STUDIES ON THE ASSOCIATION OF *Escherichia coli* WITH ANIMALS.

THESIS

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by

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STUDIES ON THE ASSOCIATION OF *Escherichia coli* WITH ANIMALS.

EXORDIUM.

Escherich (1885) described as *Bacterium coli commune* an organism which he had isolated from the stools of two healthy breast-fed infants. Subsequently, the same organism was found in the intestines of animals including the horse, the cow, the sheep, the goat, the pig, the dog, the cat, domestic and wild fowl and various species of rodents (Jensen, 1891; Dyar and Keith, 1893; Fremlin, 1893; Smith, 1895; Eyre, 1904; Houston, 1904).

Escherich (*loc. cit.*) observed that *Bact. coli commune* was a Gram-negative bacillus which grew on gelatin medium without any apparent liquefaction, exhibited fermentative ability in glucose solution and was feebly motile in hanging-drop preparations. Smith (1890) recorded the production of both acid and gas in glucose medium and Chantemesse and Widal (1891) noted that *Bact. coli* formed gas from a variety of carbohydrates that included glucose, sucrose, maltose, rhamnose, glycerol, erythritol and mannitol but not from starch or

from glycogen. To that list Jensen (1897) added arabinose, galactose, fructose, raffinose and sorbite and concluded that, although fermentative varieties of B. coli occur, the individual forms are fairly constant in their saccharolytic behaviour. However, Wilson (1929) remarked that, while fermentative tests are of great service in differentiating the typhoid-paratyphoid-colon bacteria, when they are applied to the B. coli group and when carbohydrates are employed in adequate number, the result is an almost infinite number of varieties.

Refik (1896) made use of the property of indole-production as a means of distinguishing coli-bacilli. That ability, however, is not absolute. Wilson et al. (1935), for example, reported that of two hundred and nineteen cultures of Bact. coli 18 per cent. failed to form indole.

Orlowski (1897) first suggested the use in media of iron or lead salts to distinguish B. typhosus, which produces hydrogen sulphide, from Bact. coli which does not so behave.

Voges and Proskauer (1898) described a colour reaction given by certain enteric bacteria, but not by

Bact. coli, when a few drops of potassium hydroxide solution are added to a culture that has been grown in dextrose medium. A positive reaction was explained by Harden and Walpole (1906) as due to the formation of acetyl-methyl-carbinol which, when mixed with potassium hydroxide in the presence of peptone, slowly gives rise to a pink colour.

Eijkman (1904) demonstrated that, after incubation at 46°C., Bact. coli fermented glucose with the production of both acid and gas. Wilson et al. (1935) emphasized the need for a water-bath maintained at a temperature of 43-45°C. and recommended that MacConkey's lactose broth replace Eijkman's medium.

Clark and Lubs (1915) showed that the difference in hydrogen-ion concentration produced by the growth of Bact. coli and of related organisms in a suitable carbohydrate medium was easily recognizable after the addition of the indicator, methyl red. Development of a red colour betrayed the low pH that is characteristic of Bact. coli.

Further to an observation by Brown (1921) that Bact. coli communis failed to break down available citrate, Koser (1924) described the use of a synthetic

medium in which sodium citrate was the sole source of organic carbon. Simmons (1926) modified that medium by the addition of agar and bromo-thymol-blue. Bact. coli was unable to flourish on those media.

Mitchell and Levine (1938) reported that Escherichia coli was unable to utilize urea, which finding was confirmed by Christensen (1946).

Since haemolysin-production by B. coli was observed by Kayser (1903), haemolytic strains have been recovered on many occasions and Smith (1963) has shown that they are common in healthy man and animals.

Extreme antigenic heterogeneity on the part of E. coli was recognized for many years but poor agglutinability was a peculiarity of that organism until Kauffmann (1943) explained the phenomenon. He demonstrated the presence in E. coli of two types of antigen, namely, a heat-labile 'L' surface one which caused 'O'-inagglutinability, as well as a heat-stable somatic 'O' antigen. By heating the organism to 100°C., he was able to destroy the 'L' antigen, thus allowing the 'O' portion to react with the homologous serum and to produce agglutination to high titre.

Kauffmann (1944) observed that some strains of E. coli were not rendered 'O'-agglutinable, even, after they had been exposed to 100°C. for two-and-a-half hours. He designated those variants as 'A' forms. Knipschildt (1945a & b) continued the study of 'A' forms and showed that their thermoresistance was due to thermostable capsular antigens which he called 'A' antigens. He described the growth of 'A' forms on solid media as denser and more voluminous than that of the usual strains. Vahlne (1945) showed that 'A' forms became 'O'-agglutinable after they were autoclaved at 120°C. for two hours.

Knipschildt (1945a, 1946) described a new thermolabile surface antigen which he named 'B' antigen. Heating the 'B' form at 100°C. was found to destroy the agglutinability of the antigen but not to affect the agglutinin-binding property.

Kauffmann and Vahlne (1945) suggested the use of the group term 'K' antigen to cover the different 'L', 'A' and 'B' antigens of E. coli.

Edwards and Ewing (1962) recorded that one hundred and forty-five 'O'-antigenic groups had been distinguished, eighty-six 'K' antigens had been recognized and forty-nine 'H' antigens were known.

The results of serological studies on E. coli have revealed that the population of the organism in the normal mammalian intestine is usually distinguished by a high degree of stability and that the latter must depend on means of strict control. The investigation undertaken by the writer was designed to extend knowledge of E. coli in the bowel of animals and of factors which determine the composition of that flora. The work was carried out during the period from March 1963 to September 1966 and was divided into the following two parts:

Part I. The composition of the Escherichia coli flora of the gut of the normal domestic chicken (Gallus domesticus) and of the Syrian hamster (Mesocricetus auratus).

Part II. An investigation into factors associated with the control of the Escherichia coli population of the bowel of animals.

PART I.

THE COMPOSITION OF THE *Escherichia coli* FLORA OF THE GUT OF THE NORMAL DOMESTIC CHICKEN (*Gallus domesticus*) AND OF THE SYRIAN HAMSTER (*Mesocricetus auratus*).

HISTORICAL.

Smith (1899) appears to have been the first person to carry out a serological examination of *Bact. coli commune* procured from a series of faecal samples taken from the same individual. During a period of approximately seven weeks, he examined forty-eight cultures, that had been isolated from four different platings of the stools of one normal infant, with an antiserum prepared against one of those cultures. All but two of the cultures were agglutinated by that serum.

Totsuka (1903) plated his own faecal specimens once a week for twelve consecutive weeks and selected thirty-two colonies of *Bact. coli* from each plating. He employed two antisera prepared against the first cultures from the first two platings. His results indicated that, although the number of cultures from each successive plating agglutinable by those sera progressively diminished, at the end of the period of twelve weeks

there were still some which reacted serologically.

Wallick and Stuart (1943) collected specimens at regular intervals from one human subject during a period of fifteen months and examined ten colonies from each. Their findings suggested that, at any particular time, the intestinal population of E. coli consists of a single dominant antigenic type which persists over a long period of time together with several other forms of quite transitory occurrence.

Kauffmann and Perch (1943) reported a study of two persons conducted over a period of six months and involving fortnightly examinations of faecal samples. Their results from one individual agreed well with the findings of Wallick and Stuart (loc. cit.) but the other subject was shown to possess a less stable E. coli bowel flora. Perch (1944), however, recorded that faecal samples from the latter individual examined one year later contained, at least, two of the previously identified types, which finding suggested that some strains may persist in the gut over a considerable period of time.

Sears et al. (1950) investigated the occurrence of E. coli 'O'-groups in the faeces of two persons for

two-and-a-half years and, in the case of two other individuals, during a period of less than three months. They, too, concluded that, at any particular time, the E. coli flora of the human intestinal tract consists of strains that persist over relatively long periods together with more than three or four others that survive for only a few days or weeks. Those two types were designated ' residents ' and ' transients ', respectively. Sears and Brownlee (1952) described further observations on the resident population of two of the subjects of the previous investigation and included an account of a similar examination of three infants. They demonstrated that the pattern of residents and transients may be shown by very young babies.

Emslie-Smith (1961) reported observations concerning the secular succession of types of E. coli found in the faecal flora of an adult human being and Robinet (1962) stated that a survey of six healthy persons, examined monthly for six months, confirmed the periodic fluctuations of antigenic types.

In the case of animals, Sears et al. (1956) studied the E. coli flora of two separately caged dogs and found that they showed the same general pattern of

strain composition as did human beings. Sakazaki and Miura (1956), who gave an account of the enteric bacterial flora of healthy horses, observed that E. coli occurred as residents and transients similar to those encountered in the human bowel.

Smith (1960) examined the E. coli composition of the faeces of healthy cows and calves by bacteriophage-typing techniques and ascertained that some types prevailed for a week or so to be succeeded in dominance by others. In some animals, one type might remain ascendant for a considerable period of time but Smith did not offer any information on the serological relationships of those bacteriophagic types.

In respect of the distribution of E. coli among members of the same community, Sears and Brownlee (1952) noted that infants as intimately associated as are twins are likely to harbour the same residents as well as freely to share the transient strains. Orskov (1952) reported that investigations of the coli-flora of healthy children in institutions have shown that each establishment is characterized by few coli-types demonstrable in a great number of children, with variations from place to place. Taylor and Charter (1952) described the

epidemiology of three 'O'-groups among infants in two residential nurseries. The serogroups were shown to persist in those communities for periods of several months and to appear in the stools of new members soon after arrival.

Wood (1955) recorded findings from studies of the incidence of E. coli types in groups of calves. At the beginning of the season when the animals were first gathered together, the E. coli population of the community was very heterogeneous but, later, particular serological types became widely established. Wood observed the appearance of a succession of those dominant types.

In contrast, Sears et al. (1956) examined weekly faecal samples from a man and his wife during a period of four hundred and fifty-one days but failed to find any indication that they ever exchanged their peculiar strains of E. coli.

In order to assess the relative ability of E. coli to become established in the bowel of animals, the author of the thesis under report chose the domestic chicken (Gallus domesticus) and the Syrian hamster (Mesocricetus auratus) as suitable for experiments

involving oral administration of new strains. The degree of success of such experiments is measurable by the resemblance borne by the pattern of excretion of the strain under test by the host to that of the normal resident population. Implantation of E. coli has been claimed by some workers (Rauss and Ketyi, 1960; Ashburner and Mushin, 1962) following the demonstration of a prolonged association of the test organism with the gut of the experimental host but, apart from work reported by Sears et al. (1950, 1956) and by Sears and Brownlee (1952), interpretation of the findings does not appear to have been based on a previous study of the regular enteric flora of the species of animal employed.

Exploration of the available literature failed to reveal any record pertaining to the normal population of E. coli that prevailed in the gut of either the domestic fowl or the Syrian hamster. Accordingly, the author was not inclined to accept the flora as necessarily similar to that of other animals already studied but preferred to obtain proof of the normal constitution of the E. coli present in the intestine of the chosen species. In consequence, a detailed study was made of strains of E. coli recovered from the faeces of three domestic chickens and a like, but less intensive,

investigation was undertaken into the same species of micro-organism obtained from two Syrian hamsters.

MATERIALS.

Three Leghorn bantams, about three months of age, were selected from a group of chickens which had been hatched on the premises from one setting of eggs that had been fertilized by the same male progenitor. The three birds had been reared together in the animal house under identical conditions. Two were cockerels, one black and one white, and the third was a grey pullet. They were conveniently designated as Chickens 'B', 'W' and 'G', respectively. From the beginning of the investigation, they were kept solitarily on bedding of peat moss litter in rabbit cages that were cleaned out twice weekly. They were fed on commercial meal and fresh water was constantly available. From each chicken, a cloacal swab was taken weekly until fifty-one weeks had elapsed. By that time, only bird 'B' remained under observation and from it faecal samples were thereafter procured at, approximately, monthly intervals.

Two adult Syrian hamsters, one female and the other male, numbered '11' and '12', respectively, were obtained from a breeder. After arrival, they were

caged separately in mouse boxes on bedding of peat moss litter and wood-wool and were fed on Diet 18. Water was always available. The cages were cleaned twice weekly when fresh bedding was also supplied. Faecal samples were collected weekly at eighteen to twenty-four hours after the cages had been cleaned. By means of flamed forceps, fresh faecal pellets were picked out of the bedding and consigned to sterile bijou bottles. Six, or more, pellets were selected to form each sample.

'Oxoid' MacConkey 3 agar was employed in the examinations for the isolation of lactose-fermenting coliform bacteria from faecal material. In addition to the normal ingredients, that medium contains crystal violet, at a concentration of 0.001 gm. per litre, that effectively suppresses Gram-positive cocci. The resultant isolates were subcultivated on Dorset egg slopes.

Antisera used in the course of the investigation comprised:

(a) Burroughs Wellcome Escherichia coli monovalent 'OB' diagnostic antisera for groups O26:B6, O55:B5, O111:B4, O119:B14, O125:B15, O126:B16, O127:B8 and O128:B12 and Escherichia coli polyvalent I antiserum which was declared to contain both 'O' and 'B' antibodies for all eight of the preceding strains and

(b) antisera prepared by the author inclusive of one against a culture of E. coli O103:K? isolated from a Syrian hamster and seven against strains of E. coli removed from the chickens under experiment. The latter micro-organisms were later examined by Dr. Joan Taylor, of the Salmonella Reference Laboratory, Colindale, for their identity within the International Scheme of Classification. Her findings were as follows:

Strain number	International classification	Strain number	International classification
B1	O1:K?	G33	O73:K?
B31	O37:K?	G62	O18ac:K77
B51	Not identifiable	G107	O2:K?
B233	Not identifiable		

METHODS.

The investigation into the composition of the faecal flora of Chickens 'W' and 'B' was begun on 3/8/64 and continued until 7/6/65 and 10/5/66, respectively. The examination of Chicken 'G' was commenced on 17/8/64 and sustained until 21/12/64. The study of Hamster '11' was initiated on 5/3/65 and lasted until 28/10/65 while that of Hamster '12' prevailed from 20/4/65 until 21/7/65.

The incidence of serological types of E. coli

in the bowel of the experimental animals was traced thus:

(A) Cultures of lactose-fermenting coliform bacteria were procured from faecal samples.

(B) The cultures were examined for reactions to the following tests:

(1) Eijkman test, (2) Production of indole, (3) Methyl-red reaction, (4) Voges-Proskauer reaction, (5) Citrate-utilization test, (6) Production of hydrogen sulphide, (7) Liquefaction of gelatine, (8) Utilization of urea and (9) Fermentation of carbohydrates. In addition to biochemical examinations, the cultures were tested for motility. The findings from the Voges-Proskauer and methyl-red tests were regarded as critical but failure of a strain to give the expected reaction in any one of the other examinations did not preclude its identification as E. coli.

(C) All of the strains which were identified as E. coli on the basis of biochemical activity were tested for serological behaviour with specific E. coli antisera. The serological procedures involved were:

(1) Preparation of antisera in rabbits to strains of E. coli isolated from animals under observation.

(2) Quantification of antibodies in the sera to 'O' antigen of the homologous E. coli strain. Standard

suspensions which had been heated at 100°C. for two hours and at 120°C. for two-and-a-half hours, respectively, were employed in parallel for that purpose.

(3) Examination of E. coli isolates from the experimental animals for 'O'-agglutinability in the presence of specific antiserum. In the case of the chickens, an antiserum was prepared in a rabbit to the first strain of E. coli which was isolated from faecal material from Chicken 'B' and used to test the agglutinability of cultures procured from the platings from all three birds. From those cultures that failed to react with the foregoing antiserum was selected another strain which was employed for the production of antiserum and, in that way, the incidence of seven different 'O' serogroups was revealed among E. coli procured from the creatures. Cultures obtained from hamsters were titrated against an antiserum produced to a strain of E. coli O103:K? recovered from a hamster received from the same source as those under investigation. All of the E. coli cultures from chickens and hamsters which were not identifiable by means of the foregoing sera were examined for both 'O'- and 'B'-agglutination by Burroughs Wellcome 'OB' diagnostic antisera for groups O26:B6, O55:B5, O111:B4, O119:B14, O125:B15, O126:B16, O127:B8 and O128:B12.

(4) Finally, individual cultures of the resident

serogroups of long standing were selected at, approximately, fortnightly intervals for investigation of their ability to reduce the agglutinating property of the type serum for its homologous antigen.

(A) ISOLATION OF BACTERIAL CULTURES FROM FAECAL SAMPLES.

In the case of chickens, each cloacal swab was used to inoculate a plate of ' Oxoid ' MacConkey 3 agar. The plate was incubated at 37°C. for eighteen hours whereupon ten lactose-fermenting colonies were selected at random and subcultured on Dorset egg slopes. The cultures were given the designative letter of the bird from which they were isolated and were numbered serially, from one upwards, in the order in which they were selected, e.g., B1.

A few drops of sterile nutrient broth were added to each of the faecal samples from hamsters and the material was mashed with the aid of a flamed glass rod. A loopful of the faecal suspension was sown thinly over the surface of an ' Oxoid ' MacConkey 3 agar plate and, after incubation at 37°C. for eighteen hours, five to ten lactose-fermenting colonies were selected at random for subculture on Dorset egg slopes. The cultures were labelled with the identifying number of the animal of origin, the date of preparation and other numerals, from

one to ten, that accorded with the sequence of their isolation, e.g., 11/5:3:65/1.

(B) BIOCHEMICAL METHODS.

1. Eijkman test. A ' Universal ' bottle containing ten millilitres of ' Oxoid ' MacConkey lactose broth together with a Durham tube was inoculated with the organism under test and incubated in a water-bath at 44°C. for eighteen hours, after which time it was examined for growth with production of acid and gas.

2. Production of indole. The test strains were inoculated into four millilitres of four per cent. peptone water, contained in bijou bottles, and subjected to incubation at 37°C. for eighteen hours. To each tube, approximately, 0.5 ml. of ether was then added and, after thorough shaking, the culture was allowed to stand until the ether had collected at the surface, whereupon a few drops of Ehrlich's rosindole reagent were introduced at the interface of the fluids. The development of a deep pink colour in the ethereal layer or at the junction of the latter with the culture was regarded as a positive reaction. If a strain proved negative for indole-production after incubation for eighteen hours, the test was repeated with another culture which had been incubated for seven days.

3. Methyl-red reaction. Two drops of an 0.04% alcoholic solution of methyl red were added to 2.5 ml. of a glucose-phosphate-peptone water culture of the test organism after the latter had been grown at 37°C. for three days. A red colour denoted a positive reaction, characteristic of E. coli.

4. Voges-Proskauer reaction. For this work, the modification of the test according to Barritt (1936) was preferred on account of the velocity of the reaction. To 2.5 ml. of a glucose-phosphate-peptone water culture, after incubation at 37°C. for forty-eight hours, was added 1.5 ml. of a 5 per cent. alcoholic solution of α-naphthol and 0.5 ml. of 40 per cent. potassium hydroxide solution. A positive result was indicated by the development of a deep pink colour within one hour.

5. Citrate-utilization test. Because of its convenience, the medium devised by Simmons (1926) was employed in this investigation. A straight needle bearing a minimal inoculum was used to stroke the surface of a citrate-agar slope and the culture was examined for growth after incubation at 37°C. for twenty-four and forty-eight hours.

6 and 7. Production of hydrogen sulphide and

Liquefaction of gelatine. The medium used in this investigation was ferric chloride gelatine (Kauffmann, 1954). A bijou bottle containing four millilitres of that preparation was inoculated with a drop of a peptone-water culture of the test organism and incubated at 37°C. for seven days. Darkening of the medium indicated the production of hydrogen sulphide. The bottle was then placed in the refrigerator at 4°C. for half-an-hour. Liquefaction of the medium was denoted by its failure to re-solidify.

8. Utilization of urea. A bijou bottle containing 2.5 ml. of fluid urea medium (Christensen, 1946) was inoculated with one drop of a peptone-water culture of the test organism, after which it was incubated at 37°C. for up to seven days. The culture was examined for the development of a red colour, indicative of an alkaline reaction resulting from the breakdown of urea.

9. Fermentation of carbohydrates. This was determined in respect of two sugars, namely: dextrose and lactose. One per cent. solutions of carbohydrate in one per cent. peptone water containing one per cent. Andrade's indicator were distributed in 4 ml. amounts in bijou bottles, each provided with a Durham tube. After inoculation with the test strain, the cultures were

incubated at 37°C. and inspected at intervals during a period of seven days for evidence of acid and gas production.

Motility. Motility proved so difficult to observe by means of the hanging-drop method that the procedure was abandoned in favour of the method according to Craigie (1931). After inoculation, the Craigie tube was incubated at 37°C. for twenty-four hours and inspected for growth down and into the main mass of the medium, characteristic of a motile bacterium.

(C) SEROLOGICAL PROCEDURES.

The methods are reported under four headings:

(1) Preparation of antisera;

(2) Titration of antisera;

(3) Serological identification of *E. coli* cultures and

(4) Absorption of agglutinins.

1. PREPARATION OF ANTISERA.

To ensure that the selected strain of *E. coli* was in the 'smooth' form, the stock culture was sown thinly on a 5 per cent. horse-blood-agar plate and, after incubation at 37°C. for eighteen hours, portions of a representative colony were tested for ability to produce

stable suspensions in both normal saline and a solution of 1/500 acriflavine in normal saline. The remainder of the colony was transferred to the surface of a Dorset egg slope and the resultant culture became the source of organisms required for the production of antigenic suspensions.

The antigenic suspension consisted of the growth from a culture on sloped nutrient agar which had been incubated at 37°C. for eighteen hours. The bacteria were suspended in 0.5% formol saline solution and density was adjusted to an opacity equal to that of Brown's tube, No. 3. For each injection, a fresh suspension was prepared.

The suspension was administered intraperitoneally to a rabbit in doses of 0.2 ml., 0.4 ml., 0.8 ml., 1.6 ml. and 4.0 ml. at intervals of three to four days. Before it was bled, the rabbit was rested for seven to ten days when the serum was collected and preservation effected by addition of phenol, to an amount of 0.5 ml. of a 5 per cent. solution of phenol in normal saline for every ten millilitres.

Altogether, antisera were prepared to seven strains of E. coli isolated from the experimental

chickens and labelled according to the designation of the homologous strain, namely, 'B1', 'B31', 'B51', 'B233', 'G33', 'G62' and 'G107'. An antiserum was similarly prepared to a strain of E. coli 0103:K? obtained from a hamster.

2. TITRATION OF ANTISERA.

In a sequence of bijou bottles were prepared serial dilutions of antiserum in saline that ranged from 1/100 to 1/25,600. An equivalent number of Dreyer tubes was set up and 0.3 ml. of each dilution of serum was transferred to the corresponding tube.

The standard antigenic suspension consisted of a nutrient broth culture of the homologous organism which had been incubated at 37°C. for eighteen hours and steamed at 100°C. for two hours. To each tube of diluted serum was added 0.3 ml. of antigen whereby the final dilutions of antiserum came to range from 1/200 to 1/51,200. A control tube, containing 0.3 ml. of saline and 0.3 ml. of antigenic suspension, was also included.

Mixture of the serum and antigen was effected by manual agitation before the tubes were placed in a water-bath at 50°C. Eighteen hours later, the rack was removed to be left on the bench at room temperature for

half-an-hour. For detection of agglutination, the tubes were scrutinized in the light emanating from a hooded microscope lamp. The titre of the serum was taken to be the last dilution in which some fifty per cent. of clearing was appreciable by the naked eye.

A like examination was performed on a nutrient broth culture of the homologous organism which had been incubated at 37°C. for eighteen hours and heated at 120°C. for two-and-a-half hours in an autoclave. In each case, it was observed that the titre of the serum was the same with both antigenic suspensions and, therefore, the homologous strain was not characterized by the production of an 'A'-type surface antigen.

3. SEROLOGICAL IDENTIFICATION OF E. coli CULTURES.

Two different techniques were employed:
(a) an investigation into 'O'-agglutinability of cultures in antisera prepared in rabbits by the author and
(b) an examination of both the 'O' and 'B' antigens of strains not identifiable by the preceding method by means of Burroughs Wellcome 'OB' diagnostic antisera.

(a) Serial dilutions of known antiserum in normal saline were prepared in the same manner as that employed for titration but use was made only of the three highest

dilutions which had induced agglutination of the standard suspension of the homologous organism and were numbered 1, 2 and 3 in order of increasing dilution.

The test antigenic suspension consisted of 10 ml. amounts of nutrient broth culture which had been incubated at 37°C. for eighteen hours, steamed at 100°C. for two hours and preserved by the addition of 0.5 per cent. formalin. That amount provided stock antigen for several tests.

Dreyer tubes were arranged in rows of four in racks, one row for each test antigen. Into the fourth tube of each row was pipetted 0.3 ml. of normal saline solution and the same volume of serum dilutions 3, 2 and 1 were added to the third, second and first tubes, respectively. The antigenic suspension under test was pipetted in 0.3 ml. amounts into all four tubes in the row. A similar array of four tubes containing the same serum dilutions and the standard homologous antigen was always included.

The tubes were then incubated at 50°C. in a water-bath for eighteen hours and thereafter for half-an-hour at room temperature ere they were examined for gross agglutination in the illumination provided by a microscope

lamp. Non-specific agglutination of an antigenic suspension was indicated by clearing and sedimentation in all four tubes in the row. If agglutination was observable in any of the serum dilutions, but not in the control tube, the result was recorded and compared with that noticeable in the set of tubes containing the standard homologous antigen. Provided that it gave fifty per cent. agglutination to within one tube of the titre obtained with the homologous organism, a culture was presumed to belong to the same serological group. On the few occasions on which the result was dubious, the test was repeated with a freshly prepared suspension of the test organism.

(b) When the cultures had been tested with the antisera prepared against selected strains, the remainder of unidentified suspensions was screened by means of a simple tube agglutination test dependent on the use of Burroughs Wellcome Escherichia coli polyvalent 'OB' diagnostic antiserum. The latter contains both 'O' and 'B' antibodies for groups O26:B6, O55:B5, O111:B4, O119:B14, O125:B15, O126:B16, O127:B8 and O128:B12. The serum was diluted to 1/50 and to an amount of 0.3 ml. of it contained in a Dreyer tube was added a like volume of stock 'O' suspension of the organism under test.

Thereby, a final serum dilution of 1/100 accrued. For each antigenic suspension, a saline control tube was also included. The tubes were incubated in a water-bath at 50°C. for eighteen hours and, after a further half-hour at room temperature, were inspected in the illumination of a microscope lamp for evidence of agglutination. Strains which exhibited any degree of agglutination were set aside for more detailed study.

A culture of each of those strains was prepared subsequently on a five per cent. horse-blood-agar plate and incubated at 37°C. overnight. By means of a straight needle, portions of the resultant growth were transferred and suspended in a small drop of saline and also in a similar drop of E. coli polyvalent 'OB' diagnostic antiserum on a glass slide. The test was observed under a low-power objective. If the strain agglutinated in the drop of serum but not in the saline, portions of the growth from the solid medium were suspended in a series of small drops of 'OB' antisera monovalent for the eight 'OB' groups. If clumping occurred in one of those sera, it was concluded that that manifestation was probably the result of a reaction between 'B' antibody and the homologous surface antigen of the bacterium. The identity of the somatic 'O' antigen was investigated by

a tube agglutination test with the same monovalent 'OB' antiserum.

Although the nature of the standard antigen was not described, the 'O' titre of those commercial antisera was given as 500, which figure was interpreted by the author to mean five hundred agglutinating units per millilitre of serum. The test organisms were examined in two dilutions of monovalent serum. The first was prepared by the addition of 0.1 ml. of serum to 4.9 ml. of normal saline, so to yield a concentration of ten units per millilitre. One part of the mixture was then diluted with two parts of saline to provide a second dilution containing approximately three units per millilitre. Racks were prepared in which Dreyer tubes were set out in rows of three. Into the third tube of each row was pipetted 0.3 ml. of normal saline and similar volumes of the second and first serum dilutions were added to the second and first tubes. The first tube, therefore, contained three agglutinating units and the second tube approximately one unit. To all three tubes in the row were added 0.3 ml. amounts of a nutrient broth culture of the test organism which had been incubated at 37°C. for eighteen hours and then exposed to live steam at 100°C. for two hours. Thereafter, the tubes were incubated for

eighteen hours at 50°C. in a water-bath and at room temperature for another half-hour before the result was read in the illumination provided by a microscope lamp. If a culture exhibited agglutination that was complete in the first, and was complete or partial in the second, dilution of serum, it was considered to belong to that serological 'O'-group. Strains deemed to have given positive results were forwarded to Dr. Joan Taylor for confirmation.

