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SALMONELLA AND PARACOLON SPECIES ISOLATED FROM THE WILD BROWN RAT, *RATTUS NORVEGICUS*, IN THE CITY OF RICHMOND, VIRGINIA

by

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Richmond College
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a
THESIS

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of the Requirements for the Degree of
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INTRODUCTION

The importance of bacteria as a cause of serious intestinal diseases in man and other animals has been recognized since the early days of the science of bacteriology. Of those forms usually associated with intestinal disturbances in man, the species considered most important are those belonging in the Salmonella group, the Paracolon group, and the Shigella group. Also, it has been known since the early part of this century that the common rat serves as an important vector for the dissemination of infectious organisms. For example, such standard textbooks of Bacteriology as those written by Wilson and Miles (41), Jordan and Burrows (20), Frobisher (16), Smith and Martin (35), and others list rats as primary vectors of enteric bacteria. However, there has been a surprisingly small amount of work done to determine the actual percentage of infection in wild rats. There have been numerous investigations concerning outbreaks of Salmonellosis in mouse populations, and in mouse colonies in breeding laboratories, but few surveys have been made on wild rodents in recent years.

Shortly after the First World War, and during the early twenties, there were numerous papers published

concerning the use of so-called "rat virus", a preparation first used in Germany and later throughout Europe and England as a rodenticide. This poison consisted of a bait, such as grain, that was inoculated with Salmonella enteritidis. It was shown by the work of Savage and Read (29) in 1913 that apparently three possible conditions could affect the rat that ate such a bait: it would die of gastroenteritis from the effect of the Salmonella; the animal, if partially immune, could become ill but recover and become a carrier of the organisms; or the animal could have a complete immunity and be unaffected by the organisms yet pass them in a viable state with the feces. It is apparent that either of the latter two conditions would lead to the dissemination of Salmonella organisms that were potential human pathogens. Savage and Read (29) in 1913, Savage and White (30) in 1922 and the excellent work of the Medical Research Council (25) in England all cast suspicion on the use of a potentially dangerous human pathogen for rat poison and many of the earlier papers were written in an attempt to discourage the widespread sale and use as rodenticides of such bait inoculated with Salmonella. Savage and White (30) were also among the first to demonstrate the occurrence of Salmonella organisms in wild rats. They

trapped 96 rats in a slaughterhouse and upon examination of the livers, spleens and intestinal contents of these animals found six to be infected with Salmonella enteritidis. Of these six positive animals only three were found to carry the organisms in the feces. In addition, they found that the blood of a large percentage of the animals studied carried agglutinins for Salmonella enteritidis. On the basis of this work, Savage and White suggested the possibility of a natural and widespread infection of rodents from Salmonella organisms.

Verder (39) in 1927 investigated 114 rats trapped in a slaughterhouse in Chicago. She was successful in isolating ten strains of Salmonella from six rats, indicating a double infection in several animals. However, she found that all the animals gave negative results from the contents of the gut and, presumably, did not eliminate the organisms in the feces. In the same year, Meyer and Matsumura (26) made the first extensive investigation using a large number of wild rats. Of 775 rats examined, they found 58 positive animals. Both Salmonella enteritidis and Salmonella aertrycke (now Salmonella typhi-murium) were reported from the group of 58 positive animals. They found that only 2% of the total infections occurred in the gut of the rats, and also con-

cluded that the percentage of positives were higher in those animals taken in the vicinity of the slaughter-houses of the city than in those from other areas.

Kerrin (23), in 1938, studied the occurrence of Salmonella in wild rats in Liverpool, England. He was among the first to use tetrathionate enrichment techniques for his isolations and this may account, in part, for his high recovery rate of Salmonella organisms. He examined 750 rats over a nine month period and isolated the following species: Salmonella enteritidis, Salmonella aertrycke (now Salmonella typhi-murium), Salmonella newport and Salmonella thompson. So far as is ascertainable, this seems to be the first published report of the occurrence of either Salmonella newport or Salmonella thompson in rats. Khalil's work indicated a high percentage of infections in the liver and spleen of the animals but in five animals the organisms were isolated from the gut as well as the body organs. The results of one phase of Khalil's work are summarized in the following table:

SEASONAL INCIDENCE OF SALMONELLA INFECTION IN RATS

	Jan.-Mar.	Apr.-June	July-Sept.
Animals examined	250	250	250
Animals infected	44	10	1
Percentage infected	17.6	4	0.4

Reference to the table indicates a clear-cut seasonal incidence in the infection rate of wild rats with organis-- of the Salmonella group. It should be pointed out that Khalil gave no information relative to the extent of the total rat population at the time of sampling and without such information no definite conclusions may be drawn. If, however, it is true that there is a pronounced seasonal difference in the percentage of rats infected, this would contribute to the explanation of the variation between the various reports made by other workers concerning the percentage of infected animals.

The most recent survey in this country of Salmonella found in wild rats was reported by Bartram, Welch and Ostrolenk (4) in 1940 and 1941. They showed in two papers that 15 out of 24 artificially infected rats passed the Salmonella organisms through the digestive tract and eliminated them along with the feces. However, in 800 uninoculated laboratory animals they did not find a single infected animal. In 1941 the same authors made a wide collection of rat and mouse feces in the United States and examined a total of 340 rat feces and 80 mouse feces. All of these samples consisted of droppings that were collected amd forwarded to the writers for their work, but the precise methods used for collection, shipping and preservation of the speci-

mens were not specified. Among these fecal samples only five contained species of Salmonella. These five samples were derived from four rats and one mouse and consisted of the following species: Salmonella typhi-murium, Salmonella san diego, Salmonella newington, and Salmonella anatum. In addition to these species, two samples possessed a species of Proteus which was formerly classified as a Salmonella. Thus, Bartram, Welsh and Ostrolenk reported a total infection of 1.2 %. These writers explained the very low percentage of positive results in their work by maintaining that previous surveys of Salmonella infections in rats had been made after epidemics of Salmonellosis or in animals trapped in a suspected locality. Although this is true of some of the reported surveys, it should be pointed out that this explanation is not applicable to the work of Khalil or Meyer, since both of these workers were careful to report their findings on random samples. Although it is quite true that any given animal is not a vector unless it excretes organisms in its feces or urine, the excretion is quite likely to be intermittent in nature. Consequently, a checking of a single dropping will give no true indication as to whether or not the animal is infected with Salmonella organisms. It is possible that an animal may be infected with Salmonella organisms and not excrete them at the time

a dropping is collected. On the other hand, the finding of Salmonella organisms in the gut or body organs would indicate a strong possibility that the animal will excrete the organisms at some time during its life.

In areas other than the United States and England some recent surveys have been reported. Ghosal (17) in 1941 investigated 364 rats trapped from slaughterhouses, markets, and street areas in Calcutta, India. He reported 49 positive animals, 3.5% or 13 rats positive for _Salmonella enteritidis_ amd 9.8% or 36 rats positive for _Salmonella typhi-murium_. Of the 49 positive animals, 18 were found to be positive in the gut. Assumpcao and Ribas (1), working in Brazil, found _Salmonella schottmuelleri_ and _Salmonella typhi-murium_ in a series of 950 rats. Hulphers and Hinricson (18) working in Sweden found Salmonella species in 46 out of 186 rats examined. These consisted of 32 _Salmonella typhimurium_, 2 _Salmonella enteritidis_, var. _Dublin_ (now _Salmonella dublin_) and 14 _Salmonella enteritidis_. In 33 of the positive cases the organisms were isolated from the intestinal tract only. The report in this paper of 74 "paratyphus-like" organisms from the 186 animals examined is of some interest. So far as has been determined, this is the first report of the probably occurrence of Paracolon species in rats.

The above review of the literature reveals that the following species of Salmonella have been reported from rats:

Salmonella enteritidis

Salmonella aertrycke (*S. typhi-murium*)

Salmonella newport

Salmonella thompson

Salmonella san-diego

Salmonella newington

Salmonella anatum

Salmonella schottmuelleri

Of the reported species *S. enteritidis* and *S. typhi-murium* far outnumber any of the other species in their rate of occurrence.

With the single exception of the report by Hulphers and Hinricson (18) there seems to be no mention of the Paracolon group occurring in rats. This, however, is understandable since it is only within the last two or three years that information has been accumulating which indicates that the Paracolon group may certainly show pathogenicity under certain conditions. Even so, there is still considerable doubt concerning the pathogenic behavior of the Paracolons.

From this review of the literature it is evident that there is yet a need for more extensive study of animal vectors of the enteric diseases affecting man. Since a survey of the enteric pathogens of the rats in the city of Richmond has never been made, and in view of the sporadic outbreaks of enteric fevers in the city, it was felt that a project of this nature would be worthwhile. Accordingly, an effort has been made to determine several facts concerning the infection with enteric pathogens of wild rats in the city: first, the percentage of wild rats in the city of Richmond infected with Salmonella; second, the species of Salmonella involved; and third, the distribution of the infected animals within the areas that were trapped. In addition, it was thought wise to give some attention to Paracolon organisms encountered in the study. This preliminary study will continue until a sufficient number of living animals have been examined from all parts of the city and an accurate statistical evaluation of the results can be made.

MATERIALS AND METHODS

The methods used in this study have been used so long and are so well known that in most cases it is only necessary to list them. A brief summary of the whole procedure is as follows: all of the animals used in this study were caught by the use of regular No. 1 muskrat traps and brought into the laboratory alive. The animals were secured by the personnel of the Division of Rodent Control, Bureau of Sanitation, City of Richmond Health Department for use in typhus control program. The areas of the city trapped are plotted on the map included here as Figure 1. Roughly, three to four hundred animals were caught to secure the approximately one hundred living animals that were used as the basis of this study. Animals caught alive in traps were often killed by passers-by, cats or dogs, or drowned by heavy rains. The animals brought into the laboratory were placed under ether and a sample of heart blood drawn for typhus titer. Then, the abdomen was opened and a large segment of the gut removed and placed in ten percent sterile ox bile. Usually the segment of the large intestine was split before dropping it into the bile. After remaining in bile for twenty-four hours the culture was streaked

very heavily on SS medium (31) and at the same time about three to five ccs. of the bile culture was inoculated into Selenite F medium (2). After 24 hours incubation the Selenite F culture was streaked out on another SS plate. At this time the first SS plate inoculated was scanned for non-lactose fermenting colonies, and those that were found were inoculated into Krumwiede's Triple Sugar agar. Those cultures taken from the first SS plate were given the suffix of the Roman numeral I to distinguish them from the colonies taken from the second plate carrying the suffix II. Those cultures showing a positive reaction on Krumwiede's agar, i.e., acid and gas in the butt and no change on the slant, were next inoculated into the primary differential media: urea broth (9), A.A.S.S. medium (8) and lactose.

On the basis of the results found in the above preliminary media, the cultures were identified tentatively as Proteus species, Coliform species, Paracolon species, and Salmonella species. Chilton's (8) A.A.S.S. medium was used for the detection of Paracolons and a positive reaction on this medium with the absence of action on urea and on lactose within 24 to 48 hours was considered presumptive evidence of a Paracolon organism. In theory, any culture carried over

from these media was either Salmonella or Paracolon. However, there were exceptions such as in species of Alcaligenes that would be inactive on the media used and thus simulate a Salmonella at that stage. In like manner, certain anaerogenic Coliform organisms would simulate Salmonella. Those cultures suspected of being Salmonella or Paracolon species, as separated by the above screening test, were run through a series of biochemical tests. Sucrose and lactose media were inoculated and incubated for ten days in order to detect slow fermentation of these carbohydrates. Salicin, maltose, dextrose, xylose, and mannitol were also inoculated and incubated for 24 hours, or occasionally 48 hours. Production of hydrogen sulphide and the motility of the organisms were determined by the use of T.L.I. agar (2). Indol was determined by growing the organisms in one percent peptone, incubated for 24 hours and checked with Kovac's reagent.

