THE FATE OF INGESTED *PSEUDOMONAS AERUGINOSA* IN NORMAL PERSONS

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A NUMBER of sources of *Pseudomonas aeruginosa* have been suggested that may be of importance in ward infection. These are contaminated solutions of disinfectants (Lowbury, 1951; Plotkin and Austrian, 1958; Lee and Fialkow, 1961), aspirators, respirators and incubators (Barrie, 1965; Bassett, Thompson and Page, 1965; Phillips and Spencer, 1965; Rubbo, Gardner and Franklin, 1966) and severely infected patients, especially burned patients (Lowbury and Fox, 1954; Sutter and Hurst, 1966) and patients with urinary infections (Pyrah *et al.*, 1955; Gillespie *et al.*, 1964). In general medical and surgical wards another source of importance may be the gastro-intestinal tract of the patients (Darrell and Wahba, 1964; Shooter *et al.*, 1966).

It is possible that pseudomonas in the bowel come from ingested food, but it is not known whether ingested pseudomonas will establish themselves in the bowel of normal people, what numbers if ingested will be detectable in the faeces and for how long they will remain in the bowel. The experiments described here were performed in an attempt to answer these questions.

METHODS

Volunteers. These were two men and one woman who were members of the laboratory staff. Their ages were between 29 and 35 and they had no known abnormality of the gastro-intestinal tract. During the period of the investigation the volunteers ate no cold food at midday and all cold food and drinks taken at home were sampled for the presence of pseudomonas. On three occasions one of the volunteers had a 4-day course of oral ampicillin, 250 mg being taken 4 times a day, commencing 5 hr before the ingestion of the organisms.

Strains. The doses of pseudomonas to be ingested were prepared from either laboratory strains or fresh isolates. The sources, the phage-types and serotypes of the seven strains used are shown in table I.

Preparation of strains of pseudomonas for ingestion. The strains were grown in nutrient broth (Oxoid) at 37°C for 5 hr and then either this culture or dilutions of it were added to approximately 200 ml of milk containing 4 ml of aluminium hydroxide gel. The numbers of organisms ingested ranged from 5×10^2 to 2×10^8 , determined from surface viable counts of the broth cultures on cetrимide-chloramphenicol plates. The organisms were ingested in the late afternoon approximately 4 hr after the last meal.

Composition of the media used. Cetrимide broth: Oxoid nutrient broth no. 2 containing 0.2 mg cetrимide per ml. Cetrимide agar: Oxoid nutrient agar containing 0.3 mg cetrимide per ml. Cetrимide-chloramphenicol agar: as for cetrимide agar but with the addition of 2.5 µg chloramphenicol per ml.

Examination of food. A few grammes of each type of cold food or cold drink eaten during a 24-hr period were pooled and emulsified. The mixture was used to inoculate a cetrимide agar plate and also a tube of cetrимide broth, which was subcultured to cetrимide agar after

Received 12 Apr. 1969; accepted 2 May 1969.

overnight incubation. Preliminary experiments had shown that by these methods 10 pseudomonas per ml of food suspension could be detected.

Examination of faeces. Specimens of faeces were examined as soon as they were passed, except on a few occasions when they were stored at 4°C for periods not exceeding 24 hr before they were examined. Three samples each of approximately 2 g were taken from different parts of the stool and emulsified in water to form a thin paste. This paste and ten-fold dilutions in quarter-strength Ringer's solution were used to inoculate cetrinide-chloramphenicol plates for surface viable counts. By these methods the lower limit of detection of pseudomonas in the faeces was 10 organisms per g. A single colony of pseudomonas from each specimen was stored for phage- and sero-typing.

TABLE I
Pseudomonas strains

Strain	Origin	Phage-type	Serotype
1	Environment*	21/68/119X/1214/Col 21	10
2	Faeces*	7/31/M4/Col 11	10
3	Jelly*	7/31/73/109/M4	6
4	Faeces*	7/21/68/+	1
5	Faeces†	24/68/F7/Col 11	Not typable
6	Tracheal swab†	7/21/24/68/Col 11	3
7	Faeces†	21/1214/Col 18	11

* Laboratory stock strain.

† Fresh isolate.

RESULTS

Examination of food

The cold food consumed was examined continuously in one household for 13 wk and in another for 4 wk. On no occasion was pseudomonas isolated.

Examination of faeces

The results of the ingestion of pseudomonas by the three volunteers are shown in table II. Doses of less than 10^4 organisms were not detectable in the faeces, but regular recovery was obtained with doses above 10^6 organisms.

The highest counts were usually obtained in the first specimen passed, provided that at least 12 hr had elapsed since ingestion of the organisms. Counts then fell steadily in subsequent specimens and after 6 days organisms were no longer detectable, except on one occasion when ampicillin was also taken and colonisation occurred. In this case excretion persisted steadily for 14 days before it ceased spontaneously. Subsequent administration of this pseudomonas and also of a different serotype together with ampicillin failed to establish colonisation in the same volunteer. The total number of organisms recovered from the stools was only a small percentage of the ingested dose, except on the occasion colonisation occurred, when the total number recovered exceeded the ingested dose.

