dicate no G. I. alteration of the H^3-stearic acid compared to C^{14}-stearic acid.

During chloroform-methanol lipid extraction, the possibility was considered that H^3 might exchange with H_2O washings. This possibility was checked by testing portions of the non-lipid water-methanol fractions for radioactivity; none was found. From this it would appear that the label deposited in both liver and epididymal fat pad remained in the lipid as extracted.

Due to previously demonstrated aberrations in phospholipid fractions during fatty acid studies(1,7) in which a fatty acid molecule was altered by a label, the remainder of this work dealt with comparisons of H^3 and C^{14} within the lipid fractions as separated by silicic acid columns. Variation was most evident in the fat pad, especially for both fraction c and fraction d. Finding the label in fraction c, especially for the fat pad, was of interest. It has been shown that fat tissue produces little cholesterol(8,9). This then represented incorporation of the labels into monoglycerides and diglycerides, deposition of liver cholesterol in the fat pad, or was of some significance in generation of cholesterol. Because of this finding plus the fact that H^3 seemed to show this phenomena more than C^{14}, we considered the possibility that this resulted from alterations to the H^3 label brought on by the silicic acid columns. 8.6% of the stock H^3 labeled stearic acid turned up in fraction c (Table III) when processed through the column. Here, then, was the first glaring example of apparent inaccuracy in use of the H^3 labeled stearic acid. Even accounting for H^3 stearic acid contamination, the count in fraction c is impressive. According to the studies in rat liver of Dittmer and Hanahan(3) using stearic-1-C^{14}, stearic acid has a propensity to incorporate into both phospholipid and esterified cholesterol. What we observed, then, may be an extension of this phenomenon to fat tissue, may represent some form of cholesterol transport, or may indicate incorporation of H^3 into monoglycerides and diglycerides. Further investigation already under way seems to indicate the last possibility.

Summary. The stability of orally administered stearic-9, 10-H^3 appears comparable to that of stearic-1-C^{14} in its incorporation into lipid in rat liver and epididymal fat pad. However, column fractionation of tissue lipids and stock labeled fatty acids suggests a difference in behavior of stearic acid labeled in these 2 ways.

1. Van Handel, E., Zilversmit, D. B., *J. Lab. and Clin. Med.*, 1958, v52, 831.

2. Colowick, S., Kaplan, N., *Methods in Enzymology III*, 1955, Academic Press, 310.

3. Dittmer, J. C., Hanahan, O. J., *J. Biol. Chem.*, 1959, v234, 1976.

4. *Proceedings of Symposium on Advances in Tracer Applications of Tritium, N. Y.*, Boston, Sheldon Press, 1958.

5. Blomstrand, R., Dahlback, O., Linder, E., PROC. SOC. EXP. BIOL. AND MED., 1959, v100, 768.

6. Glascock, R. T., Reinius, L. R., *Biochem. J.*, 1956, v62, 529.

7. Artom, C., Peretti, G., *Boll. Soc. Ital. Biol. Sper.*, 1932, v7, 930.

8. Feller, D., *J. Biol. Chem.*, 1954, v206, 171.

9. Miller, J. P., Cooper, J. A., *Biochem. Biophys. Acta,*

Received July 20, 1960. P.S.E.B.M., 1960, v105.

Growth of Feline Viral Rhinotracheitis Virus in Cultures of Feline Renal Cells. (26042)

