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Replacement coliform flora in carriers of intestinal pathogens

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There are many antibacterial drugs now available, antibiotic and synthetic, which are strongly inhibitory to intestinal pathogens of the Salmonella and Shigella groups and to the *Escherichia coli* serotypes. Most of these drugs can be used singly or in combination with reasonable success in the alleviation of the symptoms and signs of infection, but none can be relied upon to eradicate the infecting organisms. In about 50 % of cases treated in our hospital, the pathogen disappears spontaneously, but in the remainder it persists for weeks despite intensive and even repeated courses of treatment. Of the various drugs now in use, neomycin, paromomycin and ampicillin are probably as active as any in killing these pathogens, but even these drugs used in combination or successively fail to clear a proportion of carriers (Coles & Stewart, 1961; Stewart, Coles, Nixon & Holt, 1961).

For some years past we have explored the possibility of preventing or treating the carrier state by giving bacterial suspensions concomitantly with antibacterial drugs. At first yoghourt was used, then suspensions of *Bacillus subtilis* and lactobacilli, without any success. More recently it was decided to try another approach based on the fact that during treatment with an antibacterial drug the patient's natural coliform flora is suppressed at least as strongly as is the pathogen. We therefore selected a number of cases immediately before treatment was instituted and assumed that some of them would relapse after standard courses of treatment. From the original faecal samples submitted from these patients we isolated the principal strain of *E. coli* and kept it in subculture. This organism was rendered resistant to a drug (either neomycin or paromomycin—see below). When in due course a proportion of these cases relapsed, the drug selected was then given together with the artificially resistant coliform to see if this organism would multiply during treatment, establish itself in the gut and 'crowd out' the pathogen. The present paper relates our experience with eight cases thus treated.

METHODS

Clinical

This trial was carried out in the Gastro-Enteritis Unit of this hospital. When patients were first admitted their stools or faecal swabs were submitted in accordance with routine practice to the laboratory. A number of cases from whom a definitive pathogen was isolated were selected for trial. Along with the pathogen, the principal strain of *E. coli* was isolated and made resistant to a drug as described

below. Meanwhile, pending the bacteriological result, all cases of gastro-enteritis when admitted were treated with either neomycin or paromomycin (each at 40 mg./lb. daily in four doses by mouth). The strains of *E. coli* isolated from patients receiving paromomycin were made resistant to neomycin, and the strains from those receiving neomycin were made resistant to paromomycin. The object here was to treat cases relapsing after paromomycin with neomycin, and vice versa, and to give the resistant organism as a replacement coliform flora fully resistant to the appropriate drug at the same time. Of twelve cases thus selected, eight relapsed and were given the replacement flora. Two of these cases (1 and 2) were well-established chronic carriers; the remainder (3–8) were convalescent carriers.

Bacteriological

Strains of *E. coli* isolated as described above were plated out to ensure purity, a number of colonies being sampled and tested for biochemical reactions. Several colonies were then subcultured into broth and passaged successively on gradient plates (Szybalski, 1952) of agar containing rising concentrations of neomycin or paromomycin from 20 to 200 μg./ml. Strain M.J. was made resistant to both drugs. Passage was ended when complete resistance to 200 μg./ml., confirmed by titration in liquid media, was attained, the original untrained strain being titrated at the same time. One strain (D.S.) attained this level of resistance in four passages; one (A.T.) required nine passages; the usual number was six or seven (Table 1). When passaged, each strain showed cross-resistance to other related drugs, each to almost the same level. At their final passage the trained strains usually grew more slowly into smaller colonies than the parent strain and showed some decrease or loss of certain biochemical reactions. After four subcultures on drug-free media, normal growth and biochemical activity returned and resistance to the drugs was maintained.

For administration to the patients the resistant strains were grown overnight in nutrient broth, the resulting cultures were centrifuged, twice washed in sterile saline, and then resuspended in sterile saline at densities of 2×10^7, 4×10^7 per ml. and upwards. These suspensions were checked for purity and viable counts.

During the period of administration of the drug together with the drug-resistant replacement coliform the faeces were examined as frequently as possible by plating on McConkey agar containing 20 μg./ml. of the appropriate drug. The replacement coliform was isolated selectively on this medium. At the same time, faeces were plated for identification of the pathogen and other organisms on drug-free McConkey agar, deoxycholate citrate, nutrient agar and Czapek-Dox agar, and in selenite or tetrathionate enrichment media. The anaerobic flora was not examined in this investigation.