Finally, all of the cultures that appeared biochemically typical were agglutinated with Polyvalent Salmonella Serum, furnished by the Communicable Disease Center, Chamblee, Georgia, through the cooperation of Dr. P. R. Edwards. Those cultures that agglutinated with the Polyvalent Sera were group agglutinated with Groups B, C_1, C_2, D, and $E_{1,2,3}$ sera supplied by the Lederle Laboratories, Pearl River, New York

and all cultures that appeared to be Salmonella, or Paracolon species with common Salmonella somatic antigens, were forwarded to the Communicable Disease Center, Chamblee, Georgia, where Dr. P. R. Edwards kindly checked the determinations.

Special methods, media and techniques used are discussed in connection with the experiments in which these are concerned.

ISOLATION AND STUDY OF THE SALMONELLA AND PARACOLON SPECIES FROM THE GUT OF THE WILD RAT

The biochemical and morphological classification of the Salmonella group is fairly constant as a result of the work of the International Congress of Microbiologists and students of the bacteria such as Bergey, Topley and Wilson, Kauffmann, and Edwards. A composite description of the genus Salmonella as based on the work of these students of the group may be stated as follows: the genus Salmonella consists of non-spore forming, usually motile, gram negative rods measuring 1 to 3 microns in length by 0.5 to 0.7 microns in width. The organisms produce acid and gas from glucose, maltose, mannitol, and sorbitol. A few species, the most important being *Salmonella typhosa* and *Salmonella gallinarum*, produce acid only when cultured in these sugars. The organisms of the genus are unable to metabolize lactose, sucrose, salicin, and adonitol. Hydrogen sulphide is usually produced, but indol is never formed nor is gelatine liquified. All known species are pathogenic for animals. All the species are very closely related to each other by somatic and flagellar antigens. To this description one may add that in view of recent tabulations by Seligmann et als. (33,34) of species occurring in human

infections it appears as though there is little host specificity in the species. With few exceptions, all of the species reported in animals are gradually being found in man.

From the above description of the genus Salmonella it is evident that a combination of biochemical and serological characteristics is necessary for the distinction of species in the group.

As pointed out in the preceding section, the majority of the methods and materials used in determining the biochemical characteristics of the Salmonella, Paracolon, and Proteus genera have been standardized for some time. The primary differential medium used is Difco SS medium. This medium was developed in the laboratory of the Digestive Ferments Company and no account of the development of the medium appears except in their publication, The Difco Manual (31).

The production of an enzyme, urease, by Proteus species which enables them to utilize urea has been known for some time, but it is only quite recently that the characteristics have been fully investigated by Rustigian and Stuart (28); Stuart, van Stratum and Rustigian (38); Christensen (10); Cook (11); and Elek (15) and widely used for the separation

of the Proteus group. Most of the above workers have also studied the urease positive strains in the so-called intermediate and aerobacter groups of the Paracolons. All of the Paracolons hydrolysed urea somewhat more slowly than did the Proteus species.

The diagnostic use of urease production in the laboratory has characteristically fallen into one of two patterns. Those who are interested in the Proteus only have evolved a very highly buffered medium on which Proteus alone will grow; while those whose interest in the Proteus group was simply to eliminate it from a mixed culture have used a slightly buffered medium that screened out not only the Proteus, but also some of the Paracolon. Both types of media are commercially available. The strongly buffered medium of Stuart, van Stratum and Rustigian (38) is considered by Cook (11) to be suitable for the study of the Proteus group since none of the Paracolon cultures that he studied hydrolysed the medium and consequently distinguished them immediately from the Proteus species. On the other hand, the weakly buffered medium of Christensen (10) was recommended by Cook (11) for the simple elimination of all cultures other than Salmonella and Shigella from enteric cultures since it gave reactions with most of the Paracolons tested as well as the Proteus species. The formula of the two media are as follows:

Stuart's, et als., Strongly Buffered Medium

Yeast Extract	0.1 gm.
Monopotassium phosphate	9.1 gm.
Disodium phosphate	9.5 gm.
Urea	20.0 gm.
Phenol red	0.01 gm.
pH 6.8	Water q.v. 1000 ml.

Christensen's Weakly Buffered Medium

Peptone	1.0 gm.
Dextrose	1.0 gm.
Sodium chloride	5.0 gm.
Monopotassium phosphate	2.0 gm.
Urea	20.0 gm.
Phenol red	0.12 gm.
pH 6.8	Water q.v. 1000 ml.

Neither of these media may be sterilized by heat because of the danger of the hydrolysis of the urea. They are heavily inoculated, incubated at 37°C. and read at 8, 12, 24 and 48 hours. A positive reaction is quite evident from the release of the ammonia by the hydrolysis of the urea and its effect on the indicator. Since the Paracolons were to be maintained, a broth of Stuart's formula was used to eliminate Proteus species from the cultures.

The A.A.S.S. medium developed by Chilton and Fulton (8) was used throughout this study to detect Paracolons. The medium is composed of:

Aesculin	5.0 gm.
Adonitol	5.0 gm.
Salicin	5.0 gm.
Sucrose	5.0 gm.
Brom-cresol purple	0.015 gm.
Ferric ammonium citrate (brown scale)	0.05 gm.

Water q.v. 1000 ml.

Due to the expense and unavailability of certain of the sugars, the medium was used sparingly, usually 2 to 5 ml. in Kolmer tubes. After incubation the medium turned yellow if adonitol, salicin, or sucrose were fermented, while the medium blackened if aesculin was utilized. Kovac's test for indol can also be superimposed on the medium, and this is routinely done on all negative tests since one can occasionally eliminate a culture that is negative on the A.A.S.S. medium but indol positive. The medium often gives positive tests with Coliform organisms as well as with Proteus, but is very valuable when used in conjunction with lactose and urea media.

The biochemical tests described above are fairly satisfactory for the delineation of the Salmonella group but they

are useless for speciation within the group since there are not sufficient variations within a group of similar organisms to differentiate them. Consequently, identifications of distinct species within the group is dependent almost entirely upon serological methods. Based on the Kauffmann-White Schema (19), there are from 150 to 160 serological types in the genus Salmonella that are accorded the rank of species by most workers. There was a tendency, for a short period, to base the whole classification upon serological methods as included in Kauffmann's (21) suggested definition of the Salmonella group. Kauffmann (21) defined the genus as "gram negative bacteria which, on the grounds of their antigenic structure, can be included in the Kauffmann-White Schema". If this definition were to be followed closely one would be forced to include organisms from widely separated groups since the somatic antigens of the Salmonella are commonly found in many widely separated genera of bacteria. Common antigens are reported frequently in Paracolons and have been reported even in such forms as a strain of Flexner Shigella by Bornstein (7) and a strain of Pasteurella by Schutz (32). These common somatic antigens are widely recognized now as being present and nearly all workers attempt to use a combination of biochemical and serological methods for classification of the Salmonella

group. In view of the peculiar complexity and size of this group, it is obvious that a given culture may be identified tentatively as a member of the Salmonella on the basis of its biochemical reactions, but it must be confirmed by serological methods.

The serological classifications of the Salmonella has reached astounding proportions since the first classification in 1934 and has now reached the point where the establishment of National Salmonella Typing Centers is necessary for the actual speciation of a culture. The serological classification as based on the Kauffmann-White Schema first published in 1934 by the Salmonella Sub-committee of the Nomenclature Committee of the International Society for Microbiology (19) and later revised by Kauffmann (21). Most commonly used in this country for identification of the Salmonella is Edward's and Bruner's (12) method for the serological identification of the Salmonella. An antigenic formula as applied to one of the species of Salmonella will consist of the following notations: somatic antigens represented by Roman numerals, flagellar antigens are represented by Arabic numerals and phase 2 flagellar antigens are represented by Arabic numerals or lower case letters. If the somatic antigens are enclosed in parentheses it indicates that they are of variable occurrence.

and a series of dots indicates that portions of the formula have been omitted. The twenty-six letters of the alphabet have long since been exhausted in these formulae and so, by common agreement, letters used for flagellar antigens after the letter "z" carry a numerical subscript, e.g., z_2. Thus the formula for a complete antigenic complex such as that for Salmonella typhi-murium might be expressed as:

Salmonella typhi-murium (I), IV, (IV), XII
i, 1, 2, 3 ....

while that for Salmonella anatum would be written:

Salmonella anatum III, X, XXVI, e, h, 1, 6 ...

Fortunately the somatic antigens of the species Salmonella seem to form a natural taxonomic scheme at least consistent enough to allow the grouping together of certain serological types. Thus, the species of Salmonella possessing the somatic antigen IV are placed in Group D and so on. There are now Groups A, B, C_1, D, E_1, E_2, E_3, and F.

Diagnostic antisera may be very easily made for these groups by choosing a strain of Salmonella carrying the desired somatic antigen and inactivating the H antigens of the strain by boiling for two hours. The suspensions of boiled bacteria are then preserved with formalin and rabbits

are given a series of intra-venous injections of the bacteria. The rabbits are bled when their blood has reached a sufficient titer and the serum from the blood used as antisera (12). The organisms commonly used for this purpose are as follows:

Kauffmann and Edwards Group Specific Antigens (22)

Group	Organism	Somatic Antigen
A	<u>Salmonella paratyphi A</u>	I, II, XII
B	<u>Salmonella paratyphi B</u>	IV, V, XII
C	<u>Salmonella thompson and newport</u>	VI, VII, VIII
D	<u>Salmonella gallinarum</u>	IX, XII
E	<u>Salmonella anatum</u> and <u>newington</u>	III, X, XV

In addition to the above an extremely useful screening polyvalent antiserum can be made by injecting a rabbit with all of the above cultures after they have been treated to inactivate the H antigens.

Under normal laboratory technique speciation of an isolated Salmonella is not attempted beyond the group agglutinations. Further identification is done by the Salmonella Typing Center using flagellar antigens and an absorption technique for identification of specific antigens.

In a fairly recent and very comprehensive review of the Salmonella problem by Bornstein (7) several criteria were suggested for the determination of the status of a doubtful Salmonella. He suggested that if the culture under consideration possesses the complete antigenic formula of one species of Salmonella but differs in one of the accepted biochemical criteria, then the culture should be considered a cultural variety.

A strain that fulfills the biochemical definition of the group and possesses a new combination of Salmonella antigens should be considered a new serological type. Furthermore, a culture that has an antigenic formula typical of Salmonella will be recognized as a new serological type even if it shows minor deviations from the accepted biochemical criteria.

Finally, a strain that fits Salmonella biochemically but has no antigens common to any of the species of Salmonella cannot be recognized unless it is pathogenic; conversely the presence of minor O or H antigens alone cannot classify an organism as a species of Salmonella.

As in most attempted schemes the classification of the genus Salmonella shows certain points of error. In all proba-

bility the most difficult of the organisms encountered are the groups classified as Paracolons. They will often show typical Salmonella chemical reactions as well as an assortment of common antigens.

Some attempt has been made in this study to carry through to identification those Paracolons that have common antigens with the Salmonella and consequently agglutinate polyvalent Salmonella serum. The group itself, however, is to date almost impossible to define. It is considered by most workers to be intermediate between the Salmonella and Escherichia and possesses some of the characteristics of each group. Thus, one of the primary criteria of the group is the slow fermentation of lactose. Yet this is an entirely relative sort of thing since it is well known that the speed of utilization of a sugar may be hastened by rapid transfer through the sugar, and many organisms classified in the group never attack lactose.