4. ABSORPTION OF AGGLUTININS.

Each test culture was sown thickly over twenty nutrient agar plates and incubated at 37°C. for eighteen hours. To each plate was then added two millilitres of sterile normal saline and the growths suspended and collected in two ' Universal ' bottles. The latter were subsequently heated at 100°C. for two hours before the contents of one were centrifugalized at 2,500 r.p.m. for thirty minutes and the supernatant discarded. 1.0 ml. of a 1/10 dilution of the group antiserum was then added to the sediment and, after thorough mixing had been effected, the container was stored in a refrigerator at 4°C. overnight, after which it was again centrifugalized for half-an-hour at 2,500 r.p.m. The supernatant fluid was used to resuspend the sediment obtained after centri-

fugalization of the contents of the second ' Universal ' bottle and the process of absorption was allowed to continue in the refrigerator for a further twenty-four hours. The suspension was again spun at 2,500 r.p.m. for half-an-hour when the supernatant fluid was carefully decanted and retained.

0.3 ml. of absorbed serum was pipetted into each of two Dreyer tubes and to one of those was added the same volume of a standard suspension of the absorbing culture while the other was augmented with 0.3 ml. of standard homologous antigen. Saline control tubes were prepared for both antigenic suspensions and a control consisting of 0.3 ml. amounts of 1/10 unabsorbed serum and standard homologous antigen was also included in the series. The latter was designed to reveal any prozone reaction which might occur in serum at such concentration and which might still interfere with the test in the event of incomplete absorption. The final dilution of all the sera was 1/20. When the tubes had been agitated in order to mix the contents, the rack was placed in a water-bath at 50°C. for eighteen hours. After a further half-hour at room temperature, the tubes were inspected for agglutination in the usual way. Since the original titres of the antisera ranged from 1/6,400 to 1/12,800,

failure of the absorbed serum at a dilution of 1/20 to produce any agglutination of either the absorbing culture or the standard antigen was regarded as reasonable endorsement of the view that both of those E. coli strains belonged to the same serogroup.

RESULTS.

The findings from the investigations into the faecal E. coli flora of chickens and hamsters are described separately.

CHICKENS.

The outcome in relation to excretion of eight serogroups by individual birds was as follows:

CHICKEN 'B' (v. Table 1, pp. 38-49).

During the period of eighty-one weeks, beginning on 3/8/64 and ending on 22/2/66, Chicken 'B' was observed to excrete E. coli belonging to Group No. B1. Altogether, four hundred and ninety-three (87.4%) of the five hundred and sixty-four cultures examined were of that type and from only one plating was it not isolated. Between 22/2/66 and 5/4/66, the residency of Group No. B1 terminated and four subsequent examinations failed to reveal its presence.

Group No. B31 strains were demonstrated in the

second plating and that type persisted for at least ten weeks, although its occurrence was not detected in six of the specimens taken during the period.

The three other serological groups isolated from Chicken 'B' were noticeable for short tenures. Group No. B51 was obtained from the sixth sample only but Group No. B233 was found at the twenty-first, twenty-second, twenty-fourth and twenty-fifth selections, indicative of a tenure of four weeks. The last group, No. G33, was recovered from the twenty-eighth and thirtieth samples, which finding was suggestive of a tenure of two weeks.

CHICKEN 'W' (v. Table 2, pp. 41-42).

During a period of forty-five weeks, four hundred and forty-nine cultures of E. coli were isolated. Four hundred and thirty-seven of those strains (97.3%) proved to belong to Group No. B1, which serological type was not absent from any of the platings. The only other demonstrable serological group was No. B31, to which belonged five cultures from the sixth plating and one from the twenty-sixth isolation that was effected twenty weeks later.

The bird was sacrificed on account of progressive

loss of condition extending over several weeks and the gross post-mortem findings indicated the existence of avian leucosis.

CHICKEN 'G' (*v.* Table 3, p. 43).

Over a period of eighteen weeks, one hundred and seventy-three cultures of *E. coli* were procured from this source. For the first four weeks, Groups Nos. B1 and G33 were present simultaneously. Between the fifth and sixth platings, however, the bird developed a generalized eczematous condition for which it was given an empirical course of antibiotic therapy consisting of five daily injections of 20 mgm. of streptomycin together with oral doses of 20 mgm. of griseofulvin. That therapy was associated with the last appearance of Group No. B1, although Group No. G33 was recovered from one sample which was examined two weeks after treatment was withdrawn.

Two new resident groups, Nos. G62 and B31, were detected during the remaining thirteen weeks of study, although there was an interval of eight weeks during which Group No. G62 was not isolated. During the tenure of those groups, between the ninth and tenth and the sixteenth and seventeenth samples, the bird was given two courses of 'Betsolan' therapy in respect of the cutaneous condition which had failed to respond to the

antibiotics. The treatment consisted of five daily intramuscular injections of 1.0 mgm. of ' Betsolan '. Those courses of treatment co-incided with the disappearance of Group No. G62 and its recovery eight weeks later.

The other three antigenic groups procured, namely Nos. B51, G107 and 026:B6, were noteworthy for very short tenures which did not last for more than one week.

Both courses of ' Betsolan ' therapy were accompanied by temporary resolution of the cutaneous disorder but, when the condition recurred for a third time, the bird was sacrificed. Routine autopsical investigation failed to reveal the cause of the disease.

Results of agglutinin-absorption tests on strains secured from chickens.

Twenty-five E. coli cultures isolated from Chicken 'B' at approximately fortnightly intervals and twenty-three similarly procured from Chicken 'W', all of which were agglutinated to titre by Group No. B1 antiserum, were shown by agglutinin-absorption to reduce completely the agglutinating property of the group antiserum for the standard homologous antigen.

HAMSTERS.

The distribution of E. coli between the two animals was as follows:

HAMSTER '11' (v. Table 4, p. 44).

From twenty-four faecal samples, examined during a period of thirty-four weeks, one hundred and seventy cultures of E. coli were isolated. Of the latter, one hundred and fifty-four (90.5%) were found to belong to Group 0103, members of which were detectable in all but one of the specimens.

Fourteen cultures obtained at approximately fortnightly intervals, which were agglutinated to titre by Group 0103 antiserum, were shown by agglutinin-absorption to reduce completely the agglutinating property of Group 0103 antiserum for the standard homologous antigen.

The examination was terminated by euthanasia of the animal because of gradual loss of condition, the causation of which was not revealed by autopsy but was possibly related to advanced age.

HAMSTER '12'

Seven faecal samples, taken during the first five weeks of the investigation, failed to yield any

coliform colonies. The specimen procured in the sixth week furnished two colonies of E. coli and three of aerogenes-type but the seventh and eighth weekly samples again proved negative. At nine, ten and eleven weeks, only aerogenes-type colonies were encountered but five colonies of E. coli were forthcoming during the subsequent week and two of similar identity were recovered a fortnight later. Aerogenes-types were isolated from the intervening sample. The examination ceased after fourteen weeks, during which only nine cultures of E. coli were isolated. Although this animal came from the same source as did Hamster '11', none of the cultures was agglutinable by Group 0103 antiserum.

Table 1.

CHICKEN 'B'.

Date of sample	No. of *E. coli* isolated	Number belonging to serogroups					
		B1	B31	B51	B233	G33	*
3/ 8/64	10	10					
10/ 8/64	10	9	1				
17/ 8/64	10	10					
24/ 8/64	10		9				1
31/ 8/64	10	10					
7/ 9/64	10	3	3	3			1
14/ 9/64	10	8	1				1
21/ 9/64	10	10					
28/ 9/64	10	10					
5/10/64	10	10					
12/10/64	10	10					
19/10/64	10	6	2				2
26/10/64	10	10					
2/11/64	10	10					
9/11/64	10	9					1
16/11/64	10	10					
25/11/64	10	10					
30/11/64	10	8					2
7/12/64	10	10					
14/12/64	10	10					

* Serologically unidentified.

(contd. overleaf)

Table 1 (contd.).

CHICKEN 'B'.

Date of sample	No. of *E. coli* isolated	Number belonging to serogroups					
		B1	B31	B51	B233	G33	*
21/12/64	10	8			1		1
28/12/64	10	9			1		
4/ 1/65	10	8					2
11/ 1/65	10	4			6		
18/ 1/65	10	8			1		1
25/ 1/65	10	10					
1/ 2/65	10	9					1
8/ 2/65	10	3				1	6
15/ 2/65	10	10					
22/ 2/65	9	7				1	1
1/ 3/65	10	8					2
8/ 3/65	10	2					8
15/ 3/65	10	10					
22/ 3/65	10	10					
29/ 3/65	10	10					
5/ 4/65	10	10					
12/ 4/65	10	10					
19/ 4/65	10	8					2
26/ 4/65	5	5					
3/ 5/65	10	10					

* Serologically unidentified.

(contd. overleaf)

Table 1 (contd.)

CHICKEN 'B'.

Date of sample	No. of *E. coli* isolated	Number belonging to serogroups					
		B1	B31	B51	B233	G33	*
10/ 5/65	10	10					
17/ 5/65	10	10					
24/ 5/65	10	10					
31/ 5/65	10	10					
7/ 6/65	10	10					
14/ 6/65	10	10					
21/ 6/65	10	10					
28/ 6/65	10	10					
5/ 7/65	10	8					2
12/ 7/65	10	10					
19/ 7/65	10	10					
26/ 8/65	10	10					
29/ 9/65	10	7					3
2/11/65	10	10					
7/12/65	10	9					1
20/ 1/66	10	6					4
22/ 2/66	10	10					
5/ 4/66	10						10
12/ 4/66	10						10
26/ 4/66	10						10
10/ 5/66	3						3

* Serologically unidentified.

Table 2.

CHICKEN 'W'.

Date of sample	No. of *E. coli* isolated	Number belonging to serogroups		
		B1	B31	*
3/ 8/64	10	10		
10/ 8/64	10	10		
17/ 8/64	10	10		
24/ 8/64	10	10		
31/ 8/64	10	10		
7/ 9/64	10	6	4	
14/ 9/64	10	10		
21/ 9/64	10	10		
28/ 9/64	10	9		1
5/10/64	10	9		1
12/10/64	9	9		
19/10/64	10	10		
26/10/64	10	10		
2/11/64	10	10		
9/11/64	10	10		
16/11/64	10	10		
25/11/64	10	10		
30/11/64	10	10		
7/12/64	10	10		
14/12/64	10	10		
21/12/64	10	9		1
28/12/64	10	10		
4/ 1/65	10	10		

* Serologically unidentified.

(contd. overleaf)

Table 2 (contd.).

CHICKEN 'W'.

Date of sample	No. of E. coli isolated	Number belonging to serogroups		
		B1	B31	*
11/ 1/65	10	10		
18/ 1/65	10	9		1
25/ 1/65	10	9	1	
1/ 2/65	10	10		
8/ 2/65	10	10		
15/ 2/65	10	10		
22/ 2/65	10	10		
1/ 3/65	10	8		2
8/ 3/65	10	10		
15/ 3/65	10	10		
22/ 3/65	10	10		
29/ 3/65	10	10		
5/ 4/65	10	10		
12/ 4/65	10	10		
19/ 4/65	10	10		
26/ 4/65	10	10		
3/ 5/65	10	10		
10/ 5/65	10	10		
17/ 5/65	10	9		1
24/ 5/65	10	10		
31/ 5/65	10	10		
7/ 6/65	10	10		

* Serologically unidentified.

Table 3.

CHICKEN 'G'.

Date of sample	No. of E. coli isolated	Number belonging to serogroups							
		B1	G33	B51	G62	B31	G107	026 :B6	*
17/ 8/64	10	6	3						1
24/ 8/64	10	10							
31/ 8/64	10	6	4						
7/ 9/64	10	4	4						2
14/ 9/64	10	7	3						
21/ 9/64	1			1					
28/ 9/64	10			4	5	1			
5/10/64	10		8		2				
12/10/64	10				8	1			1
20/10/64	7					2			5
26/10/64	5					1			4
2/11/64	10					10			
9/11/64	10						10		
16/11/64	10					9			1
25/11/64	10					10			
30/11/64	10					9		1	
7/12/64	10				10				
14/12/64	10				8				2
21/12/64	10								10

* Serologically unidentified.

Table 4.

HAMSTER No. 11.

Date of sample	No. of *E. coli* isolated	Number agglutinated by 'O'-group 103 antiserum	Not agglutinated by 'O'-group 103 antiserum
5/ 3/65	10	10	
17/ 3/65	10	10	
22/ 3/65	7	7	
29/ 3/65	10	10	
5/ 4/65	10	10	
12/ 4/65	10	10	
19/ 4/65	5	5	
27/ 4/65	5	5	
5/ 5/65	5	5	
11/ 5/65	5	5	
18/ 5/65	5	5	
25/ 5/65	5	2	3
1/ 6/65	5	5	
8/ 6/65	5	5	
16/ 6/65	5	5	
22/ 6/65	5	4	1
29/ 6/65	5	3	2
6/ 7/65	5	4	1
13/ 7/65	5	4	1
21/ 7/65	3		3
8/10/65	5	5	
18/10/65	10	9	1
26/10/65	10	9	1
28/10/65	20	17	3

DISCUSSION.

In reference to residency of E. coli in the bowel, Sears and Brownlee (1952) wrote: "... the antigenic diversity of E. coli strains found in human faeces is so great that, when a number of cultures isolated from the same person at short intervals over a period of time belong to the same group, they can be assumed to be all the progeny of the same strain, ...". It was upon the same hypothesis that the author of this work interpreted the results of the serological examinations of E. coli cultures recovered from animals under his investigation. Ordinarily, agglutination of a culture to titre by specific antiserum was taken to be proof of identity but, in the case of selected isolates belonging to two serogroups, the findings were verified by means of agglutinin-absorption.

In the instance of the chicken, there was revealed a pattern of resident and transient strains similar to that which has been described for the human bowel (Wallick and Stuart, 1943; Sears et al., 1950; Sears and Brownlee, 1952); the canine gut (Sears et al., 1956) and the intestine of the horse (Sakazaki and Miura, 1956). The difference between resident and transient types was well defined in the results from Chickens 'B'

and 'W' but not so evident in those from Chicken 'G' which was studied for only a short period.

In the latter subject, the termination of the tenure of Strain No. B1 during the fifth week of the investigation was probably related to a course of antibiotic therapy. A resident population was soon re-established but the period of study was insufficient to reveal whether, or not, the succeeding strains, Nos. G62 and B31, became firmly implanted in the gut. The relative variability of the enteric E. coli population of the bird during the period following the elimination of the former residents, Nos. B1 and G33, may be related to the observation by Sears et al. (1956) that, in the human being, loss of a resident is frequently succeeded by a relatively unstable flora until a new resident strain becomes fully established.

Aside from the case of Chicken 'G', the resident strain was found to be excreted by the birds with great constancy. In the case of Chicken 'B', only one faecal sample taken during the tenure of Strain No. B1 failed to yield that group while all those of Chicken 'W' proved positive. Those findings closely resembled the report given by Wallick and Stuart (1943) following

examination of a human subject. Those authors recorded the dominance of the resident strain in man and the same prevalence was apparent in the chicken.

In respect of strains of E. coli, residencies have been recorded for up to two-and-a-half years in man (Sears and Brownlee, 1952), for more than one year in the dog (Sears et al., 1956) and for, at least, five months in the horse (Sakazaki and Miura, 1956). The investigation now under report revealed that, in chickens, E. coli strains may remain in the bowel for comparable periods.

At the outset, all three chickens were found to harbour the same serogroup as their resident and, although they were separately caged during the period of study, two of them continued to excrete that strain for a long time. Other groups of more transient occurrence were encountered in more than one bird, sometimes simultaneously. Those findings were in accord with the observation made by Sears and Brownlee (1952) that infants, as intimately associated as are twins, are likely to harbour the same residents as well as freely to share the transient strains. Some exchange of strains may have ensued among the birds but it was notable that,

after Strain No. B1 had been lost by Chicken 'G', it was not re-acquired by that creature, even, after maintenance in close proximity for thirteen weeks.

Prolonged absence of E. coli strains was noted in Chickens 'W' and 'G' and involved Groups Nos. B31 and G62, respectively. Sears and Brownlee (1952) remarked on a similar condition when they described the E. coli flora of a pair of infant twins. In that instance, the re-appearance of a strain in one baby was deemed to be the result of re-infection from the other. In the case of the chickens, a definite opinion is impossible to record since another source of infection may have been involved. In that respect, it was notable that the transient recurrence of Group No. B31 in the gut of Chicken 'W' after an interval of twenty weeks happened eight weeks after the micro-organism was last isolated from either of the other birds. Group No. G62, the residency of which in Chicken 'G' was interrupted for a period of seven weeks, was never procured from Chickens 'B' or 'W'. It is not altogether inconceivable that a strain may persist for so long in the intestine undetectable by weekly examinations. Alternatively, manifest re-appearance may have emanated from infection by a new strain belonging to the same serological group.

In respect of the E. coli bowel flora, it was found that the hamster, too, may harbour a resident strain for a long period. In one animal studied, the resident was excreted in constancy equal to that shown by the chickens. The dominance of the resident in the hamster, too, was clearly shown.

On the other hand, over a period of fourteen weeks, the other hamster was shown rarely to excrete E. coli in the faeces, a finding that is in accord with reports by Sheffield and Beveridge (1952), Hagen et al. (1965) and others.

The results of the work reported in this section are deemed to provide knowledge of the normal composition of the E. coli bowel flora of the domestic chicken and of the Syrian hamster sufficient to advance the interpretation of any results that may attend later efforts to establish new strains subsequent to oral administration.

SUMMARY.

Altogether, the findings from the investigation into the composition of the normal E. coli enteric population of the domestic chicken and of the Syrian hamster indicates a close similarity to those described for other animal species, including man, and lead to the

singular conclusion that, in those animals also, a resident strain may persist over a long period.

PART II.

AN INVESTIGATION INTO FACTORS ASSOCIATED WITH THE CONTROL OF THE *Escherichia coli* POPULATION OF THE BOWEL OF ANIMALS.

HISTORICAL.

In view of the multiplicity of serological types of *E. coli* and the probable daily ingestion of many different serogroups by an animal, the normal stability of the resident flora indicates the existence of some mechanism of strict control. Factors which have been investigated by various workers involve (a) the host, (b) the micro-organism itself and (c) the presence in the gut of other bacteria or their products. Those need here to be separately reviewed.

(A) THE INFLUENCE OF THE HOST AND ITS ENVIRONMENT ON *E. coli* IN THE GUT.

A relationship exists between the species of host and the composition of the bacterial population of the gut. Smith and Crabb (1961) examined human infants, calves, lambs, piglets and one rabbit from birth onwards and found that, although the faecal florae were initially alike, quantitative and qualitative differences occurred

until, after several months, those of the different species became grossly dissimilar. Smith and Crabb found few E. coli in faecal samples from guinea-pigs and rabbits and the scarcity of that micro-organism in the gut of the Syrian hamster has been commented on by Rogosa et al. (1957), by Sheffield and Beveridge (1962) and by Hagen et al. (1965).

Racial peculiarity of the host in relationship to the number of E. coli in the bowel has been reported on, at least, two occasions. Results of a study of adult mice by Smith and Crabb (1961) revealed that the excretion of E. coli by those animals was not markedly different from that by man, the pig, the sheep and the fowl. However, Schaedler and Dubos (1962) noted that mice belonging to one particular strain seldom excreted E. coli in the faeces, even, when maintained in premises shared by other animals. Again, Jensen et al. (1963) observed that mice belonging to one line of breeding harboured 10-100 times more coliform bacteria in the intestinal contents than did mice belonging to another. Individual aberration of a similar nature was recorded by Young et al. (1960) as a result of an investigation among normal human subjects.

Several are the reports concerning the development of the enteric bacterial population of young animals and indicating that establishment and persistence of the various microbial species is related to the age of the host (Smith and Crabb, 1961; Smith, 1965; Huhtanen and Pensack, 1965; Schaedler et al., 1965a). E. coli, which is one of the first organisms to invade the bowel after birth, multiplies rapidly to attain its maximum number within a few days but, as the age of the host advances, a gradual reduction in the E. coli content occurs.

Direct control of the intestinal population by the host has been investigated by a number of workers. Thus, in addition to the antibacterial action attributable to the stomach by virtue of its strongly acid secretion, it has been shown that other gastric inhibitory substances may obtain. Smith (1966) described the formation of anti-microbial agents in the stomach of the suckling rabbit as a result of enzymic action on the chloroform-soluble fraction of rabbit's milk, which derivatives proved active against a variety of bacteria, including E. coli. On the other hand, claims in support of the existence of antibacterial substances in the mucosa and the contents of the normal small intestine were critically

reviewed and rejected by Dixon (1960) who carried out a study of rats, with E. coli as one of his test organisms, but failed to demonstrate any bactericidal activity. Dixon as well as Dack and Petran (1934) concluded that the normal absence of microbes, including coliforms, from the anterior small intestinal tract was due to active peristaltic movements in that part of the bowel. Dixon reviewed reports of a similar nature concerning various species of animals and concluded that the findings did not indicate any differences between them.

Sears et al. (1950) reported that some of their results upheld, while others refuted, the belief expressed by Stuart (1949) that change in the resident flora of man is associated with diarrhoeic attacks or other intestinal disorders. Emslie-Smith (1961), too, noted a temporary loss of "exclusive predominance" on the part of one resident strain in his subject during, and just after, an acute intestinal disturbance. On the other hand, Sears et al. (1956) studied for a prolonged period faecal samples from a man and his wife, both of whom were subject to diarrhoeic attacks, without demonstrating any striking departure from the pattern encountered in the normal person.

Emslie-Smith (1961) suggested that humoral antibody may influence the resident E. coli population in man but Robinet (1962) found that high levels of normal serum antibody did not deter the organisms from establishing themselves in the bowel nor did antibody titre for E. coli strains determine whether they continued as residents over several months or occurred as transient flora. Copro-antibodies have been demonstrated against such pathogens as Vibrio cholerae (Burrows et al., 1947) and Shigella flexneri (Cooper and Pillow, 1959) and Park (1959) detected them in low titre in human volunteers who had been infected experimentally with E. coli O111:B4. However, Ashburner and Mushin (1962) searched, without success, for copro-antibodies in mice which had been infected experimentally with E. coli O111:B4. Hence, in the absence of more positive findings in this field of study, it appears unlikely that antibodies are involved in the normal fluctuations of E. coli in the gut.

Diet has been shown to affect, quantitatively, the E. coli population of the gut. Haenel et al. (1957) demonstrated that proliferation of E. coli occurred in human volunteers sustained on an extreme protein diet and Smith (1961) found a considerable increase in the coli-flora of pigs when large quantities of food were

ingested following enforced periods of fasting. On the other hand, at least two workers have reported the inhibitory effects of diet on the E. coli of the alimentary tract. Thus, Sieburth (1961) demonstrated that the absence of E. coli in the gastro-intestinal tract of polar birds was due to the presence of acrylic acid derived from the algae consumed. Another anti-bacterial outcome was detected in rats by Bergheim et al. (1941) in association with ingestion of unusually large quantities of butter-fat and was deemed referable to release of butyric acid in the intestinal lumen.

So far, in the instance of E. coli, there has not been any systematic examination of the effect of diet on the persistence of individual serogroups in either the human or the animal bowel although a selective action exerted by available carbohydrate on the incidence of a fermentative type has been shown by Ozawa and Freter (1964) to prevail in the intestine of the mouse. Sears and Brownlee (1952) noted that the appearance of one serological type in an infant was coincidental with the first feedings of fruit and vegetables. Taylor and Charter (1952), too, remarked that one serotype appeared in the stools of eleven out of twelve infants only after they were put on to bottle feeding. Another possible

selective action of diet on the E. coli of the intestine was observed by Kenworthy and Crabb (1963). Although the numbers of haemolytic E. coli were low in piglets prior to early weaning, most counts were composed almost entirely of haemolytic strains thereafter and the transition was quite abrupt. However, serological examinations of those micro-organisms were not reported. Later, as the result of a study of E. coli isolated from piglets both before and after weaning, Kenworthy and Allen (1966) concluded that there is a variation in the capacity of serogroups to respond to the new environment created by dietary change.

Yuill and Hanson (1965) observed multiplication of E. coli in the intestine of rabbits as a result of intraperitoneal, but not of oral, administration of endotoxin. That, together with evidence of the influence of environment, suggests the existence of a measure of control of E. coli which may become impaired by treatments which affect the physiological state of the host.

Sears and Brownlee (1952) considered that, in the case of man, change of location may be followed by alteration of the composition of the resident E. coli. Schaedler and Dubos (1962) reported that environmental

factors, such as variation in temperature of the surroundings, crowding or handling of mice, exercised a profound influence on the general microbial flora of the gut of the animals, commonly accompanied by an increase in the number of Gram-negative bacilli. Yuill and Hanson (1965) observed that chilling of rabbits was followed by an increase in the E. coli population of the intestine and associated the same phenomenon with the death of a young rabbit following forcible restraint. They also remarked that greater susceptibility to environmental influences was shown by juvenile animals.

(B) THE INFLUENCE OF E. coli ITSELF ON ESTABLISHMENT IN THE INTESTINE.

Schaedler and Dubos (1960) observed a close bond between a resident strain of E. coli and the gut of its murine host. Thorough washing of the intestinal mucosa sufficed to remove experimentally administered organisms but not the resident. They concluded that a resident strain becomes implanted in the intestinal wall. Evidence in support of this peculiar relationship between host and resident was supplied by Ketyi (1965). The findings of those workers may be connected with the observation by Florey (1933) of some unidentified bacteria in the depths of the crypts of the colon of the

cat.

The dosage, or number of cells ingested by a potential host, is apparently not a significant influence on the ability of a strain to establish itself in the bowel. When Sears and Brownlee (1952) re-established a former resident in one of their subjects, they administered the organism in numbers of the same order as those which had been used in unsuccessful experiments reported two years previously (Sears *et al.*, 1950). On the other hand, Sears *et al.* (1956) employed repeated massive doses when they failed to implant new strains in the canine gut and similar results were reported by Erskine *et al.* (1956) to have followed the feeding of large amounts of broth cultures of *E. coli* to pigs. Park (1959), in the course of a study of experimental infection of human subjects with *E. coli* O111:B4, observed that there was not a special relationship definable between the number of organisms ingested and their appearance in the faeces. Likewise, reports by other workers, who have administered to adult human volunteers graded amounts of cultures of strains of *E. coli* isolated from cases of infantile gastro-enteritis, do not indicate that increased dosage is to be associated with longer periods of excretion (Ferguson and June, 1952;

June et al., 1953; Koya et al., 1954a & b). Illyutovich et al. (1962), however, administered E. coli O111:B4 to cats and recorded that the longest period of excretion occurred in animals in receipt of the largest dose.

Other reports suggest that the actual number of cells sufficient for implantation of a strain may be quite small. An account by Rauss and Ketyi (1960) indicated that, if the resident E. coli flora of mice is first eliminated by means of streptomycin, the number of organisms necessary for stable implantation of a new strain is not significant and they considered that a similar proviso may apply in cases of spontaneous infection. Ashburner and Mushin (1962) noted that, after suppression of the normal intestinal flora of mice by antibiotics, an oral dose of ten, or fewer, cells may result in infection whereas over three hundred micro-organisms were required for more regular colonization.

Failure of strains of E. coli to become established after oral administration under normal conditions was explained by Rauss and Ketyi (1960) as due to "implantation antagonism" exhibited by E. coli already resident in the bowel, a view which was based on evidence

that the presence of an artificially settled strain effectively prevented implantation of another. That observation was supported by a report by Spaulding et al. (1961) that antagonism existed between serological types of E. coli in continuous-flow (CF) cultures. On the other hand, Ozawa and Freter (1964) did not detect any conflict to obtain in mixtures of E. coli strains growing in association in 'CF' cultures and Dixon (1959) had already recorded that, when paired strains are grown together in culture over a period of up to seven days, initially extreme inoculum ratios progressively change to approach equality.

Bacteriophagic activity has been suspected as a regulating mechanism of the E. coli population of the gut. Blacklock et al. (1937), however, were unable to show such activity on the part of filtrates of human duodenal or jejunal contents towards coliform strains isolated from lower levels of the intestine of the same individual and, although Wallick and Stuart (1943) thought it possible that a bacteriophage might be instrumental in bringing about the disappearance of a resident strain, their single attempt to demonstrate such a phage was unsuccessful. Emslie-Smith (1961) observed that, three weeks after oral administration of phage to

his subject, the resident was lost but Zakharova (1963) reported that lysis of E. coli O111:B4 by bacteriophage was only partial and did not lead to complete elimination of the organism from mice.

Production in vitro of colicins by some strains of E. coli (Gratia and Fredericq, 1946) has been considered by a number of workers in relation to the composition of the normal E. coli flora. A high incidence of antibiotic strains is indicated by the remark of Fredericq (1957) that, by the use of selective techniques, it is possible to reveal the presence of colicinogenic strains in practically all human and animal stools studied. Emslie-Smith (1961) reported that a resident strain was not recovered from his subject after the appearance of others producing a colicin active against it. During a study of that individual over a period of thirty months, three successive residents were found and all of them proved to be colicinogenetic. Branche et al. (1963) studied the E. coli flora of five healthy individuals for six months in an effort to evaluate the effect of colicin-production on E. coli in the normal human intestine and found that resident serogroups elaborated colicin more consistently than did transients. In consequence, it was suggested that the

ability of some E. coli strains to prevail in the human gut as dominant residents is ascribable to availability of colicins.

