Limitations of the Infant Mouse Test for Escherichia coli Heat Stable Enterotoxin

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ABSTRACT

A study was undertaken to evaluate the response of different test systems to preparations of heat-stable enterotoxin (ST) derived from Eschericihia coli strains recovered from diarrheal disease of humans, pigs and calves. Sterile broth culture supernatants of enterotoxigenic strains of E. coli were heated at 65°C for 30 minutes and tested for the presence of heat-stable enterotoxin. Three test systems, namely, ligated intestine of weaned pigs, ligated intestine of rabbits and the infant mouse test were used in attempts to detect ST in the culture supernatants. Two patterns of reaction were observed in response to ST-containing preparations: either the preparation elicited a response in the three tests or the preparation elicited a reaction only in the ligated pig intestine. A response in all three tests was observed for 5/5 human ST-producing E. coli, 5/5 bovine enterotoxigenic E. coli, 5/5 "atypical" porcine enterotoxigenic E. coli, 3/3 St^+LT^- porcine E. coli of serogroup O138:K81 and 4/24 LT^+ST^+ porcine E. coli. A response only in the ligated pig intestine was obtained with 5/5 ST^+LT^- porcine E. coli belonging to serogroups other than O138:K81 and to 20/24 ST^+LT^+ E. coli from pigs. The results are consistent with the view that there are two kinds of ST, one of which (ST1) reacts in all three tests and the other (ST2) which reacts only in the ligated pig intestine. The findings underscore the limitations of the infant mouse test as a means of detecting ST in porcine isolates of E. coli, since the test fails to detect ST produced by a large number of these E. coli strains. There appeared to be a relationship between kind(s) of ST produced and the animal species from which the producing organism was recovered.

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RÉSUMÉ

Cette étude visait à analyser la réaction des diverses méthodes d'épreuve à l'endroit d'échantillons de l'entérotoxine thermostable provenant de souches d'Escherichia coli isolées de cas de diarrhée, chez des humains, des porcelets et des veaux. On soumit le surnageant stérile de cultures de souches entérotoxinogènes d'E. coli à la température de 65°C, durant 30 minutes, et on y rechercha la présence de l'entérotoxine thermostable, à l'aide des trois méthodes suivantes: la ligature d'anses intestinales de porcelets récemment sevrés et de lapins, ainsi que l'épreuve des souriceaux.

On nota deux types de réaction, lorsque le surnageant recelait l'entérotoxine thermostable; il réagissait de façon positive avec chacune des trois méthodes d'épreuve, ou bien il ne réagissait de façon positive qu'avec celle de la ligature d'anses intestinales de porcelets. Les trois méthodes d'épreuve donnèrent des résultats positifs avec les cinq souches humaines d'E. coli qui produisaient l'entérotoxine thermostable, les cinq souches bovines d'E. coli. entérotoxigènes, les cinq souches porcines d'E. coli entérotoxinogènes atypiques, les trois souches porcines d'E. coli du sérogroupe O138:K81, qui élaboraient l'entérotoxine thermostable, mais non la thermolabile, et quatre des 24 souches porcines qui élaboraient l'entérotoxine thermostable et la thermolabile. Seule la ligature d'anses intestinales de porcelets donna des résultats positifs avec les cinq souches porcines d'E. coli qui ne produisaient que l'entérotoxine thermostable et qui appartenaient à des sérogroupes autres que O138:K81, ainsi qu'avec 20 des 24 souches porcines d'E. coli qui produisaient l'entérotoxine thermostable et la thermolabile.

Ces résultats concordent avec la notion selon laquelle il existe deux types d'entérotoxine thermostable dont le premier réagit avec les trois méthodes d'épreuve, tandis que le deuxième ne réagit qu'avec celle de la ligature d'anses intestinales de porcelets. Ils

soulignent aussi les limites de l'épreuve des souriceaux, comme moyen de déceler l'entérotoxine thermostable des souches porcines d'E. coli, puisque cette épreuve ne permet pas de la déceler, chez un grand nombre de souches porcines d'E. coli. Il semble exister une relation entre le ou les types d'entérotoxine thermostable produits par certaines souches d'E. coli et l'espèce animale de laquelle on isole ces souches.