The question of the pathogenicity of the Paracolon group is still questioned by many workers, but there are reports in the literature by Plass (27), Stuart and Rustigian (36), Christensen (10), Barnes and Cherry (3) of enteric infections caused by Paracolon organisms and agglutinines are occasionally demonstrable in the sera of patients.

Attempts have been made for a number of years to classify the Paracolons, but to date no satisfactory scheme of taxonomy has been worked out. Stuart, Wheeler, Rustigian and Zimmerman (37) presented an elaborate attempt at biochemical classification, but it has not been widely accepted. This scheme has been used, in so far as possible, on the cultures in this study. The identifying characteristics are summarized in Table I.

Attempts at serological classifications have also been disappointing. Some of the conflicting results have perhaps been due to the lack of a definite limiting biochemical definition. One of the better defined groups of the Paracolons was examined by Edwards, et als. (13) and is better defined biochemically than most of the other Paracolons. Since the so-called Bethesda group occurs frequently in this study, it may be worth while to list its characteristics. These are summarized as follows:

Lactose	Usually slow fermentation
Methyl red	Positive
Voges-Proskauer	Negative
Indol	Negative
Hydrogen sulphide	Positive

TABLE I

BIOCHEMICAL CHARACTERISTICS OF THE PARACOLON GROUP

PARACOLONS

PARACOLON AEROBACTER

FIRST DIVISION:
V.P. + (STRONG) — 30% GAS IN 24 hrs, 100% IN 48 hrs. (Glucose)
CITRATE AGAR +
CELLOBIOSE + (24 hrs)
SS - USUALLY NO GROWTH

SECOND DIVISION
V.P. WEAK OR NEGATIVE
CITRATE AGAR —
CELLOBIOSE —
GAS VOLUMES LOW
SS - USUALLY GOOD GROWTH

PARA. INTERMEDIATE

LACTOSE - OFTEN ACID BUT NO GAS.
V.P. - NEGATIVE
MANY REDUCE B-C-P INDICATOR IN LACTOSE, MALTOSE, AND OCCASIONALLY SALICIN. NEVER GLUCOSE, SUCROSE, OR MANNITOL.

PARA. ESCHERICHIA

MOST PRODUCE INDOL.
LOW MOTILITY.
GROW WELL ON SS.
+ INDOL
- CITRATE, USUALLY
- V.P.

ANAEROGENIC P.

TYPICALLY INACTIVE ON CARBOHYDRATES

TYPE NO.	DAYS OF INCUBATION	GLUCOSE GAS (%)	LACTOSE	SUCROSE	UREA	SALICIN	MALTOSE	MANNITOL	INDOL	V.P.	CITRATE	CELLOBIOSE	GELATIN	MOTILITY	LEAD ACETATE
PARA. AEROBACTER - 1st DIV.															
4611	1 / 2 / 3-10	+30 / +100 / ++	− / − / +	+ / + / +		+ / + / +	+ / + / +	+ / + / +	−	+	+	+	− / − / +	+	
1721	1 / 2 / 3-10	+30 / +100 / ++	− / − / +	+ / + / +		− / − / +	+ / + / +	+ / + / +	−	+	+	+	− / − / +	+	
19111	1 / 2 / 3-10	+30 / +100 / ++	− / − / +	− / − / +		+ / + / +	+ / + / +	+ / + / +	−	+	+	+	− / − / +	+	
PARA. AEROBACTER - 2nd DIV.															
721	1 / 2 / 14-40	+20 / +40 / +	− / − / +	− / − / +		+ / + / +	+ / + / +	+ / + / +	−	+	− / − / +	− / − / +	− / − / −	+	
32011	1 / 2 / 14-40	+20 / +40 / +	− / − / −	− / − / +		± / ± / ±	A / + / +	B / + / +	−	+	− / − / +	− / − / +	− / − / −	±	
37711	1 / 2 / 14-40	+30 / +100 / +	− / − / ±	− / − / +		− / − / ±	+ / + / +	+ / + / +	−	+	− / − / +	− / − / +	− / − / −	+	
35611	1 / 2 / 14-40	+20 / +40 / +	− / − / −	− / − / +		− / − / −	+ / + / +	+ / + / +	−	+	− / − / +	− / − / +	− / − / −	+	
37211	1 / 2 / 14-40	+20 / +60 / +	− / − / ±	− / − / −		− / − / −	+ / + / +	+ / + / +	−	+	− / − / +	− / − / −	− / − / −	+	
37511	1 / 2 / 14-40	+20 / +60	− / − / −	− / − / −		− / − / −	+ / + / ±	+ / + / +	−	+	− / − / ±	− / − / −	− / − / −	+	
32821	1 / 2 / 14-40	+20 / +40 / +	− / −	− / − / +		− / − / ±	+ / + / +	+ / + / +	−	+	− / − / ±	− / − / ±	− / − / −	±	
PARACOLON INTERMEDIATE															
13311	2 / 40	+ / +	A / A+	− / −		+ / +	+ / +	+ / +	+	−	+	+	−	+	−
11411	2 / 40	+ / +	A / A+	− / −		A / A	+ / +	+ / +	+	−	+	+	− / +	+	−
8011	2 / 40	+ / +	A / A+	− / −		+ / +	+ / +	+ / +	−	−	+	+	− / +	+	−
14011	2 / 40	+ / +	A− / A	− / −		− / +	+ / +	+ / +	−	−	+	A / ±	− / −	+	+
12611	2 / 40	+ / +	A− / A−	− / −		− / −	+ / +	+ / +	−	−	+	− / A	− / −	+	+
1421	2 / 40	+ / +	− / −A+	+ / +		− / −	+ / +	+ / +	−	−	− / +	− / A	− / −	+	±
PARACOLON ESCHERICHIA															
28221	1 / 7-40	+ / +	− / +	+ / +		+ / +	+ / +	+ / +	+	−	−	−		++	
5511	1 / 7-40	+ / +	− / +	+ / +		− / A+	+ / +	+ / +	+	−	− / −	− / −		+	
6611	1 / 7-40	+ / +	− / +	+ / +		− / A+	+ / +	+ / +	+	−	− / −	− / −		+	
2611	1 / 20-40	+ / +	− / +	A+ / A+		− / +	+ / +	+ / +	+	−	− / −	− / −		±+	
31611	1 / 7-40	+ / +	− / +	+ / +		− / +−	+ / +	+ / +	+	−	− / −	− / −		++++	
311	1 / 20-40	+ / +	− / +	− / −		− / +−	+ / +	+ / +	±	−	− / −	− / −		—	
111	1 / 20-40	+ / +	− / +	− / +−		− / −	+ / +	+ / +	+	−	− / −	− / −		+	
15411	1 / 7-40	+ / +	− / +	− / −		− / A+	+ / +	+ / +	+	−	− / −	− / −		++	
1811	1 / 20-40	+ / +	− / +	− / −		− / −	+ / +	+ / +	+	−	− / −	A / −		+++	
16911	1 / 20-40	+ / +	− / A+	− / −		− / +	+ / +	+ / +	+	−	− / +	− / −		—	
17611	1 / 20-40	+ / +	− / A+	− / −		− / +	+ / +	+ / +	+	−	− / +	− / −		—	
4361	1 / 20-40	+ / +	− / A	− / −		− / −	+ / +	+ / +	+	−	− / −	− / −		+++	
33811	1 / 7-40	+ / +	− / +−	− / −		− / −	− / +	+ / +	+	−	− / −	− / −		++++	
ANAEROGENIC PARACOLON															
19811	1 / 40	A / A	A / A	− / −	−	− / −	A / A	A / A	+	−	− / −	−		++++	
33111	1 / 20-40	AB / AB	− / −	− / A	+S	−A / −A	− / −	A / A	+	−	+	− / −		++	
29911	1 / 20-40	AB / AB	− / −	− / A	+W	− / −	− / −	− / −	+	−	+	− / −		+++	
8911	1 / 20-40	A / A	− / −A	−A / A	−	−A / −A	− / A	A / A	+	−	− / −	− / −		+1	

+ : POSITIVE REACTION, ACID + GAS
− : NEGATIVE REACTION
A : ACID
B : BUBBLE OF GAS
± : SOME STRAINS NEGATIVE, SOME +.

−A : SOME NEGATIVE, OTHERS ACID
AB : SOME ACID, SOME A BUBBLE OF GAS
S = STRONG
W = WEAK

J.M. SHARPLEY, 1950
AFTER STUART, et al

d-Tartrate	Positive
Simmon's Citrate	Positive
Urea	Negative by Rustigian's and Stuart's method. Positive by Christensen's method.
Dulcitol	Usually fermented promptly, negative strains occur
Sucrose	Generally negative

From the above discussion of the present taxonomic schemes, it must be obvious that any attempt to identify random strains of the Paracolons isolated during a survey is a hopeless task. However, because of the current interest in the group, the cultures have been preserved in the hope that they may be of value to other workers.

From a total of 105 rats, 125 cultures of organisms suspected of being enteric pathogens were isolated for further study. These cultures and a preliminary study of their biochemical characteristics are summarized in Table II. It will be seen that these 125 cultures may be divided into 48 possible members of the Salmonella or Paracolon groups, while the remaining 77 cultures may be classified either as Proteus or Coliforms.

TABLE II

PRELIMINARY CLASSIFICATIONS OF CULTURES

PRELIMINARY CLASSIFICATION OF CULTURES

Number	Urea				A.A.S.S.				Lactose			
	8	12	24	48	8	12	24	48	24	48	72	10d.
597*I	-	P	P		-	-	-	-	-	-	-	-
597-I-A	-	P	P		-	+			-	-	-	+
597-II	-	-	-	-	-	-	-	-	-	-	-	-
598-I	-	-	-	+	+	+	+	+	-	-	-	+
598-II	-	P	P	P								
U-I-A	-	P	P	P								
U-I-B	-	P	P	P								
599-I	-	-	-	-	±	+	+	+	AG	AG	-	-
600-I	-	P	P	P								
600-II	P	P	P	P								
601-II	-	-	-	-	+	+			A	A	A	A
602-I	-	-	-	-	+	+			AG			
602-II	-	-	-	-	-	-	-	-	-	-	-	-
602-A(S)	-	-	-	-	-	-	-	-	-	-	-	-
603-II	-	-	-	-	-	-	-	-	-	-	-	-
603-A(S)	-	-	-	-	-	-	-	-	-	-	-	-
604-I	-	P	P	P								
P-I-I	-	-	-	-	-	+	+		-	-	-	AG
P-1-II	-	-	-	-	-	+	+		-	-	-	AG
P-2-I	-	-	-	-	-	+	+		-	-	-	AG
P-2-II	-	-	-	-	-	+	+		-	-	-	AG
606-II(1)	-	P	P	P								
606-II(2)	-	P	P	P								
606-I	-	P	P	P								

PRELIMINARY CLASSIFICATION OF CULTURES

Number	Urea				A.A.S.S.				Lactose			
	8	12	24	48	8	12	24	48	24	48	72	10d.
607-II	-	P	P	P								
608-I	-	-	-	-	-	+	+		-	-	-	-
608-II	-	P	P	P	-	+	+		-	AG		
610-I(L)	-	P	P	P								
610-II(S)	-	-	-	-	-	-	-	-	-	-	-	-
610-II	-	P	P	P	-	+						
D-I-2	P	P	P	P	-	+						
612-I	P	P	P	P	-	+						
612-II	P	P	P	P	-	+						
614-I	-	-	-	-	-	-	-	-	-	-	-	-
614-II	-	P	P	P	+	+	+	+	*	-	-	-
615-I-W	-	P	P	P	-	-	-	-	-	-	-	-
615-I-B	-	-	P		+	+			AG	AG	AG	AG
616-I-A	-	-	P		+	+	+	+	A	A	A	AG
616-I-B	P	P	P	P	+	+	+	+	AG	AG	AG	AG
616-II	-	P	P	P	-	-	-	-	-	-	-	-
617-II	P	P	P	P	+	+	+	+	-	-	-	-
618-IB	-	-	-	-	-	-	-	-	-	-	-	A
618-II	P	P	P	P	+	+	+	-	-	-	-	-
619-I	-	P	P	P	-	+			-	-	-	-
620-I	P	P	P	P	-	-	-	+	-	-	-	+
621-I	-	P	P	P	-	-	-	-	-	-	-	-

