RELATION OF RABBIT GUT REACTION TO ENTEROPATHOGENIC *ESCHERICHIA COLI*

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PREVIOUSLY we published an account of factors influencing the reaction of the ligated rabbit gut to injections of strains of *Escherichia coli* (Taylor, Maltby and Payne 1958), and showed that positive reactions were obtained only with living cultures. Bokai (1888) described a somewhat similar technique. Using dogs, he tested various organisms including *Esch. coli*, and from the results obtained suggested that skatol and other protein break-down products caused the reaction. He was able to show that skatol and lactic acid products, caused increased gut movements, and believed that these substances were also produced by the organisms tested. De and Chatterje (1953) were the first to describe the use of ligated rabbit gut and investigated strains of *Vibrio cholerae* by this method ; in later communications De (1959) and De, Ghose and Sen (1960) reported that from strains of *V. cholerae* producing positive reactions a sterile filtrate could be obtained which would produce a similar reaction. Jenkin and Rowley (1959, 1960) also reported results with *V. cholerae* ; in addition, they showed that dilatation of the rabbit gut was caused by lactic acid and further suggested that this may be the toxic product produced by the strains of *V. cholerae* and *Esch. coli*.

We have extended our original studies and have examined a large number of strains of *Esch. coli* of various serotypes isolated from human, animal and miscellaneous sources. The object of this part of our work was to discover (*a*) if positive and negative results in the rabbit gut test were in accord with the history of the patients or animals from which the strains had been isolated, (*b*) whether human enteropathogenic serotypes isolated from animal or water sources produced a reaction, and (*c*) whether a positive reaction was caused by *Esch. coli* isolated from infections in which diarrhoea was not a symptom.

The second part of our work was devoted to determining the number of organisms, colonial form, the effect of the method of keeping cultures, media, and immunity on the results obtained.

PART 1

Results of tests on strains of Esch. coli *of human origin*

The methods used for testing strains on ligated loops of rabbit gut, and for reporting histological results, were the same as those described previously (Taylor *et al.*, 1958).

Strains were isolated from the faeces of babies whose clinical history was well-known. It was found that some strains which had been tested in previous years and which had given a positive reaction, were negative when re-checked. For example, the strain of *Esch. coli* O.111 : K.58 (B.4) : non-motile (nm.), Table I, was culture D.433 which was tested by De, Bhattacharya and Sarkar (1956) and found to be positive. This strain was tested by

us in 1955, when we sometimes obtained a positive reaction, sometimes a negative. It was tested on 2 subsequent occasions in 1957, 10 yr. after its isolation from faeces, and gave negative results. The culture appeared to be smooth. Results on this and other strains are given in Table I.

TABLE I.—Esch. coli *from Babies; Effect on Ligated Rabbit Gut*

Esch. coli			Ligated gut		
Serotype	History	Number of strains	Dilatation	Exudate	Histology
O.4 : nm.	Diarrhoea	1	—	—	O
H.5	,,	1	—	—	,,
O.20 : H.34	,,	2	—	—	Mild
O.26 : K.60 : nm.	,,	1	+	+	Moderate
O.55 : K.59 : H.7	,,	4	+	+	Moderate or severe
H.6	,,	1	+	+	Moderate
O.78 : K.80 : nm.	,,	2	+	+	O
O.111 : K.58 : nm.	,,	1	+	+	Moderate
O.119 : K.69 : H.6	,,	1	+	+	,,
	,,	1	—	Trace pus	,,
O.128 : K.67 : nm.	,,	1	+	+	,,
H.2	,,	1	+	+	,,
H.8	,,	1	+	+	,,
Biotype I	,,	3	—	—	Mild–moderate
O.26 : K.60 : H.11	Symptomless*	1	+	+	Moderate
O.78 : K.80 : H.12	,,	1	—	—	Mild–moderate
O.111 : K.58 : H.2	,,	1	—	—	,, ,,
H.4	,,	1	—	—	,, ,,
O.128 : K.67 : nm.	,,	1	—	—	O
H.8	,,	1	—	—	Mild
H.8	,,*	1	+	+	Moderate
Biotype I	,,	3	—	—	Mild–moderate

* Patients were contacts of patients with diarrhoea who excreted same serotype.
O = not done. nm. = non-motile.