Stool strains were always identical in serotype and phage-type with the strain ingested immediately before their recovery.

None of the volunteers experienced any untoward reactions as a result of taking pseudomonas and when colonisation occurred it was not accompanied by any gastro-intestinal tract upset.

TABLE II
Oral administration of pseudomonas and recovery in the faeces

Subject	Strain of pseudomonas	Oral dose of pseudomonas	Time (hr) between administration of pseudomonas and first appearance in the faeces	Time in days between first and last positive specimen of faeces	Total number of pseudomonas recovered from the faeces
A	1	5.0×10^2	—	0	0
B	1	5.0×10^2	—	0	0
A	1	7.5×10^3	—	0	0
B	1	7.5×10^3	—	0	0
A	1	7.5×10^7	24	3	—
B	1	7.5×10^7	72	2	—
A	1	1.5×10^6	18	3	3.0×10^4
A	2	1.0×10^7	18	3	2.5×10^5
A*	2	3.0×10^6	24	14	$>4.0 \times 10^6$ †
A	2	2.0×10^8	18	2	7.5×10^5
A*	2	1.5×10^8	24	6	3.5×10^5
A	2	3.0×10^6	24	2	1.0×10^4
C	2	3.0×10^6	24	2	3.5×10^3
A	3	7.0×10^7	24	4	1.5×10^7
A	3	1.0×10^7	18	2	2.0×10^5
A*	3	7.0×10^7	18	5	2.0×10^6
A	4	5.0×10^7	18	6	2.0×10^6
C	4	5.0×10^7	30	1	2.0×10^3
A	4	2.0×10^8	24	2	1.0×10^4
C	4	2.0×10^8	—	0	0
A	5	1.0×10^8	24	2	4.0×10^4
A	6	1.5×10^8	18	2	6.5×10^5
A	7	1.0×10^8	42	2	2.5×10^6

* Oral ampicillin administered.

† Colonisation achieved. Not all specimens were counted although they all contained pseudomonas.

DISCUSSION

The results show that if 10^6 or more pseudomonas are ingested the organism can be recovered from the faeces. This dose did not lead to colonisation; the organisms passed through the bowel and excretion soon ceased.

Although the domestically prepared food studied did not contain pseudomonas, work in progress in our laboratory has shown that institutionally prepared food may do so. It is possible that this source may account in part for the faecal carriage rate observed in this hospital (Shooter *et al.*, 1966).

The interesting points observed in these experiments about the passage of pseudomonas through the bowel were the speed at which the organisms passed

through and the fact that excretion was transient. In any studies in which the relation of faecal carriage to food contamination is being examined the timing of the examination of specimens will be important.

Other organisms that have been experimentally or therapeutically ingested by normal people and by patients with disease of the bowel are *Escherichia coli* and lactobacilli. It has been shown that large numbers of *E. coli* administered to patients with ulcerative colitis will displace the strain previously present in the bowel (Cooke, 1966). *E. coli* O111 has been administered to adults and has caused diarrhoea (Ferguson and June, 1952) and a non-enteropathogenic strain of *E. coli* has been established in the bowel of infants (Lodinová, Jouja and Lanc, 1967). For many years lactobacilli have been administered to patients for the treatment of a variety of conditions (Portis and Albus, 1931; Winkelstein, 1956; and Settel, 1962). In particular they have been administered with broad-spectrum antibiotics to prevent colonisation of the bowel with staphylococci (Gordon, Macrae and Wheater, 1957; Török and Turay, 1960).

In all this work large numbers of organisms have been administered, and in the work reported here 10^6 organisms were administered before they were regularly detected in the faeces. However, though the proportion of the ingested dose present in the faeces was usually small, it did vary considerably, so that among a larger group of people there would be probably some in whom on occasion smaller doses could be detected. Frequent small oral doses might also be more readily detectable.

The results indicate one factor that may be of importance in establishing colonisation—the administration of antibiotics. Ampicillin was used in the study, but an antibiotic such as tetracycline with a wider spectrum of activity against the normal bowel flora might have been more effective. However, it is known that colonisation may occur in the absence of antibiotics. Whether the establishment of colonisation is due to a particular property of the strain of organisms or to some unidentified characteristic of the carrier is not known.

SUMMARY

Cultures of *Pseudomonas aeruginosa* in milk were ingested by three normal people. The organisms were recovered from the faeces if 10^6 or more were ingested. They were present in the greatest number in the first specimen passed, provided this was 12 hr or more after administration of the organisms. The numbers excreted were less than those ingested and excretion did not continue longer than 6 days. Colonisation was achieved on one occasion when ampicillin was also administered.

We are grateful to Dr M. T. Parker who typed the strains of pseudomonas and to Professor R. A. Shooter for his advice.

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