PRELIMINARY CLASSIFICATION OF CULTURES

Number	Urea				A.A.S.S.				Lactose			
	8	12	24	48	8	12	24	48	24	48	72	10d.
621-II	-	P	P		-	-	-	-	-	-	-	-
622-I	-	-	-	-	-	-	-	-	-	-	-	-
623-I	-	-	-	-	-	-	-	-	-	-	-	-
623-II	-	-	-	-	-	-	-	-	-	-	-	-
624-I	-	-	-	-	-	-	-	-	-	-	-	-
624-II	-	-	-	-	-	-	-	-	-	-	-	-
625-II	-	-	-	-	-	-	-	-	-	-	-	-
637-I	-	-	-	-	-	+	+		-	-	-	A
638-I	-	-	-	-	-	-	-	-	-	-	-	-
639-I	-	-	-	-	-	-	-	-	-	-	-	-
639-II	P	P	P	-	+	+			AG	AG		
640-I	-	-	-	-	-	-	-	-	-	-	-	+-
640-II	-	P	P	P	+	+	+	+	AG	AG		
642-II	-	-	-	-	-	-	-	-	-	-	-	-
643-I	-	-	-	-	-	-	-	-	-	-	-	-
643-II	-	-	-	-	-	-	-	-	-	-	-	-
644-I	-	-	-	-	+	+			AG	AG		
645-I	-	-	-	-	-	-	-	-	-	-	-	
646-I	P	P	P	P	-	-			-	+-		
646-II	-	-	-	-	-	-	-	-	-	-	-	-
647-I	-	-	P	P	-	-	-	-	-	+-		
647-II	-	-	-	-	+	+	+	+	-	-	-	-

BRELIMINARY CLASSIFICATIONS OF CULTURES

Number	Urea				A.A.S.S.				Lactose			
	8	12	24	48	8	12	24	48	24	48	72	10d.
648-I	-	-	-	-	+	+	+	+	-	AG		
648-II	-	-	-	-	+	+	+	+	-	AG	AG	
649-II	P	P	P	P	+	+						
650-I	-	-	-	-	-	-	-	-	AG	AG		
650-II	-	-	-	-	-	-	-	-	-	-	-	-
651-I	-	-	-	-	+	+			-	-	-	-
651-II	-	-	-	-	-	-	-	-	-	-	-	-
652-I	-	P	P	P	+	+	+	+	-	-	-	-
652-II	-	P	P	P	+	+	+	+	-	-	-	-
653-I	-	P	P		+	+	+	+	A	A		
653-II	-	P	P		+	+	+	+	-	-	-	-
654-I	-	P	P		-	+	+		-	-	-	+-
654-II	-	P	P		-	+	+		-	-	-	-
655-IB	-	P	P		-	+	+		-	-	-	-
655-II	-	-	-	-	-	-	-	-	-	-	-	-
656-IB	-	-	-	-	-	-	-	-	-	-	-	-
656-II	-	-	-	-	-	+-	+		-	-	-	-
657-II	-	-	-	-	-	-	-	-	-	-	-	+-
658-II	-	-	-	-	-	-	-	-	-	-	-	-
659-II	-	-	-	-	-	-	-	-	-	-	-	-
660-II	-	P	P		-	+	+		-	-		
661-I	-	-	-	-	-	-	-	-	+-	+-	B	

PRELIMINARY CLASSIFICATION OF CULTURES

Number	Urea				A.A.S.S.				Lactose			
	8	12	24	48	8	12	24	48	24	48	72	10d.
661-II	-	-	-	-	-	-	-	-	-	-	-	-
663-I	P	P	P	P	-	-	-	-	-	+		
663-II	-	P	P		-	-	-	-	-			
664-I	-	P	P		-	-	-	-	-			
664-II	-	P	P		-	-	+					
665-I	-	-	-	-	-	-	-	-	-	-	-	-
665-II	-	-	-	-	-	-	-	-	-	-	-	-
666-I	P	P	P		-	+-	+		-	+		
667-I	-	-	-	-	-	-	-	-	+-	+-	A	
668-II	-	+	P		-	+	+		-	+		
671-II	-	P	P		-	-	-	-	-	-	+	
672-I	-	P	P		-	-	-	-	-	-	+	
672-II	-	P	P		-	-	-	-	-	-		
673-II	-	-	P		-	-	-	-	-	+		
674-I	-	-	-		-	-	+-	+-	-	-	-	-
674-II	-	P	P		-	-	+		-	-		
675-I	-	P	P		-	-	-	-	-	-		
675-II	-	P	P		-	-	-	-	-	-		
677-I	-	P	P		-	-	-	-	-	-		
680-I	-	P	P		-	-	-	-	-	-		
682-I	-	-	-	-	-	-	-	-	-	-	-	-
682-II	-	P	P		-	-	-	-	-	-	-	-
683-I	-	-	-	-	-	+	+		-	-	-	-

PRELIMINARY CLASSIFICATION OF CULTURES

Number	Urea				A.A.S.S.				Lactose			
	8	12	24	48	8	12	24	48	24	48	72	10d.
690-I	-	P	P		-	-	-		-	-	-	
690-II	-	P	P		-	+	+		-	-	-	
691-I	-	P	P		-	-	-		-	-	-	
693-I	-	-	-		-	-	-	-	-	-	-	-

TABLE III

ISOLATIONS OTHER THAN PROTEUS AND COLIFORMS

SUGAR FERMENTATIONS

ISOLATIONS OTHER THAN PROTEUS AND COLIFORMS

Number	Salicin		Maltose		Dextrose		Xylose		Mannitol	
	24	48	24	48	24	48	24	48	24	48
597-II	--	--	--	--	--	--	--	--	--	--
598-I	AG		A		AG		AG		AG	
601-II	A		A		A		A		A	
602-I	--	--	A		AG		AG		AG	
602-A(S)	--	--	AG		AG		AG		AG	
603-II	--	--	--	--	--	A	--	--	--	--
603-A-S	--	--	AG		AG		AG		AG	
P-1-I	AG		+-	AG	AG		A		AG	
P-1-II	AG		--	A	AG		--	--	A	
P-2 -I	AG		AG		AG		AG		AG	
P-2-II	AG		AG		AG		AG		AG	
608-I	A		--	A	A		+-	A	A	A
610-IIS	--	--	--	--	--	--	--	--	--	--
614-I	--	--	--	--	AG		A		--	--
618-IB	--	--	AG		AG		AG		AG	
622-II	--	--	AG		AG		AG		AG	
623-II	--	--	AG		AG		AG		AG	
623-I	--	--	AG		AG		AG		AG	
624-I	--	--	AG		AG		AG		AG	
624-II	--	--	AG		AG		AG		AG	
625-II	--	--	AG		AG		AG		AG	
637-I	--	--	AG		AG		AG		AG	
638-I	--	--	AG		AG		AG --	--	AG	
639-I	--	--	AG		AG		AG		AG	

ISOLATIONS OTHER THAN PROTEUS AND COLIFORMS

Number	Salicin		Maltose		Dextrose		Xylose		Mannitol	
	24	48	24	48	24	48	24	48	24	48
640-I	-	-	-	-	-	-	+-	+-	-	-
642-II	-	-	AG		AG		-	-	AG	
643-I	-	-	-	AG	A	A	-	-	-	-
643-II	-	-	-	AG	AG		A		A	
645-I	-	-	AG		AG		AG		AG	
646-II	-	-	AG		AG		AG		AG	
647-II	A		+-	A	A	A	A		A	A
650-II	-	-	+-	A	A	A	A		A	A
651-I	-	-	-	+-	AG		+-	+-	-	-
651-II	-	-	A		AG		AG		AG	
655-II	-	-	A		AG		A		A	
656-I-B	-	-	AG	AG	AG		AG		AG	
656-II	-	-	-	-	-	A	-	-	-	-
657-II	-	-	-	AG	AG		A		+-	A
658-II	-	-	AG		AG		AG		AG	
659-II	-	-	AG		AG		AG		AG	
661-I	-	-	AG		AG		AG		AG	
661-II	-	-	AG		AG		AG		AG	
662-II	-	-	AG		AG		A		+-	AG
665-I	-	-	AG		AG		A		A	
665-II	-	-	AG		AG		A		AG	
667-I	A		A		A		A		A	
674-I	A		AG		AG		AG		AG	
682-I	-	-	-	-	AG		+-	+-	-	-
683-I	A		A		A		A		A	
693-I	-	-	AG		AG		AG		AG	

TABLE IV

ISOLATIONS OTHER THAN PROTEUS AND COLIFORM

LACTOSE FERMENTATION

LACTOSE FERMENTATION

Number	Days 1	2	3	4	5	6	7	8	9	10
597II	-	-	-	-	-	-	-	-	-	-
5981	-	-	-	-	-	-	-	A		
601II	A	A	A	A	A	A				
602II	-	-	-	-	-	-	-	-	-	-
602 A (s)	-	-	-	-	-	-	-	-	-	-
603 II	-	-	-	-	-	-	-	-	-	-
603 A (s)	-	-	-	-	-	-	-	-	-	-
P-1-I	-	-	-	AG	AG	AG				
P-1-II	-	-	-	AG	AG	AG				
P-2-I	+-	AG	AG	AG	AG	AG				
P-2-II	-	AG	AG	AG	AG	AG				
6081	-	-	-	-	-	-	-	-	-	+-
610 II (s)	-	-	-	-	-	-	-	-	-	-
614 I	-	-	-	-	-	-	-	-	-	-
614 I B	-	-	-	A	A	A				
622 II	-	-	-	-	-	-	-	-	-	-
623 I	-	-	-	-	-	-	-	-	-	-
623 II	-	-	-	-	-	-	-	-	-	-
624 I	-	-	-	-	-	-	-	-	-	-
624 II	-	-	-	-	-	-	-	-	-	-
625 II	-	-	-	-	-	-	-	-	-	-
637 I	-	-	-	A	A	A				
638 I	G	-	-	-	-	-	-	-	-	-
639 I	-	-	-	-	-	-	-	-	-	-

LACTOSE FERMENTATION

Number	Days 1	2	3	4	5	6	7	8	9	10
640 I	-	-	-	-	-	-	-	-	-	-
642 II	-	-	-	-	-	-	-	-	-	-
643 I	-	-	-	-	-	-	-	-	-	-
643 II	-	-	-	-	-	-	-	-	-	-
645 I	-	-	-	-	-	-	-	-	-	-
646 II	-	-	-	-	-	-	-	-	-	-
647 II	-	-	-	-	-	-	-	-	-	-
650 II	-	-	-	-	-	-	-	-	-	-
651 I	-	-	-	-	-	-	-	-	-	-
651 II	-	-	-	-	-	-	-	-	-	-
655 II	-	-	-	-	-	-	-	-	-	-
656 I (b)	-	-	-	-	-	-	-	-	-	-
656 II	-	-	-	-	-	-	-	-	-	-
657 II	-	-	-	-	-	-	-	-	-	-
658 II	-	-	-	-	-	-	-	-	-	-
659 II	-	-	-	-	-	-	-	-	-	-
661 I	-	-	-	-	-	-	-	-	-	-
661 II	-	-	-	-	-	-	-	-	-	-
662 II	-	-	-	-	-	-	-	-	-	-
665 I	-	-	-	-	-	-	-	-	-	-
665 II	-	-	-	-	-	-	-	-	-	-
667 I	+-	+-	A	A	A					