Two strains of *Esch. coli* O.4 were tested, this serotype is not accepted as an enteropathogenic type; both strains gave negative results.

It will be seen that 2 strains of *Esch. coli* O.20 were tested, which were isolated from 2 babies with mild diarrhoea in an outbreak affecting about 8 babies in a maternity unit. These gave negative reactions to the test, but they had been kept on Dorset's egg medium for 2 yr. prior to testing. *Esch. coli* O.20 is not generally accepted as a cause of diarrhoea.

The strain of *Esch. coli* O.26 : K.60 (B.6) : H.11 which gave a positive result was isolated from faeces which were investigated in a survey of healthy children. Nothing is known of the clinical history of this patient, except that he was symptomless at the time the specimen was taken.

A strain of *Esch. coli* O.78 : K.80 was tested, as it causes white scours in calves, but is rarely isolated from human material. The strains from cases of infantile diarrhoea were positive, whereas the strain from a healthy baby was negative.

Two strains of *Esch. coli* O.119 : K.69 (B.14) : H.6 were tested. These were isolated from babies in 2 unconnected outbreaks. One strain caused dilatation of the gut with production of exudate, the second failed to cause dilatation of the gut although pus was present on the mucosal surface; in both instances the histological picture was one of a moderate degree of inflammation.

Esch. coli O.128 : K.67 (B.12) : H.8 was isolated from a baby with diarrhoea, from a healthy baby who, having been in contact with a case, was admitted to a residential nursery, so starting a small outbreak, and from a healthy baby unconnected with cases. The results of

the gut test were in accordance with the history of the patients from whom the strains were isolated, the strains from the baby with diarrhoea and from a healthy contact who was the cause of the outbreak giving positive reactions whereas the strain from the healthy baby failed to cause a reaction.

A study of a residential nursery was in progress in which all strains of *Esch. coli* were typed according to their fermentation reactions to lactose, maltose, sucrose, salicin, dulcitol, inositol, sorbitol, raffinose and rhamnose. During this study, an outbreak of diarrhoea occurred which was believed to be caused by a virus, though this was unproven. It was thought that a biotype I which was found in the healthy and the affected children should be tested as it was possible that this type which was common in the nursery during the whole period of study of 3 yr. might have acquired enteropathogenic properties as the result of passage from baby to baby. Three strains of biotype I from healthy babies, and 3 strains from babies with diarrhoea, were tested. All gave a negative reaction.

The results given in Table I indicate that there is good correlation between the ability of a strain of *Esch. coli* to cause a positive gut reaction and its ability to cause diarrhoea in babies. The tests reported suggest that the ability to cause dilatation of the gut may be reduced and finally lost in strains kept in the laboratory. We suspect that, although some strains produce a positive test after keeping for a number of years, others may lose this property fairly quickly.

The possibility that a positive gut reaction might be caused by any pathogenic strain of *Esch. coli* and not confined to those strains causing diarrhoea, was considered, so 4 cultures isolated from human urinary infections were tested. All gave negative results.