INTRODUCTION

Escherichia coli heat stable enterotoxin (ST) was first detected in enteropathogenic *E. coli* strains of porcine origin by injecting cell-free preparations into ligated segments of pig intestine (24). This method of detection of *E. coli* ST has since been used by several workers (11, 13, 23, 26). A similar technique has been applied to calves to demonstrate ST produced by bovine enteropathogenic strains of *E. coli* (18,22). In 1972, Dean and his colleagues (3) reported a new test in which infant mice were used for assay of *E. coli* ST of human origin. This test has become widely used (5,8,10,14,16,19,21,22) and has come to be regarded as a definitive test for *E. coli* ST. In many studies, it is the only test employed to determine whether an *E. coli* strain produces ST (5,10,14,19).

In more recent years, the ligated intestine test in rabbits, which has been used for detection of *E. coli* heat-labile enterotoxin (LT) (7,10) has been modified by Evans and her colleagues (7) so as to provide another test for *E. coli* ST. The modification consisted of reading the test at 6 h rather than at 18 h.

The inconvenience and the costs associated with the ligated intestine tests have led most researchers to use alternative tests to demonstrate the presence of the two classes (ST and LT) of *E. coli* enterotoxins. The usual alternatives are the tissue culture systems (4,9) or immunological tests (2,27) for LT and the infant mouse test for ST. These indirect tests for *E. coli* enterotoxins are often preferable to the ligated intestine test but may record activity which is not enterotoxic or may fail to detect enterotoxic activity.

This paper is concerned with the failure of the infant mouse test to detec ST produced by some strains of enterotoxigenic *E. coli.* One of the first indications of a possible discrepancy between the infant mouse test and the ligated intestine test in pigs was the report by Jacks and Wu (14) who claimed that the porcine *E. coli* strain P307 which had previously been shown to be ST^+ (23) was ST^-. Jacks and Wu (14) had used the infant mouse test for ST, while Smith and Gyles (23) had used the ligated intestine test in pigs. Subsequently, tests of ST preparations from several porcine enteropathogenic *E. coli* in this laboratory (C. Gyles, unpublished observations, 1977) were negative in the infant mouse test but positive in the pig intestine. There is also one report (10) in which the authors indicated that preliminary evidence suggested that the six-hour rabbit ileal loop test may have detected ST in preparations that were negative in the infant mouse test. On the other hand, there is evidence (18,22) that the ST from bovine strains of enterotoxigenic *E. coli* is consistently reactive in both the calf intestine and the infant mouse tests. It is also known that certain strains of porcine enteropathogenic *E. coli* are very similar to the bovine strains in their morphological, serological and enterotoxigenic characteristics (17,22,25).

The object of this study was to determine the response of three test systems, the infant mouse test, the ligated intestine test in weaned pigs, and the six-hour rabbit ileal loop test to preparations of *E. coli* ST from selected classes of enterotoxigenic *E. coli.*

MATERIALS AND METHODS

E. COLI STRAINS

Strains of *E. coli* were obtained from a variety of sources and arranged on the basis of the host species from which the organisms had been isolated, as follows:

Human — Ten strains of *E. coli* of human origin were collected (Table I). Strains TD427 and 52005 were received from Dr. Grace Thorne and strains H10407, 334 and 339 from Dr. S. Gorbach, both of the

New England Medical Center Hospital, Boston. The remaining five strains were provided by Dr. David Sack of Baltimore City Hospitals, Baltimore.

Bovine — Five *E. coli* strains of bovine origin (Table II) were obtained from Dr. L.L. Myers of the Veterinary Research Laboratory, Bozeman, Montana.

