reduction of glyceryl trinitrate intake on verapamil was 16.1 tablets a week, suggesting that glyceryl trinitrate was used rather arbitrarily and not only for angina. Neither trial corresponds to the ordinary conditions of ambulant treatment.

Summary and Conclusions

A double-blind trial is reported of verapamil in 20 outpatients with angina. The dose of verapamil was 80 mg. three times daily for a fortnight, with a similar period on placebo tablets. No significant relief of angina by verapamil was found.

Preliminary Communications

Hospital Study of Transferable Drug Resistance

Brit. med. J., 1968, 2, 741-742

Transferable drug resistance in enteric pathogens was first discovered in Japan by Ochiai *et al.* (1959) and Akiba *et al.* (1960). It was subsequently reported in London (Datta, 1962). Since then enterobacteria with transferable resistance factors, or R factors, have been isolated in many parts of the world. The subject has been reviewed by Watanabe (1963, 1966), Datta (1965), and Anderson (1967).

There is more published information about the incidence of R factors in enteropathogenic bacteria than in normal flora. Multiresistant non-pathogenic *Escherichia coli* strains carrying R factors (R+) were isolated from a large number of patients in a Dublin children's hospital (Moorhouse, 1966). Resistance to two and three drugs was transferred to a sensitive *E. coli* K12 strain in mixed culture. These R+ *E. coli* strains were part of the intestinal flora of children who had been in hospital for some time. We did not know if the strains had been present in the intestinal tract of the children before admission or had been acquired in hospital. A small investigation was planned in an attempt to answer this question. The findings are the subject of this paper.

MATERIAL AND METHODS

Twenty-two infants were selected for the investigation because they had faeces in their napkins on arrival at the hospital and had no recent history of diarrhoea. Faecal swabs were taken in the admission unit to ensure that the bacteria isolated were not acquired within the hospital. Faecal swabs were then obtained at intervals of one or two days until the infants were discharged from hospital.

Culture and Sensitivity Tests.—Each faecal swab was plated on a MacConkey agar plate for single colonies; it was also rubbed evenly over the whole surface of a second MacConkey plate to obtain a confluent bacterial lawn and a Multodisk (Oxoid 1141 E) was applied. The Multodisk contained the following drugs: ampicillin 25 µg., streptomycin 25 µg., tetracycline 50 µg., chloramphenicol 50 µg., and neomycin 30 µg. Any clearing around the discs was considered to indicate drug sensitivity. Lactose-fermenting colonies growing in the proximity of any of the discs were purified on a MacConkey agar plate and identified as *E. coli* from the following results: fermentation of lactose, glucose, maltose, and mannitol with the production of acid and gas; no fermentation of inositol;

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methyl-red positive; Voges-Proskauer negative; indole positive; and no growth in Koser's citrate medium. Single colonies were then tested for antibiotic sensitivity with the same Multodisk. At least 10 colonies were tested for slide agglutination with enteropathogenic *E. coli* antisera.

Resistance Transfer.—The recipient organism used was a strain of *E. coli* K12, E.711F⁻, that was resistant to nalidixic acid but sensitive to the five antibiotics which we tested. It is easily distinguished from other *E. coli* strains because it does not ferment lactose and requires histidine, tryptophan, and proline for growth. Overnight broth cultures from a single colony of the donor strain and the recipient E.711 strain were prepared. Donor culture 0.2 ml. was mixed with recipient culture 1.8 ml. and the mixed culture was incubated overnight. Next day the mixed culture was centrifuged and the whole deposit was streaked out on MacConkey agar plates, each containing 20 µg. of nalidixic acid per ml. plus one of the drugs to which the donor strain was resistant. The concentration of the other drug in each plate was 25 µg./ml. On the following day any non-lactose-fermenting colony on the plates was picked off and identified as E.711 by its inability to ferment lactose and its amino-acid dependence. The resistance acquired was confirmed by testing with the same Multodisk as was used for the screening sensitivity tests.

RESULTS

The majority of the swabs yielded an apparently pure growth of *E. coli*. The remainder yielded mixtures of *E. coli* and *Proteus*, of *E. coli* and *Streptococcus faecalis*, and of *E. coli*, *Proteus*, and *Str. faecalis*. None of the *E. coli* strains isolated were known enteropathogenic serotypes.

Sensitivity Tests on Admission Swabs.—The sensitivity tests on the 22 admission swabs showed that 15 (68%) of the 22 infants excreted resistant R+ *E. coli* strains, and eight of these strains were resistant to all five antibiotics (see Table).

Resistance Patterns of *E. coli* Strains Isolated from Admission Swabs

Resistance pattern . .	A S T C N	A S T C	A S T N	A S T	A T C S T
No. with each pattern	8	3	1	1	1 1 1

A = Ampicillin. C = Chloramphenicol. S = Streptomycin. N = Neomycin. T = Tetracycline.

Nine of the 22 infants were admitted from other hospitals; seven of these were excreting resistant R+ *E. coli*. The other 13 came direct from their own homes and eight of them were excreting resistant R+ strains.