TABLE II.—Esch. coli *from Farm Animals, Effect on Ligated Rabbit Gut*

Animal	Disease	Serotype	Ligated gut: Dilatation	Ligated gut: Exudate	Ligated gut: Histology
Calf	White scours	O.9 : K ? : H.19	—	—	Mild
,,	,, ,,	O.26 : K.60 : H.11	+	+	Mild–moderate
,,	,, ,,	O.78 : K.80 : nm.	+	+	Moderate
,,	,, ,,	O.137 : K.79 : H.41	+	+	Mild
,,	,, ,,	4 unclassified serotypes	—	—	Mild–moderate
Pig	Swine oedema	O.4 : K ? : H.5	—	—	Not done
,,	,, ,,	O.138 : K.81 : H ?	—	—	,, ,,
,,	,, ,,	O.139 : K.82 : H.1	—	—	Mild
,,	,, ,,	O.8. : K? : H.19*	—	—	Moderate
Pig	Swine enteritis	O.4 : K ? : H.5	—	—	Not done
,,	,, ,,	O.8 : K ? : H.19	—	—	Moderate
,,	,, ,,	O.8 : K ? : H.19	—	—	Mild
,,	,, ,,	O.86 : K.61 : H.10	—	—	Not done
,,	,, ,,	O.141 : K.85 : H.4	—	—	,, ,,
Chicken healthy		O.55 : K.59 : H.11	—	—	Mild

H ? = flagella antigen unidentified. K ? = surface antigen unidentified.
* Strain similar to one identified by Dr. F. Ørskov.

Results of tests on strains and extracts of Esch. coli *of animal origin*

Strains of *Esch. coli* of animal origin were tested, the results are shown in Table II. The 2 serotypes O.78 and O.137, commonly found in calf scours, and the rarer type O.26, gave positive results. The 4 unclassified serotypes isolated from calves had been identified by serological methods by Rees (1958), and the same types had been isolated from a number of sick animals. The correlation of strains producing a positive gut test and diarrhoea in the calves from which they were isolated is not as close as in human strains ; this might be due to the strains having been kept in the laboratory for a number of years. None of the strains isolated from either swine oedema or swine enteritis caused dilatation of the rabbit gut. The swine oedema strain O.139 : K.82 : H.1 was isolated by Timoney (1957), who prepared an extract, half of which he injected into a pig, thereby causing swine oedema ; the second half

we injected into a loop of rabbit gut, with negative results. This finding shows that the agent in the extract which caused swine oedema failed to cause dilatation of the rabbit gut. A culture of the living organism was also tested, but failed to cause dilatation. In our previous paper we gave results showing that extracts of strains of *Esch. coli* causing infantile enteritis failed to cause dilatation of rabbit gut, whereas the living strains gave positive results.

The strains from swine enteritis were tested soon after isolation, some within 10 days, but all gave negative results.

The strain from chicken was tested because of its close antigenic similarity to strains causing infantile diarrhoea. Types in the O.55 : K.59 group, commonly isolated in human disease, are non-motile, H.6 and H.7 serotypes, though others also occur. We have identified 3 strains of O.55 : K.59 : H.11, which were isolated from babies, but the clinical history of these cases was not known. These results support the view that the agent causing dilatation of the rabbit gut may be present or absent in strains of identical serotypes.

Results of test on strains of Esch. coli *isolated from water.*

Strains of *Esch. coli* were isolated from farm well waters which were undergoin groutine testing, seven of which were chosen for tests as their somatic antigens were identical with those causing infantile diarrhoea. The strains were one each of O.26 : K.60 : H.32, O.55 : K.59 : H.7 and H.27, O.119 : K.69 : nm., 2 strains of O.127 : K.63 (B.8) : H.4, and one O.128 : K.67 : H unidentified. These strains failed to cause a reaction in the ligated rabbit gut. Serotypes O.26 : K.60 : H.32, O.55 : K.59 : H.7 and O.119 : K.69 : nm. cause infantile diarrhoea. Reference to Table I shows that strains of O.55 : K.59 : H.7 isolated from human cases caused dilatation and inflammation of ligated rabbit gut.