Porcine — Thirty-eight *E. coli* strains of porcine origin were selected (Tables III, IV and V). The five strains presented in Table III are representative of *E. coli* strains which have been described as "atypical" (25) or Class II (17) porcine enteropathogens. Strain PA15 is the "Troyer" strain obtained from Dr. E. Kohler of the Ohio Agricultural Research and Development Center, Wooster, Ohio. Strains PA431 and PA637 were recevied from Dr. H.W. Moon, National Animal Diseases Laboratory, Ames, Iowa. The two remaining strains were Ontario isolates.

The nine strains listed in Table IV consist of eight field isolates and one laboratory-derived strain, 711(2176), all of which are part of the departmental collection and had previously been shown to produce ST but not LT. Strain 711(2176) was produced by transfer of the enterotoxin plasmid from the field isolate P2176 to the *E. coli* K12 strain, 711 (12). The 24 strains shown in Table V are strains which had previously been shown to produce both ST and LT and were a part of the departmental collection. The departmental collection consists of strains isolated in Ontario as well as strains donated by researchers in several countries.

A porcine isolate, P3350, from a normal pig was used as a source of negative control preparations in the tests for ST.

Preparation of E. coli ST

The *E. coli* strains had been stored on tryptic soy agar[1] slants kept at room temperature. Each strain was subcultured by streaking on a blood agar plate. Five colonies from the blood agar plate were used to inoculate 20 mL of brain heart infusion[1] broth in a 125 mL volume baffle flask which was incubated with vigorous shaking (200 rpm in a rotary water bath) at 37°C for 18 hours. The cells were then removed from the broth culture by centrifugation at 27,000 x g for 20 min. The supernatant was passed through a membrane filter of mean pore diameter 0.45 μm and then heated at 65°C for 30 minutes. This constituted the *E. coli* ST preparation. Three batches were prepared from each strain of *E. coli* and each batch of ST was tested at least three times in each of three test systems.

Ligated Intestine Test in Pigs

The method is essentially as described previously (23). The pigs were six to eight weeks old and were starved for 24 hours prior to surgery. A laparotomy was carried out under barbiturate anaesthesia and ligated segments were prepared in the anterior small intestines. The number of segments in a pig ranged from 15 to 20. Sixteen to eighteen hours after injection of the intestine with 12 mL volumes of ST preparations the pigs were killed and the volume of fluid and length of intestine measured for each ligated segment. The response to the ST preparation was recorded as the volume of fluid in mL per cm of ligated intestine. Values of 1 or greater were considered positive. The number of tests for each ST preparation ranged from three to six.

Ligated Intestine Test in Rabbits

The method used was similar to that described by Evans and coworkers (7). Adult rabbits weighing approximately 2 kg were starved for 24 hours then anaesthetized with sodium pentothal. The small bowel was flushed with 10 mL of 0.1M phosphate-buffered saline, pH7.2, and ligated segments approximately 5 cm in length were prepared by applying a single tie of surgical silk between segments. In each rabbit, 12 to 15 segments were produced and each injected with a 3 mL volume of an *E. coli* ST preparation. Six hours after injection of the segments, the rabbits were killed and the volume (mL) of fluid per cm of ligated segments were recorded for each segment. Values 0.5 and greater were considered positive. The number of tests

[1]Difco Laboratories, Detroit, Michigan.

for each ST preparation varied from three to five.

Infant Mouse Test for ST

The infant mouse test for ST was carried out as previously described (22). Swiss Webster mice[2], three to five days old, were inoculated orally with 0.1 mL of ST preparation containing one drop per mL of 2% Evans blue dye. Four mice were inoculated with each ST preparation. The mice were kept at room temperature for 3 h, then euthanized with chloroform. The intestines from the four mice were removed, pooled and weighed. The remaining carcasses were weighed together and the ratio of gut weight to remaining carcass weight (G/C ratio) was determined. Three tests were conducted for each ST preparation and the mean value determined for the three tests. Mean G/C ratios of 0.07 or less were considered negative, those greater than 0.07 but less than 0.09 were considered questionable and those 0.09 or greater were considered positive.