LACTOSE FERMENTATION

	Days									
Number	1	2	3	4	5	6	7	8	9	10
674 I	-	-	-	-	-	-	-	-	-	-
682 I	-	-	-	-	-	-	-	-	-	-
683 I	-	-	-	-	-	-	-	-	-	-
693 I	-	-	-	-	-	-	-	-	-	-

TABLE V

ISOLATIONS OTHER THAN PROTEUS AND COLIFORM

SUCROSE FERMENTATION

SUCROSE FERMENTATION

	Days									
Number	1	2	3	4	5	6	7	8	9	10
597 II	-	-	-	-	-	-	-	-	-	-
598 I	AG	AG	AG	AG	AG	AG				
601 II	A	A	A	A	A	A				
602 II	-	-	-	-	-	-	-	-	-	-
602 A (s)	-	-	-	-	-	-	-	-	-	-
603 II	-	-	-	-	-	-	-	-	-	-
603 A (s)	-	-	-	-	-	-	-	-	-	-
P-1-I	-	-	-	-	-	-	-	-	-	-
P-1-II	-	-	-	-	-	-	-	-	-	-
P-2-I	AG	AG	AG	AG	AG	AG				
P-2-II	AG	AG	AG	AG	AG	AG				
608 I	-	+-	AG	AG	AG	AG				
610 II (s)	-	-	-	-	-	-	-	-	-	-
614 I	-	-	-	-	-	-	-	-	-	-
618 I (B)	-	-	-	-	-	-	-	-	-	-
622 II	-	-	-	-	-	-	-	-	-	-
623 II	-	-	-	-	-	-	-	-	-	-
624 I	-	-	-	-	-	-	-	-	-	-
624 II	-	-	-	-	-	-	-	-	-	-
625 II	-	-	-	-	-	-	-	-	-	-
637I	-	-	-	-	-	-	-	-	-	-

SUCROSE FERMENTATION

Number	Days 1	2	3	4	5	6	7	8	9	10
638 I	-	-	-	-	-	-	-	-	-	-
639 I	-	-	-	-	-	-	-	-	-	-
640 I	-	-	-	-	-	-	-	-	-	-
642 II	-	-	-	-	-	-	-	-	-	-
643 I	-	-	-	-	-	-	-	A		
643 II	-	-	-	-	-	-	-	A		
645 I	-	-	-	-	-	-	-	-	-	-
646 II	-	-	-	-	-	-	-	-	-	-
647 II	-	-	-	-	-	-	-	-	-	-
650 II	-	-	-	-	-	-	-	-	-	-
651 I	-	-	-	A	A	A				
651 II	-	-	-	-	-	-	-	-	-	+-
655 II	-	-	-	-	-	-	-	-	-	-
656 I (B)	-	-	-	-	-	-	-	-	-	-
656 II	-	A	A	AG	AG	AG				
657 II	-	-	-	-	-	-	-	-	-	+-
658 II	-	-	-	-	-	-	-	-	-	-
659 II	-	-	-	-	-	-	-	-	-	-
661 I	-	-	-	-	-	-	-	-	-	-
662 II	-	-	-	-	A	A				
665 I	-	-	-	-	-	-	-	-	-	-
665 II	-	-	-	-	-	-	-	-	-	-
667 I	-	-	-	-	-	-	-	-	-	-

SUCROSE FERMENTATION

Number	Days									
	1	2	3	4	5	6	7	8	9	10
674 I	-	-	-	-	-	-	-	-	-	-
682 I	-	-	-	-	-	-	-	-	-	-
683 I	-	-	-	-	-	-	-	-	-	-
693 I	-	-	-	-	-	-	-	-	-	-

TABLE VI

ISOLATIONS OTHER THAN PROTEUS AND COLIFORMS

HYDROGEN SULPHIDE, INDOL, AND MOTILITY

ISOLATIONS OTHER THAN PROTEUS AND COLIFORMS

Number	Hydrogen Sulphide	Indol	Motility
597 II	-	-	+
598 I	-	+	+
601 II	-	+-	+
602 II	-	-	+
602-A(S)	+	-	+
603 II	-	-	+
603-a(S)	+	-	+
P-1-I	-	-	+
P-1-II	-	-	+
P-2-I	-	+	+
P-2-II	-	+	+
608 I	-	-	+
610 II S	-	-	+
614 I	-	-	+
618 I B	+	-	+
622 II	+	-	+
623 I	+	-	+
623 II	+	-	+
624 I	+	-	+
624 II	+	-	+
625 II	+	-	+
637 I	+	-	+
638 I	+	-	+
639 I	+	-	+

ISOLATIONS OTHER THAN PROTEUS AND COLIFORM

Number	Hydrogen Sulphide	Indol	Motility
640 I	-	-	+
642 II	+	-	+
643 I	-	-	+
643 II	-	-	+
645 I	-	-	+
646 II	+	-	+
647 II	-	-	+
650 II	-	-	+
651 I	-	-	+
651 II	-	-	+
655 II	-	-	+
656 I B	-	-	+
656 II	-	-	+
657 II	-	-	+
658 II	-	-	+
659 II	-	-	+
661 I	-	-	+
661 II	-	-	+
662 II	-	-	+
665 I	-	-	+
665 II	-	-	+
667 I	-	-	+
674 I	-	-	+
682 I	-	-	+
683 I	-	+	+
693 I	-	-	+

TABLE VII

SALMONELLA ANTISERA AGGLUTINATIONS

ISOLATIONS OTHER THAN PROTEUS AND COLIFORM

SALMONELLA ANTISERA AGGLUTINATIONS

ISOLATIONS OTHER THAN PROTEUS AND COLIFORMS

Number	Polyvalent Sera	Grouping Sera					Species
		B	C_1	C_2	D	E_{1-3}	
597-II	-						Alcaligenes species
598-I	+-						Paracolon species (Escherichia)*
601-II	+-						Paracolon species (Escherichia)*
602-II	-						Paracolon species*
602-A(S)	+	+					Salmonella typhimurium*
603-II	-						Alcaligenes species
603-A(S)	+	+					Salmonella typhimurium*
P-1-I	-						Paracolon species*
P-1-II	-						Paracolon species
P-2-I	+-						Paracolon (Escherichia)*
P-2-II	±						Paracolon (Escherichia)*
608-I	-						Paracolon (Anaerogenic)
610-II(S)	-						Alcaligenes species
618-IB	+						Bethesda Paracolon
622-II	+	+					Salmonella typhimurium*
623-I	+	+					Salmonella typhimurium*
623-II	+		+				Salmonella newport*
624-I	+	+					Salmonella typhimurium*
624-II	+					+	Salmonella typhimurium**
625-II	+	+					Salmonella typhimurium*
637-I	+-						Bethesda Paracolon *
638-I	+		+				Salmonella newport*
639-I	+					+	Salmonella anatum*

* ---- Indicates species confirmed by Communicable Disease Center, USPH.

** --- Author not in agreement with this identification made by the Communicable Disease Center.

SALMONELLA ANTISERA AGGLUTINATIONS

ISOLATIONS OTHER THAN PROTEUS AND COLIFORM

Number	Polyvalent Sera	Grouping Sera					Species
		B	C_1	C_2	D	E_{1-3}	
640-I	+-						Bethesda Paracolon*
642-II	+					+	Salmonella anatum*
643-I	+-						Bethesda Paracolon*
643-II	+-						Bethesda Paracolon*
645-I	+-						Bethesda Paracolon*
646-II	+	+					Salmonella typhimurium*
647-II	-						Paracolon (Anaerogenic)
650-II	-						Paracolon (Anaerogenic)
651-I	+						Paracolon species *
651-II	+						Paracolon (Aerogenes)*
655-II	+-						Ballerup Paracolon*
656-IB	+						Aerogenes-like Paracolon*
656-II	-						Alcaligenes species
657-II	-						Paracolon (Aerobacter)*
658-II	+						Paracolon (Bethesda)
659-II	+						Paracolon (Bethesda)*
661-I	-						Biochemically typical of Salmonella
661-II	-						Biochemically typical of Salmonella
662-II	-						Paracolon (Anaerogenic)
665-I	-						Paracolon (Aerobacter)
665-II	-						Paracolon (Aerobacter)
667-I	-						Paracolon species
674-I	+						Paracolon (Bethesda)*
683-I	-						Paracolon (Anaerogenic)*
693-I	-						Biochemically typical for Salmonella

The 48 cultures suspected of being Salmonella or Paracolon were studied more thoroughly in an effort to separate them into one or the other group. First, each was inoculated into a series of sugar broths to determine their ability to metabolize salicin, maltose, dextrose, xylose, mannitol, lactose and sucrose. The results of this phase of the study are shown in Tables III, IV, and V. The same cultures were then inoculated into T.L.I. agar and peptone broth for the determination of their ability to produce hydrogen sulphide and indol. Motility as evidenced by swarming of the organisms through the semi-solid T.L.I. agar was noted. The results are recorded in Table VI. Having thus determined the biochemical characteristics of the organisms, the serological characteristics were determined next. These reactions are summarized in Table VII.

The above tables reveal certain points of interest. First, 12 isolates were classified as being definite species of Salmonella; second, 27 were classified as Paracolons; third, 4 were classified as Alcaligenes species; and fourth, 3 species resemble Salmonella biochemically but because of the negative reaction in poly-valent serum could not be classified. It is of interest to note that the twelve

cultures identified as Salmonella were found in a total of ten animals and that in one of these, No. 623, there was a double infection, the organisms being _Salmonella typhi-murium_ and _Salmonella newport_.

Having determined the identity of the 48 species studied, it seemed worthwhile to determine the distribution of the rats from which the cultures were isolated and to ascertain if there were any correlation between distribution and the positive carriers of Salmonella. Accordingly, the areas of the city from which these rats were trapped are shown in Figure 1. Each of the areas plotted is shown in greater detail in figures marked 2 through 10. The distribution of all animals trapped, and those positive for Salmonella or Paracolon are shown thereon with appropriate markings. The positive Salmonella cultures isolated in this study were found in three areas of the city; Figure 3 (plot 2), Figure 4 (plot 3), and Figure 10 (plot 9). Four positive animals, Nos. 622, 623, 624 and 625 were concentrated in an area of one square block, as shown in Plot 2. There were a total of 23 live animals captured in this area. Five positive animals were isolated in Plot 3 within a total distance of four blocks. A total of 29 animals were trapped in

FIGURE I

AREAS OF THE CITY IN WHICH ANIMALS WERE TRAPPED

CITY OF

RICHMOND

VIRGINIA

AND

ENVIRONS

DEPARTMENT OF PUBLIC WORKS

PLOT 2

PLOT 3

PLOT 6

PLOT 4

PLOT 1

PLOT 7

PLOT 8

PLOT 5

JAMES

RIVER

FIGURE II
(PLOT 1.)