PART 2

Numbers of organisms in ligated gut loops

Results already published showed that large numbers of the *Esch. coli* type which had been injected were present in loops of rabbit gut, but it was suggested that larger numbers might be present in the positive loops, which would account for the reaction. This point was investigated by the following method ; a loop of ligated gut was injected, as described in our previous paper, with 1 ml. of a peptone-water culture containing a known number of organisms. This loop was separated by an uninoculated portion of gut from a second test loop into which 1 ml. of sterile peptone water was inoculated as control ; then another uninoculated portion of gut separated the control loop from the third test loop into which peptone water culture was inoculated. The following day at post-mortem the small gut was removed aseptically. Each test loop was placed in a separate sterile Atomix cup with 100 ml. of sterile normal saline and homogenized. Bacterial counts were made by the Miles and Misra (1938) method. Typical results on 1 of 3 rabbits used are given in Table III, which shows that similar numbers of

TABLE III.—*Number of* Esch. coli *in Loops of Ligated Gut*

Inoculum		Gut	
Serotype	Number injected	Dilatation	Number organisms
O.111 : K.58 : H.12	14×10^3	—	50×10^8
O.26 : K.60 : nm.	6×10^3	+	10×10^8
Peptone water	Sterile	—	3 aerobic sporing organisms

Esch. coli were present in positive and negative loops, and that the loops inoculated with sterile peptone water contained only a few aerobic sporing bacilli ; the latter material was incubated both aerobically and anaerobically. These results prove that the failure of some strains to cause dilatation of the gut is not due to their failure to multiply in that situation.

Effect of colonial variation on results obtained in rabbit gut

We have been investigating *Esch. coli* for a number of years, and noticed that some strains which had been tested many times, and had given positive results for a number of years,

became unreliable in that they gave sometimes positive and sometimes negative results. This effect was observed in strains which appeared to be smooth as well as in those showing smooth-rough change. Some strains, on keeping, show a change to a typical rough colonial form in which the organisms cannot be identified by serological methods. Some strains of *Esch. coli* show a change to a mucoid colony which is of the same serotype as the parent strain, but has an additional mucoid antigen, which was the same in the *Esch. coli* " O " group studied by Taylor and Charter (1955). Smooth, rough and mucoid colony variants were tested. A summary of the results is given in Table IV. It was found that rough variants gave negative results, and that the mucoid variant of a negative strain was also negative. The mucoid variant of O.128 : K.67 : H.2 gave positive results, although the smooth non-mucoid culture was negative.

TABLE IV.—*Effect of Colonial Form of* Esch. coli *on the Gut Test*

Esch. coli		Gut	
Serotype	Colonial form	Dilatation	Histology
O.26 : K.60 : nm.	Smooth	+	Moderate–severe
Variant	Rough	—	Moderate
O.111 : K.58 : H.12	Smooth	—	,,
Variant	Mucoid	—	,,
O.128 : K.67 : H.2	Smooth	—	,,
Variant	Mucoid	+	,,

nm. = non-motile.

Effect of method of storing strain on gut test

Kirby *et al.* (1950) using adult volunteers tested the effect of administering a culture of *Esch. coli* O.111 : K.58 by mouth. The strain used had been isolated from a case of infantile diarrhoea, and had been kept on 2 egg slopes, one stored at 4°, the other at room temperature. They found that symptoms were produced only by the culture which was stored at room temperature. As a result of this observation we decided to test strains which had been stored by different methods. A strain of *Esch. coli* which repeatedly produced a positive reaction was stored at room temperature on Dorset's egg medium, and was freeze-dried by 3 methods ; unfortunately, this Dorset's egg culture was used for other purposes, so a second batch of cultures on Dorset's egg medium were made from the same parent strain as had been used for the preparation of the freeze-dried cultures.

Method 1.—A small amount of 7 per cent glucose broth was added to an over-night culture on an agar slope. This suspension was then freeze-dried.

Method 2.—Culture freeze-dried in 5 per cent Evans' peptone + 5 per cent glucose.

Method 3.—Culture was pre-freezed at −40° in 5 per cent Evans' peptone.

Method 4.—Culture was freeze-dried in mist desiccans, Fry and Greaves (1951).