RESULTS

E. coli Strains of Human Origin

The effects of ST preparations from the ten enterotoxigenic *E. coli* strains of human origin are summarized in Table I. All four strains which had previously been shown to produce ST elicited a moderate but distinctly positive response in the ligated pig intestine, a markedly positive reaction in the infant mouse test and a positive reaction in the ligated rabbit intestine. Among the six strains designated as ST^-, one (strain K326) gave a pattern of response similar to that seen for the ST-positive isolates.

E. coli Strains of Bovine Origin

Table II shows the reaction of ST preparations from five isolates of bovine enterotoxigenic *E. coli* in the three tests for ST. All five isolates had previously been demonstrated to be enterotoxigenic in ligated segments of calf intestine (C. Gyles, 1977, unpublished observations). The ST preparations were positive in all three tests.

E. coli Strains of Porcine Origin

The results of tests of ST preparations from five "atypical" porcine enteropathogenic *E. coli* are presented in Table III. Heat-stable enterotoxin preparations from all five strains were positive in all three tests.

The response to ST preparations obtained from eight porcine enteropathogenic *E. coli* and one laboratory-derived strain, all of which produce only ST, is shown in Table IV. There are two patterns of response — one in which a positive result was obtained in all three test systems and one in which a positive result was obtained only in the ligated pig intestine. It is noteworthy that all strains whose ST preparations reacted in all three test systems were of the O138:K81 serogroup or derived from a strain of this serogroup.

The reactions of ST preparations from 23 LT^+ST^+ strains of *E. coli* of serotypes associated with diarrhea in pigs and of one laboratory-derived strain are shown in Table V. All strains of O groups 18, 138, 147 and 157 produced ST which reacted only in the ligated pig intestine. Among the strains of O group 141, ST from one of four strains reacted in all three systems, and among the seven strains of O group 149, ST from two strains reacted in all three systems. Heat-stable enterotoxin from the remaining O141 and O149 isolates gave a positive reaction only in the ligated pig intestine. Of the 24 strains of *E. coli*, only four produced ST which reacted in the ligated rabbit intestine and the infant mouse.

Heat-stable enterotoxin preparations from the negative control *E. coli* strain were consistently negative with the three methods for detection of ST.

DISCUSSION

Of the three tests for ST that were used in this study only the ligated pig

[2]Connaught Laboratories, Toronto, Ontario.

TABLE I. Response of Three Test Systems to Preparations of Heat-stable Enterotoxin (ST) from *E. coli* Strains of Human Origin

E. coli Strain	Serogroup	Enterotoxins Produced[b]	Response[a] to ST Preparation in Ligated Pig Intestine	Ligated Rabbit Intestine[c]	Infant Mice
52005	— [d]	LT, ST	1.2	0.6	0.15
H10407	O78:K ?	LT, ST	1.5	0.5	0.12
334	O15:K ?	LT, ST	1.5	0.5	0.16
339	O15:K ?	LT, ST	1.4	0.7	0.13
TD427	—	LT	0.2	0.2	0.07
K100	O64:H$^-$	LT	0.2	0.1	0.06
K121	O8:K(A):H$^-$	LT	0.0	0.1	0.06
K206	O141:H$^-$	LT	0.3	0.2	0.07
K207	O141:H$^-$	LT	0.2	0.2	0.06
K326	O25:H42	LT	0.9	0.5	0.14

[a]Mean value for at least three tests. The ligated intestine test results are given as volume (mL) of fluid per cm of intestine and the infant mouse test results are shown as ratios of gut weight to body weight
[b]As indicated by the donor of the culture
[c]The test was read at 6 h
[d]Not known

TABLE II. Response of Three Test Systems to Preparations of Heat-stable Enterotoxin (ST) from *E. coli* Strains of Bovine Origin