Legend:

Red - Salmonella
Green - Paracolon
Yellow - Negative

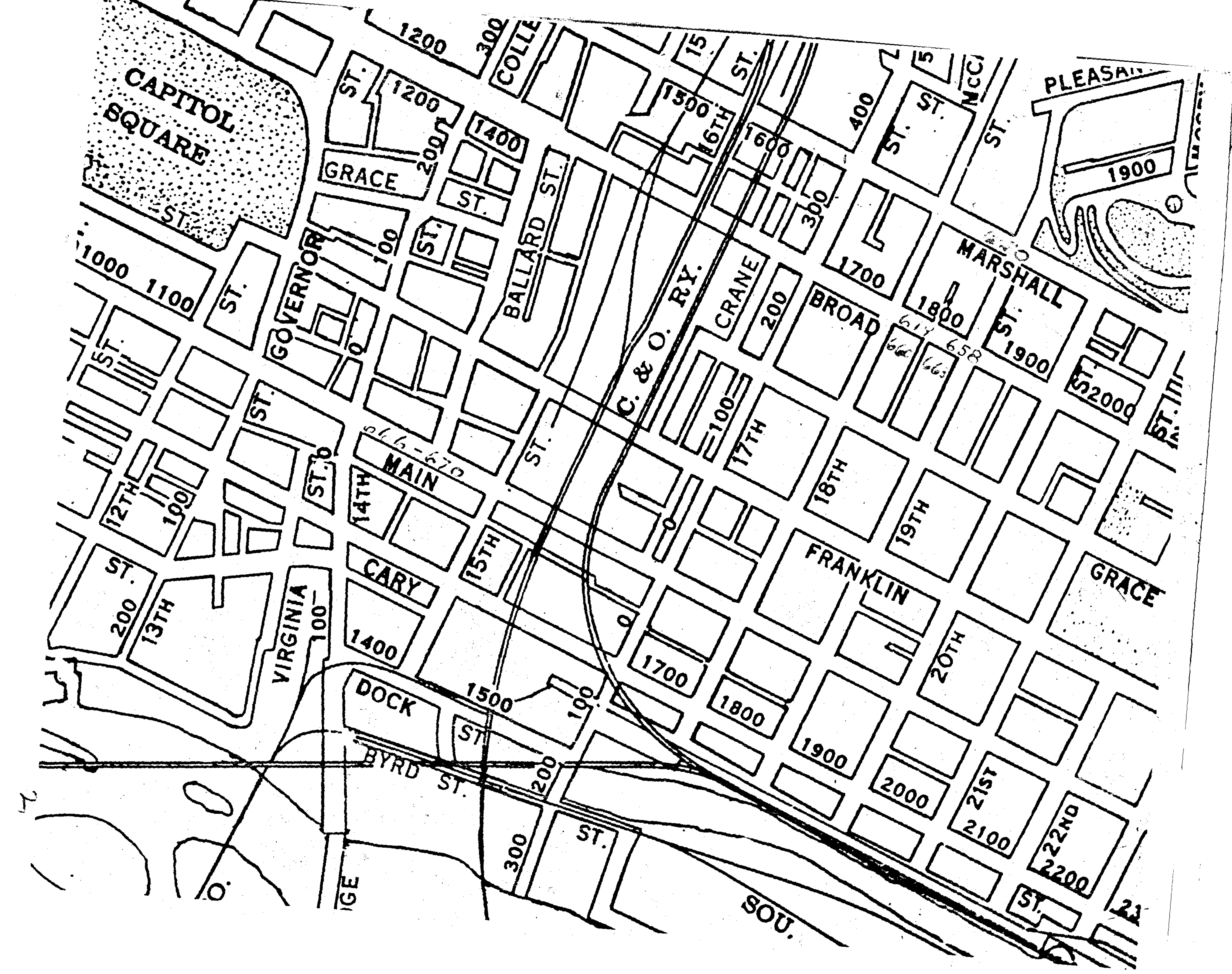
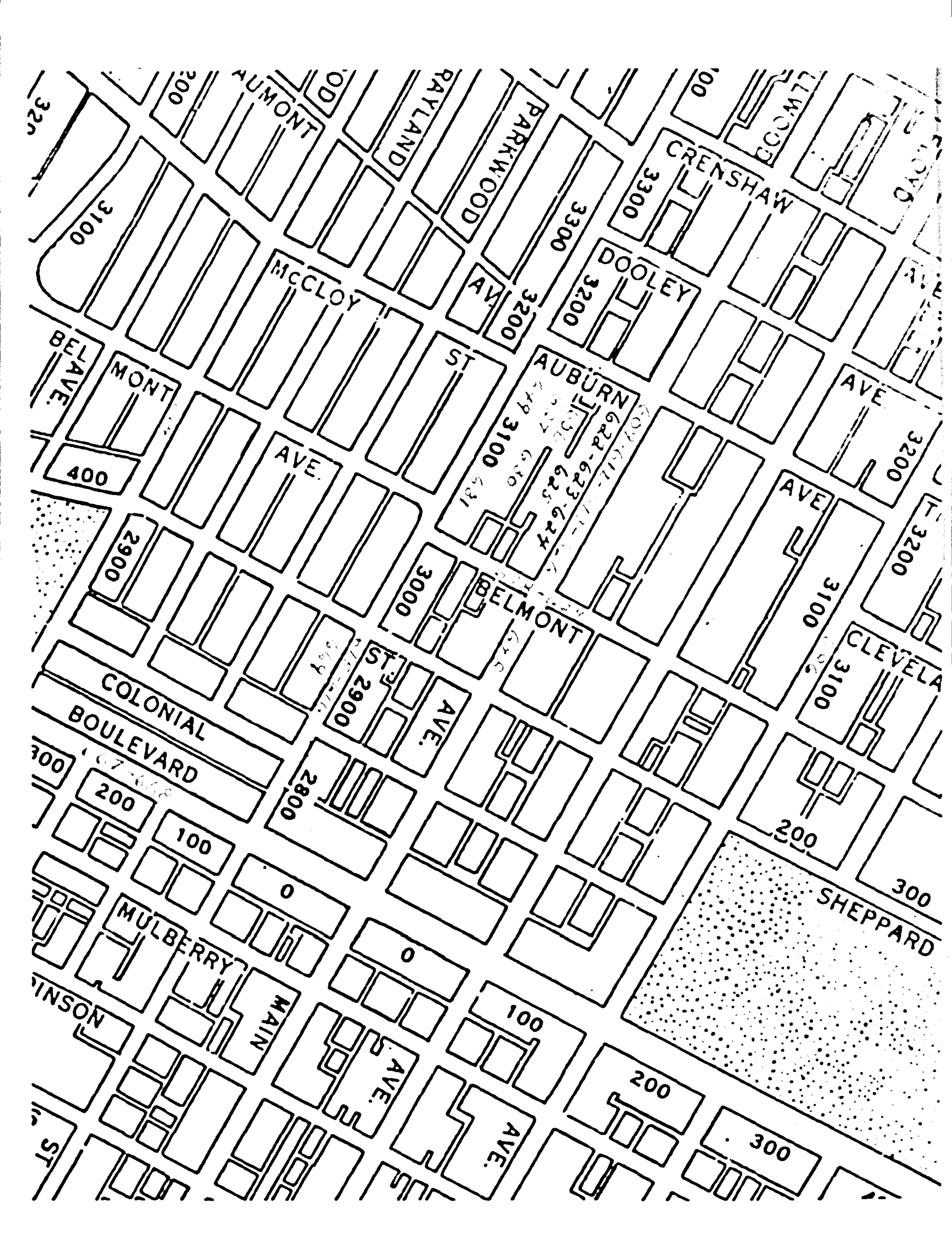

CAPITOL SQUARE
1200
300
COLLE
1200
1400
GRACE
BALLARD ST.
1500
16TH
1600
400
PLEASAN
1900
1000
1100
GOVERNOR
100
300
1700
MARSHALL
CRANE
200
BROAD
1800
1900
C. & O. RY.
2000
100
17TH
18TH
19TH
MAIN
14TH
12TH
15TH
CARY
FRANKLIN
GRACE
13TH
200
VIRGINIA
100
1400
1700
1800
20TH
DOCK
1500
100
1900
2000
21ST
2100
22ND
2200
BYRD ST.
200
300
SOU.

FIGURE III
(PLOT 2.)

Legend:
Red - Salmonella
Green - Paracolon
Yellow - Negative



RAYLAND
PARKWOOD
CRENSHAW
3300
3300
3100
MCCLOY
AV
3200
DOOLEY
3200
BEL AVE.
MONT
ST
AUBURN
3100
AVE
3200
400
AVE.
AVE.
T 3200
2900
3000
BELMONT
3100
ST.
2900
CLEVELA
3100
COLONIAL
BOULEVARD
AVE.
300
200
2800
100
200
0
300
SHEPPARD
MULBERRY
0
MAIN
100
AVE.
200
AVE.
300
ST

FIGURE IV
(PLOT 3.)

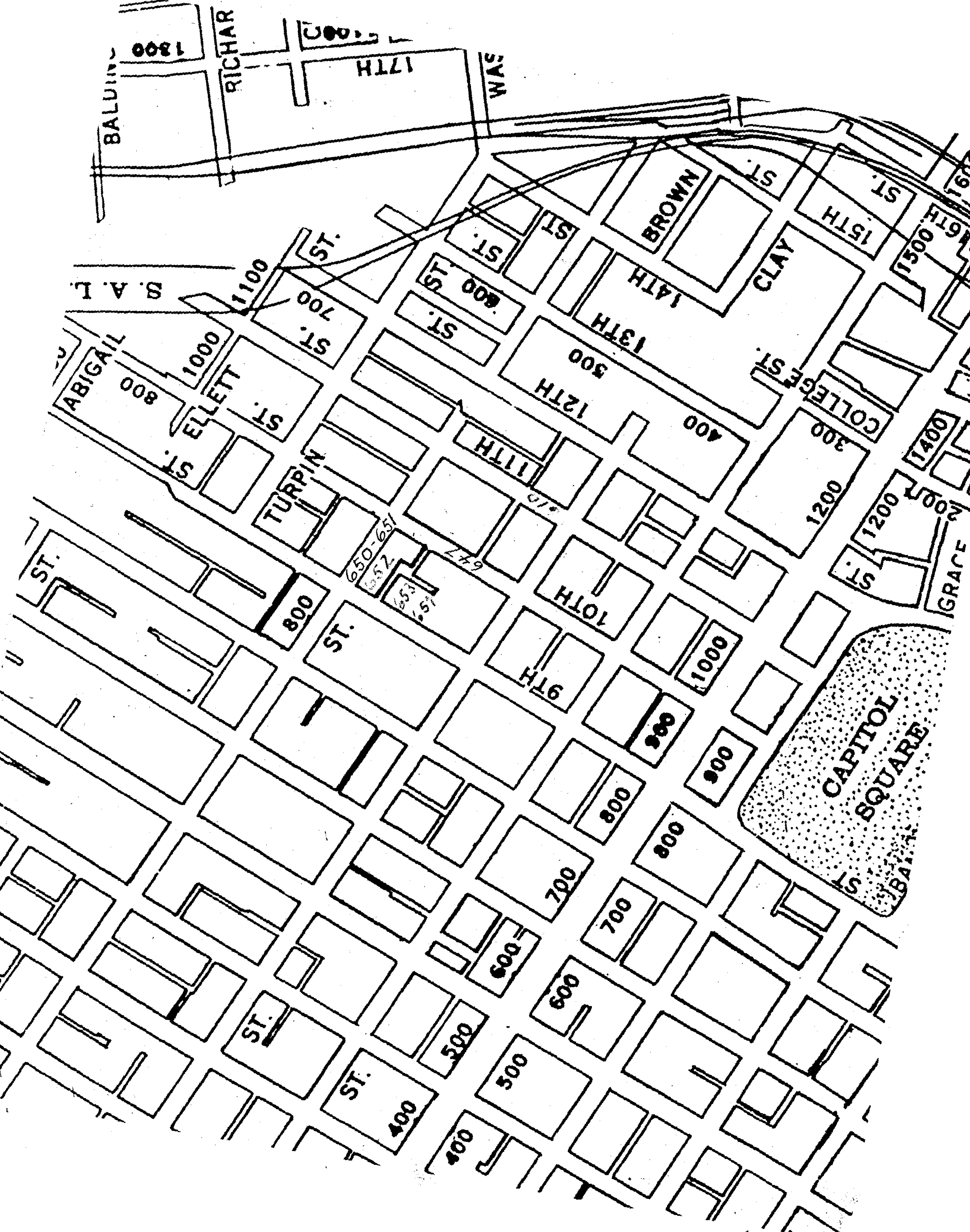
Legend:

Red - Salmonella
Green - Paracolon
Yellow - Negative

MEADOW
700
2000
SHIELDS
WINDER
BLAIR
CLAIBORNE
AVE.
AVE.
ROWLAND
ST.
ALLEN
GRANBY
ST.
ST.
KEMPER
JACQUELIN
IDLEWOOD
PARKWOOD
1800
1800
TEMPLE
1700
1700
AVE
ST
ST
1600
1600
VINE
ST.
700
600
500
ST.
LOMBARDY
LADY
1500
1500
GROVE
ST.
AVE.
1200
DANCE
1300
WALLACE
AVE.
GRAYLAND
ST.
CARY
1400
PLUM
1400
MAIN
FLOYD
AVE.
ST.
1200
900
HARVIE
ST.
1300
BRUNSWICK
1300
100
ST.
1400
1500
WEST
HARRISON
400
300
AVE.
200
1200
1200
ST.
ST.
200
BIRCH
ST.
DOBSON ST.
100
MORRIS
0
ST. 1100
1000
AVE.
1100
1100
AVE.
BOYD
FRANKLIN
BEECH
1000
ST.
ST.
AVE. 1000
300
200
1000
AVE.
1000
HARRISON
LINDEN
900
IDLEWOOD
CUMBERLAND
ST.
AVE.
900
900
400
ALBEMARLE
ST.
ST.
200
800
SHAFER
800
300
900

FIGURE V
(PLOT 4.)