The cultures were kept at room temperature for 9 months. The ampoules opened and a few drops of peptone water mixed with the material which was then transferred to 4 ml. of peptone water, incubated at 37° for 24 hr. Peptone water was added to this culture until the opacity matched the standard, then a standard loop used to transfer one loopful to 4 ml. of peptone water, 1 ml. was injected into the gut loop. Material was taken from the Dorset's egg slope and inoculated into peptone water ; this culture was treated in a similar manner. Tests were repeated three times, the results are shown in Table V. A culture of *Esch. coli* O.55 : K.59 which had been freeze-dried in mist desiccans and stored at room temperature was also tested. Freeze-drying methods may have reduced the ability of *Esch. coli* to produce a positive test, though results after 2 and 7 years storage were quite good.

Effect of medium on the ability of Esch. coli *to produce a positive gut test*

Many of these tests were made with D.5301, a strain of *Esch. coli* O.128 : K.67, as it had been found that the results were sometimes positive, sometimes negative. The method used was to subculture daily, except at weekends, in the medium under test. Usually, the strains

TABLE V.—*Effect of Freeze-drying Cultures on Gut Test*

Number of rabbits	Serotype	Culture	Period	Gut	
				Dilatation	Histology
2	O.26 : K.60 : H–	Freeze-dried (1)	9 mth.	–	Mild–moderate
		,, ,, (2)	9 ,,	–	Moderate
		Egg slope	6 ,,	+	,,
1	O.26 : K.60 : H–	Freeze-dried (1)	9 ,	+	Mild
		,, ,, (2)	9 ,,	+	Moderate
		Egg slope	6 ,,	+	,,
1	O.26 : K.60 : H–	Freeze-dried (2)	2 yr. 4 mth.	+	,,
		,, ,, (3)	2 yr. 4 mth.	+	,,
		Egg slope	1 yr.	+	Mild–moderate
1	O.55 : K.59	Freeze-dried (4)	7 ,,	+	Moderate
		Egg slope	6 ,,	+	,,

Figures in brackets indicated method of culture storage (see text).

were tested after about 10–15 passages, when the organism was grown in the test medium for 18 hr. If the medium itself were transparent, then the culture was diluted with the same medium to standard opacity ; the volume added was used as a guide for the dilution of opaque media. A new tube of the test medium was inoculated with a standard loopful of diluted culture and 1 ml. of this was inoculated into the loop. Bacterial counts were made on all inocula. The media tested were cooked meat (veal) broth, tested after aerobic and anaerobic incubation, 5 per cent horse blood in nutrient broth, 2 per cent hog gastric mucin in peptone water, 5 per cent human ovomucin in peptone water, cow's milk. In each instance the sterile medium was tested and failed to cause dilatation or exudate or to affect the histological findings. The results given in Table VI suggest that 2 findings might be important, firstly

TABLE VI.—*Effect of Media on Strains Used in the Gut Test*

Strain	Medium	Total number tests	Results		Histology
			Number dilated	Number normal	
D.5301	Peptone water	9	3	6	Moderate
	Cooked meat (aerobic)	3	1	2	Moderate–severe
	,, ,, (anaerobic)	1	0	1	Mild–moderate
	Ovomucin	1	0	1	,, ,,
	Gastric mucin	4	0	4	Moderate
	5 per cent horse blood broth	3	2	1	,,
	Milk	4	4	0	Moderate–severe
D.2101	Peptone water	100	0	100	Mild
	Milk	2	0	2	Very mild
E.65	Peptone water	100	100	0	Moderate
	Gastric mucin	2	2	0	,,

that milk enhances the pathogenic effect, and secondly that hog gastric mucin reduces the effect. The details of the 2 experiments show these effects particularly clearly (Table VII). Both these rabbits gave typical gut reactions with the control strain E.65. Rabbit 95 gave a strongly positive reaction with the peptone water culture of D.5301, but failed to react to the same organism in hog gastric mucin. Rabbit 97 failed to give a positive test with a peptone water culture, but gave a good positive reaction with the milk culture.