On the other hand, Sears et al. (1950) examined the colicin activity of several hundred cultures from their subjects but did not find the antagonistic action of resident strains to be greater than that of the transients or of those obtained from extra-intestinal sources. Friedman and Halbert (1960) observed that a colicinogenic strain of E. coli was not more efficacious than a non-antibiotic culture in antagonizing the lethal effect on mice of dosage with a pathogenic strain of E. coli and Rauss and Ketyi (1960) were unable to superimpose a colicin-producing strain on a previously established E. coli O111:B4 in the gut of the same species of animal. Freter (1956) observed that E. coli strongly antagonized strains of Shigella flexneri and of Vibrio cholerae in the intestine of mice and guinea-pigs but was unable to demonstrate colicin-production by the strain of E. coli in vitro. Likewise, Hentges and Freter (1962) failed to show any correlation between in vitro and in vivo antagonism of E. coli and other enteric bacteria towards a strain of Sh. flexneri and expressed the opinion that colicin-like substances are not likely to be evolved in

the gut under the strongly reducing conditions normally prevalent there.

Gage *et al.* (1961) studied the distribution of strains of *E. coli* among a group of premature infants in a nursery and observed that some, which they described as ' strong ', were more likely to persist in the intestine of those individuals. That observation is supported by results of studies of other communities (*v. s.*) but the reasons for that 'strength' have not been discussed.

Taylor (1961) and Sojka (1965) have commented on the association of certain serological types with particular animal hosts. There is little experimental evidence in support of host-specificity but Schaedler and Dubos (1960) noted that, in the case of the gut, whereas a strain of murine origin persisted in those mice to which it had been fed, laboratory cultures of undisclosed origin were soon lost. In some instances in which success in establishing strains from a different species of host has been reported, the work was carried out under very artificial conditions (Rauss and Ketyi, 1960; Ashburner and Mushin, 1962).

Ozawa and Freter (1964) studied 'CF' cultures of mixed growths of *E. coli* and observed that, when an

'invader' strain is introduced at an equal concentration into a population of an already-established culture of E. coli, it undergoes a process of adaptation lasting from one to three days. During that time, its numbers steadily decrease but, thereafter, rise again to a fixed level which is considerably below that of the resident. A similar picture was found when E. coli was administered to mice from which the normal enteric flora had been eliminated as a result of antibiotic treatment and an artificial resident E. coli strain substituted. The duration of the period of adaptation was considerably reduced in 'CF' cultures when the invader was offered a source of fermentable carbohydrate which it alone could utilize whereby its population quickly came to exceed that of the resident. A similar, but less striking, result was obtained in experiments carried out on mice. The authors concluded that analogous processes of adaptation and competition for carbon sources under reduced conditions prevail in the animal bowel and that transient strains of E. coli are expelled from the gut by normal physiological movements while they are still undergoing adaptation.

A like theory propounded by Freter (1962) to explain the resistance displayed by the normal murine

gut to the establishment therein of Sh. flexneri was criticized by Meynell (1963) on the grounds that an abundance of available food material obtains in the liquid intestinal contents and by Bohnhoff et al. (1964) who reported that heat-treated suspensions of colonic contents remain inhibitory to Salmonellae. The latter authors suggested that growth of the normal flora is controlled, not by lack of nutriment, but by metabolic derivatives from other organisms in the bowel.

(C) THE RELATIONSHIP OF E. coli TO OTHER INTESTINAL MICRO-ORGANISMS AND THEIR PRODUCTS.

Miller and Bohnhoff (1963) observed a direct correlation to exist between antagonism to the establishment of Salmonella in the gut of the mouse and the presence therein of members of the genus, Bacteroides, which latter, incidentally, occur numerously in the bowel of other animal species, including chickens and hamsters (Smith, 1965). Meynell (1963) related that anti-salmonellar activity to the presence in the murine caecum of large amounts of volatile fatty acids, mainly acetic and butyric, produced by other members of the intestinal flora. In the following year, Bohnhoff et al. (1964) confirmed those findings and referred the production of volatile fatty acids to anaerobic organisms of the

Bacteroides group.

Ketyi (1964) examined the influence exerted upon *E. coli* by a number of organisms of enteric origin including *Klebsiella*, *Proteus*, *Lactobacillus*, *Streptococcus faecalis* and *Bacteroides*. Individually, none of those showed typical implantation antagonism towards experimentally administered *E. coli* in the murine gut, observations that were later supported by the results of a study by Schaedler *et al.* (1965b). The latter authors noted that germ-free mice fed successively cultures of an anaerobic streptococcus, two strains of *Lactobacillus*, one of *Bacteroides* and one of a coliform continued to harbour each of those bacterial species for four months in numbers equal to those that obtained when the organismal type was administered alone. When the animals were subsequently exposed to faecal material from 'NCS' mice (which are peculiar in that they consistently yield few coliform bacilli in the excrement), within two days the number of coliform bacilli in the intestine steeply declined. In consequence, antagonism to the coliform was attributed to a transmissible agent, the identity of which was not discovered.

In spite of the work which has been reported, it is obvious that information concerning the regulation of the E. coli population of the intestine is still very incomplete. Factors which merit investigation are host specificity, 'strength' of individual strains of E. coli, colicinogenesis on the part of E. coli and the effect of the physiological state of the host. The work about to be described was undertaken in order to test those properties inasmuch as they affected the E. coli flora of the gut of animals. It consists of the following four studies:

(1) the composition of the Escherichia coli flora of the bowel of a group of dogs experimentally infected with the virus of canine hepatitis,

(2) a series of experiments designed to establish Escherichia coli in the bowel of chickens and hamsters,

(3) a study of the association between colicinogenesis in vitro and the composition of the Escherichia coli flora of the gut of animals and, finally,

(4) an investigation into the influence of other organisms, mainly of enteric origin, on colicin produced in vitro by a strain of Escherichia coli.

SECTION I.

THE COMPOSITION OF THE *Escherichia coli* FLORA OF THE BOWEL OF A GROUP OF DOGS (*Canis familiaris*) EXPERIMENTALLY INFECTED WITH THE VIRUS OF CANINE HEPATITIS.

INTRODUCTION.

Relatively little is known of the relationship between *E. coli* and viruses but it has been shown that the virulence of *E. coli* as a cause of respiratory disease in chickens under experimental conditions is enhanced by the presence of the virus of infectious bronchitis (Fabricant and Levine, 1962). Cramblett and Siewers (1965) reviewed the aetiology of gastro-enteritis in infants and children with emphasis on the occurrence of mixed viral-bacterial infections, including infection by enteropathogenic types of *E. coli*, but did not point to any particular association. On the other hand, Amstutz (1965) in a review of the aetiology of infectious calf diarrhoea stated that, while various viruses may produce infectious diarrhoea alone, for the most part there appears to be some synergistic action between *E. coli* and viruses in the production of the typical disease.

Should synergism obtain between a virus and E. coli in the intestine, it is conceivable that such activity might consist of rendering conditions suitable for selective multiplication of a strain of E. coli either already resident there or subsequently introduced but any study of that sequence has not been reported. The close association shown by Schaedler and Dubos (1960) and Ketyi (1965) to exist between the resident E. coli flora and the mucosa of the gut and the possible influence of the physiological state of the host on the enteric population provided the stimulus to investigate whether, or not, a viral infection of the host might affect the qualitative composition of the E. coli in the intestine. A comprehensive study of Rubarth's disease of dogs provided the opportunity to test that theory. The investigation formed part of a joint experiment in which clinical, histopathological and virological examinations were undertaken by other members of staff.

MATERIALS AND METHODS.

An entire litter of seven farm-bred dogs, eight weeks of age, was used. After arrival, the dogs were maintained in separate cages in the animal house under the care of one attendant. The cages were thoroughly

cleansed daily but special attempts were not made to isolate the inhabitants from one another. Nourishment consisted of milk and a proprietary canned dog food. The dogs were given distinguishing numbers that ran from seven to thirteen.

During the first four weeks of observation, the dogs were tested for the presence of maternal antibodies to canine viral hepatitis (CVH) by means of neutralization tests in tissue-culture but were found to be negative in that respect. Thereupon, the animals were infected by oral administration of $10^6 TCID_{50}$ of virus. Approximately two weeks later, and at intervals of one to four days, euthanasia was effected by means of intravenous injection of ' Nembutal ' and autopsy immediately carried out.

From each of the animals, a rectal swab was taken twice weekly and material therefrom was seeded on an ' Oxoid ' MacConkey 3 agar plate. The latter was incubated at 37°C. for eighteen hours, after which four lactose-fermenting colonies were subcultivated on five per cent. sheep-blood-agar plates. Those preparations were also incubated at 37°C. for eighteen hours ere the resultant growths were inspected for haemolysis. From each of the subcultures, one well isolated colony was inoculated over the surface of a Dorset egg slope, to

provide the material for future biochemical and serological examinations. Post-mortem, a like examination was performed on samples of gut contents taken at five levels of the alimentary tract, namely: three from the small intestine, one from the caecum and one from the colon. From each of those samples, five lactose-fermenting colonies were subsequently subcultured, via sheep-blood-agar plates, on Dorset egg slopes.

Within the limits imposed by the small number of colonies selected from the samples, an attempt was made to represent the colonial types in the proportions in which they occurred in the primary cultures from the swabs.

To the first group of bi-weekly samples was applied the group designation 'A' and each like set thereafter was accorded the subsequent letter of the alphabet. Individual cultures were distinguished by means of the group letter of the original sample together with the distinguishing number of the animal and, in parenthesis, followed the number of the colony, from 1-4, according to the order in which it had been selected from the primary culture, for example, A7(1). Strains isolated from post-mortem cultures bore the number of

the dog, the approximate situation in the bowel from which they were recovered (duodenum, jejunum, ileum, caecum or colon) and another numeral, 1-5, indicative of the order of selection, for example, 7 Duodenum (1).

Biochemical examination of the strains was carried out as described in Part I except that the organisms were tested for fermentation of eight carbohydrates comprising dextrose, mannitol, lactose, adonitol, inositol, dulcitol, salicin and sucrose.

Antisera were prepared against a series of fifteen antigenically different cultures by the method that was employed for the strains from the chickens and the procedure of serological identification of cultures was identical with that which has already been described.

Of the fifteen strains which were selected for the production of antisera, only five were identifiable by Dr. Joan Taylor thus:

Designation of type strain	International classification
7 Ileum (1)	O111:B4
D7(1)	069:K?
N8(4)	071:K?
N11(1)	02:K?
O11(3)	049:K?

The other ten strains comprising Nos. A7(3), A8(1), A9(3), A7(1), D8(2), J8(4), D11(1), 11 Jejunum (4), K13(1) and O8(1) were not found to belong to any of the groups for which Dr. Taylor possessed antisera.

Strain No. 11 Jejunum (4) was characterized by the production of an 'A'-type surface antigen. In consequence, agglutination tests involving the use of No. 11 Jejunum (4) antiserum were performed on suspensions of E. coli which had been heated at 120°C. for two-and-a-half hours.

A tonsillar swab procured from each of the dogs on the sixth day after infection with virus was submitted to a general bacteriological examination.

RESULTS.

The results are reported under two headings, (a) findings from the dogs relative to infection by the virus of canine hepatitis and (b) the incidence of biochemical and serological varieties of E. coli among the group of animals before and after exposure to virus.

(A) THE FINDINGS FROM THE DOGS RELATIVE TO INFECTION BY THE VIRUS OF CANINE HEPATITIS.

The temperatures of the animals were all elevated

on the third day after infection and generally remained high until the seventh day but, by the ninth day, they all returned to normal. The dogs were depressed on the fourth day but their appetite remained good throughout the period of clinical illness. Virus was recoverable from the tonsils up to the sixth day after infection and viraemia was detectable from the fifth until the seventh days.

Haematological changes first became prominent in some of the dogs on the sixth day after infection and were generally evident twenty-four hours later. The pattern was remarkably similar in all of the animals. In general, a leucopenia accompanied the viraemia and pyrexia and this stage, which lasted for approximately three days, was followed by a marked lymphocytosis which persisted for about six days. There was a moderate increase of the erythrocyte-sedimentation-rate but the haemoglobin content of the blood, the packed cell volume and the mean corpuscular haemoglobin concentration remained relatively constant throughout.

(B) THE INCIDENCE OF BIOCHEMICAL AND SEROLOGICAL VARIETIES OF *E. coli* AMONG DOGS BEFORE AND AFTER EXPOSURE TO VIRUS.

Altogether, six hundred and twenty coliform

cultures were examined and five hundred and ninety-nine of them were identified as E. coli on biochemical grounds. Nineteen were deemed to be Aerobacter aerogenes and two others were identified as E. freundii. None of the cultures was found to exhibit haemolysis on 5 per cent. sheep-blood-agar plates.

All of the E. coli strains fermented dextrose, lactose and mannitol to produce acid with, or without, gas. Five hundred and thirty-five of the isolates split salicin, three hundred and ninety-eight reduced dulcitol, eighty-seven fermented sucrose, seventy-two decomposed adonitol and twelve affected inositol. Altogether, nineteen different fermentative varieties were distinguishable (v. Table 5, p. 89).

Varieties Nos. 1, 3 and 4 were present in most of the bi-weekly groups of samples procured throughout the period of the investigation. On the other hand, Varieties Nos. 6 and 7 were found mainly in faecal samples belonging to Groups 'A' to 'H' and Varieties Nos. 2, 5, 9, 10 and 14 were obtained most frequently from samples in Groups 'I' to 'Q'. The incidence of other varieties was not so definite. The composition of biochemical varieties observed in cultures derived, post-mortem, from the

intestine of the animals corresponded with that in samples taken during the latter part of the experiment. The distribution of varieties among samples of faeces and of gut contents from individual dogs is described in Table 6 (v. pp. 90-94).

Four hundred and seventy-six *E. coli* cultures fell into sixteen different serological groups. Group No. A8(1), and probably also Group No. N11(1), were found to be widespread throughout the community over the entire period of investigation. Groups Nos. D11(1), D7(1) and N8(1) occurred simultaneously, or in rapid succession, in more than one animal but the individual tenures were brief and the organisms seemed to be quickly eliminated. Each of Groups Nos. 7 Ileum (1), 11 Jejunum (4) and K13(1) was first demonstrated in the case of a single animal and, over a period of some weeks, it was possible to trace their subsequent progress through all the other experimental subjects but the period of study was too short to allow of exegesis of their peculiar roles. The distribution of Groups Nos. D8(2), J8(4), O8(1) and of O126:B16 was less well defined but the micro-organisms prevailed in the community for relatively long periods, apparently in singular association with one animal and with sporadic occurrence in the case of the others. Groups Nos. A7(3),

A7(1) and O11(3) were isolated only from single specimens, which finding suggests that their association with the host was of extremely short duration. Since Groups Nos. A7(3) and A7(1) were obtained from one of the first set of faecal samples, it may be that both cultures represented the final appearance of former residents and a similar interpretation may also attach to the remaining Group No. A9(3). The incidence of serogroups among individual dogs is described in Table 7 (v. pp. 95-98).

Apart from Groups Nos. A7(3) and O11(3), of which only single strains were identified, all of the serological types proved to include two or more biochemical varieties. Serogroups Nos. A8(1) and O8(1) belonged predominantly to Variety No. 1; Groups Nos. D8(2) and 11 Jejunum (4) consisted largely of Variety No. 3 and Groups Nos. D11(1) and N11(1) were composed mainly of Variety No. 4. Serogroups Nos. 7 Ileum (1) and D7(1) comprised mostly Varieties Nos. 9 and 6, respectively. In the case of Group No. K13(1), the majority of the isolates was equally distributed between Varieties Nos. 2 and 3. The sixteen serological groups, the number of strains belonging to each and the incidence of biochemical varieties within them are listed in Table 8 (v. p. 99).

A tonsillar swab procured from each of the dogs on the sixth day following oral infection with virus was examined bacteriologically. Each of the samples yielded E. coli, among a variety of other organisms. A representative member of each E. coli colonial type was selected from the plate and retained for serological examination. The swab from Dog '13' yielded E. coli belonging to Group No. A8(1). Those from Dogs '7', '8', '9' and '10' contained E. coli of Group No. 11 Jejunum (4) and, in addition, Dogs '8' and '10' were shown to harbour E. coli Group No. 7 Ileum (1), i.e., O111:B4, on the tonsils.

DISCUSSION.

Altogether, the clinical findings revealed considerable physiological disturbance on the part of the dogs as a result of infection by virus. However, examination of the incidence of serological and fermentative varieties in individual animals and the community as a whole failed to associate change of the E. coli composition of the intestine with the presence of the virus.

The demonstration of rapid changes in the E. coli population involving several or all of the animals during

the earlier part of the investigation prior to infection with virus revealed that a similar occurrence in association with the presence of the latter might be purely co-incidental and that only the isolation of a serogroup already incriminated as the cause of canine disease might be interpreted as indicative of a synergistic relationship. The sole record of the serology of E. coli isolated from diseased dogs is the account by Mansi (1962) of experimentally-reproducible enteritis caused by 'O'-group 42. That serogroup was not found to be represented in any of the strains identified in the dogs during the investigation under report. Nevertheless, the results are deemed worthy of comment.

An outbreak of diarrhoea occurred in the community between one week and ten days after infection with virus when the animals were, once more, clinically normal except for enlargement of tonsils and superficial lymph-nodes but there were not any findings from faecal samples to indicate a radical change in the E. coli composition during those attacks. However, two of the tonsillar swabs procured from the dogs on the sixth day after infection, the last on which virus was recoverable from that site, yielded E. coli O111:B4 although that serogroup was not obtainable from rectal swabs from any of

the creatures until the diarrhoeic phase was over. The relation between that 'OB' group and the bowel disorder, therefore, remains indefinite. E. coli O111:B4 is commonly associated with enteric disease in human beings (Bray, 1945; and others) but, although the micro-organism has been recovered from dogs by other workers (Jacalne and Aragon, 1958; Mian, 1959), it is not a noteworthy cause of disease in those animals. Possibly, in the case under report, the virus may have formed a synergistic association with E. coli O111:B4 by reducing the resistance of the dogs to an organism not usually pathogenic to them. More frequent sampling of the dogs may have revealed the presence of the organism in the bowel at an earlier date but, on the other hand, its seeming absence may have concurred with a process of adaptation in the gut (Ozawa and Freter, 1964). The findings are classifiable only as inconclusive although isolation of the O111:B4 strain for the first time so soon after experimental viral infection and coincidental diarrhoea suggests a connection between the three.

E. coli O111:B4 was isolated from all seven animals and tenures of up to nineteen days were demonstrated. Another 'OB' group, O126:B16, which has been recovered from cases of human disease (McDonald and

Charter, 1956), was procured from two dogs during the investigation under report and one possible residency of twenty-five days was observed. The source of infection of those two serogroups was not established but it may have been a human one. Such long natural tenures in dogs suggest that animals may yet be shown to form a potential reservoir of human infection. Tonsillar infection may occur in dogs and the habit of the latter of licking the face and hands of man provides an opportunity for transmission of E. coli.

Sears et al. (1956) commented on the relative instability of the E. coli population of dogs compared with that of human beings. In the investigation under report, it was observed that the flora of the dogs was generally much less stable than that of the chickens and of the hamster, the findings from which animals are reported in Part I of this thesis. That inconstancy may be characteristic of the canine species. On the other hand, the microbial instability may have been connected with maintenance of the animals since it has been shown that external factors may affect the enteric population (Schaedler and Dubos, 1962), especially in the case of young animals (Yuill and Hanson, 1965). For more than half of the duration of the experiment, the dogs were

subjected to various clinical tests including temperature readings, tonsillar swabbing and removal of urine and blood samples, which interferences may have affected the E. coli of the gut. The chickens and hamsters, on the other hand, were not handled oftener than three times weekly when the cage was cleaned or a faecal sample was obtained. Another possible influence involved adaptation of the animals to the new environment which adjustment may have affected the E. coli flora especially during the earlier part of the investigation.

The period of examination of the dogs was too short to allow exact comparisons with work reported by other investigators but it was evident that, in the community, there prevailed a common resident strain and that a great degree of sharing of other serogroups also obtained. In that respect, the results were similar to findings by Orskov (1952) in the case of children housed in institutions. Again, dissemination of some strains throughout the dogs followed a pattern similar to that described for the spread of enteropathogenic serogroups through communities of infants (Taylor and Charter, 1952).

The incidence of fermentative varieties among

the dogs was investigated primarily to ascertain any association that may have obtained between their prevalence and the period of virus-induced disease or the attacks of diarrhoea. Although such a connection was not proven, the results are deemed worthy of deliberation.

When the presence of biochemical varieties was examined in individual animals, a pattern was observed closely resembling that normally found with serogroups. Two or three varieties were recoverable from faecal samples procured during most, or all, of the period of study while others were demonstrable in successive samples over short periods or were to be obtained only on isolated occasions. In view of the potential variety of biochemical types, the picture in each of those cases was one of remarkable stability indicating some form of biochemical control over E. coli in the bowel. That opinion was strengthened by the not infrequent discovery that an apparently biochemically homogeneous E. coli flora in single or successive samples from the same animal comprised serologically unrelated members.

As in the case of serogroups, abrupt changes in the incidence of biochemical varieties were observed in successive samples from the same animal. Ozawa and

Freter (1964) showed that availability of fermentable carbohydrate increases the incidence of E. coli which can utilize it in the intestine. Throughout the period of the investigation under report, a diet of one proprietary brand of canned dog food was administered but, probably, the contents were not of standard quality, a factor that may have effected temporary changes in the proportions of biochemical varieties in the gut. On the other hand, some less common varieties were recovered from faecal samples from individual animals over a short, but well defined, period lasting from one to two weeks, which finding did not suggest an alteration associable with temporary dietary variation.

When the animals were viewed as a community, a more definite succession of biochemical varieties became manifest. Sharing of some less frequent biochemical varieties by more than one animal occurred more or less simultaneously, as happened in the case of serogroups. Three varieties were present in most of the bi-weekly groups of samples but others were found to predominate during the earlier or later part of the investigation, which periods were separated, approximately, by the date of administration of virus. It was impossible, however, to refer the occurrence of any

biochemical variety to the disease resulting from the viral infection.

The relation between the distribution of serogroups and of biochemical varieties in the dogs was not clearly defined. In the case of all but one of the more common serogroups, the members belonged predominantly to one variety and usually the latter was one of the 'resident' types, Nos. 1, 3 or 4. Varieties Nos. 9 and 6 comprised mainly isolates of serogroups Nos. 7 Ileum (1) and D7(1), respectively, and, in those instances, the incidence of biochemical variety and serogroup closely corresponded. On the other hand, varieties also characterized by a relatively restricted distribution (Nos. 2, 7, 10 and 14) each contained several antigenic forms. The author considers that biochemical activity rather than serological structure is more likely to affect the establishment of E. coli in the intestine. Manifest fluctuations of serological types are probably a result of an alteration in the enteric environment, which difference favours the proliferation of a serogroup with a particular biochemical potential. At the same time, ability on the part of E. coli to produce more than one biochemical variety may render a serogroup more adaptable to temporary changes arising in the bowel and

thus, help to maintain its residency therein. On the other hand, in the cases under report, it must be emphasized that the 'resident' biochemical varieties were not found to be the most actively saccharolytic.

The need for further work in this field is, thus, indicated. Fluctuations of biochemical types may come to be revealed as a natural phenomenon associable with age, diet, health or other factor. A comparative examination of different animal species may disclose variations in the incidence of biochemical varieties and, thereby, explain observations by other workers concerning a host-specific relationship. On the other hand, negative findings may indicate the feasibility of transmission of E. coli from one species of host to another.

Altogether, the results of this investigation failed to prove that experimental infection with canine viral hepatitis was responsible for changes in the enteric E. coli population of dogs despite considerable concurrent physiological disturbance. Nevertheless, the outcome served to indicate the need for further studies of the same type in order to determine the significance of the physiological state and environment of the host on the composition of the resident microbiota.

SUMMARY.

The E. coli flora of the gut of a litter of seven young dogs was examined over a period of approximately two-and-a-half months, in the course of which the animals were infected experimentally with the virus of infectious canine hepatitis. Variations occurred in the incidence of biochemical varieties and serological types in the faeces but they were not referable to the period of clinical disease arising from the viral infection. Excretion of biochemical varieties of E. coli was found to follow a pattern similar to that of serogroups inasmuch as a few 'resident' types prevailed throughout the period of study whereas others were eliminated by the dogs for relatively short intervals. An outbreak of diarrhoea involving all of the animals may have been associated with infection by E. coli O111:B4. Two cases of tonsillar localization were detected several days before animals were observed to excrete that 'OB' group. The isolation of the latter organism and of E. coli O126:B16 from the dogs has been discussed in relation to animals as a potential reservoir of human infection.

TABLE 5.

Number of Biochemical Variety	Carbohydrates fermented							
	Dextrose	Mannitol	Lactose	Adonitol	Inositol	Dulcitol	Salicin	Sucrose
1	+	+	+	-	-	+	+	-
2	+	+	+	-	-	-	+	-
3	+	+	+	-	-	-	+	+
4	+	+	+	-	-	+	+	+
5	+	+	+	-	-	-	-	-
6	+	+	+	-	-	+	-	-
7	+	+	+	-	-	+	-	+
8	+	+	+	-	+	+	+	-
9	+	+	+	+	-	-	+	-
10	+	+	+	+	-	-	+	+
11	+	+	+	-	-	-	-	+
12	+	+	+	-	+	+	+	+
13	+	+	+	+	+	-	+	+
14	+	+	+	+	-	+	+	-
15	+	+	+	+	+	+	+	+
16	+	+	+	+	-	-	-	-
17	+	+	+	+	-	+	+	+
18	+	+	+	+	-	+	-	-
19	+	+	+	-	+	-	+	+

- = failure to ferment the carbohydrate.

+ = production of acid or acid and gas.

TABLE 6.

Biochemical variety	Dog No.	Incidence of variety in faecal or intestinal material procured during Mar A	B	C	D	E	April F	G	H	I	* J	K	L	May M	N	O	P	Q
1	7	+	+				+	+	+	+	+	+	+	+	+	+		
	8	+	+			+		+		+	+	+	+	+	+	++		
	9	+	+	+		+	+	+		+			+		+	+	+	
	10	+	+		+		+	+	+	+				+		+	++	
	11	+	+				+	+	+	+	+		+		+		+	+
	12	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+
	13	+	+		+	+			+	+	+		+	+	+		++	
2	7	+														+		
	8	+												+		+		
	9										+	+			+		+	
	10																	+
	11													+		+		
	12												+					
	13			+								+		+			+	
3	7	+												+	+	+		
	8	+			+	+			+	+	+	+	+	+		+		
	9						+	+	+	+	+	+	+	+				
	10					+				+		+				+		+
	11									+	+	+						
	12										+							
	13					+			+	+	+	+				+	+	
4	7		+	+	+	+					+	+	+		+			
	8			+		+	+	+			+	+	+					
	9		+	+	+	+		+				+		+				
	10			+			+	+			+	+	+		+			+
	11			+	+		+	+			+	+			+	+	+	
	12	+	+	+	+				+			+						
	13			+	+				+									

(contd.)

TABLE 6 (contd.).

Biochemical variety	Dog No.	Incidence of variety in faecal or intestinal material procured during Mar A... B..C...D..E...F..G...H..I...*J. .K...L..M...N..C...P..Q..... April May
5	7	+ +
	8	+
	9	+ + + +
	10	
	11	+ + +
	12	+
	13	
6	7	+ + +
	8	
	9	+
	10	
	11	+
	12	+
	13	+ + + +
7	7	+ +
	8	
	9	+ + + +
	10	+
	11	+
	12	+
	13	
8	7	+
	8	
	9	
	10	
	11	
	12	
	13	

(contd.)

TABLE 6. (contd.)

Biochemical variety	Dog No.	Incidence of variety in faecal or intestinal material procured during Mar A... B..C...D..E...F..G...H..I...J. .K...L..M...N..O...P..Q..... April May
9	7	+
	8	*
	9	+ +
	10	+ + +
	11	+ + ÷
	12	+ + +
	13	*
10	7	+
	8	
	9	+ +
	10	
	11	+ +
	12	
	13	
11	7	
	8	+ +
	9	+
	10	
	11	
	12	+
	13	
12	7	
	8	+
	9	+
	10	+ +
	11	
	12	
	13	

(contd.)

TABLE 6 (contd.).