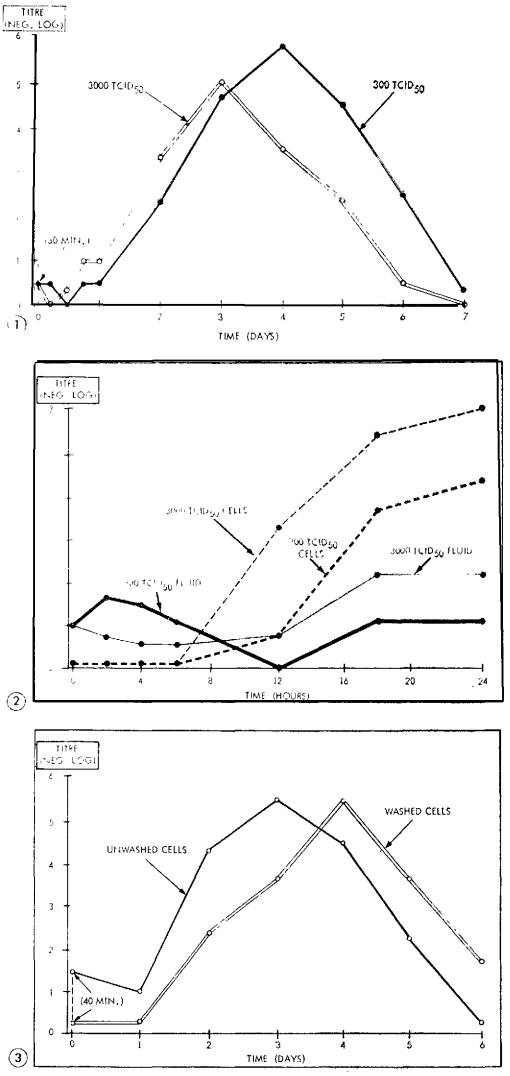
FORD F. EBNER AND ROBERT A. CRANDELL (Introduced by Fred D. Maurer)
Armed Forces Institute of Pathology, Washington, D. C.

The isolation of a virus which induces rhinotracheitis in cats and produces a cytopathic change in cultures of feline renal cells has been reported(1,2). This report deals with the growth of feline viral rhinotracheitis (FVR) virus in cultures of feline renal cells and the

correlation between release of infective virus and appearance of inclusion bodies.

Materials and methods. Methods for preparation and maintenance of feline renal cell cultures have been described(2). The maintenance medium at time of virus inoculation was 0.5% lactalbumin hydrolysate in Hanks balanced salt solution fortified with 3% lamb serum. The 5th tissue culture passage of the original isolate that had a $TCID_{50}$ of 10^{-5} 0.1 ml was used throughout. One group of culture tubes was inoculated with 0.1 ml of virus containing a calculated 300 $TCID_{50}$ dose. A second group received 0.1 ml of virus containing a calculated 3,000 $TCID_{50}$ dose. At selected time intervals following inoculation, the fluid from 3 tubes at each dosage level was harvested and pooled. The cells were removed from the glass surface with a rubber "policeman" and washed 3 times in maintenance medium. The fluid and cell harvests were stored at −20°C until assayed. At the time the fluid and cell harvests were made, collodion sections were prepared from replicate tubes and stained with hematoxylin and eosin for cytologic studies. For virus assay, fluid and cell samples were thawed at room temperature. The cells were disrupted by addition of 0.2-mm glass beads and shaken on the Mickle tissue disintegrator for 3 minutes. For titrating the cellular and extracellular virus, serial 10-fold dilutions were prepared, and 0.1 ml of each dilution was inoculated into each of 4 feline renal culture tubes, which were observed for 6 days. Virus titres were calculated by the method of Reed and Muench and are expressed as the negative logarithm per 0.1 ml of that dilution which caused cytopathic change in 50% of the cultures. Fluid and cells collected during the first 18 hours were also tested for viral content in the undiluted form.

In another experiment, two 32-oz. prescription bottles containing a monolayer of feline renal cells were inoculated with 500,000 $TCID_{50}$ doses of virus in a total volume of 50 ml of maintenance medium to determine(1) when maximum titre would occur if large quantities of virus were used, and (2) the effect on the growth curve of washing the cell



layer. The fluid from 1 bottle was removed after 30 minutes of incubation, and the monolayer was washed 3 times with maintenance medium. The original volume was replaced with the latter medium. The first fluid sample was harvested from both bottles 40 minutes following inoculation with the virus. One milliliter of fluid was then removed at 24-hour intervals from each bottle for a period of 6 days and stored at −20°C until assayed. These samples were titrated as described above.