Sensitivity Tests on Hospital Swabs.—Eleven of the 15 infants with resistant R+ strains on admission continued to excrete these strains while in hospital, though some were in

hospital for a considerable time—for example, 49, 40, and 20 days. After four days in hospital only fully sensitive strains or strains resistant to one antibiotic were isolated from the other four infants who had R+ strains on admission: two of them received antibacterial therapy in hospital; one was a hydrocephalic with meningitis and received courses of cloxacillin, streptomycin, and nalidixic acid; the other, also a case of meningitis, received ampicillin, sulphonamides, chloramphenicol, and nalidixic acid. The nalidixic acid was given to both infants for concurrent urinary tract infections. The seven infants from whom we isolated only fully sensitive strains on admission excreted multiple resistant R+ strains within a week. Four of these infants had no antibacterial therapy in hospital; three received one antibiotic only. We thought that these seven infants might have acquired their R+ strains in the hospital from other children. We therefore selected three of the infants, each in a different ward, and we took faecal swabs from all the children in these wards. The *E. coli* strains isolated were tested for sensitivity to the five antibiotics and for slide agglutination with enteropathogenic *E. coli* antisera. Faecal swabs from 34 (82%) of the 41 children in the three wards yielded multi-resistant R+ *E. coli* strains, none of which were known enteropathogenic serotypes.

DISCUSSION

Nine of the 22 infants were transferred from other hospitals, and seven of these were excreting resistant R+ *E. coli* strains which could have been acquired in those hospitals. The other 13 infants came direct from their own homes; exclusion from this group of two infants who had recently been in another hospital and of two who had previous antibacterial therapy left a total of nine whose intestinal flora might be expected to be normal. Of these six were excreting R+ *E. coli*. Little is known of the frequency with which R factors occur in the intestinal bacteria of normal people, and it is likely to vary considerably in different communities. Smith and Halls (1966) examined faeces from a small number of healthy human beings as well as from healthy farm livestock. They isolated R+ *E. coli* from the faeces of 15 out of 24 human beings, a figure comparable to ours of six out of nine. If our figure reflects the true incidence of R+ enterobacteria in the infant population, then R factors are widely disseminated in this country.

All 22 infants on admission were routinely screened for the presence of enteropathogenic *E. coli*, shigellae, and salmonellae. As none of them carried any of these pathogens they were admitted to open wards. Writing on the Tees-side outbreak of infantile enteritis caused by multiresistant R+ enteropathogenic *E. coli*, Anderson (1968) suggested that the R factors had probably been transferred from non-pathogenic *E. coli*. If R+ *E. coli* strains are as common in infants' faeces as our investigation suggests, there would be a strong likelihood of such transfer. The possession of non-pathogenic R+ *E. coli* is potentially dangerous, and the dangers have already been emphasized by others (Watanabe, 1963, 1966; Datta, 1965; Anderson, 1965, 1967). A most disturbing feature in our investigation was the high percentage (80%) of R+ *E. coli* strains we examined that were resistant to both ampicillin and chloramphenicol, when one considers possible infection with *Salmonella typhi*.

Four of the infants who were admitted with multiple resistant R+ *E. coli* excreted either fully sensitive strains or strains resistant to only one antibiotic after three to four days in hospital. As two of these infants were receiving multiple drug

therapy from the time of their admission, selection of resistant *E. coli* was to be expected. Yet we isolated only fully sensitive *E. coli* strains from their faecal swabs within a few days of admission. One explanation is that the resistant R+ strains were replaced by sensitive *E. coli* strains, acquired soon after admission to hospital; another is that the resistant strains lost their resistance genes. We have not differentiated between these two possibilities, but we believe that the first explanation is unlikely in view of the preponderance of resistant *E. coli* isolated from other children in the hospital. Spontaneous loss of resistance from R+ bacteria can occur (Watanabe, 1963), and this fact is of considerable importance in routine diagnostic bacteriology. Might it not be necessary to do more frequent sensitivity tests on Gram-negative enteric bacilli we isolate in our diagnostic laboratories? A multiple-resistant pathogen isolated from the first specimen of material we receive may within days become antibiotic-sensitive, thus giving the clinician a much wider choice of antibacterial drugs.

The high percentage (82%) of children within the hospital who were excreting R+ *E. coli* is a striking figure. It is not surprising that seven of the infants from whom we isolated only sensitive *E. coli* on admission excreted resistant R+ *E. coli* within a week. Presumably they acquired their R+ *E. coli* from other children in the wards.

Our total number of cases was small, but the findings answered our question. While some of the infants acquired their R+ *E. coli* within the hospital, the majority were admitted already carrying R+ *E. coli* in their intestinal tract, indicating the widespread existence of R factors in this country.

SUMMARY

Fifteen out of a total of 22 infants excreted multiple antibiotic-resistant strains of *Escherichia coli* on admission to hospital. These strains transferred part or all of their resistance to sensitive *E. coli* K12 in mixed culture. During their stay in hospital all 22 infants at some time excreted R+ *E. coli* strains.

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