Biochemical variety	Dog No.	Incidence of variety in faecal or intestinal material procured during Mar A	B	C	D	April E	F	G	H	I	*J	K	May L	M	N	O	P	Q
13	7																	
	8				+													
	9																	
	10																	
	11																	
	12																	
	13																	
14	7																	
	8																	
	9												+					
	10														+		+	
	11																	
	12													+		+	+	+
	13																	
15	7																	
	8																	
	9																	
	10								+		+						+	
	11																	
	12																+	
	13																	
16	7																	
	8																	
	9																	
	10																+	
	11																	
	12																	
	13																	

(contd.)

TABLE 6 (contd.)

Biochemical variety	Dog No.	Incidence of variety in faecal or intestinal material procured during Mar A...	B..	C...	D..	April E...	F..	G...	H..	I...	* J.	May .K...	L..	M...	N..	O...	P..	Q.....
17	7																	
	8																	
	9																	
	10																	+
	11																	
	12						+											
	13																	
18	7																	
	8																	
	9																	
	10																	+
	11																	
	12																	
	13																	
19	7																	
	8																	
	9																	
	10																	
	11		+															
	12																	
	13																	

* Sample No. J was obtained immediately before oral dosage with the virus of contagious canine hepatitis.

\+ Indicates the recovery of the biochemical variety from the sample of material.

TABLE 7.

Number of serogroup	Dog No.	Incidence of serogroup in faecal or intestinal material procured during Mar A...	B..	C...	D..	April E...	F..	G...	H..	I...	*J.	K...	L..	M...	May N..	O...	P..	Q.....
A7(3)	7	+																
	8																	
	9																	
	10																	
	11																	
	12																	
	13																	
A8(1)	7		+				+	+	+	+		+	+	+		+		
	8	+						+		+			+				+	
	9	+	+			+							+				+	+
	10	+	+		+		+	+	+	+	+		+	+		+		++
	11	+	+			+	+	+	+	+			+		+			+
	12	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+
	13	+	+		+	+			+	+	+		+	+	+		++	
A9(3)	7																	
	8																	
	9	+																
	10						+											
	11																	
	12	+	+															
	13	+																
A7(1)	7	+																
	8																	
	9																	
	10																	
	11																	
	12																	
	13																	

(contd.)

TABLE 7 (contd.).

Number of serogroup	Dog No.	Incidence of serogroup in faecal or intestinal material procured during Mar A	B	C	D	April E	F	G	H	I	J*	K	L	M	May N	O	P	Q
7 Ileum (1)	7														+			
	8															+		
	9																+	
	10														+	+	+	+
	11													+			+	+
	12																+	+
	13															+	+	
D8(2)	7														+			
	8				+	+	+		+		+							
	9																	
	10					+												
	11																	
	12																	
	13																	
J8(4)	7		+															
	8									+	+							
	9																	
	10																	
	11																	
	12																	
	13																	
D11(1)	7					+												
	8			+														
	9				+													
	10			+														
	11				+													
	12				+													
	13			+	+													

(contd.)

TABLE 7 (contd.).

Number of serogroup	Dog No.	Incidence of serogroup in faecal or intestinal material procured during Mar A	B	C	D	April E	F	G	H	I	*J	K	L	M	May N	O	P	Q
11 Jejunum (4)	7										+		+		+ +			
	8								+	+	+	+	+	+				
	9				+		+	+	+	+	+	+	+	+		+		
	10									+	+						+	
	11									+	+	+						+
	12										+	+						+
	13								+	+	+						+	
D7(1)	7				+	+												
	8																	
	9																	
	10				+													
	11					+												
	12					+												
	13						+	+										
N8(4)	7																	
	8														+			
	9																	
	10														+			
	11																	
	12																	
	13																	
N11(1)	7														+			
	8					+	+											
	9			+								+	+		+			
	10																	
	11			+			+					+			+	+		
	12		+															+
	13																	

(contd.)

TABLE 7 (contd.)

Number of serogroup	Dog No.	Incidence of serogroup in faecal or intestinal material procured during Mar A	B	C	D	April E	F	G	H	I	* J	K	L	May M	N	O	P	Q
K13(1)	7														+	+		
	8															+		
	9													+				
	10																+	
	11													+	+			
	12												+					
	13								+			+		+		+		
08(1)	7		+	+					+									
	8															+		
	9						+											
	10																	
	11																	
	12															+		
	13																	
011(3)	7																	
	8																	
	9																	
	10																	
	11															+		
	12																	
	13																	
0126 :B16	7		+	+						+								
	8																	
	9																	
	10								+									
	11																	
	12																	
	13																	

* Sample No. J was procured immediately before oral dosage with the virus of CCH.

TABLE 8.

Serological group	No. of strains	Number of strains belonging to biochemical variety No.																		
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
A7(3)	1			1																
A8(1)	236	202	3	2	18		4		1	1			1		2	1		1		
A9(3)	6	2			2			2												
A7(1)	2		2																	
7 Ileum (1)	38	1								30	2				4				1	
D8(2)	18			14	1						1	1		1						
J8(4)	3	2			1															
D11(1)	19				18								1							
11 Jejunum (4)	70	4	9	37	14	1					1	1	2			1				
D7(1)	19	1			1		16	1												
N8(4)	2	1											1							
N11(1)	23	5			15			2							1					
K13(1)	26	1	11	11	3															
08(1)	8	7			1															
011(3)	1		1																	
0126:B16	4	1			1		1	1												
Unidentified	123	37	17	5	10	21	2	3		14	3	2	1		2	2	2	1		1
Totals	599	264	43	70	85	22	23	9	1	45	7	4	6	1	9	4	2	2	1	1

SECTION II.

A SERIES OF EXPERIMENTS DESIGNED TO ESTABLISH *Escherichia coli* IN THE BOWEL OF CHICKENS AND OF HAMSTERS.

HISTORICAL.

Apart from observations on its incidence in normal subjects, much of the information concerning regulation of *E. coli* in the intestine has been acquired as a result of attempts to establish new strains in man and animals. The mechanism of such control, however, is still improperly understood.

Sears, Brownlee and Uchiyama (1950) were probably the first workers who endeavoured experimentally to establish a new resident strain of *E. coli* in the human bowel. The bacterial cells were suspended in water to be accepted either between meals or gradually with food. From each of the cases so treated, the strains were subsequently recovered from the faeces for a few days only or not at all. Repeated trials were attended by similar results.

Sears and Brownlee (1952) considered that they had achieved success when, in one of their subjects, they

re-established a former resident strain which was not demonstrable in faecal cultures during a period of forty days after return from a vacation lasting thirty-five days.

Sears *et al.* (1956) reported failure to establish new resident *E. coli* 'O' groups of both human and canine origin in two dogs after ingestion of cultures contained in milk or in 'enteric-coated' capsules.

Halbert and Swick (1950) observed excretion of a strain of human origin by mice for a few days only after it had been administered *per os* and Rauss and Ketyi (1960) obtained similar results with both a human and a murine culture.

Attempts to reproduce pathological conditions in man and animals as a result of feeding with cultures of *E. coli* have, generally, yielded similar findings. Koya *et al.* (1954a & b) either fed a strain of *E. coli* O111:B4 to human volunteers or introduced the organism directly into the bowel and found that that 'OB' group was detectable in faecal samples for ten days, at most. Wentworth *et al.* (1956) observed that ingestion of *E. coli* O127:B8 was followed by its elimination for twelve days by one individual and for five days by another.

Stasilevich (1958) infected monkeys with various serotypes and reported consequent carriage of the organisms for periods of up to sixteen days. Erskine et al. (1956) administered to weaned pigs for several days large doses of serotypes commonly encountered in 'oedema disease'. During that period, rectal swabs yielded pure growths of those serotypes but, twenty-four hours after dosing had ceased, the latter were usually not any longer so detectable. On the other hand, Illyutovich et al. (1962) fed a strain of E. coli O111:B4 to cats and recorded excretion of the organism for periods of up to one hundred and sixty days.

Administration of E. coli to new-born mice, too, has proved not to result in permanent implantation. Thus, Friedman and Halbert (1960) found that a strain of human origin was excreted by the majority of animals for from thirteen to nineteen days, while Mushin and Dubos (1965) reported that a strain of similar source was eliminated for approximately twenty-eight days. When the latter authors infected mice aged twelve or twenty-four days with the same strain, they again recorded carriage of the organism by the animals until only the twenty-eighth day of life. Adult animals from the same colony resisted colonization even when large amounts of bacterial

culture were added to the drinking water for three consecutive days. Mushin and Dubos (loc. cit.) discussed the possibility of a physiological explanation of that phenomenon but concluded, instead, that it was a result of the development of an inhibitory microflora in the gut at about twenty-eight days of age, i.e., at weaning.

Clearly, then, there have been but few successful efforts to establish a new resident strain of E. coli as a result of administration to a potential host under normal conditions and, manifestly, such a strain has first to overcome resistance to its presence in the bowel. The different, and probably inter-related, factors proffered to explain the persistence of some strains of E. coli in the intestine and resistance to establishment of others have already been reviewed in the introduction to this part. The multiplicity of those factors indicates that maintenance of residency may be a very complicated process involving environmental and physiological conditions of the host, the degree of antagonism offered to a strain of E. coli by organisms of the same species and the presence of metabolic products of other bacteria normally found in the intestine.

The ensuing report presents the results of a series of attempts to introduce, as residents in the gut

of newly-hatched and of adult chickens as well as of adult hamsters, strains of E. coli of partly known role in the host of origin. Because of the measure of success attained as a result of dosage of E. coli to day-old mice (Friedman and Halbert, 1960; Mushin and Dubos, 1965), it was decided to test the performance of strains in day-old chicks and to compare it with that in adult animals. Chicks of such tender age provide a gut free of established bacterial population whereby information on the fate of E. coli ingested during the first day of life becomes available.

Some procedures were designed to test the ability of strains of known tenure in a particular species of host to repeat that behaviour in creatures of the same type whereby it was hoped to test the observations by Gage et al. (1961), namely, that strains of E. coli vary in ability to persist in the bowel and so provide an explanation for residence or transience in the normal intestine. Other experiments, in which strains of E. coli isolated from a specific host were fed to animals belonging to the same and/or different species, were intended to test observations on host-specificity made by other workers (Taylor, 1961; Sojka, 1965). The inclusion of three known colicinogenetic cultures among

the strains of E. coli under test enabled the author to assess the significance of antibiosis in the establishment of organisms in the gut of animals.

MATERIALS.

Six serologically different strains of E. coli were chosen for the tests. They were as follows:

(1) B1, a colicinogenetic former resident strain of E. coli O1:K? procured from a chicken,

(2) G143, a transient strain of E. coli O26:B6 isolated from a chicken,

(3) G107, a colicinogenic transient strain of E. coli O2:K? recovered from a chicken,

(4) H11(1), a former resident strain of E. coli O103:K? obtained from a hamster,

(5) A8(1), a former resident of unknown serological type isolated from a dog and

(6) A7(1), a colicinogenetic transient from a dog.

Aside from two birds acquired from an outside source, use was made of young adult fowls aged from about six months to one year which had been reared in the animal house. Two were White Leghorns but the others belonged to bantam breeds. The choice of the latter was deliberate and was made on account of small size and ease of housing. They were maintained in rabbit cages bedded

with peat moss litter, were fed on commercial chicken meal and fresh water was always available. The birds were disposed in groups of two per cage or, in the case of larger stock, they were housed in adjoining cages. Each pair comprised birds with a similar background.

Chicks were hatched in small batches in an egg-incubator in the department. Again, preference was given to bantam breeds when they were available. The chicks, identified with coloured dye, were reared in guinea-pig cages under heat provided by an infra-red lamp. They were fed with commercial chicken meal and fresh water was constantly supplied. As the birds grew, the groups were transferred to rabbit cages.

Five hamsters obtained from a local dealer and, later, Hamster No. 12 (v. Part I) were maintained separately in metal mouse-boxes with peat moss litter and wood wool as bedding. They were fed with Diet 18 and fresh water was constantly available.

METHODS.

The procedures followed were (a) to allow an animal to consume ad lib. for a standard period of time diluted broth culture of the strain of E. coli under investigation and (b), thereafter, to examine faecal

specimens at intervals until it was determinable whether the strain had become firmly established in, or it had been eliminated from, the bowel. Failure to detect the strain in faecal samples procured during a period of two weeks or more was considered indicative of loss of the organism from the gut. Before an animal was exposed to the strain under test, faecal samples were examined on one, or more, occasions to ensure that the serogroup of E. coli was not already in residence.

(A) ADMINISTRATION OF E. coli TO ANIMALS.

For a period of twenty-four hours, the supply of water was with-held from the day-old chicks as well as from the adult birds and replaced by a mixture consisting of one volume of nutrient broth culture of the test strain of E. coli grown at 37°C. for eighteen hours and three volumes of sterile distilled water. The birds were seen readily to consume the proffered suspension.

Hamsters were presented with a similar culture-in-water mixture but, since they were seldom observed to drink it and to overcome possible reluctance on their part, those animals were provided with freshly prepared mixture supplied daily for five consecutive days. During that period, the supply of fresh water was withdrawn.

(B) RECOVERY OF THE STRAIN UNDER TEST.

Cloacal swabs from chickens and faecal samples from hamsters were cultivated on ' Oxoid ' MacConkey 3 agar plates whence, after incubation at 37°C. for eighteen hours, five to ten lactose-fermenting colonies were selected. Each colony was subcultivated to a 5 per cent. horse-blood-agar plate and into four millilitres of nutrient broth. For convenience, the isolates from each sample were accommodated on one agar plate. Both plate and broth cultures were incubated at 37°C. for eighteen hours. After it had been heated at 100°C. for two hours, the broth culture provided the 'O' suspension for a tube agglutination test against dilutions of the specific antiserum prepared against the administered strain. The technique of the test and the mode of interpretation were the same as those described in Part I of this thesis.

Of the cultures agglutinable to titre by the specific antiserum, at least one from each positive sample was subcultivated from the blood agar plate and tested for indole-production, for citrate-utilization and for reactions to the Voges-Proskauer and the methyl-red tests.

INVESTIGATIONS AND RESULTS.

The ensuing report describes the findings from seven studies, designated Experiments Nos. 1-7, each

involving the examination of a single E. coli serogroup.

Experiments Nos. 1, 2 and 3 were designed to test the ability of avian resident Strain No. B1 and transient Strains Nos. G143 and G107, respectively, to establish themselves in the gut of chickens whereby information might be forthcoming concerning 'strength' of E. coli. In Experiment No. 4, Strain No. B1 was offered to hamsters and, in Experiments Nos. 5 and 6, Strains Nos. H11(1) and A8(1) were fed to both chickens and hamsters in order to provide data on host-specificity. In Experiment No. 7, an actively colicinogenetic culture, No. A7(1) of canine origin, was given to chickens for the purpose of comparing the findings with those of Experiments Nos. 1 and 3, in which the cultures employed were also of antibiotic type.

EXPERIMENT No. 1.

Introduction.

As a result of their studies of a group of human infants, Gage et al. (1961) concluded that certain strains of E. coli, which they termed 'strong', are more able than others to establish themselves and to persist in the intestine. However, there is as yet scant experimental evidence to support that conjecture. Sears et al. (1950) and Sears and Brownlee (1952) reported on the administration of former resident strains to human beings and claimed one instance of success but not yet is available information concerning the preterite role of test cultures in experiments conducted by other workers. The generally high rate of failure of attempts to implant a new strain in the bowel may be attributable to the use of organisms unsuited for the purpose.

That possibility provided the author of this thesis with the stimulus to investigate the ability of a former resident strain isolated from a chicken to colonize other members of the same species.

Materials.

Strain No. B1 was fed to (a) two adult Scots Greys bantam pullets (Chickens Nos. 3 and 4) and (b) a

group of four day-old White Leghorn chicks (Chicks Nos. 11, 12, 13 and 14).

Results.

(a) (v. Table 9, p. 115).

Twenty-four hours after Chicken No. 3 had been fed the culture, a faecal sample yielded colonies of B1 type in the proportion of eight out of ten examined. The following day, nine out of ten colonies again belonged to Group No. B1 but, on the third day, none of the ten subcultures proved agglutinable by B1 antiserum. On the fifth day, two colonies of Group No. B1 were again present in the ten that were examined and from the sixth until the ninety-sixth day thereafter the organism was constantly found, usually as the dominant strain.

Similar results prevailed in the case of Chicken No. 4. Twenty-four hours after the bird had received B1 culture, a cloacal swab yielded ten out of ten colonies of B1 type. On the second and third days, the numbers of positive colonies fell to five and one, respectively, but by the fifth day the number of B1 colonies had once more risen to four out of the ten examined. From the sixth until the sixty-eighth day the organism continued to be excreted, usually as the dominant strain.

(b) (v. Table 10, p. 116).

All four chicks continued to excrete the B1 serogroup for up to one hundred and twenty-two days, when the experiment was discontinued.

Discussion.

Although residencies of only ninety-six and sixty-eight days were maintained by the B1 strain in adult birds, it may be confidently asserted that the organism established itself in those creatures. Aside from one negative faecal sample obtained from Chicken No. 3 at the third day of the experiment, the B1 organism was recoverable from all of the swabs procured from the birds during its tenure in the latter. Those experimentally-produced residencies were not as stable as the one exhibited in Chicken 'W' but, while they lasted, they were not unlike that of the same strain in Chicken 'B' (v. Part I).

Chickens Nos. 3 and 4 were housed in adjoining cages but excretion of the B1 serogroup by both birds followed the same pattern. For from twenty-four to forty-eight hours after culture had been substituted for the fresh water supply, the creatures were observed to void many B1 organisms but a marked fall in the incidence of the latter ensued until, from the sixth day onwards,

the B1 serogroup resumed the dominant role in the excremental E. coli flora. Those findings may signify a process of adaptation by the B1 invader similar to that propounded by Ozawa and Freter (1964). The method of examination of faecal samples employed in this investigation was not designed to estimate actual numbers of E. coli but, probably, the preponderance of the test strain relative to other coliforms encountered in the initial specimens represented a process of elimination while the secondary rise indicated proliferation of the same organism adapted to survive in the intestinal environment of the new host.

The B1 strain implanted itself readily in day-old chicks and persisted in the gut for one hundred and twenty-two days. As in the case of adult experimental fowls, the organism assumed dominance and was excreted by the chicks with a constancy equivalent to that recorded in Chicken 'B', the original host of the organism under test.

Summary.

Implantation of the former resident B1 strain was successful in two adult chickens despite resistance offered to it by the existing intestinal flora. The organism also established itself in a group of four day-

old chicks. In the case of both adult and newly-hatched birds, the pattern of excretion of the artificially introduced resident resembled that of the same serogroup in its original host.

TABLE 9.

Date of sample	No. of Bl colonies/No. of colonies examined	
	Chicken 3	Chicken 4
Pre-infection		
17/2/65	0/10	0/3
22/2/65	0/10	0/5
24/2/65	0/10	0/10
	Allowed to drink 25% broth culture of Bl <u>ad lib</u>. for 24 hours.	
25/2/65	8/10	10/10
26/2/65	9/10	5/10
27/2/65	0/10	1/10
1/3/65	2/10	4/10
2/3/65	9/10	9/10
5/3/65	9/10	9/10
8/3/65	9/10	2/10
11/3/65	10/10	10/10
15/3/65	10/10	10/10
22/3/65	10/10	2/10
29/3/65	4/4	6/10
5/4/65	3/7	9/10
12/4/65	9/10	10/10
19/4/65	10/10	8/10
26/4/65	10/10	-*
3/5/65	8/10	9/10
10/5/65	5/5	0/5
17/5/65	3/5	0/5
24/5/65	3/5	0/2
31/5/65	4/5	0/5
7/6/65	0/5	0/5
14/6/65	0/5	0/5
21/6/65	0/10	0/10
28/6/65	0/5	0/5
5/7/65	0/5	0/5
19/7/65	-*	0/5

* Absence of growth by coliform bacteria.

TABLE 10.

Date of sample	No. of Bl colonies/No. of colonies examined			
	Chick 11	Chick 12	Chick 13	Chick 14
26/4/65*	0/0	0/0	0/0	0/0
27/4/65	5/5	5/5	5/5	5/5
29/4/65	5/5	5/5	5/5	5/5
1/5/65	5/5	5/5	5/5	5/5
5/5/65	5/5	5/5	5/5	2/5
7/5/65	5/5	5/5	5/5	5/5
10/5/65	5/5	5/5	4/5	2/5
17/5/65	5/5	5/5	5/5	2/5
24/5/65	5/5	5/5	5/5	5/5
31/5/65	4/5	5/5	5/5	5/5
7/6/65	4/5	5/5	3/5	5/5
14/6/65	5/5	3/5	4/5	3/5
21/6/65	4/5	5/5	5/5	5/5
28/6/65	5/5	5/5	4/5	5/5
5/7/65	3/5	0/5	3/5	1/5
12/7/65	4/5	0/5	3/5	0/5
19/7/65	2/5	5/5	4/5	3/5
	Not examined during Summer vacation.			
26/8/65	3/10	8/10	8/10	9/10

* Sample procured immediately before broth culture mixture was offered for 24 hours.

EXPERIMENT No. 2.

Introduction.

The results of the previous experiment indicated that a strain, resident in a chicken, was capable of repeating that role in other birds of the same species. If 'strength' (Gage *et al.*, 1961) is a property of the micro-organism, a strain unable to persist in the gut of one bird may be expected likewise to perish in another. The work about to be reported was undertaken in order to test that hypothesis.

Materials.

Strain No. G143, a transient organism isolated from a chicken and a member of *E. coli* Group 026:B6, was administered to (a) two adult White Leghorn pullets (Chickens Nos. 5 and 6) and (b) a group of five day-old Millefleur bantam chicks (Chicks Nos. 19, 20, 21, 22 and 23).

Results.

(a) (*v.* Table 11, p. 121).

Chicken No. 5 was twice fed the culture but none of fifty colonies isolated from five samples during the six days after the first exposure, or any of thirty-five

from four samples during the six days following the second treatment, proved to belong to the G143 type. When the experiment was repeated, sixty colonies were isolated from six samples taken during the subsequent nine days but none of them was found to be positive. At this point, the bird was supplied daily with fresh culture mixture in place of drinking water for two consecutive periods of four and five days, respectively, separated by an interval of two days. Not at any time throughout the spell of continuous exposure to culture or during the five days after cessation of administration were any isolates identifiable as belonging to Group No. G143. In the course of the final stage of the experiment, a total of one hundred and fifteen colonies was examined.

An identical investigation pertaining to Chicken No. 6 yielded similar results.

(b) (v. Table 12, p. 122).

Subsequent to its administration to five day-old chicks, Nos. 19, 20, 21, 22 and 23, the G143 serogroup was recovered over a period of twenty-one, fourteen, forty, twenty-three and sixteen days, respectively.

Discussion.

The findings from the tests with adult birds

confirmed expectations, namely, that the strain under examination would be unable to persist in the avian gut. Even continuous feeding with culture was not attended by recovery of the organism from faecal samples collected during, or immediately after, treatment. In that respect, the results are not unlike those recorded by Sears et al. (1950, 1956) and by Sears and Brownlee (1952) subsequent to attempts to establish new resident serogroups in human beings and in dogs, in the course of which trials repeated doses of organisms were administered. Unfortunately, in most cases, the authors did not specify the role of the strains in the previous host.

The greater ease experienced in establishing the G143 strain in day-old chicks was associated with periods of excretion ranging from sixteen to forty days. The findings from the chicks were not unlike those reported by Friedman and Halbert (1960) and by Mushin and Dubos (1965) following administration of strains of human origin to new-born or day-old mice, in consequence of which the animals continued to eliminate the organism in the faeces for periods of from nineteen to twenty-eight days. Nevertheless, the results from chicks, when compared with the outcome of Experiment No. 1, indicated that strains of E. coli differ in ability to remain in the intestine of a

particular species of host.

Summary.

A transient strain of E. coli isolated from a chicken failed to establish itself after administration to two adult chickens. When it was offered to a group of day-old chicks, the organism was found to be excreted by the latter for periods ranging from sixteen to forty days.

TABLE 11.

Date of sample	No. of G143 colonies/No. of colonies examined	
	Chicken 5	Chicken 6
Pre-infection		
8/6/65	0/10	0/10
11/6/65	0/10	0/10
15/6/65*	0/10	0/10
	Allowed to drink 25% broth culture of G143 ad lib. for 24 hours.	
16/6/65	0/10	0/10
17/6/65	0/10	0/10
18/6/65	0/10	0/10
19/6/65	0/10	0/10
21/6/65*	0/10	0/10
22/6/65	0/10	0/10
23/6/65	0/10	0/10
24/6/65	0/10	0/10
28/6/65*	0/5	0/5
29/6/65	0/10	0/10
30/6/65	0/10	0/10
1/7/65	0/10	0/10
3/7/65	0/10	0/10
5/7/65	0/10	0/10
7/7/65*	0/10	0/10
8/7/65*	0/10	0/10
9/7/65*	0/10	0/10
10/7/65*	0/10	0/10
12/7/65	0/5	0/5
13/7/65*	0/10	0/10
14/7/65*	0/10	0/10
15/7/65*	0/10	0/10
16/7/65	0/10	0/10
17/7/65	0/10	0/10
19/7/65	0/10	0/10
21/7/65	0/10	0/10
22/7/65	0/10	0/10

* Sample procured immediately before broth culture mixture was offered for 24 hours.

TABLE 12.

Date of sample	No. of G143 colonies/No. of colonies examined				
	Chick 19	Chick 20	Chick 21	Chick 22	Chick 23
26/5/65*	0/0	0/0	0/0	0/0	0/0
27/5/65	5/5	5/5	5/5	5/5	5/5
28/5/65	5/5	5/5	5/5	5/5	5/5
31/5/65	5/5	5/5	5/5	5/5	5/5
4/6/65	5/5	5/5	5/5	5/5	5/5
7/6/65	5/5	5/5	5/5	5/5	4/5
9/6/65	5/5	5/5	5/5	5/5	5/5
11/6/65	5/5	-	5/5	5/5	5/5
14/6/65	5/5	0/1	5/5	5/5	0/5
16/6/65	7/10	0/10	10/10	5/10	0/5
18/6/65	0/10	0/10	2/10	4/10	0/6
21/6/65	0/10	0/10	2/10	0/10	0/10
28/6/65	0/5	0/5	0/5	0/5	0/5
5/7/65	0/10	0/10	1/10	0/10	0/10
12/7/65	0/10	0/10	0/10	0/10	0/10
19/7/65	0/10	0/10	0/10	0/10	0/10

* Sample taken immediately before broth culture mixture was offered for twenty-four hours.

\- Absence of growth by coliform bacteria.

EXPERIMENT No. 3.

Introduction.

Of the respective strains of E. coli employed for Experiments Nos. 1 and 2, Strain B1 was found subsequently to be poorly colicinogenetic but Strain G143 was not observed to be so active. The antibiotic character of the former organism may be associated with its peculiar ability to establish itself and to persist in the gut of experimental animals. The significance of colicinogenesis in the control of the enteric E. coli population has not yet been elicited. Studies by other workers on the incidence of antibiotic strains in relation to their residency in normal subjects have yielded conflicting results and attempts to implant colicin-producing strains in the intestine of animals have not been successful (Rauss and Ketyi, 1960; Friedman and Halbert, 1960). However, Friedman and Halbert noted that, when they gave to new-born mice a mixture consisting of a colicinogenic strain and a non-antibiotic one, the former came to constitute the dominant organism of the bowel.

A colicinogenetic transient strain of E. coli of avian origin was selected for dosing to chickens further to test the theory of variation in 'strength' and to

compare the performance of an actively antibiotic organism with that of those of the cultures which were employed for Experiments Nos. 1 and 2.

Materials.

Strain No. G107 was administered to (a) two adult Millefleur bantam cockerels (Chickens Nos. 9 and 10) and (b) a group of three day-old Millefleur bantam chicks (Chicks Nos. 24, 25 and 26).

Results.

(a) (v. Table 13, p. 127).

During the nineteen days following the first exposure of Chicken No. 9 to culture, eight faecal samples were examined but none of the eighty colonies investigated was assignable to Group No. G107. The test was repeated and, out of ten colonies procured from a cloacal swab twenty-four hours after the close of the period of exposure to culture, one was found to belong to the G107 group. Examination of ten samples during the following forty-one days proved negative for the presence of colonies belonging to the homologous serogroup.

The first sample from Chicken No. 10, retrieved twenty-four hours after exposure to Strain No. G107, failed to yield any colonies characteristic of that serogroup but three samples examined during the subsequent

four days proved so related. Four other samples obtained during the succeeding fourteen days proved negative. The bird was offered the culture for a second time with the result that six of the seven samples secured during the first thirteen days yielded organisms referable to Group No. G107. During the twenty-eight days thereafter, four samples failed to yield organisms belonging to the serogroup.

(b) (v. Table 14, p. 128).

After the feeding of Strain No. G107 to three day-old chicks, the birds were observed to excrete organisms belonging to that serogroup during periods ranging from eight to eleven days.

Discussion.