Throughout the study all cultures were incubated at 37°C. A simultaneous titration of the virus pool was performed each time the

tubes and bottles were inoculated and $TCID_{50}$ dose calculated.

Results. The growth curves shown in Fig. 1 represent results obtained in cultures inoculated with 300 $TCID_{50}$ or 3,000 $TCID_{50}$ doses of virus. The titre at 30 min for the 300 $TCID_{50}$ curve was less than 1 log. At 12 hr no virus could be demonstrated, but newly released virus was found at 18 hours. This steadily increased to a maximum virus titre of $10^{-5.6}$ on the 4th day. The logarithmic decrease following the peak titre corresponded to the release of cells from the glass surface.

The level of measurable virus at 30 minutes with the 3,000 $TCID_{50}$ level was not significantly different from the 300 $TCID_{50}$ dose curve. At 6 hours there was no detectable fluid virus, but the 12-hr sample indicated early multiplication. The titre between 18 and 24 hours was 10^{-1} before ascending rapidly to a peak level of 10^{-5} on the 3rd day. The logarithmic decrease was earlier with the 3,000 $TCID_{50}$ dose than with the lower dose. About 3 days after the maximum titre in both curves, the titre was almost undetectable.

The amount of cellular virus demonstrated during the first 24 hours is shown with the extracellular (fluid) virus in Fig. 2. Cellular virus infective for tissue culture was not detected during the first 6 hours but was present at 12 hours. With the 300 $TCID_{50}$ inoculum a small amount of virus was demonstrated intracellularly at 12 hours, but none was detected in the fluids at this time. Virus content of the cells which had received the higher dose was more than 1 log higher than that in the fluid at 12 hours. Beyond 12 hours until approximately at 48 hours, cellular virus concentration remained considerably higher than extracellular virus. The extracellular virus then exceeded the cellular virus by about 1 log in titre.

Results of replicate growth curves demonstrating extracellular virus for the 2 levels of inoculum during the first 12 hours, sampled at 30, 50, 70, 90, and 110 minutes and at 2-hour intervals thereafter, were in agreement with the curves shown in Fig. 2. Although there were differences in amount of detectable virus for each curve during the first 12 hours, it was always less than 0.5 log.

The effect upon the growth curve of washing the monolayer 30 minutes following inoculation is shown in Fig. 3. The fluid titre 40 minutes following virus inoculation from the culture bottle in which the monolayer was not washed was $10^{-1.5}$. This decreased to 10^{-1} by the 1st day. After the 1st day, virus content increased rapidly and yielded a maximum titre of $10^{-5.5}$ on the 3rd day. Virus titre decreased steadily until it was $<10^{-1}$ by the 6th day. The amount of fluid virus in the washed culture bottle during the first 24 hours remained constant at $<10^{-1}$. Quantities of virus released into the fluid increased after the 1st day at a slower rate than in the unwashed bottle and attained a maximum titre of $10^{-5.5}$ on the 4th day. Rate of decrease of virus titre from the 4th day to the 6th day was similar to that in the unwashed bottle.

Nuclear changes suggestive of virus activity were observed in the cells inoculated with 3,000 $TCID_{50}$ doses at 18 hours, but were not observed in the cell cultures inoculated with 300 $TCID_{50}$. Well-formed intranuclear inclusion bodies were demonstrated in stained preparations of feline renal cell cultures 24 hours following inoculation of 300 and 3,000 $TCID_{50}$ doses of virus.

Discussion. When cultures of feline renal cells were infected with 2 different dosages of FVR virus, a characteristic viral growth curve was obtained; *i.e.*, following a brief latent period when little or no detectable virus was present, virus content increased to a maximum yield and then declined. The only significant difference demonstrated by comparing the 2 inocula was in time; namely, a shorter latent period, more rapid rise to approximately equal titre, and an earlier degeneration of the cell layer at the higher dosage level. The decrease in detectable virus which preceded the release of new extracellular virus can be explained as the period of attachment or absorption of virus to the cells and/or a period of eclipse when no virus was detectable in the cells. It is apparent that the virus was in a state which cannot be detected by infectivity tests for the period between 6 and 12 hours postinoculation.