E. coli Strain	Serogroup	Enterotoxins Produced[b]	Response[a] to ST Preparation in Ligated Pig Intestine	Ligated Rabbit Intestine[c]	Infant Mice
B483	O9:K35;K99	ST	2.1	0.5	0.15
B490	O101:K30;K99	ST	2.1	0.6	0.15
B505	O101:K28;K99	ST	1.9	0.5	0.15
B524	O8:K85;K99	ST	1.6	0.5	0.13
B559	O9:K25;K99	ST	3.3	0.6	0.15

[a]Mean value for at least three tests. The ligated intestine test results are given as volume (mL) of fluid per cm of intestine and the infant mouse test results are shown as ratios of gut weight to body weight
[b]As indicated by the donor of the culture
[c]The test was read at 6 h

TABLE III. Response of Three Test Systems to Preparations of Heat-stable Enterotoxin (ST) from "Atypical" Strains of Porcine Enteropathogenic *E. coli*

E. coli Strain	Serogroup	Enterotoxin Produced[b]	Response[a] to ST Preparations in Ligated Pig Intestine	Ligated Rabbit Intestine[c]	Infant Mice
PA9	O9:K(A)	ST	7.5	0.7	0.15
PA15	O9:K35	ST	6.0	0.5	0.13
PA64	O64:K(A)	ST	3.6	0.5	0.15
PA637	O64:K(A)	ST	2.4	0.5	0.14
PA431	O101:K(A)	ST	1.4	0.6	0.15

[a]Mean value for at least three tests. The ligated intestine results are given as volume (mL) of fluid per cm of intestine and the infant mouse test results are shown as ratios of gut weight to body weight
[b]As indicated by previous tests in this laboratory
[c]The test was read at 6 h

TABLE IV. Response of Three Test Systems to Preparations of Heat-stable Enterotoxin (ST) from *E. coli* Strains Recovered from Pigs and Known to Produce only ST

E. coli Strain	Serogroup	Enterotoxin Produced[b]	Response[a] to ST Preparations in: Ligated Pig Intestine	Ligated Rabbit Intestine[c]	Infant Mice
P16	O9:KA	ST	5.1	0.1	0.06
P57	O138:K81	ST	5.7	0.6	0.16
P215	O138:K81	ST	5.8	0.6	0.14
P2176	O138:K81	ST	5.6	0.6	0.16
711 (2176)[d]	O⁻:K ?	ST	5.2	0.8	0.15
P3	O139:K82	ST	5.0	0.4	0.07
P204	O139:K82	ST	5.3	0.1	0.06
P122	O141:K85a,c	ST	4.0	0.1	0.06
P95	O157:K"V17"	ST	4.1	0.3	0.08

[a]Mean value for at least three tests. The ligated intestine results are given as volume (mL) of fluid per cm of intestine and the infant mouse test results are shown as ratios of gut weight to body weight
[b]As indicated by previous tests in this laboratory
[c]The test was read at 6 h
[d]*E. coli* K12 containing the enterotoxin plasmid from *E. coli* strain P2176

TABLE V. Response of Three Test Systems to Preparations of Heat-stable Enterotoxin (ST) from Porcine Enteropathogenic Types of *E. coli* Known[a] to Produce both ST and LT