Although the birds were housed in the same cage, the findings from the adult chickens were not similar. Chicken No. 9 harboured the G107 serogroup for twenty-four hours, at most, but Chicken No. 10 continued to excrete the organism for two periods of five and thirteen days, respectively.

The strain under test did not appear to maximal number in the faeces of the latter bird until the third or

the fourth day and, thereafter, the incidence fell suddenly in one case and progressively in the other. The results suggested inability on the part of the organism to adapt itself to the new intestinal environment although the persistence of the strain in the bird contrasted with the non-recovery of the other transient strain, No. G143, from similar birds. It remains doubtful that colicinogenesis alone was responsible for the difference in performance of those transient strains in adult experimental chickens.

Day-old chicks harboured the colicinogenic Strain No. G107 for, at most, eleven days compared with periods of up to forty days during which the non-antibiotic Strain No. G143 was excreted by similar creatures. Those results, by themselves, indicate that colicin-production is not a factor likely to aid the establishment of a strain of E. coli in a new intestinal environment.

Summary.

The findings show that, despite its colicinogenic ability, a transient strain of E. coli procured from a chicken was unable to implant itself in either of two adult fowls or in newly-hatched chicks.

TABLE 13.

Date of sample	No. of G107 colonies/No. of colonies examined	
	Chicken 9	Chicken 10
Pre-infection		
30/ 8/65	0/10	0/10
2/ 9/65	0/10	0/10
16/ 9/65	0/10	0/10
17/ 9/65*	0/10	0/10
	Allowed to drink 25% broth culture of G107 *ad lib.* for 24 hours.	
18/ 9/65	0/10	0/10
20/ 9/65	0/10	2/10
21/ 9/65	0/10	10/10
22/ 9/65	0/10	1/10
23/ 9/65	0/10	0/10
29/ 9/65	0/10	0/10
5/10/65	0/10	0/10
6/10/65*	0/10	0/10
7/10/65	1/10	1/10
8/10/65	0/10	0/10
9/10/65	0/10	9/10
11/10/65	0/10	8/10
12/10/65	0/10	2/10
15/10/65	0/10	5/10
19/10/65	0/10	1/10
26/10/65	0/10	0/10
2/11/65	0/10	0/10
9/11/65	0/10	0/10
16/11/65	0/10	0/10

* Sample procured immediately before broth culture mixture was offered for 24 hours.

TABLE 14.

Date of sample	No. of G107 colonies/No. of colonies examined		
	Chick 24	Chick 25	Chick 26
13/6/66*	0/0	0/0	0/0
14/6/66	10/10	10/10	10/10
16/6/66	10/10	10/10	10/10
21/6/66	3/10	4/10	9/10
24/6/66	0/10	0/10	7/10
29/6/66	0/10	0/10	0/10
1/7/66	0/1	0/10	0/10
5/7/66	0/10	0/10	0/10
9/7/66	0/10	0/10	0/3
11/7/66	0/10	0/10	0/10

* Sample procured immediately before broth culture mixture was offered for 24 hours.

EXPERIMENT No. 4.

Introduction.

Cross-infection with E. coli has been considered by several workers with inconclusive inferences. Rees (1958) observed the presence of similar serological types in both calves and lambs. He suggested that the lambs had obtained the infection from calves and so might constitute a reservoir for re-infection of the latter. Other workers (Thomson, 1956; Jacalne and Aragon, 1958; Mian, 1959; Mackel et al., 1960; Mushin and Ashburner, 1964) have investigated the lower animals as a potential source of E. coli pathogenic to man but Ewing (1962) found that serotypes associated with infantile diarrhoea are infrequent in creatures other than primates.

Observations on the normal incidence of some serogroups are strongly suggestive of a host-specific relationship (Taylor, 1961; Sojka, 1965), which association is further supported by the report by Schaedler and Dubos (1960) on the unique ability of a strain of murine origin to establish itself in experimental mice. There are reports of the successful experimental transference of E. coli from one species to another (Rauss and Ketyi, 1960; Ashburner and Mushin, 1962) but, in those instances,

the organism was administered under abnormal conditions occasioned by antibiotic treatment.

The findings from Experiment No. 1 suggested that a strain of E. coli may possess a peculiar property which enables it to persist over a long period in the intestine of a particular species of host. That experience stimulated the author to consider if a so-called 'strong' strain may be able, also, to survive in the gut of a host belonging to a different species. The experiment about to be recorded was designed as a test of host-specificity.

Materials.

Strain No. B1, a former resident isolated from a chicken, was administered to four adult hamsters (Hamsters Nos. 7, 9, 12 and 13).

Results. (v. Tables 15 & 16, pp. 134-135).

On the first occasion on which Hamster No. 7 received the strain, the latter continued to be excreted intermittently for up to seventy-five days. Loss of the organism was associated with an abrupt fall in the number of E. coli in the faeces. Eleven faecal examinations carried out during the following eight weeks provided, altogether, only eleven lactose-fermenting colonies.

At the end of that period, B1 culture was again offered (v. Table 16) with the result that two faecal samples obtained from the animal during the feeding process yielded B1 colonies in the proportion of eight out of eight and ten out of ten examined, respectively. Faeces, selected at the fourth day after culture had been withdrawn, failed to provide the serogroup but similar material taken four days later yielded two B1 colonies out of three isolated. Thereafter, only one out of six studies of faecal material, procured at intervals up to the eighteenth day of the investigation, produced lactose-fermenting colonies and none of the latter was agglutinated by B1 antiserum.

Hamster No. 9 was exposed twice to Group B1 culture. On the first occasion, the organism was found to be present only in small numbers in a faecal sample that was procured during the period of exposure but was not encountered in any of six samples examined during the following five weeks. After a second dose, faecal samples obtained during the feeding period and at three days later both yielded organisms of Group B1 but none of four subsequent weekly faecal examinations proved so positive.

Faecal samples procured from Hamster No. 12 during, and at two days after, the first administration of the B1 strain both furnished the serogroup which was not forthcoming from any of the subsequent five weekly samples. When the animal was fed the strain for a second time, from both of the samples procured during, and at three days after, the feeding period, B1-type colonies were recovered but none of six subsequent weekly specimens was shown to contain the serogroup.

Hamster No. 13 was also given the strain twice. After the first administration, only the sample taken during the feeding period produced Group No. B1 *E. coli* whilst both during and after the second period of exposure none of the samples was found to include the serogroup.

Discussion.

The findings reveal that the avian B1 strain was able to colonize the bowel of one out of four hamsters. The serogroup persisted in the animal for seventy-five days although, for most of that time, it remained in a subordinate role. The organism survived a period of adaptation similar to that observed in the case of the avian gut (*v.* Expt. 1) but its subsequent association with the host was unstable compared with that maintained in

birds.

The termination of the tenure of the B1 serogroup in the hamster co-incided with a marked reduction in the incidence of lactose-fermenting coliform bacilli in the faeces, which state was found to prevail for eight weeks until B1 culture was again offered to the animal. Consequently, B1 organisms were recoverable from a faecal sample procured at the eighth day after treatment in circumstances which suggested that the strain had adapted itself to the intestinal habitat but six faecal examinations undertaken during the following ten days proved negative for its presence. Five out of the six investigations failed to provide lactose-fermenting colonies, which finding is attributable to the existence in the animal of an enteric environment inimical to E. coli.

Summary.

The results of the work under report indicate that, although a strain of E. coli may show a preference for a particular species of host, the relationship is not absolute. They reveal that transference from one species to another is possible under natural conditions.

TABLE 15.

Date	No. of Bl colonies/No. of colonies examined			
	Hamster 7	Hamster 9	Hamster 12	Hamster 13
8/10/65	0/10	0/5	0/5	0/10
12/10/65	0/10**	0/10**	0/5 **	0/10**
13/10/65	*	*	*	*
14/10/65	*	*	*	*
15/10/65	5/10*	1/5 *	10/10*	8/10*
16/10/65	*	*	*	*
19/10/65	1/10	0/10	10/10	0/10
26/10/65	0/5	0/10	0/1	0/10
2/11/65	5/10	0/10	0/6	0/10
9/11/65	4/10	0/10	0/2	0/10
16/11/65	2/2	0/10	0/2	0/10
23/11/65	3/10	0/10**	- **	0/10**
24/11/65		*	*	*
25/11/65		*	*	*
26/11/65	0/10	6/10*	10/10*	0/10*
27/11/65		*	*	*
30/11/65	1/10	1/10	1/1	0/10
7/12/65	0/10	0/10	0/1	0/10
14/12/65	1/10	0/10	0/2	0/10
21/12/65	0/10	0/2	0/4	0/10
28/12/65	10/10	0/10	-	0/10
31/12/65	-		-	

** Sample procured immediately before broth culture mixture was offered.

* Administration of culture continued.

- Absence of growth by coliform bacteria.

TABLE 16.

HAMSTER No. 7.

Date	No. of Bl colonies/ No. of colonies examined
1/1/66	-
7/1/66	-
11/1/66	-
14/1/66	0/5
18/1/66	0/2
25/1/66	0/2
1/2/66	-
9/2/66	0/2
16/2/66	-
22/2/66	-
2/3/66	-
7/3/66**	
8/3/66*	8/8
9/3/66*	
10/3/66*	10/10
11/3/66*	
15/3/66	0/10
19/3/66	2/3
21/3/66	-
22/3/66	0/10
24/3/66	-
25/3/66	-
26/3/66	-
29/3/66	-

** Administration of broth culture mixture begun.
* Administration of culture continued.

EXPERIMENT No. 5.

Introduction.

Although the findings from Experiments Nos. 1 and 4, in which a strain of E. coli of avian origin was offered to chickens and hamsters, revealed that cross-infection with E. coli may occur, they were also indicative of a host-specific propensity on the part of the micro-organism. For the experiment about to be reported, a culture isolated from a hamster served further to test the possibility of predilection for a particular species of host.

Materials.

Strain No. H11(1), a member of 'O'-group 103 and a former resident in a hamster, was administered to (a) five adult hamsters (Hamsters Nos. 7, 8, 9, 10 and 12), (b) two adult Millefleur cockerels (Chickens Nos. 11 and 12) and (c) a group of four day-old Millefleur bantam chicks (Chicks Nos. 27, 28, 29 and 30).

Results.

(a) (v. Tables 17 and 18, pp. 142-143).

A faecal sample taken from Hamster No. 7 during the first period of feeding yielded nine colonies of Group O103 out of ten isolated but none of eight samples

cultured during the following nine weeks yielded that serogroup. The experiment was repeated whereby six samples procured during the first five weeks proved positive for the presence of Group 0103 organisms. When six more weekly samples failed to yield identical organisms, the serogroup was considered to have been eliminated from the bowel.

Hamster No. 8 was exposed only once to the H11(1) strain. Neither of two pre-infection samples was found to yield any lactose-fermenting colonies as was the case, too, during the feeding period but a sample, obtained four days after the culture had been withdrawn, furnished a pure growth of the H11(1) strain. The tenure of the latter continued uninterruptedly for thirty-one weeks thereafter. Investigation of this animal was discontinued for almost three months when, subsequently, four out of five faecal samples examined during a period of forty-one days again proved positive.

Hamster No. 9, too, was exposed only once to the H11(1) strain. The presence of the organism in the gut was revealable for eighty-three days, during which time nine out of fourteen samples yielded E. coli assignable to 'O'-group 103.

A sample collected from Hamster No. 10 during the first period of exposure to the H11(1) culture yielded a pure growth of the homologous organism but none of the eight samples examined during the next fifty days proved to be positive for that serogroup. When the experiment was repeated, only the sample procured from the animal immediately after withdrawal of the culture yielded the H11(1) strain. None of ten samples recovered during the following eleven weeks was found to contain E. coli of the specific serogroup.

Hamster No. 12, which had been shown previously to excrete few E. coli in the faeces (v. Part I), was exposed once only to H11(1) culture. One faecal sample obtained from the animal during the period of administration failed to yield any E. coli colonies but faeces passed four days after the culture was withdrawn yielded a pure growth of the H11(1) type. The organism continued to be excreted by the animal for one hundred and twelve days when the investigation was terminated.

(b) (v. Table 19, p. 144).

During the five days after exposure of Chicken No. 11, forty colonies from four samples were examined but not one of them proved to belong to Group No. H11(1).

After a second exposure to culture, none of sixty isolates from six samples examined during the following twenty-two days was agglutinable by O103 antiserum.

Identical observations on Chicken No. 12 also gave negative results.

(c) (v. Table 20, p. 145).

After exposure of day-old chicks to culture, Nos. 23 and 24 were not shown to excrete the H11(1) organism for more than three and eight days, respectively. Chicks Nos. 25 and 26, however, continued to yield the strain intermittently for at least seventy-one and forty-three days, respectively.

Discussion.

The results of this combined experiment lend further support to the belief that strains of E. coli have a host-predilection. The H11(1) strain survived for more than a month in the intestine of four out of five adult hamsters and probably for, at least, two hundred and forty-nine days in the case of one of them. Although the intestinal environment of Hamster No. 12 and probably, also, of Hamsters Nos. 8 and 9 was highly inimical to the presence of E. coli, none the less, the strain came to be

implanted therein. In the case of Hamsters Nos. 8 and 9, the tenure of the H11(1) strain closely resembled in stability that of the parent organism in the intestine of Hamster No. 11 (v. Part I). In contrast, duplicate attempts to establish the H11(1) strain in two adult chickens all failed.

A trial with four day-old chicks yielded less definite results. It was noted that, although the birds were caged together, two continued to excrete the organism long after the latter seemed to have been lost by the others but the findings were complicated by the sudden death of one of the chicks after the thirty-sixth day of the investigation.

Summary.

A strain of E. coli formerly resident in a hamster was implanted into the gut of other four. By two of the latter, the organism was excreted with a constancy equivalent to that exhibited by the original host. Attempts failed to establish the serogroup in adult chickens but a prolonged association was effected between the micro-organism and two out of four chicks. It was again evident that, although possibly better adapted to the intestine of a particular species of host, a strain may

come to persist for a long period in an animal of a different species.

TABLE 17.

Date	No. of H11(1) colonies/No. of colonies examined			
	Hamster 7	Hamster 8	Hamster 9	Hamster 10
1/ 3/65	0/10	-	0/10	0/10
2/ 3/65	0/10**	- **	- **	0/10**
3/ 3/65	*	*	*	*
4/ 3/65	*	*	*	*
5/ 3/65	9/10*	- *	8/10*	8/8 *
6/ 3/65	*	*	*	*
11/ 3/65	-	10/10	10/10	0/5
17/ 3/65	0/10	10/10	10/10	-
22/ 3/65	-			0/10
25/ 3/65	0/8	10/10	2/10	-
29/ 3/65	0/4	2/10	6/10	0/10
5/ 4/65	0/10	10/10	0/10	0/10
12/ 4/65	0/10	10/10	8/10	0/10
19/ 4/65	0/10	10/10	4/10	0/10
27/ 4/65	0/5	2/5	0/5	0/5
29/ 4/65	*			*
30/ 4/65	*			*
1/ 5/65	*			*
2/ 5/65	*			*
3/ 5/65	*			*
4/ 5/65	4/5	5/5	1/5	4/5
11/ 5/65	3/5	2/5	0/5	0/5
18/ 5/65	5/5	5/5	0/5	0/5
25/ 5/65	3/5	4/5	0/5	0/5
30/ 5/65	5/5	5/5	1/5	0/5
1/ 6/65		5/5		
8/ 6/65	1/5	5/5	0/5	0/5
22/ 6/65	0/5	3/5	0/5	0/5
29/ 6/65	0/5	5/5	0/5	-
6/ 7/65	0/5	2/5	-	0/5
13/ 7/65	0/5	5/5	0/5	0/5
20/ 7/65	0/5	2/2	0/5	0/10
	Examinations stopped for Summer vacations.			
8/10/65	0/5	1/5	0/5	0/5
28/10/65		10/10		
11/11/65		-		
14/11/65		3/10		
18/11/65		10/10		

** Sample procured immediately before culture was offered.

* Continued administration of culture.

\- Absence of growth by coliform bacteria.

TABLE 18.

HAMSTER No. 12.

Date	No. of H11(1) colonies/ No. of colonies examined
21/12/65	0/4
28/12/65	-
31/12/65	-
3/ 1/66**	
4/ 1/66*	-
5/ 1/66*	
6/ 1/66*	
7/ 1/66*	
11/ 1/66	10/10
14/ 1/66	10/10
18/ 1/66	10/10
25/ 1/66	10/10
1/ 2/66	10/10
9/ 2/66	10/10
16/ 2/66	10/10
22/ 2/66	10/10
2/ 3/66	9/9
8/ 3/66	10/10
15/ 3/66	7/10
22/ 3/66	10/10
29/ 3/66	10/10
5/ 4/66	10/10
12/ 4/66	10/10
19/ 4/66	0/10
26/ 4/66	4/5
3/ 5/66	9/10

** Commencement of administration of culture mixture.

* Administration of culture continued.

- Absence of growth by coliform bacteria.

TABLE 19.

Date of sample	No. of H11(1) colonies/No. of colonies examined	
	Chicken 11	Chicken 12
Pre-infection		
26/8/65	0/10	0/10
30/8/65	0/10	0/10
2/9/65	0/10	0/10
	Allowed to drink 25% broth culture of Strain No. H11(1) ad lib. for 24 hours.	
3/9/65	0/10	0/10
4/9/65	0/10	0/10
6/9/65	0/10	0/10
7/9/65	0/10	0/10
	Allowed to drink broth culture mixture again ad lib. for 24 hours.	
8/9/65	0/10	0/10
10/9/65	0/10	0/10
11/9/65	0/10	0/10
13/9/65	0/10	0/10
16/9/65	0/10	0/10
29/9/65	0/10	0/10

TABLE 20.

Date of sample	No. of Hll(l.) colonies/No. of colonies examined			
	Chick 23	Chick 24	Chick 25	Chick 26
12/6/66*	0/0	0/0	0/0	0/0
13/6/66	7/10	5/10	7/10	9/10
15/6/66	7/10	9/10	6/10	5/10
20/6/66	0/10	3/10	1/10	1/10
23/6/66	0/10	0/10	0/10	1/10
28/6/66	0/10	0/10	7/10	0/10
1/7/66	0/10	0/10	0/10	4/10
5/7/66	0/10	0/10	9/10	0/4
7/7/66	0/10	0/10	6/10	0/6
11/7/66	0/10	0/10	0/10	0/8
18/7/66	0/10	0/5	0/2	0/10
25/7/66	0/10	Died.	0/7	2/10
1/8/66	0/10		0/10	0/8
8/8/66	0/10		9/10	0/10
15/8/66	0/10		7/10	0/10
22/8/66	0/10		6/10	0/5
29/8/66	0/10		0/10	7/10
5/9/66	0/10		0/10	5/10
12/9/66	0/5		0/5	10/10

* Sample procured immediately before broth culture mixture was offered for 24 hours.

EXPERIMENT No. 6.

Introduction.

Although the findings from Experiments Nos. 1, 4 and 5 were indicative of a considerable degree of host-specificity, it was considered advisable further to test that observation. Thus, a former resident strain of canine origin was selected for administration to chickens and hamsters in order to determine if a culture of that source were capable of implantation into either of the two animal species.

Materials.

Strain No. A8(1) was dispensed to (a) two adult Millefleur bantam cockerels (Chickens Nos. 7 and 8), (b) two groups of day-old White Leghorn chicks comprising Chicks Nos. 1, 2 and 3 and Nos 15 and 16, respectively, and (c) two hamsters (Hamsters Nos. 8 and 10).

Results.

(a) (v. Table 21, p. 150)

In the case of Chicken No. 7, forty colonies were isolated from four faecal swabs procured during the five days following the first exposure to culture but none proved to belong to the A8(1) type. Immediately after the fourth of those samples had been taken, the bird was

offered the culture for a second time but none of sixty colonies recovered from six samples procured during the subsequent twenty-two days was shown to appertain to Group No. A8(1).

Identical results were obtained in the case of Chicken No. 8.

(b)

Strain No. A8(1) was administered to three day-old chicks, Nos. 1, 2 and 3, with the result that the micro-organism was excreted by the birds for fourteen, sixteen and fourteen days, respectively (v. Table 22, p. 151).

When the same culture was fed to two day-old chicks, Nos. 15 and 16, both were shown subsequently to eliminate the organism for only twenty-four hours (v. Table 23, p. 152).

(c) (v. Table 24, p. 153).

During the five weeks that followed the first exposure to culture, none of seven faecal samples obtained from Hamster No. 8 yielded E. coli belonging to Group A8(1). After the animal had been again fed the test strain, the samples retrieved during the feeding period and at three days later both contained colonies of Group

A8(1) but the succeeding four weekly samples proved negative in that respect.

Only one positive colony was found among ten isolated from a sample taken from Hamster No. 10 at two days after the close of the first feeding period. None of the five subsequent weekly samples furnished E. coli belonging to the A8(1) group. When the experiment was repeated, A8(1) micro-organisms were recoverable from the samples secured during, and at three days after, the period of feeding but were not procured from any of the four succeeding weekly samples.

Discussion.

Complete failure attended attempts to establish the canine-resident A8(1) strain of E. coli in adult chickens and a tenure of but a few days was demonstrated in the case of adult hamsters. Inability of the micro-organism to persist in the bowel of those animals supports the view of a host-specific relationship.

As in the previous experiments, longer residencies were obtained by administering the strain to day-old chicks but, in the latter, the A8(1) micro-organism failed to persist for longer than sixteen days, a finding which does not indicate stable implantation. One pair of

chicks was observed to excrete the A8(1) strain for, at most, twenty-four hours. It was noted that the organism which succeeded Group No. A8(1) in those two birds belonged to Group No. B1. That finding not only further confirmed the ability of the B1 strain to implant itself in the avian intestine but also revealed failure on the part of A8(1) organisms to resist substitution there by a 'strong' strain of avian origin.

Summary.

The experiment provided evidence to support the theory of host-specificity on the part of E. coli. Failure attended attempts to implant a strain formerly resident in a dog into adult chickens and hamsters. In the case of day-old chicks, relatively short tenures were obtained and, in one instance, the A8(1) strain yielded to the B1 serogroup which has already been shown to have a strong affinity for the avian intestine.

TABLE 21.

Date	No. of A8(1) colonies/No. of colonies examined	
	Chicken 7	Chicken 8
Pre-infection		
26/8/65	0/10	0/10
30/8/65	0/10	0/10
2/9/65	0/10	0/10
	Allowed to drink 25% broth culture of Strain No. A8(1) ad lib. for 24 hours.	
3/9/65	0/10	0/10
4/9/65	0/10	0/10
6/9/65	0/10	0/10
7/9/65	0/10	0/10
	Allowed to drink 25% broth culture of Strain No. A8(1) ad lib. for 24 hours.	
8/9/65	0/10	0/10
10/9/65	0/10	0/10
11/9/65	0/10	0/10
13/9/65	0/10	0/10
16/9/65	0/10	0/10
29/9/65	0/10	0/10

TABLE 22.

Date	No. of A8(1) colonies/No. of colonies examined		
	Chick 1	Chick 2	Chick 3
29/11/63*	0/0	0/0	0/0
30/11/63	10/10	10/10	10/10
1/12/63	10/10	10/10	10/10
2/12/63	10/10	10/10	10/10
3/12/63	9/10	10/10	10/10
4/12/63	10/10	10/10	10/10
5/12/63	9/10	8/10	10/10
6/12/63	10/10	10/10	10/10
7/12/63	10/10	10/10	8/10
8/12/63	9/10	2/10	4/10
9/12/63	5/10	5/10	1/10
10/12/63	8/10	2/10	0/10
11/12/63	1/10	1/10	0/10
12/12/63	2/10	2/10	0/10
13/12/63	1/10	0/10	1/10
14/12/63	0/10	0/10	0/10
15/12/63	1/10	0/10	0/10
16/12/63	0/10	0/10	0/10
17/12/63	0/10	0/10	0/10
18/12/63	0/10	0/10	0/10
19/12/63	0/10	0/10	0/10
26/12/63	0/10	0/10	0/10

* Sample procured immediately before 25% broth culture mixture was offered for 24 hours.

TABLE 23.

Date	No. of A8(1) colonies/No. of colonies examined	
	Chick 15	Chick 16
26/4/65*	0/0	0/0
27/4/65	5/5	5/5
29/4/65	0/5	0/5
1/5/65	0/5	0/5
5/5/65	0/5	0/5
7/5/65	0/5	0/5
10/5/65	0/5	0/5
13/5/65	Drowned in water dish	0/5
17/5/65		0/5
24/5/65		0/5
31/5/65		0/5

* Sample procured immediately before broth culture mixture was offered for 24 hours.

TABLE 24.

Date	No. of A8(1) colonies/No. of colonies examined	
	Hamster 8	Hamster 10
8/10/65	0/10	0/5
12/10/65**	0/10	0/10
13/10/65*		
14/10/65*		
15/10/65*	0/10	0/5
16/10/65*		
19/10/65	0/10	1/10
26/10/65	0/10	0/10
2/11/65	0/10	0/10
9/11/65	0/10	0/10
16/11/65	0/10	0/10
23/11/65**	0/10	0/10
24/11/65*		
25/11/65*		
26/11/65*	10/10	5/10
27/11/65*		
30/11/65	8/10	4/10
7/12/65	0/10	0/10
14/12/65	0/10	0/10
21/12/65	0/10	0/10
28/12/65	0/10	0/10

** Sample procured immediately before broth culture mixture was offered.

* Administration of culture continued.

EXPERIMENT No. 7.

Introduction.

The results of Experiment No. 1 revealed that a weakly colicinogenetic former resident strain of E. coli of avian origin was able to implant itself readily in chickens. Similarly, a non-antibiotic strain of E. coli isolated from a hamster established itself in other animals of that type (v. Experiment No. 5) and the outcome of Experiment No. 3, involving a more actively antibiotic avian strain of transient occurrence, indicated that antibiosis is not an important factor in the establishment of a strain in the intestine. In order further to test those findings, another strongly colicinogenetic culture was administered to fowls. The micro-organism was recoverable from only the first of a series of faecal samples from a dog and, accordingly, it was presumed to be of transient type.

Materials.

Strain No. A7(1) was dispensed to (a) a pair of adult Millefleur bantams, the male of which was identified as Chicken No. 1 and the female as Chicken No. 2, and (b) three day-old chicks (Chicks Nos. 4, 5 and 6). One of the latter, No. 4, belonged to the White Leghorn

variety and the other two were Millefleur bantams.

Results.

(a) (v. Table 25, p. 158).

During the forty-two days following the first exposure to culture, ten faecal samples from Chicken No. 1 failed to yield the serogroup. Twenty-four hours after the second exposure, a faecal sample yielded two colonies belonging to Group No. A7(1) out of the ten that were examined but none of forty-four isolates investigated during the next twelve days were of similar relationship. Three days later, the bird was found to be paralyzed and, thus, was sacrificed by dislocation of the cervical vertebrae. The exact nature of the affection was not determined by autopsical examination and was assumed to have been neuro-lymphomatosis. Of nine colonies subsequently isolated from the small intestine, two were found to belong to Group No. A7(1).

Three A7(1) colonies were among the ten that were isolated from Chicken No. 2 twenty-four hours after first exposure to culture but none of the nine samples examined during the following forty-one days proved positive. Three colonies of A7(1) type were, again, identified among ten obtained from the faecal sample secured from the bird

twenty-four hours after a second exposure. None of forty colonies examined during the succeeding seven days proved to belong to the same group but, on the twelfth day, one colony out of ten was so identifiable. None of thirty-four colonies encountered during the subsequent twenty-three days agglutinated with A7(1) antiserum.

(b)

Chick No. 5 was found to excrete the A7(1) organism for, at least, twenty-seven days and Chick No. 6 to do so for not less than twenty-eight days (v. Table 26, p. 159).

After Strain No. A7(1) had been fed to Chick No. 4, the organism was recovered from the faecal sample taken twenty-four hours later and thereafter continued to be excreted for ninety-five days (v. Table 30, p. 187).

Discussion.

In respect of the significance of colicinogenesis on establishment of E. coli in the intestine, the above experiment failed to yield any definite findings. Although adult chickens were shown to harbour the colicinogenic A7(1) type for the relatively long periods of twelve and seventeen days, there was not any evidence to indicate that the organism had successfully established itself in

those birds.

As in the case of most of the other experiments, chicks were more easily infected than were adult birds but, although two of the former continued to excrete the strain for approximately one month, that period may not be assumed to be of unusual duration, particularly in view of the previous findings. In a third chick, however, stable implantation was achieved for more than three months but its assignment to colicinogenesis must remain indeterminate.

The results were of note inasmuch as they provided another instance of transference of E. coli from one species to another and indicated that, for cross-infection to occur, a 'strong' relationship with the former host is not essential.

Summary.

Attempts to implant an actively colicinogenic strain of E. coli of canine origin in fowls were attended by inconclusive results. The micro-organism survived for relatively long periods in the gut of two adult chickens and maintained a prolonged and stable association with one out of three chicks but that finding was not attributable to colicinogenesis per se.