Administration of replacement flora

Normally six doses were given, starting with 20 million resistant organisms, increasing in steps daily until 1000 million organisms were given on the 5th and 6th days. The organisms were added to milk or puree immediately before ingestion. The bacterial suspensions from which the doses were prepared were stored at

Table 1. *Replacement strains of* Escherichia coli *rendered resistant to neomycin or paromomycin* in vitro

Strain of *E. coli*	Rendered resistant to	Minimal inhibitory concentration (μg./ml.)		No. of passages	Cross resistance Minimum inhibitory concentration (μg./ml.)		
		Original	Final		Streptomycin	Paromomycin	Neomycin
S.W.	Neomycin	20	200	6	200	200	.
A.S.	Neomycin	20	200	6	50	200	.
J.F.	Paromomycin	20	200	7	200	.	150
A.T.	Neomycin	20	200	9	100	200	.
A.H.	Paromomycin	20	200	7	200	.	100
N.S.	Paromomycin	20	200	6	50	.	200
K.F.	Neomycin	20	200	6	100	200	.
D.S.	Paromomycin	40	200	4	100	.	200
C.F.	Paromomycin	20	200	7	50	.	100
M.J.	Neomycin and	20	200	8	100	.	.
	Paromomycin	20	200				
T.C.	Paromomycin	5	200	6	< 50	.	200
K.R.	Paromomycin	15	200	7	200	.	100

4° C. Each dose was checked by viable count at the time of administration. In all instances these viable counts showed good approximation to the estimated dose. In case 1 (S.W.) the replacement coliform was given for ten further daily doses of 1000 million organisms.

Drug treatment was started 1–3 days before administration of the replacement coliform, which was given for 3–4 days with the drug and then, for 2–3 days, alone, except in case 1.

RESULTS

The replacement coliform organism began to appear in the faeces of cases 1–7 at or towards the end of the period during which it was administered (Table 2). In case 8, through an oversight, the faeces were plated only on selective media which precluded identification of the coliform. In cases 2, 3, 6 and 8 the pathogen disappeared during the period of therapy and failed to reappear in at least five specimens examined after the suppressive drug was no longer being excreted. These cases (2 *Shigella sonnei*, 2 *Salmonella typhi-murium* infections) were regarded as being cleared of infection and were discharged from hospital. In four others (3 *Salm. typhi-murium*, 1 *E. coli* 026) the pathogen disappeared, along with the remainder of the coliform flora, during the period of administration of the drug, but reappeared a day or two later and persisted for many days thereafter, along with the replacement coliform. On re-isolation these pathogens showed no drug-resistance.

Case 1 (S.W.) was given the replacement coliform for a total of 16 days. By the 10th day it was well established and it persisted until the 25th day, after which no further stools were examined. During this time the pathogen (*E. coli* 026) disappeared from the 2nd until the 12th day, reappeared on the 13th, was not isolated on the 15th, but reappeared again on the 19th and 25th days in increasing numbers. During this time *Candida albicans* also appeared in the stool, and for this reason nystatin (500,000 units twice daily) was given by mouth.

In case 6 (M.J.) the replacement coliform had a useful 'marker' property in that it formed amidase, which was identified by its power to break the carbon–nitrogen linkage in leucinamide, in penicillin G and other selected substrates. This property enabled the organism to be identified amid the natural coliforms in drug-free media and we were therefore able to estimate the degree of replacement semi-quantitatively. On the 9th and 10th days the replacement coliform accounted for the entire coliform flora. On the 11th day the natural coliforms returned in quantity, but on the 17th and 25th days the amidase-forming replacement coliform was still present in considerable quantity.

All the children were watched carefully for untoward effects during this procedure. The coliform suspension was accepted without complaint or distaste; there was no nausea or vomiting. Case 4 showed persistent mild diarrhoea, but this was present before the replacement coliform was given. None of the other five cases showed any diarrhoea during treatment, and cases 1, 5, 6, 7 and 8 were in fact constipated after the period of treatment.