E. coli Strains	Serogroup	Response[b] to ST Preparations in: Ligated Pig Intestine	Ligated Rabbit Intestine[c]	Infant Mice
P307, P263	O8:K87;8K88ab	4.8, 4.8	0.1, 0.2	0.06, 0.06
P205	O8:K87;K88ab	3.9	0.1	0.07
P491, C662	O138:K81;K88ac	5.3, 3.3	0.2, 0.1	0.06, 0.07
P311	O138:K81	3.6	0.3	0.06
P1253, P258, P105	O147:K79;K88ac	4.3, 2.0, 2.5	0.2, 0.1, 0.1	0.06, 0.06, 0.07
P86, P V17, P164	O157: KV17;K88ac	5.3, 5.4, 5.5	0.1, 0.0, 0.0	0.06, 0.07, 0.07
P681, P91	O141:K85ab;K88ab	5.0, 4.2	0.2, 0.1	0.07, 0.06
P682	O141:K85ab-ac	4.0	0.3	0.07
P1108	O141:K85ac;K88ab	7.1	0.7	0.13
PB1[d], PB2	O149:K91;K88ac	5.9, 5.7	0.1, 0.2	0.06, 0.06
PB3, PB4		2.9, 3.0	0.2, 0.0	0.05, 0.06
15320	O148:K91	3.3	0.2	0.07
P155, A1	O149:K91;K88ac	7.2, 5.0	0.5, 0.6	0.15, 0.14
711 (P155)[e]	O⁻:K ?	5.4	0.5	0.13

[a]On the basis of previous tests in this laboratory
[b]Mean value for at least three tests. The ligated intestine results are given as volume (mL) of fluid per cm of intestine and the infant mouse test results are shown as ratios of gut weight to body weight
[c]The test was read at 6 h
[d]These four strains were recovered from calves, but belong to a serotype characteristic of porcine enteropathogenic *E. coli*
[e]*E. coli* K12 containing the enterotoxin plasmid from *E. coli* P155

intestine gave a positive response to ST preparations from all *E. coli* strains tested. The study indicates that ST preparations from suspected porcine enterotoxigenic *E. coli* should be subjected to this test instead of, or in addition to, the infant mouse test. In the earliest studies of *E. coli* enterotoxins (23) two types of enterotoxigenic *E. coli* of animal origin were recognized, namely those which produced ST only and those which produced both ST and LT. These findings were based on tests in ligated pig intestine. Subsequently, there were reports of enterotoxigenic *E. coli* of human origin which produced LT but not ST (20). The tests in this study confirm that the LT^+ST^- *E. coli* strains of human origin are not strains which produce ST unreactive in the infant mouse test but

reactive in the ligated pig intestine.

Two patterns of response to the ST preparations were noted. Either the preparations elicited a positive reaction in all three tests or they caused a positive reaction only in the ligated pig intestine. All preparations which reacted in the infant mouse test also reacted in the ligated rabbit intestine. The infant mouse test seemed preferable to the ligated rabbit intestine test on several counts; speed, simplicity, reproducibility and distinction between positive and negative results.

If the results for the enterotoxigenic *E. coli* of human origin are representative of those for human enterotoxigenic *E. coli*, then either the six hour rabbit ligated intestine test or the infant mouse test should be adequate for detection of ST in these strains. However, it may be worthwhile in initial studies of suspect enterotoxigenic *E. coli* of human origin to subject them to tests in the ligated pig intestine if they are unreactive in the other tests for ST.

The tests of ST preparations from the bovine enteropathogenic strains of *E. coli* confirm previous studies which demonstrated the value of the infant mouse test for detection of ST in these strains (1,22). It is evident that the rabbit or pig ligated intestine may also be used for determination of ST production by these types of *E. coli*. The "atypical" porcine enteropathogens produced ST which behaved like that obtained from the bovine enteropathogenic *E. coli*. These "atypical" strains have increased considerably in frequency of involvement in *E. coli* diarrhea in pigs (6 and M. Wilson and C. Gyles, unpublished) and are now often more commonly incriminated in diarrhea than are the strains previously considered "typical".

It is interesting that, among the strains of ST^+ porcine enteropathogens shown in Table IV, all those whose ST preparation reacted in the rabbit and infant mouse belonged to serogroup O138:K81. Further, it is evident that this finding is associated with the enterotoxin plasmid rather than the bacterial host since the property is transferred from strain P2176 to the *E. coli* K12 recipient. It is noteworthy that members of the O138:K81 serogroup which form both ST and LT (Table V) produced ST which reacted differently. These observations are consistent with the view that the ST-only plasmids in these organisms did not arise simply from loss of LT genes from LT-ST plasmids and that the LT-ST plasmids did not arise simply by "pick-up" of LT genes by ST plasmids.