Nuclear changes with well-formed inclusions were observed about the time that extra-

cellular virus was demonstrated. We did not, however, observe nuclear alteration at the time new virus was detectable within the cells, as reported with polio virus(3). Mature inclusion bodies have been demonstrated concomitantly with release of extracellular virus in growth studies of canine hepatitis virus (4,5).

Unpublished results in our laboratory show that the FVR virus is antigenically unrelated to the kidney cell degenerating virus (KCD) (6), which also was obtained from a cat. In addition to antigenic differences, these 2 viruses can be differentiated on the basis of their respective growth pattern and cytopathic changes in cultures of feline renal cells. FVR virus multiplied at a slower rate than the KCD virus, which, when an inoculum of 1,000 $TCID_{50}$ doses was used, reached its peak titre by the 21st hour. The main cytopathic differences between FVR virus and KCD were the presence of intranuclear inclusions and the longer time required for cytopathic change to appear in infected cultures with the FVR virus.

Summary. The growth pattern of feline viral rhinotracheitis virus in cultures of feline renal cells was investigated. Following an initial period of 18 to 24 hours during which little or no virus could be detected, virus concentration increased steadily until a maximum titre of $10^{5.5}$ was reached between the 3rd and 4th days. Intranuclear inclusion bodies were demonstrated in infected cells at the time of release of new extracellular virus.

1. Crandell, R. A., Maurer, F. D., PROC. SOC. EXP. BIOL. AND MED., 1958, v97, 487.
2. Crandell, R. A., Despeaux, E. W., *ibid.*, 1959, v101, 494.
3. Reissig, M., Howes, D. W., Melnick, J. L., *J. Exp. Med.*, 1956, v104, 289.
4. Leader, R. W., *Am. J. Vet. Res.*, 1958, v19, 152.
5. Carmichael, L. E., *Cornell Vet.*, 1959, v59, 504.
6. Fastier, L. B., *Am. J. Vet. Res.*, 1957, v18, 382.

Received July 21, 1960. P.S.E.B.M., 1960, v105.

Studies on Tetracycline-resistant *Escherichia coli* Strains and a Growth-requirement for l-Histidine. (26043)

ARTHUR R. ENGLISH
Bacteriology Laboratory, Chas. Pfizer & Co., Inc., Maywood, N. J.

In contrast to its tetracycline-sensitive parent, it was found that a laboratory-developed, tetracycline-resistant *E. coli* strain would not grow in Witkin M-9 synthetic medium(1). Additional studies indicated that the tetracycline-resistant strain had a specific growth requirement for l-histidine. Attempts were made to determine whether or not a metabolic relationship existed between histidine and tetracycline resistance in clinically-isolated, tetracycline-resistant *E. coli* strains.

Methods and discussion. The parent *E. coli* has been maintained a number of years in this laboratory by regular transfer in brain heart infusion agar. The tetracycline-resistant strain was selected by a serial transfer procedure in brain heart infusion broth(2). In the current studies, the parent *E. coli* was maintained on M-9 synthetic agar medium. Surface growth was washed off, sedimented by centrifugation, and washed 3 times with 10 ml aliquots of saline. The tetracycline-resistant strain was maintained on brain heart infusion agar. Such cells were washed as above with 3 separate aliquots of 10 ml sterile saline.

Growth was determined as percent of incident light transmitted in the Evelyn photoelectric colorimeter. This approximated 50% transmittance (660 mu filter) for the parent *E. coli* in M-9 medium after 18 hours incubation at 37°C. In contrast, the laboratory-developed, tetracycline-resistant *E. coli* failed to show any indications of growth in this medium even after 48 hours incubation at 37°C.

In an effort to supply a necessary growth factor, a variety of amino acids, vitamins, indole, and purine bases (at 10 μg/ml) in