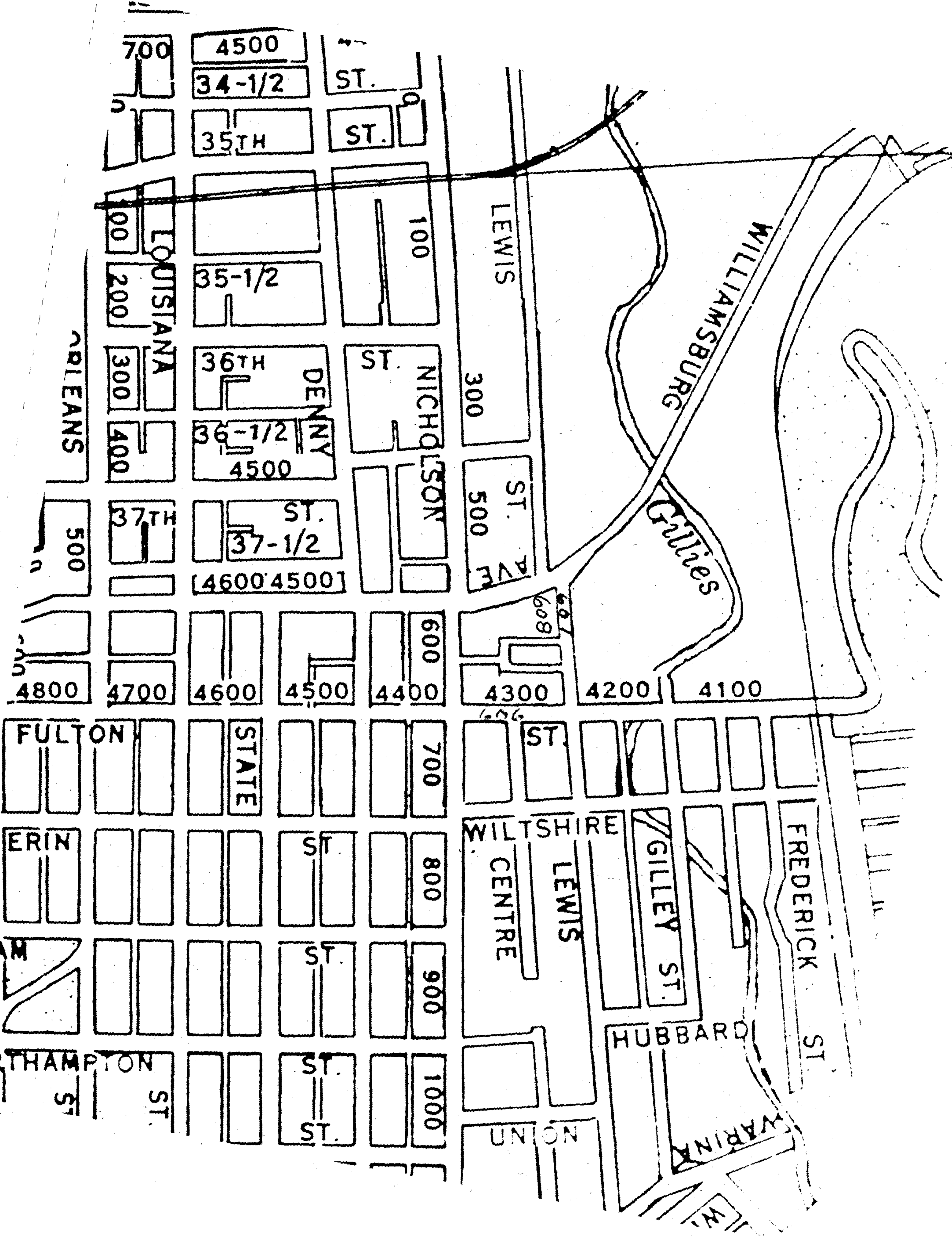
Legend:
Red - Salmonella
Green - Paracolon
Yellow - Negative



1300
RICHAR
17TH
S.A.L.
ABIGAIL
800
1000
1100
ELLETT ST.
700
ST.
TURPIN ST.
BROWN ST.
CLAY ST.
14TH ST.
13TH ST.
12TH ST.
11TH ST.
10TH ST.
9TH ST.
15TH ST.
1500
1400
1200
300
400
COLLEGE ST.
GRACE ST.
900
800
700
600
500
400
CAPITOL SQUARE
650-651
652
647

FIGURE VI
(PLOT 5.)

Legend:
Red - Salmonella
Green - Paracolon
Yellow - Negative



4500
34-1/2
ST.
35TH
ST.
LOUISIANA
100
LEWIS
WILLIAMSBURG
35-1/2
200
ORLEANS
300
36TH
ST.
NICHOLSON
DENNY
300
36-1/2
4500
400
500
ST.
AVE
37TH
ST.
37-1/2
500
4600 4500
Gillies
608
600
4800
4700
4600
4500
4400
4300
4200
4100
FULTON
STATE
ST.
700
WILTSHIRE
ERIN
ST
800
CENTRE
LEWIS
GILLEY
FREDERICK
ST.
900
ST.
HUBBARD
ST
THAMPTON
ST.
ST
1000
ST.
UNION

FIGURE VII
(PLOT 6.)

Legend:

Red - Salmonella
Green - Paracolon
Yellow - Negative

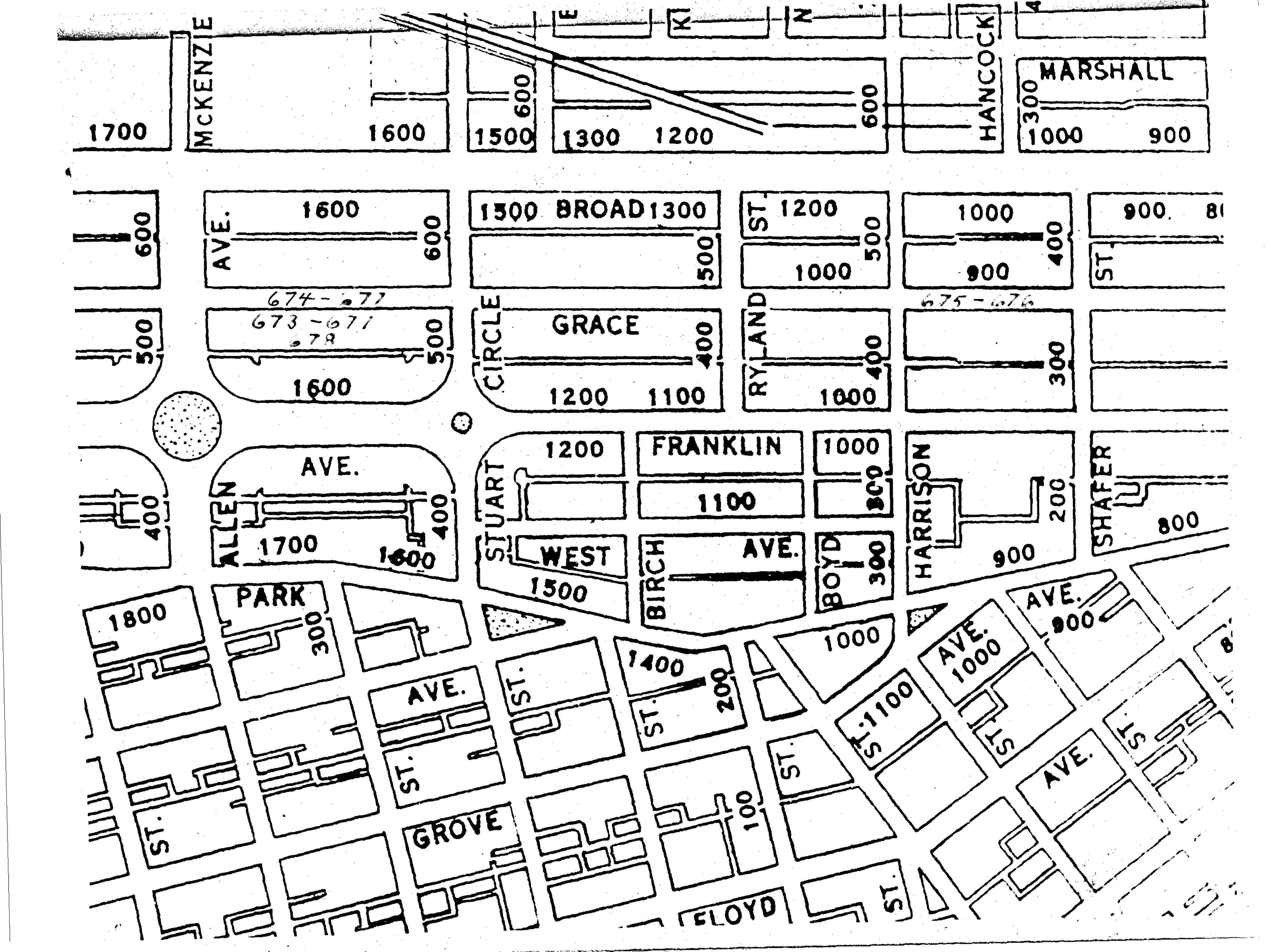
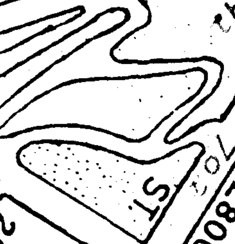

McKENZIE
HANCOCK
MARSHALL
1700
1600
1500
600
1300
1200
600
300
1000
900
BROAD
1600
1500
1300
1200
1000
900
800
AVE.
600
500
1000
400
ST.
674-677
673-677
678
675-676
GRACE
CIRCLE
RYLAND
1100
FRANKLIN
AVE.
STUART
ALLEN
1700
WEST
BIRCH
BOYD
HARRISON
SHAFER
200
PARK
1800
1400
100
GROVE
FLOYD
ST. 1100

FIGURE VIII
(PLOT 7.)