TABLE 25.

Date	No. of A7(1) colonies/No. of colonies examined	
	Chicken 1	Chicken 2
Pre-infection		
29/ 9/64	0/10	0/10
30/ 9/64	0/10	0/10
1/10/64	0/10	0/10
6/10/64	0/5	0/10
	Allowed to drink 25% broth culture of Strain No. A7(1) ad lib. for 24 hours.	
7/10/64	0/10	3/10
8/10/64	0/10	0/10
9/10/64	0/10	0/10
10/10/64	0/10	0/10
12/10/64	0/10	0/10
14/10/64	0/10	0/10
19/10/64	0/10	0/10
26/10/64	0/10	0/10
16/11/64	0/10	0/10
17/11/64	0/10	0/10
	Allowed to drink 25% broth culture of Strain No. A7(1) ad lib. for 24 hours.	
18/11/64	2/10	3/10
19/11/64	0/10	0/10
20/11/64	0/10	0/10
23/11/64	0/10	0/10
25/11/64	0/4	0/10
30/11/64	0/10	1/10
5/12/64	Sacrificed (2/9)	0/10
11/12/64		0/10
14/12/64		0/6
23/12/64		0/8

TABLE 26.

Date	No. of A7(1) colonies/No. of colonies examined	
	Chick 5	Chick 6
7/4/64*	0/0	0/0
8/4/64	0/5	1/5
9/4/64	5/5	5/5
10/4/64	4/5	4/5
12/4/64	5/5	5/5
13/4/64	5/5	5/5
14/4/64	5/5	5/5
15/4/64	5/5	5/5
16/4/64	5/5	5/5
18/4/64	5/5	5/5
19/4/64	5/5	-
22/4/64	5/5	5/5
26/4/64	5/5	4/5
1/5/64	5/5	5/5
3/5/64	0/5	1/5
4/5/64	1/5	0/5
5/5/64	0/5	1/5
7/5/64	-	0/5
8/5/64	0/5	0/5
9/5/64	0/5	0/5
11/5/64	0/5	0/5
12/5/64	0/5	0/5
13/5/64	0/5	0/5
14/5/64	0/5	0/5
15/5/64	0/5	0/5
18/5/64	0/5	0/5
1/6/64	0/10	0/10

* Sample procured immediately before broth culture mixture was offered for 24 hours.

\- Absence of growth by coliform bacteria.

DISCUSSION ON RESULTS OF SECTION II.

The aims of the investigation particularly concerned the relative ability of strains of E. coli to persist in the intestine as well as the contingency of a host-specific relationship. If they exist, such factors must be related to the resistance of the micro-organism to all of the antagonisms normally prevalent in the bowel. It was necessary, therefore, to carry out the tests under conditions as nearly as possible to natural.

Practicable methods of examination of the E. coli flora of the gut involve a study of a few isolates from a vast, heterogeneous population. Thus, failure to detect the presence of a serogroup in faecal samples obtained throughout a period of time is not proof of the loss of the micro-organism from the alimentary tract. Prolonged absence on the part of E. coli serogroups has been observed to occur naturally. In one instance, in the course of the work under report, a serogroup was isolated post-mortem from the small intestine of a bird seventeen days after the bacterium had last been recoverable from faecal samples procured from the creature. Nevertheless, the results are deemed significant in respect of the relative ability of serogroups of E. coli to persist in the bowel.

It was rendered apparent that resistance to E. coli is offered by the bowel of the domestic chicken and of the Syrian hamster just as in the case of creatures which have been investigated by other workers since, in a number of instances, administration of E. coli was attended either by failure to recover the strain from faecal samples or by isolation that was achievable for only a few days.

Certain findings have revealed that some strains are more able than others to persist in the intestine of animals. Thus, a former avian resident was established by the author with complete constancy in both day-old and adult birds but attempts to implant two transient types of similar source failed. Again, transference of a resident serogroup from one hamster to other hamsters was quite easily achieved. The results showed that the incidence of a serogroup in a host is not a fortuitous occurrence but is due to a property, peculiar to the organism, which Gage et al. (1961) designated 'strength'.

The latter, however, must be regarded a relative attribute in view of (a) the unsuccessful attempts by Sears et al. (1950) to re-establish in a human subject a former resident, (b) inability on the part of the author to achieve complete success in experiments involving Strain No. H11(1) and hamsters and (c) the failure of Strain B1

to resume its tenure in Chicken 'G' after a course of antibiotic treatment (v. Part I). Various workers have tried in vain to establish strains of E. coli in the gut of other members of the same species of host but, with the exception of Sears et al. (1950) and of Sears and Brownlee (1952), they did not provide any information about the previous role of the organism. The use of formerly transient strains might well account for the high rate of failure. Beyond doubt, the findings from the work under report emphasize that the information available about a strain must be as complete as possible ere a bacterium be put to experimental use.

A process of adaptation resembling that described by Ozawa and Freter (1964) was noted on several occasions on which E. coli cultures were administered to adult creatures. Test strains became habituated to the new host with a degree of success which was reflected in the relative numbers encountered in faecal samples. In the case of a former avian resident, adaptation to the gut of two other chickens was achieved over a period of six days and a former transient strain from a chicken was similarly effective in another bird within three to four days. An adaptive process, too, appeared to attend establishment of E. coli in hamsters but the intervals between examinations

of faecal samples were longer than in the case of the birds so that the sequence was less manifest.

There was obtained evidence to reveal that strains of E. coli may have a predilection for a particular host but that cross-infection between different species of animals is also possible. A host-specific tendency was most apparent when organisms of known origin were dispensed to adult creatures in which there was already a normal enteric population.

The reasons for host-specificity on the part of E. coli may be found to relate to the degree of resistance offered by a strain to the sum of the antagonisms encountered in the alimentary tract of a particular species of animal. Excepting the production of anti-bacterial substances in the stomach of suckling rabbits (Smith, 1966), direct action on the part of the animal host has not yet been proven (Dixon, 1960). However, other members of the enteric flora (possibly, including Bacteroides) appear to be intimately concerned with control of the number of E. coli in the bowel (Schaedler et al., 1965b) and may exercise that effect by means similar to those inhibitory of Salmonella (Miller and Bohnhoff, 1963; Meynell, 1963; Bohnhoff et al., 1964).

Since the microbial population of the gut is remarkably constant for a given kind of animal (Smith and Crabb, 1961), not improbably the quality of antagonism offered by members of a species of host to a strain of colonizing E. coli will reflect that stability. In addition, certain strains of E. coli may find selective advantages to obtain in the anatomical, physiological or dietary peculiarities of a particular species of host. Prior adaptation to such an environment ought, therefore, to favour establishment of a strain in the gut of another animal of the same species. Successful settlement, however, need not be automatic since the invader must also overcome and displace the resident E. coli population. Host-specificity on the part of E. coli may account for the high rate of failure of attempts by some workers to implant strains of human origin into animals (Sears et al., 1956; Friedman and Halbert, 1960; Rauss and Ketyi, 1960; Mushin and Dubos, 1965).

A notable feature of the investigation was the relatively long period during which chicks, compared with adult birds, continued to excrete the organisms under test. With few exceptions, in the case of chicks dosed within a day of hatching, strains were carried for fourteen days, but were usually lost by the age of twenty-eight

days. In that respect, the results were similar to those obtained by Friedman and Halbert (1960) and by Mushin and Dubos (1965) from like experiments on day-old mice.

In the case of the newly-hatched chick, the initial multiplication of E. coli in the gut was probably facilitated by the absence of an antagonistic microflora and the failure of the micro-organism to maintain a prolonged association with the juvenile host may be attributable to subsequent establishment in the intestine of other microbic species, in the presence of which latter the strain under test was unable to survive. The nature of the developing antagonism is not yet clear but, although there is some evidence to the contrary (Ketyi, 1964), anaerobic bacteria belonging to the Bacteroides group have been incriminated. Development of the enteric flora has been shown to follow a definite sequence (Riley et al., 1956; Smith, 1961; Schaedler et al., 1965a; Huhtanen and Pensack, 1965). Although Smith encountered Bacteroides in the faeces of chicks from the age of two days onwards, Huhtanen and Pensack found that the organism did not become established until between fourteen and twenty-eight days of life. The latter range coincides with the period during which most of the chicks in the investigation under report ceased to excrete the test

culture.

However, the importance of mutual antagonism on the part of E. coli must not be under-estimated. Thus, in one instance, loss of a test strain of canine origin within forty-eight hours after administration to chicks and replacement of the micro-organism by the strong B1 type suggested direct competition between those serogroups for tenancy of the bowel.

It appears probable that, once established in the gut of the chick, a strain of E. coli of any source has an advantage over other foreign types acquired during the first few days. Subsequent development of an inhibitory microflora is followed either by adaptation of the resident to the new hostile environment or by its disappearance and replacement by more versatile E. coli. In that context, strains of avian origin singularly tended to duplicate in chicks the role which they occupied in their former host. Results from this study revealed that, at least in the case of the domestic chicken, the future resident intestinal E. coli population may be determined within the first few hours of free existence.

SUMMARY.

The findings from the work under report revealed

that, in order to establish itself in the bowel of domestic chickens and hamsters, E. coli must first overcome resistance offered to it. Evidence was obtained to support the theories of 'strength' and host-specificity on the part of the organism. It was observed that, prior to implantation in the intestine of chickens and hamsters, E. coli undergoes a process of adaptation to the new environment but a successful outcome was not necessarily attended by prolonged residency therein. Despite a pronounced host-specific tendency on the part of the organism, transference of E. coli from one species of animal to another was achieved in four instances. In only one of those cases, in which a strain of canine origin had been administered to a chick, did the serogroup appear in faecal samples with the constancy shown by the resident B1 strain in chickens (v. Part I). The results of administration to animals of strains of E. coli, whether colicinogenetic or non-colicinogenic, failed to reveal a significant role on the part of antibiosis in respect of establishment of the organism within the intestine.

SECTION III.

A STUDY OF THE ASSOCIATION BETWEEN COLICINOGENESIS *in vitro* AND THE COMPOSITION OF THE *Escherichia coli* FLORA OF THE GUT OF ANIMALS.

HISTORICAL.

Nissle (1916) first reported that some strains of coliform bacteria exerted an inhibitory effect on other micro-organisms when grown together in liquid media. Gratia (1925) encountered a strain of *E. coli* which, on solid medium, produced a diffusible substance antibiotic to another micro-organism of the same species. Gratia and Fredericq (1946) demonstrated that among coliform bacilli, antibiosis is not infrequent and gave the name 'colicin' to the antibiotic substance which also proved peculiar to the strain that produced it. Some bacteria were found to provide several colicins with characteristically different spectra and antibiotic-producing cultures were observed sometimes to be susceptible to colicins elaborated by other strains of *E. coli*. Gratia and Fredericq, too, considered that such antibiotic activity may be a significant factor in the organization of the

E. coli flora of the normal bowel.

Whether, or not, colicin is forthcoming in the gut, however, is still unknown. Although Heatley and Florey (1946) were able to obtain colicin from aerated broth cultures, Halbert and Magnuson (1948) noted either complete failure of antibiotic production or the evolution of that material to very low titre after an active strain of *E. coli* had been grown in deep broth with, or without, aeration as well as under anaerobiosis. A similar result was obtained when the organism was cultivated in a thin layer of broth in an aerobic environment and high titres were obtained only when the bacterium was grown on solid medium. Hentges and Freter (1962) suggested that colicin-like substances may not be evolved in the gut under the strongly reducing conditions normally prevalent there.

The discrepant findings reported by various workers, such as Sears *et al.* (1950), Emslie-Smith (1961) and Branche *et al.* (1963) who endeavoured to relate colicin-production by *E. coli* to residency in the human intestine, prompted the author of this thesis to carry out a series of examinations of cultures *in vitro* designed to investigate the importance of such antibiotic activity in the composition of the *E. coli* of the animal gut.

METHODS.

The method preferred to test a strain for colicin production was a modification of that described by Fredericq (1957). The medium consisted of ' Oxoid ' broth solidified with 2 per cent. ' Difco ' agar and, for use, was poured into glass Petri dishes of 9 cm. diameter, in approximately fifteen millilitre amounts. After the medium had set, the plates were dried off in the incubator at 37°C. By means of a wax pencil, the base of the dish was marked into eight sections. One prospective colicinogenic strain was inoculated with a straight needle at one point on the surface of the agar within each section. Eight strains per plate were thus tested for colicinogenesis. The seeded plates were incubated at 37°C. for forty-eight hours when the resultant colonies were sterilized by exposure to chloroform vapour for one hour in a closed metal canister. 0.1 ml of a broth culture of the indicator strain, grown at 37°C. for eighteen hours, was mixed with five millilitres of molten nutrient agar at approximately 45°C., and quickly layered over the surface of each of the sterilized plates. After a further twenty-four hours in the incubator at 37°C., the preparations were examined for evidence of inhibition of growth of the indicator strain around the colonies.

The width of any zone of inhibition was measured, in millimetres by means of a transparent scale held firmly against the base of the dish. When the degree of inhibition was too slight to be so estimated, it was recorded as a 'trace'. Sometimes, the zone was found to contain colonies of the indicator strain, in which case the result was registered as 'partial'.

The method described above served to test strains of E. coli for colicin production towards selected indicator cultures. The same technique was used to investigate the colicin sensitivity of a micro-organism but, in that instance, the latter microbe was employed in place of the indicator.

Before the commencement of the investigation, irregular demonstration of colicinogenesis was found to be associated with the use of an old stock of dehydrated nutrient agar base which, when reconstituted, proved to have a low pH. Thereafter, the medium described supra was substituted with completely satisfactory results but a preparation containing a known colicinogenetic strain, No. A7(1), and a sensitive indicator, E. coli Strain No. 206, was included in every test in order to confirm the suitability of the medium.

INVESTIGATIONS AND RESULTS.

The work under report consisted of (a) an examination of both the colicinic activity and the sensitivity of twenty-eight serologically different strains of E. coli of both resident and transient type obtained from chickens, dogs and a hamster, (b) an investigation into the colicinic antagonism prevailing between the resident B1 strain in Chickens 'B' and 'W' (v. Part I) and other cultures of E. coli isolated from the intestine of those birds, (c) an inquiry into the significance of colicin production not only in the persistence of the A7(1) strain but also in the replacement of the latter by other serogroups within the gut of Chick No. 4 (v. p. and, finally, (d) a study of colicin production by successive isolates of the A7(1)-type during the residency of the latter in the bowel of Chick No. 4.

EXPERIMENT No. 1.

Introduction.

Sears *et al.* (1950) did not find that there was any correlation to obtain between the range of antagonistic activity of *E. coli* strains and the duration of their residency in the bowel of man. Emslie-Smith (1961) reported that all of three resident strains isolated from one individual were colicinogenetic and Branche *et al.* (1963) observed that resident types in man are more constantly antibiotic than are transients. The experiment under report was designed to investigate the relationship between colicinogenesis and the length of tenure of strains isolated from animals.

Materials.

A total of twenty-eight serologically different *E. coli* cultures was tested for reciprocal colicinogenesis. Fifteen strains, Nos. A7(3), A8(1), A9(3), A7(1), O10(2), D8(2), J8(4), D11(1), 11 Jejunum (4), D7(1), N8(4), N11(1), K13(1), O8(1) and O11(3), were isolated from a group of dogs during a period of approximately two-and-a-half months. Eight strains, Nos. B1, B31, B51, B233, G33, G62, G107 and G143, were recovered from Chickens 'B' and 'W' and four others, Nos. 20, 29, 42 and 162, were obtained from Chick No. 4. One, H11(1), was procured

from Hamster No. 11 (v. Part I).

On the basis of residencies lasting twenty-eight days, or less, in the original host, the strains were divided arbitrarily into 'residents' and 'transients', respectively. The former group consisted of thirteen strains, Nos. A8(1), 11 Jejunum (4), K13(1), B1, B31, B233, G33, G62, 20, 29, 42, 162 and H11(1), and the latter consisted of fifteen strains, Nos. A7(3), A9(3), A7(1), O10(2), D8(2), J8(4), D11(1), D7(1), N8(4), N11(1), O8(1), O11(3), B51, G107 and G143.

Results.

Nine (32.1%) of the twenty-eight strains were found to exhibit colicinogenetic activity. They comprised four (30.7%) of the residents and five (33.3%) of the transients. The other nineteen cultures embracing nine residents and ten transients were not revealed to be antibiotic (v. Table 27, p. 178).

Table 28 (p. 179) presents the number of cultures antagonized by individual resident and transient strains, the aggregate of antagonistic reactions for both groups and the average number of reactions per strain attributable to colicinogenesis. The latter figure was calculated at eleven for residents and fourteen for transients.

Table 29 (p. 180) lists the resident and transient strains according to the number of colicinogenetic cultures to which they were susceptible. Resident strains varied in reactivity from one which was resistant to all nine colicins to one which yielded to eight and the aggregate of sensitive reactions for the group of thirteen cultures was fifty-four. The average number of sensitive reactions per resident strain came to 4.1. In the case of transient strains, susceptibility varied from complete resistance to total sensitivity and the aggregate of positive reactions was sixty-four for fifteen cultures with an average of 4.2 for each.

Discussion.

Approximately thirty-two per cent. of strains of E. coli from animals proved to be mutually antagonistic in vitro. Among human beings, the incidence of antibiotic strains has been estimated by Levine and Tanimoto (1954) as 19% of cultures recovered from samples of water and of faeces and by Halbert (1948) as 18% among cultures procured from the intestine. Since the methods used by those investigators as well as by the author of the work under report differed, the results are not strictly comparable. However, it is evident that, in animals as in man, colicinogenic strains frequently occur.

A difference was not revealed between resident and transient forms of E. coli in respect of colicin production. Thus, the percentage of colicinogenetic strains was not dissimilar in the case of both resident and transient groups and the average number of cultures, against which types belonging to the latter groups were found to be active, did not differ greatly.

Cultures of both groups exhibited marked difference in sensitivity to colicin and resistance was not observed to be peculiar to resident types. The degree of sensitivity of resident and transient E. coli, measured in terms of the average number of colicins to which members of the groups were susceptible, was almost identical.

A small number of cultures was submitted to investigation in vitro with results which do not provide a safe estimate of the importance of colicinogenesis in the gut of animals but which clearly suggest that the property does not significantly aid the establishment of E. coli. To that extent, the findings are in complete agreement with the description given by Sears et al. (1950) in respect of an examination of E. coli isolated from human beings.

Summary.

An investigation of thirteen resident and fifteen transient strains of E. coli of animal origin revealed the same proportion of colicinogenetic organisms to obtain in both groups. The average degree of antagonism by resident bacteria did not differ greatly from that exerted by transients and resistance to colicin was not peculiar to either type.

TABLE 27.

Source	Colicinogenetic strains		Non-colicinogenetic strains	
	Resident	Transient	Resident	Transient
Chicken	G33	G107	B1	G143
	G62	B51	B31	
	29		B233	
	162		20	
			42	
Dog		A7(1)	A8(1)	A7(3)
		J8(4)	11 Jejunum (4)	A9(3)
		N8(4)	K13(1)	010(2)
				D8(2)
				D11(1)
				D7(1)
				N11(1)
				08(1)
				011(3)
Hamster			H11(1)	
Aggregate	9		19	
%	44.4	55.5	47.3	52.6

TABLE 28.

Type of strain	Number	Number of cultures antagonized	Aggregate of antagonistic reactions	Average number of cultures antagonized per colicino-genetic strain
Resident	G33	17	44	$\frac{44}{4} = 11$
	G62	6		
	29	17		
	162	4		
Transient	A7(1)	25	70	$\frac{70}{5} = 14$
	J8(4)	3		
	M8(4)	18		
	G107	16		
	B51			

TABLE 29.

Number of colicins to which strain was sensitive	Type of strain under test: Resident		Transient	
0	G62	(0)*	D8(2) B51	(0)*
1	B1	(1)	A7(1)	(1)
2	A8(1) G33 29	(6)		
3	K13(1)	(3)	J8(4) N8(4) G107	(9)
4	B233	(4)	D11(1)	(4)
5	11 Jejunum (4)	(5)	A7(3) D7(1) O8(1) O11(3)	(20)
6	H11(1)	(6)		
7	20 42 162	(21)	O10(2) N11(1) G143	(21)
8	B31	(8)		
9			A9(3)	(9)
Aggregate of sensitive reactions		54		64
Average number of sensitive reactions per strain.		$\frac{54}{13} = 4.1$		$\frac{64}{15} = 4.2$

* Figure in parenthesis = No. of strains multiplied by No. of colicins to which they were sensitive, i.e., the total of sensitive reactions.

EXPERIMENT No. 2.

Introduction.

Findings from Experiment No. 1 suggested that Strain No. B1 was not colicinogenetic but was relatively resistant to colicins produced by others. The work about to be reported was intended to test those results inasmuch as they affected the residency of Strain No. B1 in the gut of Chickens 'B' and 'W'.

Materials.

During the period of forty-four weeks from 3/8/64 to 7/6/65, in which weekly examinations were made of the E. coli bowel flora of Chicken 'W', only thirteen cultures were obtained which did not belong to the resident group, B1. Those thirteen strains, comprising five belonging to Group No. B31 and eight not serologically identifiable, were arranged into two groups according to whether they were isolated during the first or the second half of the investigation. Each of the groups was then tested for colicinogenesis towards eight cultures of resident type obtained at approximately fortnightly intervals over the same period of twenty-two weeks. The two sets of 'resident'-type cultures were, also, assayed for antagonism towards the respective group of non-B1 strains.

During the interval of forty-seven weeks from 3/8/64 to 19/4/65, from Chicken 'B' were recovered sixty-one *E. coli* cultures which were not agglutinated by B1 antiserum. The cultures included sixteen belonging to Group No. B33, three of Group No. B51, nine of Group No. B233 and two of Group No. G33. The other thirty-one were not agglutinated by any of the antisera employed in the examination. The cultures were separated into three groups according to whether they were isolated during the early, middle or last parts of the investigation and each batch was tested for colicinogenesis towards eight cultures of Group No. B1 procured fortnightly from the bird over the same period of approximately sixteen weeks.

Results.

None of the B1 cultures isolated from Chicken 'W' was found to display any colicinogenesis towards the other strains recovered from the same bird. At the same time, none of the latter was observed to antagonize the resident type.

One of the sixty-one organisms isolated from Chicken 'B' proved to be weakly antagonized by all eight B1 strains against which it was tested. The resident was not so affected by any of the non-B1 strains.

Discussion.

As a result of Experiment No. 1, the type strain of Serogroup No. B1, notable for stable residency in Chickens 'B' and 'W', was adjudged non-antibiotic. In the course of the work under report, Group No. B1 was revealed to produce a colicin *in vitro* but the activity of the latter was slight inasmuch as it was able to antagonize only one out of seventy-four cultures against which it was tested. In that instance, inhibition attributable to it was barely measurable. The performance of the serogroup *in vitro* suggested that colicinic action was unlikely to play a major part in colonization in the avian intestine.

On the other hand, although antibiosis *in vitro* by Strain No. B1 proved negligible towards other *E. coli* isolated from the same host, the findings were insufficient to establish that the residency of the B1 serogroup was not influenced by colicinogenesis *in vivo*. Sensitive serogroups may have been destroyed by Type B1 colicin in the gut to leave only resistant forms, or an encounter by a sensitive strain with colicin in the intestine may have led to emergence of resistant variants in the faeces in a manner similar to that demonstrated *in vitro* by Fredericq (1948).

In the one case in which they antagonized a strain, all eight Type B1 cultures acted uniformly. Since the latter had been obtained at intervals over a period of three months, the antibiotic activity of the resident serogroup appears to have remained constant during that time. The reactivity of the B1 serogroup, too, did not vary inasmuch as none of eight cultures procured fortnightly during a period of three months was affected by colicin elaborated by three strains of the proven colicinogenetic type, B51. In the latter instance, resistance of the B1 serogroup was unlikely to have been developed in the gut since the cultures under test included strains collected at five, three and one weeks before the first isolation from faeces of the B51 serogroup.

Summary.

The colicinogenetic ability of Group No. B1 of E. coli was established but that property did not appear to be a significant factor in the residency of the serogroup in either of two birds.

EXPERIMENT No. 3.

Introduction.

Investigation of the Group No. B1 strain, resident in Chickens 'B' and 'W', revealed that it was colicinogenetic but the inquiry failed to demonstrate any connection between antibiosis and residency in the birds. The prolonged tenure of an artificially established colicinogenic strain, No. A7(1), in a chicken offered another opportunity to assess the importance of colicin-production.

Materials and methods.

Over a period of approximately five-and-a-half months embracing the residency of E. coli A7(1) in Chick No. 4 and its natural replacement by other strains, two hundred and seven E. coli isolates inagglutinable by the homologous antiserum were collected. They were numbered from 1 upwards in the order of their selection and antisera were prepared, by the methods already described, to four, namely Nos. 20, 29, 42 and 162. All but twenty-seven of the isolates were identifiable by means of those sera.

Towards determination of antagonism to the former resident, the type cultures of the four serogroups were tested for antibiosis to forty A7(1)-type strains procured

from the bird at intervals of a few days throughout the last eight weeks or so of the tenure of the serogroup.

The same type cultures were examined for colicin-production towards each of the two hundred and seven non-A7(1) isolates to decide whether, or not, the four serogroups were antagonistic to each other and to the group of unidentified strains.

The antagonism offered by the A7(1) serogroup to strains superceding it in the intestine was measured by means of an A7(1)-type strain, No. 15/3(1), which was isolated from the bird two days after the first appearance of the succeeding resident group, No. 29. All of the isolates not agglutinated by A7(1) antiserum were tested for sensitivity to homologous colicin. In order to reveal any change in colicinogenetic activity on the part of the A7(1) serogroup that may have occurred in the course of three months residency in the fowl, the original A7(1) culture was examined simultaneously for antagonism to the same organisms.

Results.

The results of serological examination of E. coli obtained from the intestine of Chick No. 4 during the period of approximately twenty-four weeks from 26/12/63

TABLE 30.

Chick 4.

Week beginning	No. of samples	No. of *E. coli* colonies	No. of colonies belonging to Serogroup No. A7(1)	20	29	42	162	**
23/12/63	4*	30	30					
30/12/63	6	60	60					
6/ 1/64	7	70	70					
13/ 1/64	7	70	70					
20/ 1/64	7	70	70					
27/ 1/64	7	70	70					
3/ 2/64	7	70	70					
10/ 2/64	7	68	68					
17/ 2/64	7	70	70					
24/ 2/64	7	70	67			1		2
2/ 3/64	7	70	65					5
9/ 3/64	7	66	64		1			1
16/ 3/64	7	57	36	12	8			1
23/ 3/64	7	61	10	6	37	3		5
30/ 3/64	3	27	2	2	21			2
6/ 4/64	1	7		1	6			
13/ 4/64	2	18		1	17			
20/ 4/64	2	17			17			
27/ 4/64	2	19			16			3
4/ 5/64	1	9			4		2	3
11/ 5/64	1	10			4		6	
25/ 5/64	1	9			6		2	1
1/ 6/64	1	9			8		1	
8/ 6/64	1	9			4		2	3

* Inclusive of one sample from which coliform bacteria were not obtainable, procured on 26/12/63 immediately before A7(1) broth culture mixture was offered for 24 hours.

** Serologically unidentifiable strains of E. coli.

to 14/6/64 are presented in Table 30 (p. 187). Organisms belonging to Group A7(1) continued in residence for ninety-five days and, for most of that time, appeared to constitute the only E. coli serogroup in the gut. On the seventy-seventh day, a new resident serogroup, No. 29, first appeared in faecal samples and, thereafter, was excreted without interruption for the remainder of the period of observation. A tenure of four weeks was demonstrated for each of two other serogroups, Nos. 20 and 42, and the possible start of residency on the part of another serogroup, No. 162, was also disclosed.

Table 31 (vide infra) records the findings from examination of forty A7(1)-type cultures for sensitivity to colicin produced by the type strains belonging to Serogroups Nos. 20, 29, 42 and 162. No. 29 alone proved to be weakly antibiotic to Group No. A7(1).

TABLE 31.

No. of A7(1) strains	Average width of inhibition around the type strain of serogroup Nos.			
	20	29	42	162
40	nil.	trace	nil.	nil.

Table 32 lists the discoveries relating to investigation of Serogroups Nos. 20, 29, 42 and 162 for antagonism to each other as well as to twenty-seven serologically unidentified cultures obtained from Chick No. 4. Serogroups Nos. 20 and 42 were not found to be antibiotic to any of the cultures tested but proved to be slightly sensitive to colicin produced by the representative strains of Serogroups Nos. 29 and 162. The latter serogroups were each resistant to colicin formed by the other. Out of a total of twenty-seven serologically unidentified cultures of E. coli, Strain No. 29 antagonized nineteen and, of the latter, seven were also affected by colicin elaborated by Strain No. 162.