Table 2. *Results of treatment with drugs and replacement flora of resistant coliforms in eight children carrying intestinal pathogens*

Case	Pathogen	Replacement coliform made resistant to	Time table	Days of surveillance 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25	Result
(1) S.W.	*E. coli* 026	Neomycin	Drug (neomycin)	←——→	Pathogen persisted with replacement coliform
			Replacement coliform	. ←————————————→	
			Pathogen in faeces	. 0 0 0 0 (*C. albicans*) 0 0 0 + . 0 . . . – . . . . . +	
			Replacement coliform in faeces	. 0 0 0 0 0 0 0 0 + + 0 + . + . . . + ... +	
(2) A.S.	*S. sonnei*		Drug (neomycin)	←——→	Pathogen eliminated during therapy. Replacement coliform persisted
			Replacement coliform	. . ←——→	
			Pathogen in faeces	. . . . 0 0 0 0 0 0 0 0	
			Replacement coliform in faeces	. . . . 0 0 0 0 0 + + + ...	
(3) A.H.	*S. sonnei*	Paromomycin	Drug (paromomycin)	←——→	
			Replacement coliform	. . ←——→	
			Pathogen in faeces	. . . . . 0 0 0 0 0	
			Replacement coliform in faeces	. . . . . 0 0 + + + + ...	
(4) C.F.	*S. typhi-murium*	Paromomycin	Drug (paromomycin)	←——→	Pathogen persisted with replacement coliform
			Replacement coliform	. . ←——→	
			Pathogen in faeces	. . . . 0 0 . + + ... + ...	
			Replacement coliform in faeces	. . . . 0 0 . 0 + ... + ...	
(5) N.S.	*S. typhi-murium*	Paromomycin	Drug (paromomycin)	←——→	
			Replacement coliform	. ←——→	
			Pathogen in faeces	. . . . . + . . + . . 0 . . . 0 + + + . . .	
			Replacement coliform in faeces	. . . . . 0 . . + . . + . . . ++ + + + . . .	
(6) M.J.	*S. typhi-murium*	Neomycin and paromomycin	Drug (neomycin)	←——→	Pathogen eliminated during therapy. Replacement coliform persisted
			Replacement coliform*	. . . ←——→	
			Pathogen in faeces	. . . . 0 0 . . 0 0 0 . . . . 0 0 0	
			Replacement coliform in faeces	. . . . 0 0 . . ++++ 0 . . . . 0 + ... +	
(7) T.C.	*S. typhi-murium*	Paromomycin	Drug (paromomycin)	←——→	Pathogen disappeared one month later while patient was receiving streptomycin and framycetin
			Replacement coliform	. ←——→	
			Pathogen in faeces	. . . . . + . + + ...	
			Replacement coliform in faeces	. . . . . + . . . + ... + . . .	
(8) K.R.	*S. typhi-murium*	Paromomycin	Drug (paromomycin)	←——→	Pathogen eliminated
			Replacement coliform	. ←——→	
			Pathogen in faeces	. . . . . 0 0 0	
			Replacement coliform in faeces	. . . . . Not tested	

* Amidase-forming (see text).

DISCUSSION

It is difficult to explain the persistence of susceptible pathogens in the intestine after large doses of poorly absorbed, bactericidal drugs like neomycin and paromomycin. While the drugs are being administered, the entire aerobic flora is suppressed and the faeces may be sterile when cultured aerobically. Some pathogens (e.g. *Salmonella typhi* and *paratyphi* B) may linger in the biliary tract, but this is not known to occur with *Sh. sonnei* or *E. coli* serotypes, which may be equally persistent in the gut. It could be argued that the drugs act preferentially on the commensal flora, thereby entrenching the pathogen, but the results recorded above do not support this view, for the presence of a competitive drug-resistant flora was not conducive to elimination of the pathogen in four of the eight cases treated. In four other cases, including the two carriers of *Sh. sonnei*, the pathogen disappeared during this régime, but this might well have been due to chance.

It is clear therefore that a replacement flora of drug-resistant coliforms can be established in the gut, rapidly and comparatively easily, by giving a few doses of a suitably prepared bacterial suspension along with a selected drug. This flora persists after dosage ceases and does not prevent the return of part or all of the original flora.

As a therapeutic technique, the method described here may therefore be of use in maintaining a coliform flora during prolonged antibiotic therapy but cannot be relied upon to assist in the elimination of pathogenic intestinal bacteria.

SUMMARY

Eight carriers of intestinal pathogens (5 *Salm. typhi-murium*, 2 *Sh. sonnei*, 1 *E. coli* 026) were given their own coliforms, rendered drug-resistant *in vitro* to neomycin or paromomycin, together with one of these drugs. In seven cases, the drug-resistant coliform flora established itself, while the natural coliform flora and the pathogen were suppressed. In four cases only (2 *Sh. sonnei*, 2 *Salm. typhi-murium*) the pathogen was eliminated in the course of this procedure.

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