D.2101 which gave consistently negative results by the usual method was tested after 9 and 18 passages in milk ; the results were unchanged.

TABLE VII.—*Effect of Media*

Rabbit No.	Strain	Medium	Number organisms injected	Gut	
				Dilatation	Histology
95	D.5301	Gastric mucin	51×10^{11}	—	Moderate
	,,	,, ,,	O	—	Mild
	D.5301	Peptone water	20×10^{6}	+	Moderate
	E.65*	,, ,,	18×10^{6}	+	,,
97	D.5301	Milk	14×10^{5}	+	Moderate–severe
	,,	,,	O	—	Mild
	D.5301	Peptone water	5×10^{5}	—	Moderate
	E.65*	,, ,,	4×10^{3}	+	Moderate–severe

* E.65 = control positive strain of *Esch. Coli.*

E.65, which always gave positive results, was tested after 9 and 13 passages in hog gastric mucin ; the results were unchanged.

D.5301 was used to find out whether the enhancing effect of growth in milk was reversed by changing to hog gastric mucin ; also whether the depressing effect of growth in hog gastric mucin was reversed by changing to milk. The method used was to subculture serially D.5301 in 2 per cent hog gastric mucin 6 times (primary medium) from an 18 hr. culture in mucin a loopful was inoculated into 4 ml. of sterile milk, 1 ml. of this was injected into the gut loop ; the remaining 3 ml. of the milk were incubated for 18 hr. and one loopful transferred to another tube of milk (secondary medium), 1 ml. of which was inoculated into the gut loop, the rest being incubated, and tests continued in a similar manner on further serial subcultures. The results are shown in Table VIII. These results show that the negative results of the strain,

TABLE VIII.—*Reversal of Effect of Media, Strain D.*5301

Primary passage medium	Secondary passage medium	Number organisms injected	Gut		
			Dilatation	Exudate	Histology
Mucin (6)	—	13×10^{4}	—	—	Mild–moderate
	Milk	12×10^{4}	+	+	,, ,,
	,, (1)	5×10^{4}	—	—	,, ,,
	,, (11)	3×10^{4}	+	+	Moderate
Mucin (11)	—	8×10^{4}	—	—	Mild–moderate
	Milk	7×10^{4}	Trace	—	Mild
	,, (1)	2×10^{4}	+	+	O
	,, (2)	5×10^{4}	+	+	Moderate–severe
	,, (4)	4×10^{4}	+	+	O
Milk (6)	—	31×10^{4}	+	+	Severe
	—	7×10^{4}	+	+	Moderate–severe
	Mucin (1)	7×10^{4}	—	—	Mild–moderate
	,, (11)	9×10^{4}	—	—	Moderate
Milk (11)	—	15×10^{4}	+	+	Moderate–severe
	—	7×10^{4}	+	+	Moderate
	Mucin (1)	3×10^{4}	Trace	Trace	O
	,, (2)	10×10^{4}	+	+	Moderate–severe
	,, (4)	5×10^{4}	—	—	O

O = not done.
Figures ( ) = number of serial subcultures.

when inoculated in gastric mucin, become positive when the medium is changed to milk ; also that the reverse change occurs. The reversal in the gut effect is more rapid with a change from mucin to milk medium, as in one experiment the inoculation of a mucin-passaged strain, when inoculated in milk, gave a positive result in the ligated gut, whereas a milk-passaged strain needed to be cultured usually twice in mucin before a negative result was obtained.

