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Unpublished results in our laboratory show that the FVR virus is antigenically unrelated to the kidney cell degenerating virus (KCD) (6), which also was obtained from a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.

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

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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

TABLE I. Effect of Adjuvants on Growth of *Escherichia coli* (Tetracycline-Resistant) in Witkin M-9 Medium.

Adjuvant group*	Composition of group	Growth of *E. coli*, as % light transmittance, %
I	l-Leucine dl-Threonine l-Isoleucine l-Histidine l-Methionine	54
II	Cytosine l-Valine l-Phenylalanine l-Tryptophane	99 (no growth)
III	Indol Guanine l-Arginine l-Alanine	99 (no growth)
IV	Thiamin Riboflavin Pantothenate Folic acid Biotin	99 (no growth)
Individual components of Group I	l-Leucine	99
	dl-Threonine	99
	l-Isoleucine	99
	l-Histidine	50-60
	l-Methionine	99
Controls		
Parent *E. coli* (tetracycline-sensitive) in M-9 medium		52
E. coli (tetracycline-resistant) in M-9 medium		99

* 10 μg/ml of each component adjuvant.

groups of 4 or 5, were added to the M-9 medium. These data are presented in Table I. Only the group composed of l-leucine, dl-threonine, l-isoleucine, l-histidine, and l-methionine permitted growth, which was 54% light transmittance after 18 hours at 37°C. The 3 remaining groups of adjuvants failed to permit growth even after 48 hours incubation. Of the individual amino acids in Group 1, l-histidine was the only one which permitted growth to 50-60% light transmittance within 18 hours. It appeared obvious, therefore, that this tetracycline-resistant strain of *E. coli* selected by a laboratory-procedure was a histidine-requiring auxotroph. Such auxotrophic mutants of *E. coli* are not uncommon (3).

A number of diverse compounds have been shown to play a role in synthesis and/or degradation of histidine in microorganisms (4,5). A number of these, *i.e.*, urocanic acid, imidazole, 5-imidazole analogs, l-histidinol, and l-histidinal at concentrations of 1, 10, and 100 μg/ml could not replace the specific and definite requirement for l-histidine in the tetracycline-resistant *E. coli* (Table II).

It was of interest to determine whether or not l-histidine and several of the compounds mentioned above would antagonize the activity of tetracycline against the parent and the tetracycline-resistant *E. coli* strains. None of the compounds had any demonstrable effect on tetracycline activity against the parent *E. coli* (Table III). Although this strain is resistant to 100 or >100 μg/ml of tetracycline when tested in natural medium (brain heart infusion broth), MIC (minimum inhibitory concentraton) is less when tested in synthetic medium, *i.e.*, 12.5-25 μg/ml.

To determine the relative occurrence of the requirement for l-histidine among other strains of *E. coli* resistant to tetracycline, a number were obtained from clinical sources in two widely-separated parts of the United States. All were found to have minimum inhibitory concentrations of 100->100 μg/ml

TABLE II. Specificity of l-Histidine as Growth-Requirement for Tetracycline-Resistant *E. coli* in M-9 Medium.

Organism	Adjuvant	Conc., μg/ml	Growth of *E. coli*, as % light transmittance, %
E. coli (tetracycline-resistant)	None		99 (no growth)
Idem	l-Histidine	10	52
"	Urocanic acid	100	99
		10	"
		1	"
"	Imidazole	100	99
	2-(p-Nitrobenzyl-mercapto) imidazole	100	"
	2-(Phenylthio-carboxy) imidazoline	100	"
	2-(m-Nitro-henyl-thiocarboxy) imidazoline	100	"
	2-(p-Chlorophenylthiocarboxy) imidazoline	100	"
	Benzimidazole	100	"
"	l-Histidinal	100	"
"	l-Histidinol	100	"

TABLE III. Failure of Adjuvants to Alter Minimum Inhibitory Concentration of Tetracycline against *E. coli* Strains in M-9 Medium.

Organism	Medium	μg/ml of adjuvant		MIC of tetracycline
E. coli (tetracycline-sensitive)	M-9			1.56
	"	l-Histidine,	10	1.56
E. coli (tetracycline-resistant)	" + 10 μg. ml l-histidine			12.5
Idem	*Idem*	Urocanic acid,	100	12.5
			10	12.5
			1	12.5
"	"	Histidinol,	100	12.5
"	"	Histidinal,	100	12.5
"	"	l-Histidine,	100	12 5
			200	12.5
			400	12.5
"	"	Imidazole,	100	12.5

for tetracycline as determined by the 2-fold serial dilution technic in brain heart infusion broth. All the 20 strains received were able to grow in the synthetic medium approximately as well as the parent laboratory strain. Therefore, none of these tetracycline-resistant strains, which were developed naturally *in vivo,* had a requirement for l-histidine.

Summary. 1. A tetracycline-resistant *E.* coli, which had been selected by a laboratory technic, was found to have a specific requirement for l-histidine. 2. Urocanic acid, imidazole and 5 analogs, l-histidinol, and l-histidinal were unable to substitute for l-histidine, nor were they able to antagonize the activity of tetracycline against the parent *E. coli* (tetracycline-sensitive) in synthetic medium. 3. Twenty *E. coli* strains, all tetracycline-resistant and isolated from clinical sources were obtained. None had the specific requirement for l-histidine.

1. Witkin, E. M., *Cold Spring Harbor Symposia on Quantitative Biology,* 1947, v12, 256.

2. English, A. R., Gelwicks, P. C., *Antibiot. & Chemotherap.,* 1951, v1, 118.

3. Lubin, M., *Science,* 1959, v129, 838.

4. Broquist, H. P., Snell, E. E., *J. Biol. Chem.,* 1949, v180, 59.

5. Meister, A., *Biochemistry of the Amino Acids,* Academic Press, Inc., New York City, 1957, 326.

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On the Mechanism of Decrease of Aldosterone Secretion in the Dog. (26044)

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Previous experiments(1) have suggested that the integrity of the vagus nerves is necessary for the decrease in secretion of aldosterone following release of constriction of the thoracic inferior vena cava, but not for the increase in secretion of aldosterone following caval constriction. Further, vagotomy alone has no effect on aldosterone secretion in the otherwise intact animal. This suggests that the vagal pathway is strictly an inhibitory one, and that release of this inhibition does not result in increased secretion of aldosterone. Other experiments(2,3) have indicated that stretch of the right atrium results in diminished secretion of aldosterone. These experiments suggest a possible mechanism for activation of the vagal pathway.

However, several questions concerning the function of the vagus nerve in control of secretion of aldosterone have remained unan-