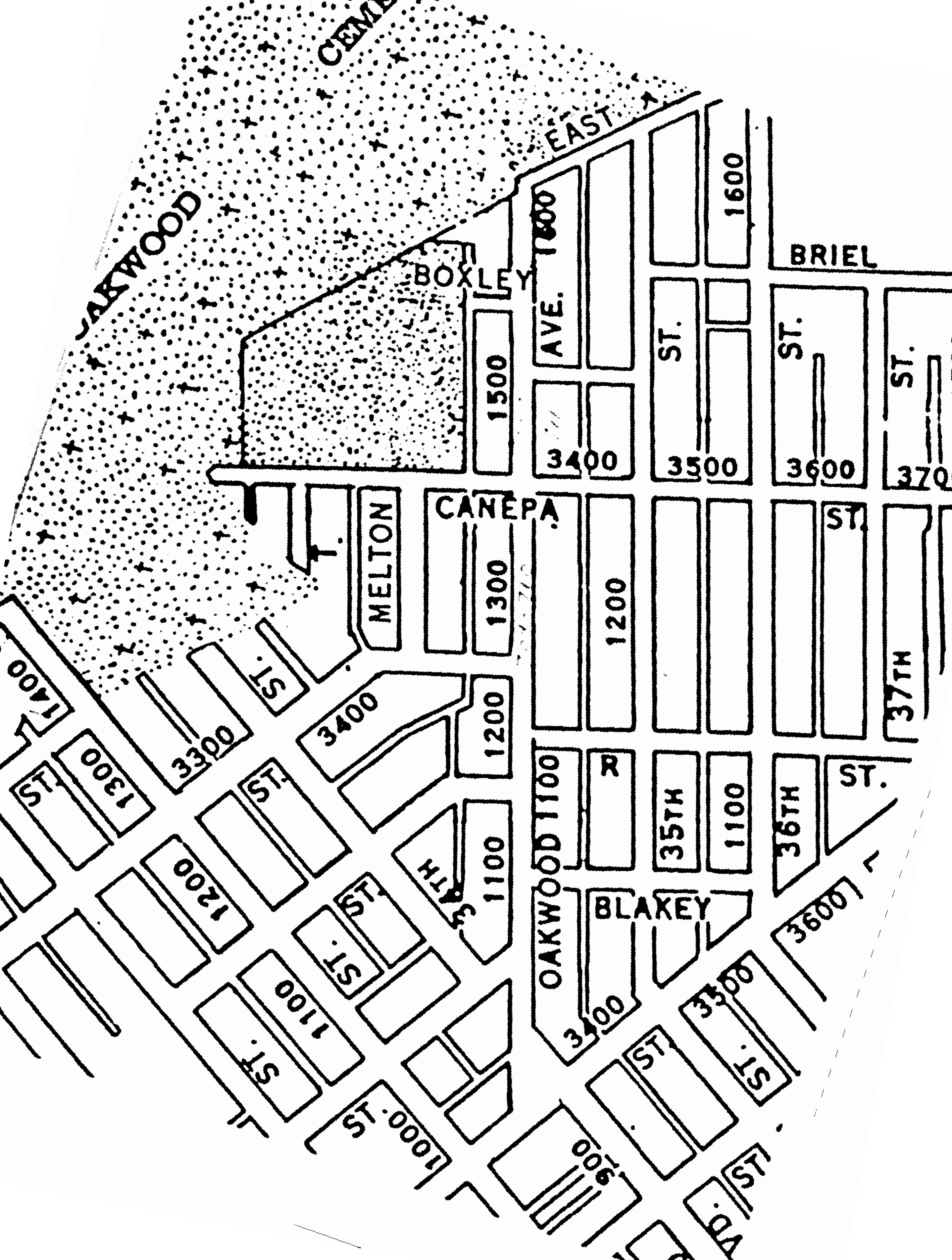
Legend:
Red - Salmonella
Green - Paracolon
Yellow - Negative



24TH
25TH
26TH
27TH
28TH
29TH
30TH
31ST
32ND
33RD
35TH
CLAY
LEIGH
M
N
AVE.
ERSON
CHIMBORAZO
ORAZO
ST
ST.
2600
2700
2800
3200
3300
3400
100
200
300
400
500
600

FIGURE IX
(PLOT 8.)

Legend:
Red - Salmonella
Green - Paracolon
Yellow - Negative

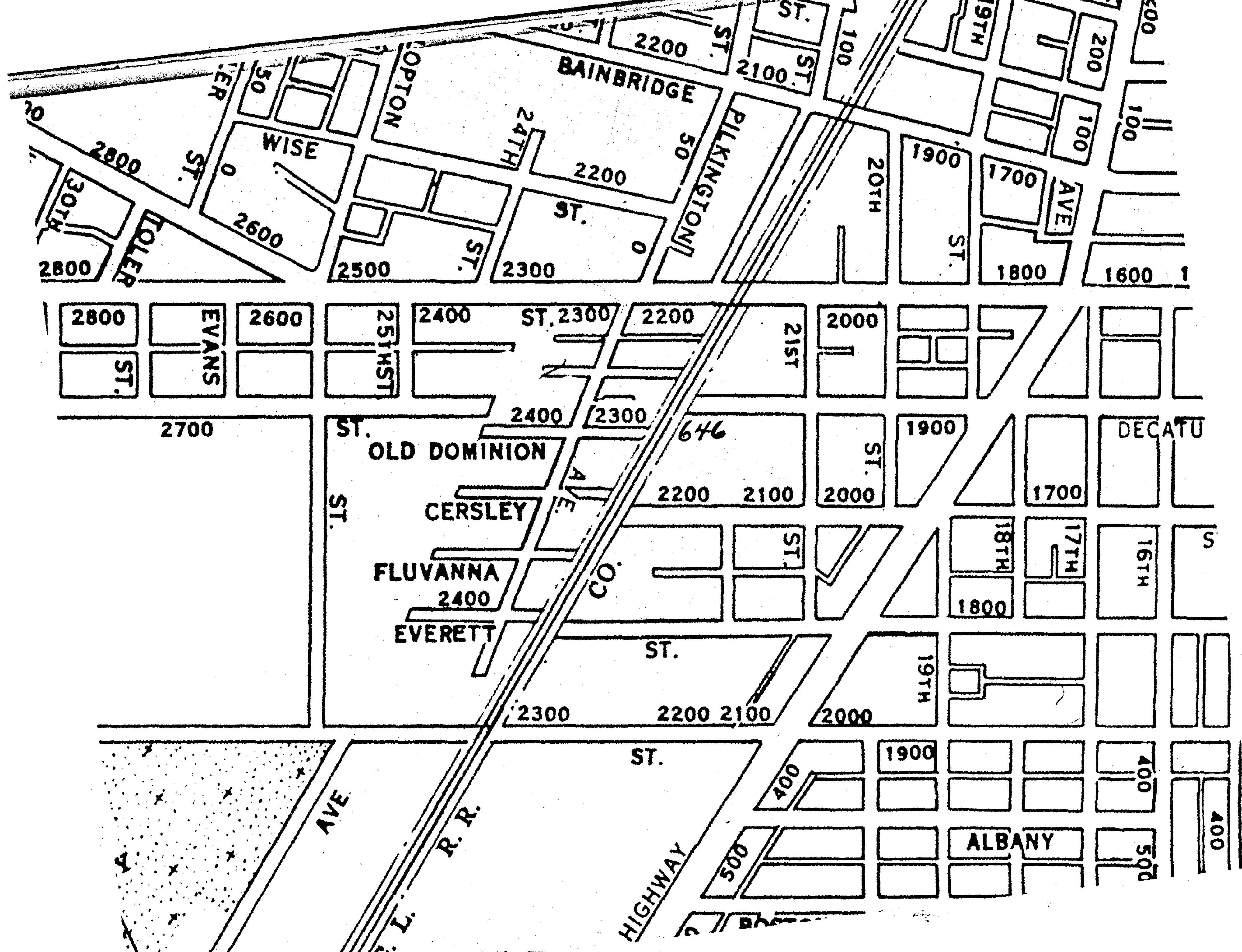


OAKWOOD
EAST
BOXLEY
AVE.
1600
BRIEL
ST.
1500
3400
3500
3600
CANEPA
ST.
MELTON
1300
1200
37TH
3300
3400
1400
1300
1200
1100
OAKWOOD
R
35TH
36TH
BLAKEY
3600
3500
3400
1000
900

FIGURE X

Legend:

Red - Salmonella
Green - Paracolon
Yellow - Negative


BAINBRIDGE
WISE
PILKINGTON
TOLER
EVANS ST.
25TH ST.
24TH
20TH
21ST
OLD DOMINION
CERSLEY
FLUVANNA
EVERETT
ST.
AVE.
CO.
646
DECATU
18TH
17TH
16TH
19TH
ALBANY
HIGHWAY
R. R.
AVE
2800
2700
2600
2500
2400
2300
2200
2100
2000
1900
1800
1700
1600
500
400
200
100

this plot. One additional positive animal was found in plot 9 but this animal was trapped at the end of this study and is the single sample from the area.

In addition to the animals found positive for Salmonella in these areas, Plot 3 also showed a very high concentration of animals positive for Paracolon species. Ten positive animals occurred in the group of 29 trapped. In this particular district, better than 50% of all animals sampled were infected with either Salmonella or Paracolon organisms.

All of the species of Salmonella that were found in this survey have been previously reported from rats, but not in this country. _Salmonella newport_ was reported by Khalil (24) in England but this species has not been reported in this country in the brown rat. The frequent occurrence of _Salmonella typhi-murium_ was expected, but _Salmonella anatum_ has not been reported many times in rats.

Of the Paracolon identified, the Bethesda and the Ballerup groups were unusual. Neither of these groups have been previously reported from rats. The Bethesda group has been reported as a possible human pathogen by Barnes and Cherry (3) and the Ballerup Paracolon as classified by Edwards (14) was formerly classified as

a Salmonella. Salmonella ballerup as classified by Breed (5) has been reported only from a case of human gastroenteritis in Denmark.

CONCLUSIONS

It seems evident from this study that the percentage of rats infected with Salmonella in Richmond, Virginia, is considerably higher than has recently been reported by others in this country, although in close agreement with reports of observations made in England.

The greater percentage of positive infections reported here may be due to several factors. First, and possibly most important, the animals used in this study were all living when brought into the laboratory. Consequently, any possible post-mortem changes were obviated. Second, the entire gut of the animal was removed and cultured using more sensitive and modern differential media than has been available in most of the previous surveys. In support of this latter point, it may be well to point out that 6, or 50%, of the positive Salmonella cultures were isolated from enrichment media and not detected at all on preliminary culture.

This investigation has confirmed the occurrence of *Salmonella typhi-murium*, *Salmonella anatum* and *Salmonella newport* in rats and has shown a rather clear-cut distribution of the infected animals.

No data, except the findings of Hulphers and Hinricson (18), have been published concerning the occurrence and per-

centage of infections with Paracolon organisms. The occurrence of the Bethesda and the Ballerup groups, with their probable pathogenicity to humans, and the frequent occurrence of other ill-defined Paracolons, with their common somatic antigens with the Salmonella, would benefit from study from the viewpoint of the transmission of human pathogens.

The large number of Paracolons reported is due primarily to delibertately isolating cultures that gave preliminary tests for the group and identifying them where possible. Previously these cultures when isolated were often simply discarded as aberrant Coliforms.

Finally, this study has served to emphasize the fact that the rat is a potentially dangerous vector of human enteric disease organisms, and that special effort should be made to control these rodents in the interest of the human population.

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Upon release from the Army I accepted a position as Bacteriologist with Froehling and Robertson, Richmond, Virginia. While working with this company I entered Richmond College in 1947, receiving the Bachelor of Arts degree in 1949. In September, 1949, I entered the Graduate School of the University of Richmond and was appointed a Graduate Fellow in the Department of Biology. In March, 1950, I accepted the Position of Principal Bacteriologist, Bureau of Laboratories, City of Richmond Department of Health.