Effect of active and passive immunization on response in ligated loop

Active immunization.—Rabbits were immunized with living suspensions of *Esch. coli* O.26 : K.60. The organism was grown on 0·1 per cent glucose in nutrient agar, the growth harvested in ¼ strength Ringer and diluted to standard opacity indicating 500 × 10^6 organisms per ml. Starting with a first intravenous injection of 0·1 ml. the volume was doubled on each succeeding occasion until a final dose of 1·6 ml. was given at the 5th injection. The interval between doses was 2 or 3 days. A test bleeding was made from each rabbit 5 days after the last injection. the titres were O = 6400–12,800, K = 400 in each rabbit. Between 5–7 days after the last injection, challenge inoculations were made using the strain used for immunization and strains of unrelated serotypes which had given positive results in ligated gut loops. At the same time the cultures were tested on a normal rabbit as a control.

Five rabbits were immunized with E.65, *Esch. coli* O.26 : K.60 ; in each a loop of gut was injected with a culture of E.65, 3 tests were negative, 2 positive ; the control normal rabbit gave positive results with the 5 cultures. These results show that immunization gave some protection against the action of the *Esch. coli* used for immunization. Rabbits which had been immunized with *Esch. coli* O.26 : K.60 were tested with *Esch. coli* serotypes O.111 : K.58 and O.128 : K.67. The results were not very satisfactory, but there was no protection against the action of serotypes different from the immunizing strain.

Passive immunization

The effect on rabbit gut of injecting known positive strains and antiserum was tested. Cultures were grown in the usual way for 18 hr., and diluted to standard opacity. Two standard loopfuls were transferred to a mixture of 1 ml. of test serum and 1 ml. peptone water ; 1 ml. of mixture was used for inoculation, and bacterial counts were made on the remainder. Controls were made by inoculating a mixture of normal rabbit serum with the test strain. The results are given in Table IX, which shows that O.26 : K.60 antiserum protected the

TABLE IX.—*Effect of Inoculating Mixtures of Immune Sera and* Esch. coli

Inoculum						
Serum			Organism		Gut	
Immunizing strain	Type	Titre	Strain	Type	Dilated	Histology
Nil	Normal rabbit serum	*Nil*	E.65	O.26 : K.60	+	Moderate
			G.193	O.55 : K.59	+	,,
			E.177	O.119 : K.69	+	Mild–moderate
E.65	O.26 : K.60	O = 12,800 K = 1,600	E.65	O.26 : K.60	—	Mild
			E.854	O.26 : K.60	—	Very mild
			G.193	O.55 : K.59	+	Moderate–severe
Aberdeen β	O.55 : K.59	O = 6,400 K = 800	G.193	O.55 : K.59	—	Moderate
E.3729	O.119 : K.69	O = 3,200 K = 200	E.177	O.119 : K.69	—	Mild

gut from the action of cultures of *Esch. coli* O.26 : K.60, but had no effect on cultures of other *Esch. coli* serotypes, and that similar results were obtained with other serotypes and the corresponding specific sera. It was thought that these results might be influenced by the ability of the organism to grow in serum mixtures injected into the gut. To investigate this point, bacterial counts were made on the loops after removal by the method described earlier. The results shown in Table X demonstrated that *Esch. coli* increased in numbers to about the same degree when inoculated with the homologous antiserum, with a heterologous antiserum, or without the addition of serum ; also that the same number of organisms were present in the positive and negative loops.

TABLE X.—*Numbers of* Esch. coli *in Mixtures with Antisera*

Rabbit	Serum	Inoculum: Strain	Inoculum: Serotype	Inoculum: Number injected	Gut: Dilatation	Gut: Total number *Esch. coli*
141	—	D.2101	O.111 : K.58 : H.12	11×10^3	—	13×10^8
	O.26 : K.60	E.65	O.26 : K.60	21×10^3	—	2×10^8
	O.26 : K.60	—	—	—	—	—
	—	E.65	O.26 : K.60	19×10^3	—	19×10^8
144	—	D.2101		19×10^3	—	11×10^8
	O.26 : K.60	G.193	O.55 : K.59	4×10^4	+	36×10^8
	—	G.193		24×10^3	—	11×10^8
	—	E.65		16×10^3	+	31×10^8