Among the ST^+LT^- strains of *E. coli* belonging to porcine enteropathogenic serotypes, most strains produced ST which reacted only in the ligated pig intestine. It may be that the occasional strain whose ST reacted in all systems carried a recombinant plasmid derived from interaction between two types of enterotoxin plasmids.

A recent publication by Burgess and coworkers (1) identified two forms of *E. coli* ST. In their studies, STa was methanol soluble and active in the infant mouse as well as the neonatal pig and STb was methanol insoluble and active in ligated intestines of older pigs as well as rabbit ligated loops. Furthermore, STb had greater heat stability than had STa.

The findings in the present study are consistent with the notion that there are two kinds of ST and that one reacts in the infant mouse while the other does not react in the infant mouse but does react in the pig intestine. The results obtained with culture filtrates of *E. coli* strains P307, PA431, PA637 and P155 in the suckling mouse test were the same in both studies. However, *E. coli* strain P16 failed to give a positive test in the infant mouse test in this study. Furthermore, in the infant mouse test, strain Abb was negative in the study by Burgess and colleagues (1) but positive in this study (Strain PA1, Table V).

Burgess and coworkers (1) obtained markedly positive reactions with ST preparations from *E. coli* P16 in the ligated rabbit intestine. Those *E. coli* strains whose ST preparation were positive in the rabbits used in this study gave weakly positive values similar to those reported by Sack and his colleagues (21) in studies of ST-producing strains of *E. coli* of human origin.

There are sufficient differences in the experiments by Burgess and colleagues (1) and those described in this paper to make certain comparisons difficult. These differences include method of preparation of ST, dose of ST and age and source of weaned pigs. For example, in this study a large dose of ST was used

in the weaned pigs. Furthermore, possible exposure of weaned pigs in Britain to an oral *E. coli* vaccine may have increased their resistance to ST (15).

In this study, the response to ST preparations in the ligated pig intestine was weakest for the *E. coli* strains of human origin, weak for the bovine strains and strong for the porcine isolates. The experiment was not designed, however, to make fine comparisons between these groups but it is likely that use of smaller doses may have resulted in negative values for the strains of human origin and possibly those of bovine origin as well.

There is evidence (Kapitany, Scoot and Forsyth, VIDO Symposium, Saskatoon, Saskatchewan, Oct. 1978) of infant mouse and pig gut loop activity in purified ST from bovine and porcine strains of *E. coli*. This evidence is consistent with the findings in this study. One of two explanations may account for the differences compared with the results by Burgess and coworkers (1). As indicated previously, effects of dosage and of susceptibility of pigs may have caused a negative response to STa preparations in the British study (1). Alternatively, there may be copurification of two molecules (STa and STb) in the studies by Kapitany and his colleagues.

If one accepts the view that one form of ST (ST1) reacts in the infant mouse and in the intestine of the weaned pig and the other (ST2) reacts in the intestine of the weaned pig but not in the infant mouse, then all the ST^+ strains tested produced ST2. The human isolates, the atypical porcine strains, the bovine strains and a small percentage of the typical porcine strains produced ST1, in addition to ST2. The situation may be even more complicated, in view of the apparent differences in degree of activity in ligated pig intestine of ST derived from *E. coli* from different animal host species. A complete resolution of this situation will require additional studies involving amino acid sequencing of purified ST and possibly nucleotide sequencing of the genes for ST. The important conclusion to be drawn from this study is that the infant mouse test is unsatisfactory as a means of detection of ST in uncharacterized strains of *E. coli*, since many *E. coli* strains which would appear to be ST^- in this test can be shown to produce ST in tests in the ligated pig intestine. Furthermore, different types of ST activity appear to be associated with *E. coli* strains which cause diarrhea in different animal host species.

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