TABLE 32.

Serogroup	No. of strains tested	Average width of inhibition in mm. around the type strain of serogroup Nos.			
		20	29	42	162
20	21	nil.	2.0	nil.	trace
29	142	nil.	nil.	nil.	nil.
42	4	nil.	2.5	nil.	1.0
162	13	nil.	nil.	nil.	nil.
Unidentified	27	nil.	2.5 (19)*	nil.	0.8 (7)*

* Figure in parenthesis refers to number of susceptible cultures out of 27 tested.

Table 33 summarizes the results of examination of strains belonging to Serogroups Nos. 20, 29, 42 and 162 together with twenty-seven serologically unidentified E. coli cultures for sensitivity to colicin produced by both the original A7(1) strain and the A7(1)-type Strain No. 15/3(1). To colicin produced by the primordial A7(1) strain, Serogroups Nos. 20 and 42 and all but four of the serologically unidentified cultures proved to be highly sensitive whereas Serogroups Nos. 29 and 162 were weakly so. Similar findings attended the examination of Strain No. 15/3(1) yet, in all but the case of Serogroup No. 162, the width of the zone of inhibition was reduced by approximately two-and-a-half to three millimetres. In most instances, inhibition of Serogroup No. 162 was not evident around Strain No. 15/3(1).

TABLE 33.

Number of serogroup	Number of strains	Average width of inhibition in mm. around A7(1) Strains Nos. Original	15/3(1)
20	21	10.7	7.5
29	142	2.8	0.2
42	4	11.0	8.0
162	13	trace	trace
not identified	27	7.8 (25)*	5.1 (23)*

* Number in parenthesis indicates strains susceptible out of twenty-seven tested.

Discussion.

On the part of the strains under test, a very close correlation was observed to exist between colicinogenic ability *in vitro*, or resistance thereto, and residency in the host. The findings suggested that, at the time of its replacement, the experimentally-established colicinogenetic resident had lost much of its antagonism but remained weakly sensitive to colicin produced by its successor, with which a resistant serogroup subsequently co-existed for, at least, five weeks before the end of the investigation. On the other hand, two serological types, which maintained relatively short tenures, were neither colicinogenetic nor resistant to antibiotics produced by the others. Most of a batch of serologically unidentified cultures, which probably contained a high proportion of transient serogroups, were susceptible to colicin produced by both of the resident strains.

Young *et al.* (1966) examined the colicinogenic activity of a series of isolates of *E. coli* from several human individuals over a period of six months and recorded that colicin-production was not constant in organisms of the same serotype obtained at different times from the one subject. In the course of the work under report, slightly different reactivity to colicin was detectable in the

course of examination of successive isolates belonging to a single serogroup. Occasionally, a culture of a slightly sensitive strain proved resistant to colicin while moderately sensitive members were sometimes demonstrated in an otherwise refractory group. Such findings may have been attributable to innate micro-organismal variability but were more probably the effect of minor differences in the conditions of the test.

Of greater note was the reduced colicinogenetic activity of the A7(1)-type strain, No. 15/3(1), which was recovered from the host after a residency by the serogroup of, approximately, three months. In this instance, the diminished activity is to be regarded as significant on account of the constancy with which it was demonstrable. On the other hand, the discovery of one less active culture to Group No. A7(1) need not have indicated general loss of antagonism on the part of the serogroup in the gut but may have been the result of the isolation of a rare mutant.

Summary.

Examination of a series of isolates of E. coli during the residency of two serogroups in the intestine of a chicken revealed a close correlation to exist between antibiosis in vitro and length of tenure of the strain.

The replacement of one resident type by another may have been associated with loss of antagonism by the former.

EXPERIMENT No. 4.

Introduction.

The findings of the previous experiment served to show that loss of colicinogenicity by Strain No. A7(1) occurred during its three-months of residency in the intestine of a chicken. On the other hand, since colicinogenetic cultures may give rise to non-antibiotic mutants (Fredericq, 1957), the possibility remained that the result may have been due to accidental selection of a relatively rare variant. The studies about to be reported were intended to reveal which of those issues was the more likely.

Materials.

Forty-four A7(1)-type cultures recovered from Chick No. 4 during the residency of the serogroup were tested for colicinogenesis towards type strains of Groups Nos. 29 and 20, both of which were isolated from the same creature. They comprised twelve collected during the last seven days of January 1964 (the fifth week of residency), fifteen obtained at intervals of a few days throughout February and seventeen isolated similarly during March. The last group included Strain No. 15/3(1) previously employed in Experiment No. 3.

For purposes of comparison, an identical examination was made of the antagonism on the part of the original A7(1) culture.

Results.

The zone of inhibition of Strain No. 29 around the original A7(1) culture extended for 4 mm. but the width around A7(1)-type cultures isolated from the chicken ranged from 5.5 mm. down to nil. There was not any progressive loss of colicinogenetic activity on the part of successive cultures but actively antibiotic organisms predominated in the earlier part of the investigation. The average width of inhibition around the first group of twelve A7(1)-type cultures was 2.3 mm. That around the second set of fifteen was 1.2 mm. and around the third collection of seventeen the width was 0.5 mm.

Strain No. 20 was inhibited for a distance of 10 mm. around the original A7(1) culture and the A7(1)-type test cultures were active within a range of 11 mm. to 6 mm. The average width for each of the three consecutive groups was 8.7 mm., 8.2 mm. and 8.1 mm., respectively.

There was not any obvious correlation observable between the zones of inhibition of Strains Nos. 20 and 29. Marked reduction in the case of the latter was often

associated with little, if any, alteration of the former.

Findings are summarized in Table No. 34.

TABLE 34.

No. of A7(1)-type strains	Month of isolation	Mean width of inhibition in mm. around Strain Nos. 29	20
12	January	2.3	8.7
15	February	1.2	8.2
17	March	0.5	8.1
Original		4.0	10.0

Discussion.

The findings from examination of groups of A7(1)-type cultures isolated from a chicken during three successive months pointed to a continual loss of antagonism on the part of the serogroup and revealed that the results of Experiment No. 3 had not been influenced by the selection of a rare mutant.

The difference observed in loss of antagonism on the part of A7(1) strains towards two test cultures may have been attributable to either (a) shedding of a colicinic fraction to which one of the cultures was particularly susceptible or (b) a reduction in output of

antibiotic substances to which both cultures were prone but manifest to greater degree in the case of the more refractory organism.

Summary.

During three months of residency, an accumulation of less antibiotically active A7(1) organisms occurred in the intestine of a chicken whereby the overall effect was one of progressive loss of antagonism by the serogroup, which phenomenon was particularly evident towards the succeeding resident strain.

DISCUSSION OF RESULTS OF SECTION III.

Fredericq (1948) reported on the "selective production" of resistant mutants *in vitro* from a sensitive strain of *E. coli* in the presence of colicin. Should colicin be present in the bowel, it is not improbable that resistant forms may be evolved as a result of an encounter with the antibiotic *in vivo*. In the course of the investigation under report, examinations of series of cultures isolated over a period of time revealed that antibiotic ability may change but failed to indicate that the sensitivity of a serogroup may alter during residency of the latter in the gut. That finding suggests that antibiotic may not be available in the intestine and agrees with the belief expressed by Hentges and Freter (1962), namely, that colicin is not formed therein.

SUMMARY.

The series of experiments reported in this section revealed a high incidence of colicinogenetic strains of *E. coli* to obtain in the intestine of dogs and domestic chickens. The findings, however, were not conclusive in respect of the importance of antibiosis in intra-intestinal establishment, although they indicated that colicin-production may sometimes enable a strain to persist in the gut.

SECTION IV.

THE INFLUENCE OF OTHER ORGANISMS, MAINLY OF ENTERIC ORIGIN, ON COLICIN PRODUCED BY *Escherichia coli* *in vitro*.

INTRODUCTION.

On four occasions during examination of strains of *E. coli*, the author observed that contaminative micro-organisms, presumably of atmospheric origin, interfered with the demonstration of colicinogenesis on the part of two antibiotic cultures but any description of that phenomenon has not been traceable in the available literature. Micro-organisms of that kind comprised a sarcina, a Gram-positive sporulating bacillus and two moulds, one of which latter belonged to the genus, *Aspergillus*. Photographs of those findings were not obtained but the diagrammatic records kept are reproduced on page 210. Those illustrations indicate that, adjacent to a colicinogenetic colony, growth of the above-mentioned organisms was accompanied by erosion of the region of colicinic activity.

Those results suggested that micro-organisms of intestinal origin may exert a similar effect attributable

to the seeming insignificance of colicinogenesis in establishment of E. coli in the gut. Accordingly, an investigation was carried out (a) to observe in vitro the effect of intestinal, and other bacteria on antibiotic activity on the part of a strain of E. coli and (b) to examine the nature of that effect.

(A) THE EFFECT in vitro OF OTHER BACTERIA ON COLICINOGENESIS BY E. coli STRAIN No. A7(1).

Introduction.

The aims of the experiment under report were, firstly, to ascertain whether bacteria mainly of intestinal origin may interfere with colicinogenesis by E. coli in vitro and, secondly, to determine the variety of organisms so capable.

Materials and methods.

Since the effect on colicinogenesis had been observed during examinations involving colicinogenic Strain No. A7(1) and the sensitive indicator Strain No. 206, the same strains came to be used for this experiment.

For their effect on antibiosis by Strain No. A7(1), bacteria belonging to the following genera were tested:

Staphylococcus (8): Streptococcus (31): Pseudomonas (4): Escherichia (8): Proteus (5): Clostridium (9): Salmonella (6): Corynebacterium (2) and three others of miscellaneous kind. The numerals in parenthesis indicate the number of strains tested. Some had been recently isolated but others were stock cultures obtained either from the National Collection of Type Cultures or in the course of routine diagnostic investigations.

Nutrient agar plates were prepared according to the method already described. A portion of the growth of Strain No. A7(1) was removed from a blood agar plate by means of a flamed wire loop and streaked once diametrically across each of those plates, several of which latter were so inoculated without recharging the loop. One plate was allotted to each test. The loop was re-sterilized, charged with a portion of a colony of the test organism and streaked at right angles across the line of the colicinogenetic strain at a point about two thirds from one end to terminate in an arrow head indicative of the direction of the cross streak. As a result, each plate came to bear (a) a line of pure Strain No. A7(1), (b) a streak of pure test culture continuing as (c) a stroke consisting of a mixture of both inocula (v. Diagram on page 211).

The plates were incubated at 37°C. for forty-eight hours when the resultant growth was sterilized by exposure to chloroform vapour for one hour. Five millilitres of nutrient agar at 50°C., made to contain 0.1 ml. of an eighteen hour nutrient broth culture of the sensitive indicator strain, No. 206, were layered over each of the sterilized plates, which latter were then returned to the incubator for a further eighteen hours. Preparations were then examined for breach of the zone of inhibition at the point where the stroke of the test strain crossed the line of the colicinogenetic culture and for lack of inhibition around the mixed growth of Strain No. A7(1) and the bacterium under test. Inspection of the line of pure test culture served to reveal any antagonism towards the indicator strain likely to interfere with demonstration of its anticolicinic activity.

A control plate, indicative of the suitability of the medium, was included in each group of tests and consisted of one nutrient agar plate, streaked with Strain No. A7(1), incubated and processed in parallel with the rest of the batch.

Results.

Around the A7(1) streak on the control plate was to be seen a broad uninterrupted zone of colicin-induced

inhibition of the sensitive indicator strain, No. 206 (v. Plate I, p. 202).

That zone of inhibition was repeated along the streak of Strain No. A7(1) on the test plates and, when a test organism did not affect colicin activity, inhibition of the indicator strain was continued along the stroke of mixed growth (v. Plate II, p. 213)

When some organisms were found to interfere with colicinic activity, the zone was broken at the junction of the crossed lines. In addition, inhibition of the indicator strain was not apparent around the streak of mixed growth of test culture and colicinogenetic E. coli (v. Plate III, p. 214).

Another form of interference in which only a trifling breach occurred where the lines crossed, without any evidence of colicin activity around the mixed culture, is portrayed in Plate IV (p. 215).

When the test culture was of swarming kind, anticolicin action was more diffuse and sometimes completely cancelled colicin effect along the streak of the colicinogenetic culture (v. Plate V, p. 216).

The results with the various bacterial genera

were as follows:

Staphylococcus.

Altogether, eight strains were examined. Of five identified as Staphylococcus saprophyticus, two had been isolated from the intestinal contents of chickens, two were obtained from the vagina of a bitch and one was an aerial contaminant procured from a blood agar plate. The remaining three were obtained from the National Collection of Type Cultures but their distinctive number was not any longer available and they were designated only as Staph. aureus, Staph. albus and Staph. citreus.

Definite anticolicin effect (v. Plate III) was shown by both of the chicken strains as well as by Staph. citreus. A less positive reaction was produced by Staph. albus but none of the others was found to be so active.

Streptococcus.

Of a total of thirty-one cultures of intestinal origin comprising nineteen from cats, eight from chickens, three from dogs and one from a lamb, five manifested anticolicin activity (v. Plate III). Three of those cultures were of feline origin and two had been procured from chickens. (One of the feline strains liquefied

gelatin but neither of the others was able to do so).

Pseudomonas.

Four cultures were tested. Three were of intestinal origin and were obtained from a sea-lion, a calf and a dog, respectively. The fourth was procured from the gastric contents of an aborted ovine foetus. Marked anticolicin effect (v. Plate III) was shown by the bovine, ovine and canine strains before delayed lysis of the indicator strain ensued due to antibiotic activity on the part of Pseudomonas. Any anticolicinic action on the part of the strain from the sea-lion was obscured by rapid antibiotic activity as denoted by a zone of inhibition around the streak of pure test organism.

Escherichia.

In all, eight strains of E. coli were tested. Three had been isolated from the intestinal contents of chickens and five were obtained from canine faecal samples. All of the avian strains and three canine ones yielded the reaction depicted in Plate IV.

Proteus.

Five cultures were examined. Two isolated from the bowel of dogs and two procured from the gut of chickens, all of which were biochemically identifiable as

Proteus mirabilis, swarmed across the surface of the plate and completely prevented the colicin effect along the length of the streak of Strain No. A7(1), as shown in Plate V. The fifth, a non-motile strain of P. vulgaris N.C.T.C. No. 4175, produced the effect illustrated in Plate III.

Clostridium.

One stock culture of each of nine species was tested. They originated from the National Collection of Type Cultures but their numbers were not any longer available. Clostridium botulinum, Cl. chauvoei, Cl. oedematiens, Cl. septicum, Cl. sporogenes, Cl. sordellii and Cl. welchii type D proved to exert definite anticolicin effect. Only a trace of reaction was given by Cl. haemolyticum and that of Cl. tetani was indefinite. The appearance of the reaction varied according to the swarming ability of the clostridial strain.

Salmonella.

Six stock cultures of N.C.T.C. origin and comprising Salmonella paratyphi A, Sal. paratyphi B, Sal. enteritidis, Sal. typhi, Sal. pullorum and Sal. gallinarum were not found to exert any effect on the colicin activity of Strain No. A7(1).

Corynebacterium.

Two strains of Corynebacterium pyogenes were examined. One was isolated from a bovine lesion and the other was N.C.T.C. No. 5225 of porcine origin. The anticolicin effect of the former was very slight but the latter was associated with a marked reaction, of the type shown in Plate III.

Miscellaneous strains.

An aerobic Gram-positive sporulating bacillus isolated from the caecum of a chicken and a stock culture of Bacillus subtilis both manifested definite anticolicin effect (v. Plate III). A strain of Aerobacter aerogenes procured from the intestine of a dog was characterized by a slight reaction similar to that of the E. coli strains (v. Plate IV)

Discussion.

The findings revealed that, at anyrate in vitro, various micro-organisms were able to reduce the potency of the colicinogenetic strain, E. coli No. A7(1), and the range of bacteria able so to act was shown to be extensive. Of cultures of known, or presumed, intestinal origin, approximately 45% proved antagonistic. Members of the Enterobacteriaceae, comprising strains of Escherichia,

Proteus and Aerobacter, were active in varying degree as were also micro-organisms belonging to the genera Pseudomonas, Clostridium, Staphylococcus and Streptococcus. The list of bacteria examined does not represent all of the bacteria of intestinal habitat and it is not unlikely that others of that source may also interfere with antibiosis by Strain No. A7(1).

Noticeably, both cultures of staphylococci of enteric origin were active, which activity contrasted with the inability of four out of six staphylococci from other, or unknown, sources. Such a finding suggested that the property may be more characteristic of faecal staphylococci, a peculiarity which merits further investigation.

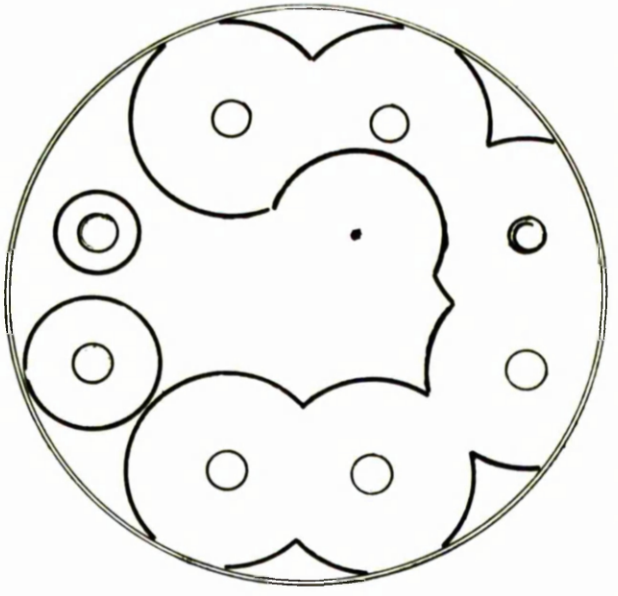
Basically, two manifestations of a positive reaction were observed. In one, strong interference with antibiosis was evident around the line of the A7(1) strain as well as around the mixed culture. That form was the more common and was displayed by most of the genera tested. In the case of the other reaction, antagonism was observed mainly around the streak of mixed growth with little effect on the zone of colicinic activity around the pure A7(1) strain. The latter type of reaction was associated

with strains of Escherichia coli and of Aerobacter aerogenes. The manifest difference may be due merely to variation in the degree of activity on the part of the test strain or to the existence of more than one mechanism.

The results indicated that colicinogenesis on the part of E. coli may be of little advantage to the micro-organism in its efforts to colonize in the gut in the presence of other bacteria commonly present there. In that respect, strains of E. coli notably interfered with antibiosis on the part of a member of the same species.

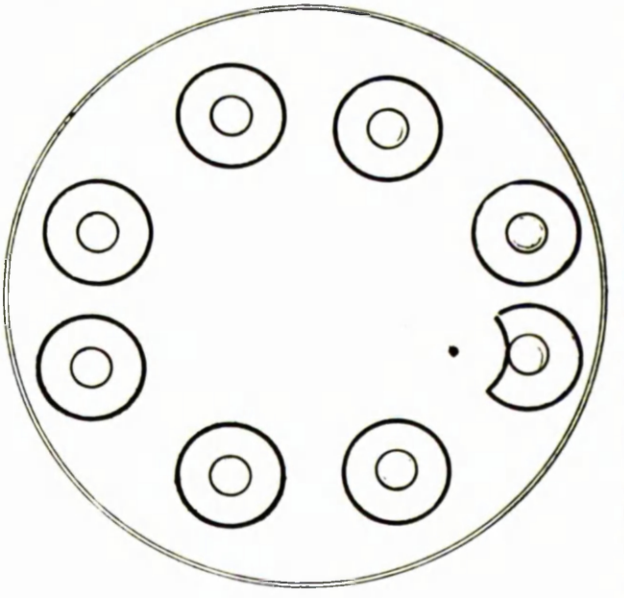
Summary.

Bacteria belonging to seven out of eight genera of intestinal habitat were observed to prevent antibiosis in vitro on the part of a colicinogenetic strain of E. coli. The incidence of cultures of that kind proved to be approximately forty-five per cent. Of the genera tested, only Salmonella constantly failed to interfere with the antibiotic reaction. The findings offer an explanation of the reported insignificance of colicinogenesis in establishment of E. coli in the gut.



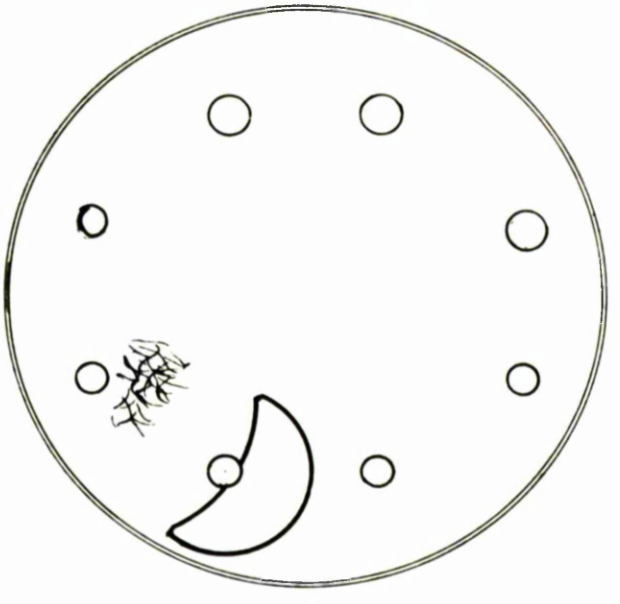
Contaminant: Gram-positive sporulating bacillus.

Colicin: A7(1).



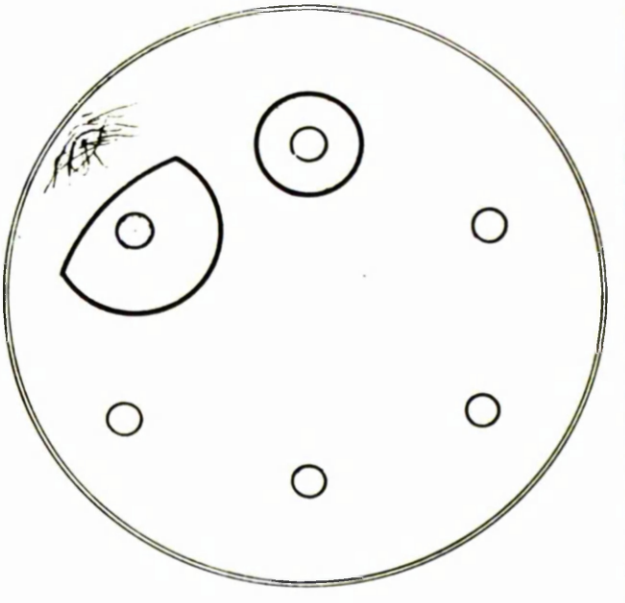
Contaminant: Sarcina.

Colicin: A7(1).



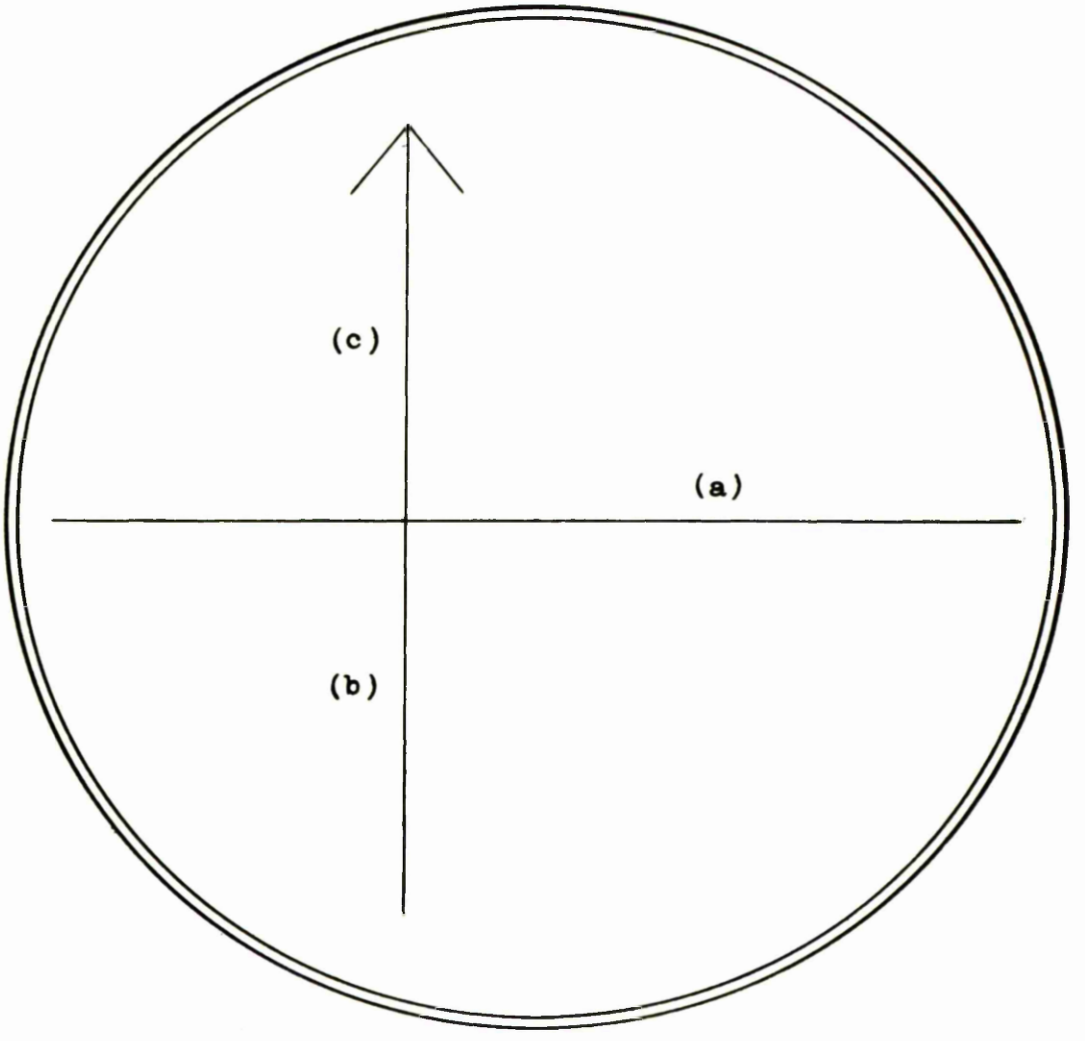
Contaminant: Mould (unidentified).

Colicin: N8(4).



Contaminant: Mould (Aspergillus).

Colicin: A7(1).

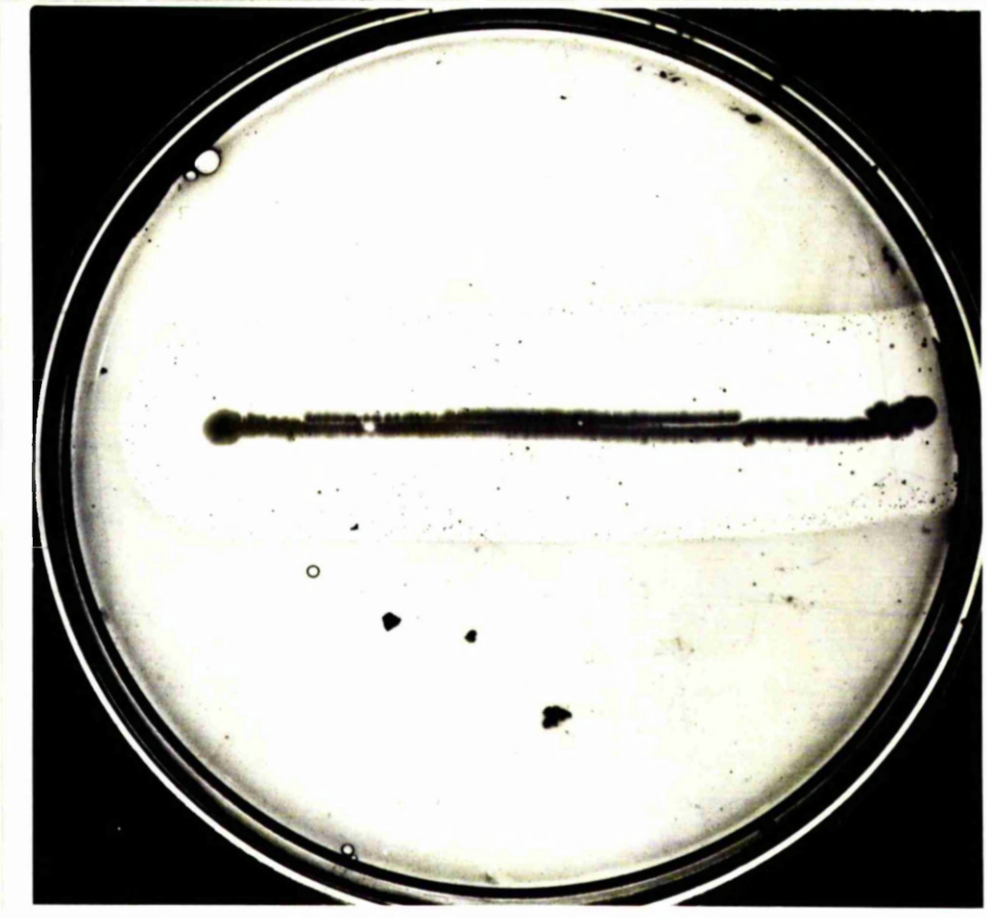


(a) Pure Strain No. A7(1).

