. Coverslips were fixed in Carnay's fluid and stained by means of a modification of the technique described by Stowell(11). These preparations were counterstained with fast green. Specificity of the Feulgen reaction was demonstrated by incubating coverslips in 95% alcohol for 18 hours (to remove lipids) prior to hydrolysis and by staining of similar preparations without hydrolysis. Hydrolysis for 11 minutes at 56°C gave the most satisfactory results and was used throughout this study. Some preparations were stained with Giemsa solution and then treated by the Feulgen technique. This permitted a comparison of the same cells and inclusions by both methods. A third set of coverslips was fixed in ethyl alcohol and ether and stained with acridine orange (AO) according to the method described by Bertalanffy(9). The stained coverslips were examined under ultraviolet light with an Osram HBO 200-watt light source and a Leitz Ortholux-VAM microscope.

Results. Staining characteristics of normal cells: With the Feulgen technique, the nuclei stained pale pink, indicating a fairly even distribution of desoxyribonucleic acid. The nucleoli appeared as green, irregularly shaped bodies. In areas of the monolayer where active multiplication occurred, as evidenced by numerous mitotic figures, there was a strong positive Feulgen reaction. Pyknotic nuclei reacted similarly.

In the May-Grunwald-Giemsa preparations the desoxyribonucleic acid appeared reddish-purple: the ribonucleic acid in the nucleoli and cytoplasm blue. The nucleus was filled predominantly with numerous small reddish-purple granules intermingled with a few blue-staining particles.

In the acridine orange preparations the cytoplasm and nucleoli stained various shades of orange. The nuclei were vivid green. Specificity of the staining reaction was confirmed by treatment of the coverslips with desoxyribonuclease or ribonuclease prior to staining with acridine orange.

Observations on infected cells: The same sequential morphological changes described previously(1) resulting from H and E were observed in the present study following the Feulgen and May-Grunwald-Giemsa techniques.

The Feulgen-positive material in the nucleus appeared to be identical with the chromatin stippling described as an early nuclear change stained red. Later, the nucleus was filled with a homogeneous mass varying between shades of pink and vivid red. In the final stage, the inclusion body stained a lighter pink. The nucleoli fragmented, marginated near the nuclear membrane, stained deep green and provided a sharp contrast to Feulgen-positive nuclear material.

With the May-Grunwald-Giemsa stain the nuclear material stained reddish-purple and the nucleoli appeared as dark-blue bodies. The intensity of the reddish-purple color was the same during all stages of inclusion body formation.

More nuclear detail was observed with the May-Grunwald-Giemsa stain than with the Feulgen or acridine orange techniques. The inclusion bodies appeared to be composed of aggregates of reddish-purple circular bodies containing a dark center.

Feulgen-positive granules were not observed in the cytoplasm of infected cells, although some granules could be seen in the cytoplasm of cells stained by the May-Grunwald-Giemsa technique. No identity could be ascribed to this material.

All infected coverslips observed with AO staining revealed morphologic patterns similar to those described for preparations stained with May-Grunwald-Giemsa stain. Various nuclear alterations were observed, from stippling to the formation of large desoxyribonucleic acid aggregates. In the early stages the cytoplasm appeared bright orange. In the later stages some desoxyribonucleic acid appeared in the cytoplasm, as evidenced by the presence of areas of intense green staining concomitant with advanced cytopathic changes.

Discussion. In general, a good correlation was observed by the three staining techniques employed in that desoxyribonucleic acid was demonstrated in the nucleus of feline renal cell cultures infected with feline viral rhinotracheitis virus. Slight differences were apparent with the 3 methods employed.

The little bodies demonstrated within the inclusion mass stained by the May-Grunwald-Giemsa method have not been observed when the other staining techniques were used.

Inclusions stained by the Feulgen technique during the later stages of infection showed a less intense reaction than that seen in the earlier stages. A decrease in staining intensity in the later stages has also been reported with viral inclusions induced by herpes virus(12) and equine abortion virus(13). This difference in staining reaction between the early and late stages was not observed with the other 2 staining methods.