DISCUSSION

For many years it has been recognised that there is a need for some method for testing the ability of an organism to cause diarrhoea. A number of different enterobacteria have been tested in human volunteers, and valuable results obtained. Strains of *Esch. coli* have been tested in babies by Neter and Shumway (1950), and in adults by Kirby, Hall and Coackley (1950) and Ferguson and June (1952), but it is difficult to get human volunteers. In addition, it is impossible to determine their immunity to a particular organism before carrying out the experiment. The ligated rabbit technique seemed to give results which could be correlated with the ability of an organism to cause diarrhoea in man. It seemed important to find out if this test could be usefully applied to organisms believed to cause diarrhoea in animals, also if *Esch. coli* causing diseases not associated with diarrhoea also produced a positive test. In other words, was this a test for pathogenicity in general or a test for enteropathogenicity? We believe that the latter is true, as, using strains of *Esch. coli* isolated from cases of infantile diarrhoea, in which the organism was believed to be the aetiological agent, good correlation was found with the ability to cause dilatation of the ligated loop of gut, whereas, using similar serotypes isolated from healthy babies, healthy animals, or well water, the results were negative. *Esch. coli* causing human urinary infections, swine oedema and swine enteritis, gave negative results, but some strains causing calf scours gave positive results. The correlation between a positive gut test and an aetiological role in animal diarrhoeal diseases was not close.

The reaction of a doubtful strain is affected by the medium in which it is grown, and in which it is inoculated into the ligated gut, milk enhancing the reaction, whereas hog gastric mucin caused a negative reaction. With a strain giving strongly positive reactions the hog gastric mucin is without effect. This finding may be related to the treatment of infantile diarrhoea, as it has been found that water only should be given by mouth until the symptoms improve.

Rabbits were actively immunized with living cultures of *Esch. coli* injected intravenously. Some rabbits were protected, and failed to give a positive test, others gave the usual positive result. Although the experiments were rather unsatisfactory there appeared to be no protection against challenge with heterologous serotypes. By injecting mixtures of positive strains with the homologous antiserum, there was complete protection, but no protection against other serotypes. This finding agrees with the findings in human infection, where babies recovering from diarrhoea due to one serotype became infected with a different serotype and developed another bout of diarrhoea (Anderson, Crockatt and Ross, 1954).

SUMMARY

Ligated loops of rabbit gut have been used to test strains of *Esch. coli* for their ability to cause dilatation. It was found that strains which caused infantile enteritis caused dilatation, but strains causing human urinary infection were without effect. As far as human strains are concerned, a positive gut test is not a test for general pathogenicity, but a test for the ability of the organism to cause diarrhoea, probably as the result of its ability to produce some toxic substance. Some strains isolated from calf scours caused dilatation, but the correlation was not absolute—possibly because some had been kept in subculture for years—possibly because the pathological processes in this disease, which is accompanied by septicaemia, are different from those in infantile enteritis, in which the infection is localised to the gut. Strains of *Esch. coli* associated with swine enteritis and swine oedema were without effect, as were *Esch. coli* serotypes which cause infantile diarrhoea, but which had been isolated from well water.

The effect of colonial variation was investigated. It was found that smooth strains of *Esch. coli* isolated from infantile enteritis caused dilatation of the ligated loop, whereas the rough variant was negative. Some smooth strains of *Esch. coli* gave variable results ; with such cultures the mucoid variant gave a positive reaction.

The effect of media was tested. Strains which gave variable results when injected in peptone water, gave positive results when injected in milk, but negative results when injected in 2 per cent hog gastric mucin in peptone water. Media had no effect on strains which invariably gave positive results, or those which gave negative reactions. The same number of organisms was found in positive and negative loops.

Active immunization of the rabbit gave partial protection, passive immunization gave complete protection, against the homologous serotype, but no protection against different serotypes.

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