(b) Pure test culture.

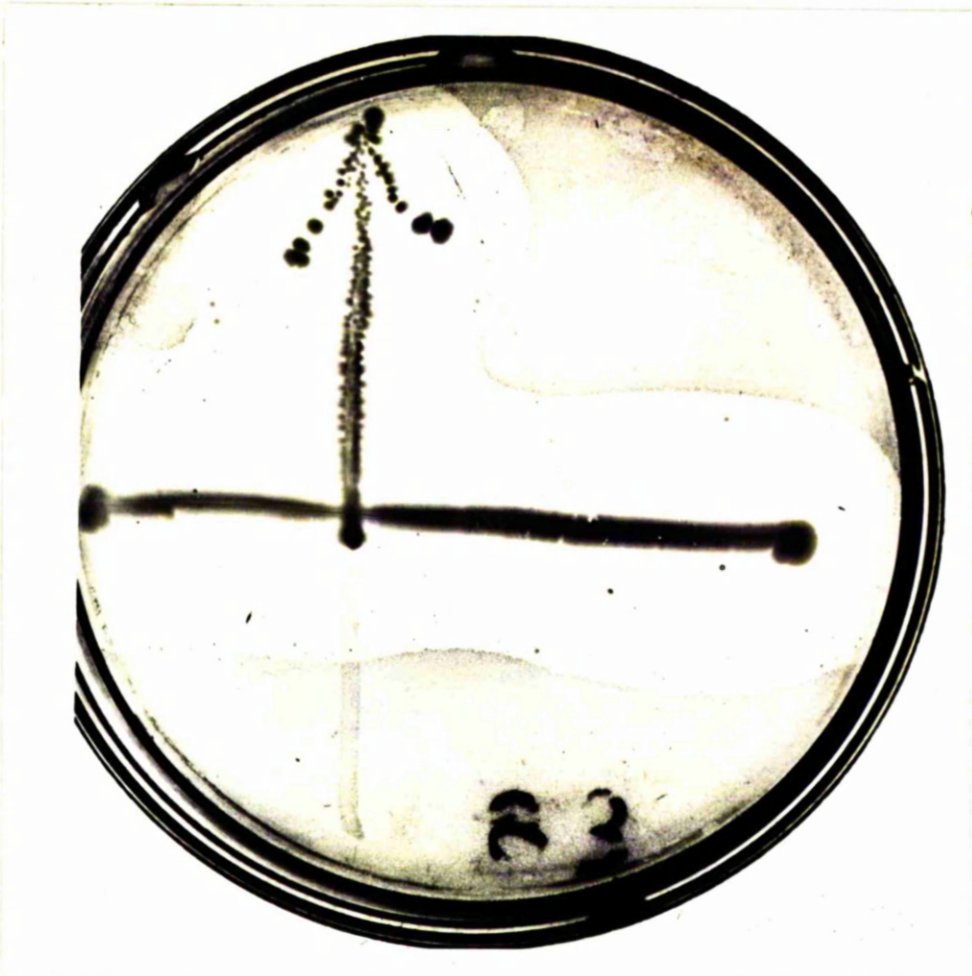
(c) Mixture of Strain No. A7(1) and test culture.

PLATE 1.



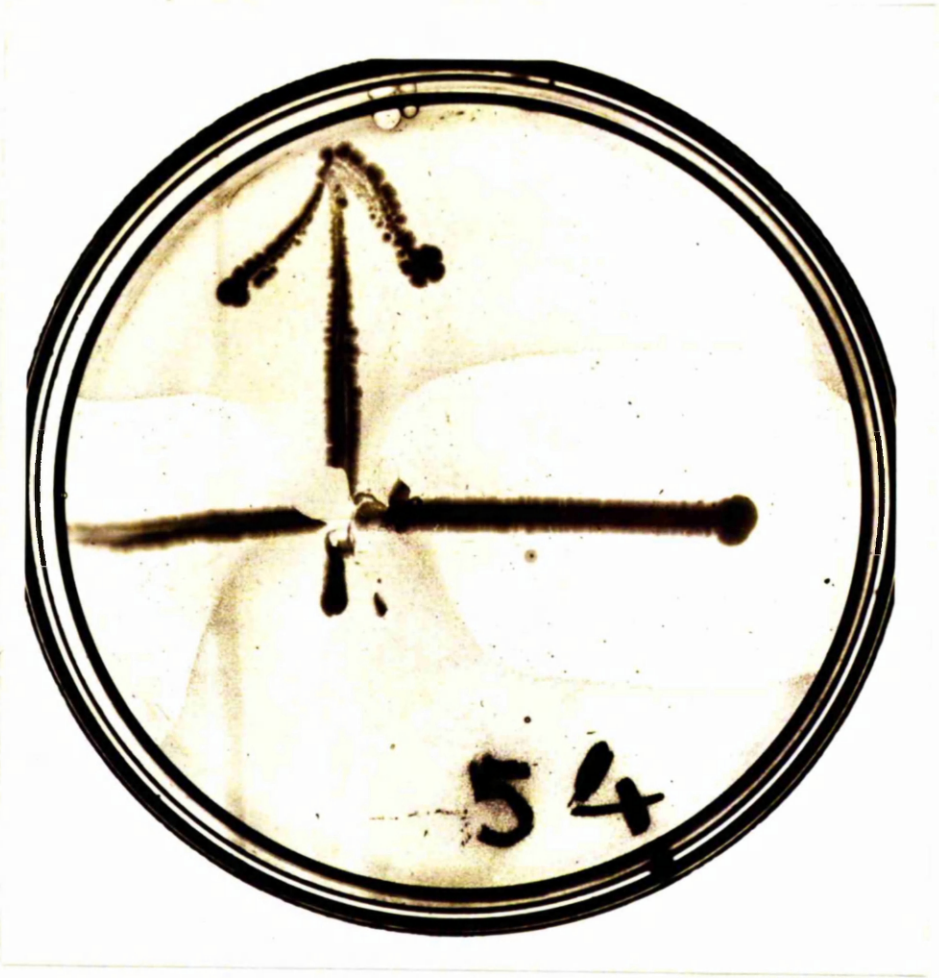
Control plate. The streak of E. coli No. A7(1) is surrounded by a broad uninterrupted zone of colicin-induced inhibition of the sensitive indicator strain, No. 206.

PLATE 2.



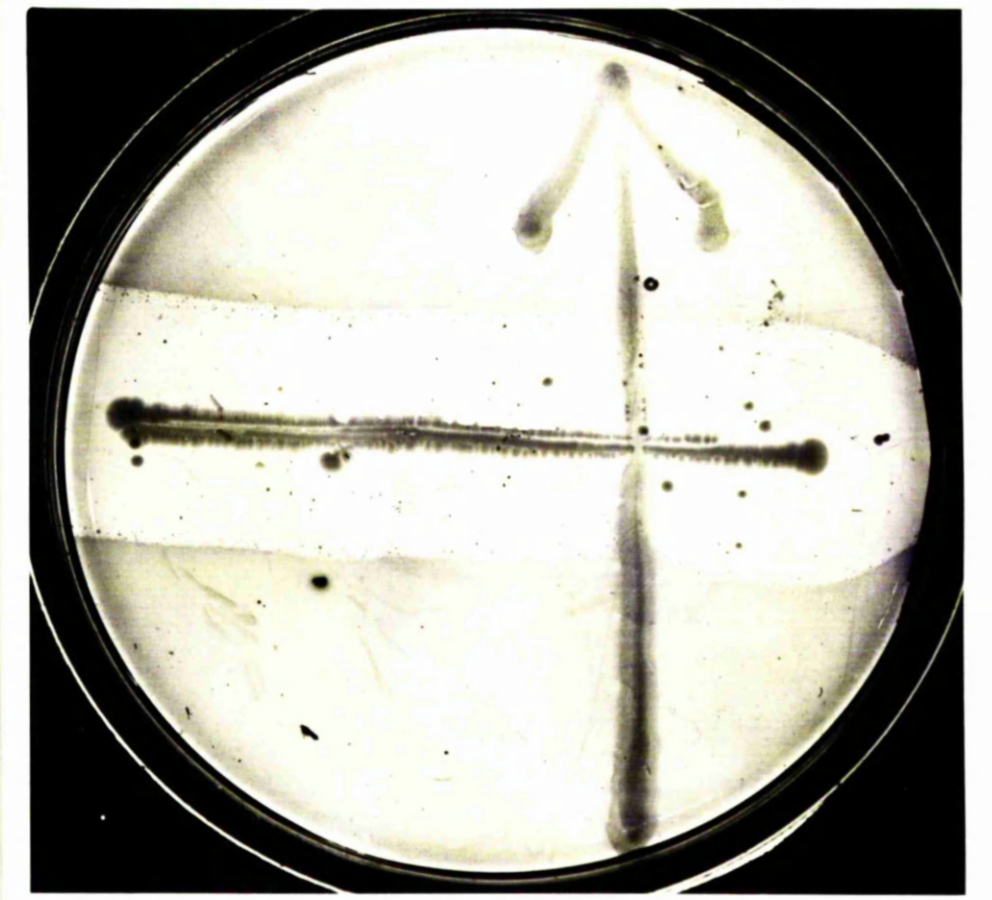
The zone of inhibition of Strain No. 206 is continued along the stroke of mixed growth.

PLATE 3.



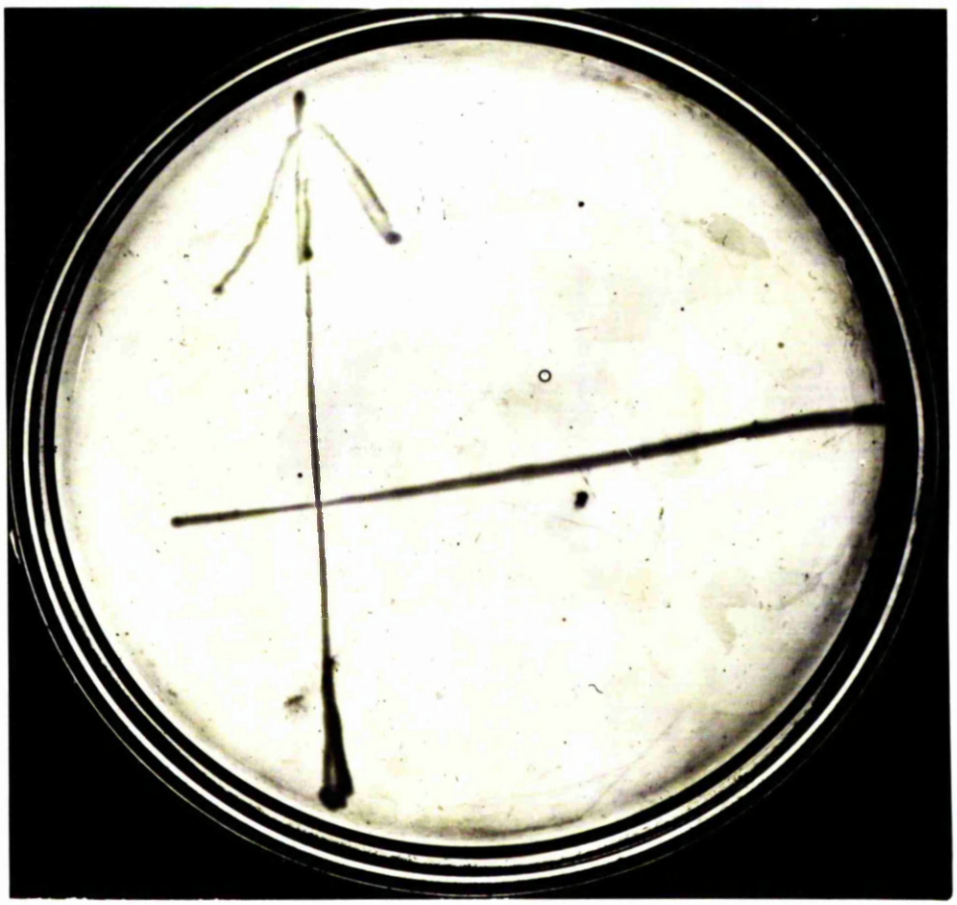
The zone of inhibition of Strain No. 206 is broken at the junction of the crossed lines of Strain No. A7(1) and of the organism under test. Inhibition of the indicator strain is absent around the streak of mixed growth.

PLATE 4.



Slight indentation of the zone of colicinic action is visible in association with the growth of the organism under test. There is not any inhibition of Strain No. 206 around the mixed growth of Strain No. A7(1) and test culture.

PLATE 5.



The colicin effect is obliterated along the length of the streak of Strain No. A7(1).

(B) AN INVESTIGATION INTO THE ACTION OF OTHER BACTERIA ON ANTIBIOSIS *in vitro* BY *E. coli*, STRAIN No. A7(1).

Introduction.

Several are the reports concerning the action of bacteria on colicin *in vitro*. Heatley and Florey (1946) noted that the supernatant fluid from a centrifugalized sample of staphylococcal pus destroyed the activity of a colicin when incubated with it. Colicinic action, however, was not affected by incubation for several hours at 37°C. in the presence of heavy suspensions of various *E. coli* cultures. The latter observation was confirmed by Halbert and Magnuson (1948). Fredericq (1957) remarked that extracts of sensitive, alone, bacteria specifically neutralize colicins by a process of fixation. He also stated, however, that certain strains may bind a given colicin and yet be insensitive to its action. Examination of the available literature has failed to reveal any report on the action of other micro-organisms on colicinogenesis or on colicinic activity.

The findings of the previous experiment revealed that members belonging to a number of different bacterial genera reduced *in vitro* the antibiotic activity of a colicinogenetic strain of *E. coli*. The work about to be

reported was designed to establish if the mechanism so involved was related to prevention of colicin-production or to interference with the antibiotic after its formation.

Materials and methods.

Nutrient agar plates were streaked once diametrically with the colicinogenetic strain of E. coli No. A7(1). After incubation at 37°C. for forty-eight hours to allow of elaboration of antibiotic substances, the plates were sterilized by exposure to chloroform vapour for one hour. Fourteen cultures, mainly of enteric origin and proved by the previous experiment capable of preventing antibiosis by Strain No. A7(1), were streaked, one to each plate, at right angles across the band of growth of the colicinogenetic organism. The cultures tested comprised five of E. coli, two each of Pseudomonas and P. mirabilis, one each of A. aerogenes, a non-haemolytic streptococcus, Staph. saprophyticus and an aerobic Gram-positive sporulating bacillus as well as C. pyogenes. The last-mentioned strain was recovered from an extra-intestinal source.

Plates were returned to the incubator for another forty-eight hours to enable test strains to propagate and to influence any colicin produced during the first stage.

Thereupon, growth was again arrested by exposure to chloroform vapour for one hour. The plates were then layered with the indicator strain, E. coli No. 206, as practised in the preceding experiment and incubated for a further period of eighteen hours before they were inspected for manifestations of anti-colicin activity.

A control plate streaked with Culture No. A7(1) was included to test both the suitability of the medium for colicin production and for the efficacy of sterilization of the A7(1) growth by chloroform vapour. Aside from the fact that, after the first exposure to chloroform, a sterile loop was drawn across the A7(1) streak at right angles to it, the control plate was treated in exactly the same way as were the test ones.

Results.

The control plate showed a wide zone of inhibition of the indicator culture around the streak of the colicinogenic Strain No. A7(1) but there was not any growth or colicinic activity along the line drawn at right angles to it.

A complete break of varying width occurred in the zone of colicinic action, not unlike that depicted in Plate III (v. previous experiment), where it was crossed

by the line of the following six test cultures, namely, the staphylococcus, the streptococcus, the Gram-positive sporulating bacillus, C. pyogenes and both strains of Pseudomonas. The reaction on the part of one strain of Pseudomonas was partly obscured by its strongly antibiotic activity towards the indicator organism.

Both strains of P. mirabilis swarmed across the surface of the plate completely to inhibit the colicinic effect along the entire length of the streak of Strain No. A7(1).

All five strains of E. coli displayed a very slight anticolicin effect as evidenced by slight indentation of the zone of colicin activity around the streak of Strain No. A7(1) at the point where the test strain crossed it. Three of the strains of E. coli were resistant to A7(1) colicin but the other two were shown to be as sensitive to be as sensitive to the antibiotic as was the indicator strain.

Discussion.

All fourteen test cultures were able in varying degree to affect the activity of A7(1) colicin after its formation. The most appreciable activity was displayed by the staphylococcus, the streptococcus, the aerobic

sporulating Gram-positive bacillus, C. pyogenes and the Pseudomonas cultures, around which the zone of anticolicin action extended for up to two centimetres. The relative anticolicinic ability of Proteus strains was not determinable because the swarming growth completely covered the plate with consequent reduction of colicin activity along the entire length of the streak of the colicinogenic organism. The effect of E. coli on the colicin was barely evident.

The disparity of effect produced by other bacteria and that caused by E. coli suggests that different mechanisms may be involved. In the instance of the former, a readily diffusible agency was obviously concerned since the anticolicin reaction was not limited to the immediate vicinity of the test strain, as it was in the case of E. coli.

The nature of the action of those, and other, test organisms on the colicin produced by Strain No. A7(1) remains obscure and the author intends to carry out a more detailed investigation of the phenomenon. None the less, several possibilities merit consideration.

Thus, Fredericq (1964) remarked that the majority of colicins are destroyed more or less rapidly by

proteolytic enzymes and, in some of the cases under report, proteolytic action may have been involved although not all of the bacteria shown to be active against the A7(1) colicin were of proteolytic nature. On the other hand, Halbert and Swick (1950) detected production of colicin by E. coli to high titre in peritoneal exudates of mice and rabbits despite the fact that proteolytic enzymes are present in such material and so concluded that the enzymes did not destroy the antibiotic at an appreciable rate.

The author found one batch of medium to be unsuitable for demonstration of colicin-production when the only detectable abnormality was a pH lower than normal. In some cases, change of pH wrought in the medium by a bacterium under test may have caused some reduction in colicinic activity although Halbert and Magnuson (1948) observed one colicin to exhibit a relatively high resistance to an acid environment, even, at 100°C.

Fredericq (1957), in a review of the properties of colicins, noted that those antibiotics attach themselves to specific receptors on the bacterial cell and that colicin may be fixed by both sensitive and resistant organisms. The latter fact may explain the similarity of

the inhibitory reaction to A7(1) colicin on the part of susceptible as well as refractory strains of E. coli. The limitation of the anticolicinic effect to the immediate vicinity of the culture under test and to the area of lowest antibiotic concentration was consistent with adsorption of colicin on to the test strain or on to its products of autolysis. In the case of organisms other than E. coli or A. aerogenes, the antagonism may involve the elaboration of diffusible substances sufficiently similar to its specific receptors to be able to block the action of the anyibiotic.

Summary.

A variety of bacteria, mainly of enteric origin, were found to interfere in vitro with the activity of colicin after its formation by one strain of E. coli. Possible reasons for that action have been discussed.

DISCUSSION OF RESULTS OF SECTION IV.

The findings from the work reported in this section are deemed significant on several grounds. Thus, the action of other microbial inhabitants of the bowel may interfere with colicin formed there and so neutralize any potential advantage offered by antibiotic production. As a result of such anticolicinic activity, the tenure of a

colicinogenetic strain of E. coli in the intestine may be partly dependent on the constitution of the other micro-organismal flora found there.

Anti-colicinic antagonism in vivo may be the reason for the inconclusive results experienced by the author, as well as other workers, in comparative studies on antibiosis by E. coli in vitro in relation to the duration of residency of strains in the host of origin. A similar cause may have attended failure artificially to establish a colicinogenetic strain of E. coli in the gut or to detect colicin in preparations from intestinal contents.

Perhaps, of greater importance is the influence by the enteric microflora on bacteriophage. As in the case of colicin, the role of bacteriophage in the formation of the E. coli population of the gut has not yet been defined. Although he concluded that the agents are fundamentally dissimilar, Fredericq (1957, 1964) described similarities to obtain between colicin and bacteriophage, including the probable possession of common receptors. On the other hand, Sandoval (1965) observed a morphological resemblance of a colicin to bacteriophagic particles and suggested that "all bacteriocins may represent various

products of defective lysogeny". Hence, it is not unlikely that, should other bacteria interfere with colicinic activity, they may do likewise in the case of bacteriophagic action.

SUMMARY.

Following observation of interference with colicinogenesis by E. coli on the part of other micro-organisms, the author carried out an investigation which served to reveal that a variety of bacteria of enteric origin may nullify the colicin produced in vitro by one strain of E. coli. The findings have been discussed with special emphasis on the role of colicinogenesis in the establishment of E. coli in the intestine.

FINAL DISCUSSION.

Enteric disturbance in infants and young animals is frequently associated with proliferation of specific serogroups of *E. coli* in the gut, which multiplication is possibly related to dietary, or other, factors. The pathogenesis of infection is not yet clear although, in the case of oedema disease and of haemorrhagic gastro-enteritis of pigs, an anaphylactic reaction on the part of the host may be involved (Thomlinson and Buxton, 1962, 1963). So-called enteropathogenic *E. coli* (EEC) do not differ markedly from strains normally to be found in the intestine.

In the course of the work under report, the author recovered from healthy chickens 'O' groups (O1, O2, O18 and O73) which have been associated with avian disease by other workers (Gross, 1957; Sojka and Carnaghan, 1961; Guinee, 1963). In the case of human beings, EEC sero-groups have been procured not infrequently from faecal samples from individuals in whom the micro-organism probably constituted part of the normal intestinal flora Neter *et al.*, 1951; Shanks and Studzinski, 1952; Bernet *et al.*, 1955; Curtin and Clifford, 1956; Thomson, Watkins and Gray, 1956; Gamble and Rowson, 1957; Cooper *et al.*, 1959; Solomon *et al.*, 1961 and others).

Examination of healthy as well as diseased animals has yielded similar serogroups (Orskov, 1951; Orskov and Fey, 1954; Englert, 1955; Ewing et al., 1955; Thomson, 1956; Sakazaki and Namioka, 1956; Jecalne and Aragon, 1958; Mian, 1959; Mushin et al., 1960; Scheibner, 1960; Glantz et al., 1959; Reuss and Ketyi, 1960; Mackel et al., 1960; Rees, 1960; Kaeckenbeeck and Thomas, 1960; Schoenaers and Kaeckenbeeck, 1960; Guinee, 1963; Glantz and Rothenbacher, 1965; Mackel et al., 1965; Smith et al., 1965 and others).

Some of the above studies were designed to investigate animals as a reservoir of infection for man. Smith et al. (1965) observed a relationship to exist between human infection and the consumption of chicken and of pork as well as a correlation with contact with household pets. The author of this thesis demonstrated natural tenures of up to four weeks on the part of two EEC serogroups in the gut of dogs and also noted two instances of tonsillar infection in the same animals, factors which may offer considerable opportunity for transmission to human beings.

Records of natural infection by EEC in infants (Taylor and Charter, 1952) and of artificial infection in

piglets (Saunders et al., 1963) reveal that transmission occurs readily among young creatures. Greater ease has been experienced by this author as well as by other workers (Friedman and Halbert, 1960; Mushin and Dubos, 1965) in establishing strains of E. coli in day-old animals than was the case in adults. In addition, the observation by Nemedi (1965) of an incidence of EEC, twice as high in normal infants as that found in healthy adults, suggests that the infantile intestine may be a particularly suitable environment for some serogroups.

The role of EEC in respect of the intestinal flora has yet to be elicited. The author believes that further research into the mechanism controlling E. coli in the normal intestine is indicated since pathogenic strains are subject to the same form of government. Investigation into the physiological state of the host is also deemed necessary because the results may explain the pathogenesis of various diseases with which some serogroups are peculiarly associated.

CONCLUSIONS.

(1) In respect of the composition of resident and transient serogroups, the normal enteric population of the domestic chicken closely resembles that of the other species of animals studied.

(2) Strains of E. coli differ in ability to establish themselves in the intestine.

(3) Strains of E. coli have a predilection for a particular species of host but cross-infection is feasible under natural conditions.

(4) Intestinal micro-organisms may interfere in vitro with colicin produced by E. coli. Similar anticolicinic activity in vivo may account for the seeming insignificance on the part of the antibiotic in establishment of E. coli in the gut.

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STUDIES ON THE ASSOCIATION OF *Escherichia coli* WITH ANIMALS.

SUMMARY.

The first part of the thesis reports an examination of the serological composition of the normal enteric *E. coli* flora of three domestic chickens and of two Syrian hamsters. In the case of the former animals, the results were similar to those described for other species, including man. The study of hamsters revealed that, in those animals too, a resident strain may be excreted with great constancy during a long period.

The second part describes an investigation into factors associated with the regulation of *E. coli* in the gut of animals, with special reference to (a) the effect of the physiological state of the host, (b) the degree of ability of the organism to become implanted in the intestine, (c) host specificity on the part of the organism and (d) colicinogenesis.

The intestinal *E. coli* flora of a litter of seven young dogs was examined over a period of approximately two-and-a-half months, in the course of which the animals were infected experimentally with the virus of infectious canine hepatitis. Variations occurred in the incidence of biochemical varieties and serological types in the faeces but they were not referable to the period of clinical disease arising from the viral infection. Accordingly, the author concluded that

physiological disturbance on the part of the host had not influenced the constituent E. coli of the bowel. Excretion of biochemical varieties by dogs was observed to follow a pattern similar to that normally exhibited by serogroups inasmuch as a few 'resident' types prevailed throughout the period of study whereas others were present for relatively short intervals. The cause of the fluctuations of biochemical varieties was not understood but may have been related to diet.

Attempts were made to establish in adult and day-old chickens or in adult hamsters, under natural conditions, six different serogroups of E. coli demonstrably resident or transient in the host of origin. As a result, two previously transient strains of avian origin failed to colonize the intestine of adult birds and the association with day-old chicks was short in comparison with the marked ability of a former resident type from a similar source to inhabit the gut of experimental birds. Thus, the author concluded that E. coli varies in its ability to persist in the intestine.

A pronounced host-specific tendency was noted on the part of E. coli. In that respect, a strain formerly resident in a fowl was more easily implanted into the gut of chickens than into that of hamsters. Likewise, a strain isolated from a hamster established itself more readily in other animals of the same species than in birds. Nevertheless, transference of E. coli from one species of animal to another was achieved in four instances but, in only one, did the

serogroup appear subsequently in faecal samples with the constancy exhibited by E. coli naturally resident in the type of animal.

Positive results were more easily obtained from experiments involving day-old chicks than from those on adult animals. That finding agreed with results of other workers who have carried out studies on day-old mice and it suggested that transmission may occur readily among infantile animals.

In respect of the significance of colicinogenesis in establishment of E. coli in the intestine, attempts to implant new strains failed to yield conclusive findings.

An investigation of thirteen resident and fifteen transient strains of E. coli of animal origin in vitro revealed the same proportion of colicinogenetic organisms to obtain in both groups. The average degree of antagonism by resident bacteria did not differ greatly from that exerted by transients and resistance to colicin was not peculiar to either type. Those results indicated that antibiosis in respect of colicin-production was not a likely factor in establishment of E. coli in the bowel.

On the other hand, examination of a series of isolates of E. coli from one out of three chickens revealed a close correlation to exist between antibiosis in vitro and length of tenure of the strain. In that instance, the replacement of one resident type by another may have been associated with demonstrable loss of antagonism by the former.

Bacteria belonging to seven out of eight genera of intestinal habitat, including Escherichia, were observed to interfere with antibiosis *in vitro* on the part of a colicinogenic strain of *E. coli*. The incidence of cultures of that kind proved to be approximately 45%. Of the genera tested, only Salmonella constantly failed to affect the antibiotic action. A further investigation revealed that the effect was exerted on colicin after its formation. The findings offer an explanation of the seeming insignificance of colicinogenesis in establishment of *E. coli* in the gut.

In brief, the investigation served to reveal that the not inconsiderable physiological disturbance accompanying a viral infection of the host may not be attended by alteration of the biochemical or serological strain composition of *E. coli* in the intestine. Some strains of *E. coli* are particularly able to inhabit the normal bowel. There is a strong host-specific tendency on the part of *E. coli* but cross-infection under natural conditions is feasible. Colicinogenesis is of doubtful value to *E. coli* in the process of implantation in the gut, possibly as a result of anticolicinic activity on the part of other bacteria normally present there. The outcome emphasized that much is yet to be discovered concerning the regulation of *E. coli* in the normal intestine.

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