Another difference noted between the Feulgen, the May-Grunwald-Giemsa, and the acridine orange techniques was the positive staining of desoxyribonucleic acid in the cytoplasm when infected cells were stained by the latter two methods. In the terminal stages of infection large masses of green material were demonstrated in the cytoplasm by the acridine orange technique.

Growth studies(14) of this virus have shown that the release of infective virus is associated with the appearance of inclusion bodies. There is no evidence from the present study that these inclusions contain virus; however, viral particles have been demonstrated in both the nucleus and cytoplasm of infected feline renal cell cultures by electron microscopy(15).

Summary. Examination of feline renal cell cultures infected with feline viral rhinotracheitis virus and stained by the May-Grunwald-Giemsa, acridine orange, and the Feulgen techniques showed that there were definite morphological alterations with biochemical changes within the cell. The evidence suggests that the cytologic changes occurred in the nucleus first. The development of the inclusion body was accompanied by an increase in staining reaction for desoxyribonucleic acid in the nucleus. The Feulgen technique revealed a decrease in staining intensity of the nucleus in the later stages of inclusion development. Such results were not obtained with the May-Grunwald-Giemsa and acridine orange stains. With these latter 2 staining methods the terminal stages of infection were characterized by an increased accumulation or redistribution of desoxyribonucleic acid-staining material in the cytoplasm.

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Relationship of K+ to H+ in Histamine-Stimulated Canine Gastric Juice.* (28621)

HELENA GILDER† AND FRANK G. MOODY (Introduced by F. Glenn)

Department of Surgery, New York Hospital-Cornell University Medical Center, New York City

A constant relationship between potassium ion (K+) and hydrogen ion (H+) in histamine-stimulated gastric juice has been noted by some observers(1) but denied by others (2,3). This investigation presents evidence from canine Heidenhain pouch juice which indicates that such a relationship exists.

Methods. Vagally denervated Heidenhain pouches were prepared in 8 mongrel dogs weighing between 10 and 16 kg. A nylon cannula served to connect the pouch to the exterior. Dogs were on regular kennel ration, and were fasted 20 hours before study. Half of the experiments were carried out on conscious dogs lying quietly on the table. In the other half, where intravenous infusions were required, light Nembutal anesthesia was used. Results were the same in the 2 groups.

The experimental procedure was as follows: The pouch was evacuated at timed intervals (10 to 20 minutes) by gentle aspiration with a 2-inch length of thin-walled, collapsible rubber tubing connected to a one-inch narrow bore glass adapter, and a 10 ml syringe. The aspirate was placed in a 15 ml graduated centrifuge tube. Four ml of distilled water were then drawn into the same syringe, instilled into the pouch and immediately withdrawn and added to the centrifuge tube. Two ml of air were next injected into the pouch and withdrawn along with the fluid remaining in the cannula. The opening was closed by flattening and clamping the rubber tubing close to the cannula.

Pouch secretion was stimulated either by subcutaneous injections of 0.5 or 1 mg histamine phosphate, singly, or intermittently every 10 minutes, or by intravenous injection of histamine phosphate in 5% glucose at a rate between 2.6 and 4.9 μg/kg/min.

The volume of the juice plus the wash was measured in the graduated tube and the volume of juice determined by deducting the volume of the wash from the total.

Na+ and K+ were measured on the Beckman DU flame photometer; Cl— was measured by the Schales method(4), and acid by titration to pH 7 with 0.01 N NaOH using phenol red indicator.

Results. Fig. 1a summarizes the typical pattern of electrolyte secretion in an experiment in which 2 injections of histamine were given sequentially, the pouch being allowed to return to base line secretion rate before the second injection. After each injection, the Na+ and K+, then the Cl—, H+ and volume had peak outputs. Fig. 1b is a plot of the K+ against H+ output showing that whereas points fell above the line in periods 1 and 2 after the first injection, and in periods 1, 2 and 3 after the second injection, all subsequent points after both injections fell on the same line.

In 6 experiments the pouch was stimulated continuously (see methods). Fig. 2 and 3 show the relation of the K+ to the H+ output in 2 such experiments. The numbers near the points indicate the sequence of the first 6 samples, and show that the first 4 in Fig. 2, and the first 3 in Fig. 3 fell above the straight line formed by all subsequent points. The lines drawn are regression lines calculated

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