QUANTITATIVE   EXPERIMENTS   IN   URINARY

ANTISEPSIS.

Ian Lawson Dick.

CONTENTS

INTRODUCTION.

Infection of the urinary tract with the
Bacillus coli may occur suddenly and without warning,
giving rise to acute pyelitis or, less commonly,
acute cystitis. The illness is an acute febrile
one but it commonly subsides under the simplest of
treatment - rest in bed and the administration of
bland fluids. When it does not subside, the
perpetuating cause may be found in some coincident
pathological process in the urinary tract of the
nature of a calculus or a hydronephrosis. Exami-
nation should be made for these or some other cause
of the chronicity of the condition, and if a gross
pathological cause is found, the further treatment
will be in the hands of the operating surgeon.
Sometimes the removal of the stone or other
obstructing agent from the renal pelvis or ureter
is sufficient to allow of the subsidence of the
infection, but the infection will not subside while
the obstructing agent is still present.

Brewer (4) has shown that acute pyelitis
is in fact acute pyelonephritis, and that there is
always some infection of the renal parenchyma in
this condition. The area of the parenchyma affected
is/

is the medulla - the part composed of the collecting
tubules. Morrison (38) showed that, in the
laboratory animal, if there is any stasis in the
renal pelvis or obstruction to the outflow of urine,
the dissemination of the infection throughout the
kidney parenchyma is widespread and rapidly gives
rise to abscess formation. This is the "acute
secondary suppurative pyelonephritis" of the patho-
logist. In the latter case, even if the patient
recovers from this serious condition, the kidney
will be reduced to a disorganised, permanently
infected, almost functionless remnant, and the
treatment indicated will be the surgical removal of
the organ.

There are three possible modes of termi-
nation of the first condition. (1) As has already
been mentioned, the condition may resolve completely.
(2) Small abscesses may form and gradually spread
until the major part of the kidney is destroyed.
The treatment indicated is again excision of the
kidney. (3) The kidney may in the main recover from
the infection, and, as judged by its function, be a
useful organ. The urine, however, coming from this
kidney is persistently infected with B. coli, and the
patient has the symptoms and shows the signs of
chronic pyelitis. This has been shown by Band (1)
and/

and others to be a chronic pyelonephritis, and to be associated with the presence of small discrete residual abscesses in the renal parenchyma, from which the infection of the renal pelvis, ureter and bladder is being constantly renewed and maintained.

This third type of condition is that which goes by the name of chronic pyelitis, and it is in this condition, and particularly in the earlier stages of this condition, that the use of urinary antiseptics is most likely to be attended with success. The large number of drugs and preparations which have been used and are used as urinary anti-septics in itself shows that no one preparation is preeminently satisfactory. Each drug or method has in its turn found its supporters, who have made for it extravagant claims which were not upheld by the general experience of the profession. The object of this work is to examine and to attempt to assess the value of some of the methods which are used in the treatment of infections of the urinary tract.

The points which have been investigated are:

1. the effect of the administration of sodium citrate on the acidity of the urine;

2. the effect of alteration of pH on the growth of B. coli in urine;

3. the effect of addition of hexamine in vitro on the growth of B. coli in urine;

4. the effect of the oral administration of hexyl resorcinol on the growth of B. coli in urine;

5/

5.   the effect of the oral administration of
        amyl meta cresol on the growth of B.
        coli in urine;

6.   the growth of B. coli in ketonurine.

1. <u>Sodium citrate</u>.   It has for long been the
practice in infection of the urinary tract to
attempt to treat the condition by alteration of
the reaction of the urine. Particularly popular
has been the administration of urinary alkalies
in the treatment of B. coli infections, because
with the achievement of alkalinity of the urine
to litmus the symptoms of dysuria and frequency
disappear. It was, however, pointed out by
Thomson Walker (50) as long ago as 1913 that
this improvement was merely symptomatic, and
that some other agent was required to sterilise
the urine. This observation has been corro-
borated by many subsequent workers. The re-
action of the urine in the subject whose meta-
bolism is normal can be influenced by (1) Diet,
and (2) Drugs.

      The reaction of the urine of a
person on an ordinary mixed diet is slightly
acid. After a meal, the secretion of HCl into
the stomach necessitates that the kidneys shall
excrete some compensatory alkali, and the urine
secreted/

secreted at this time may have an alkaline re-
action. In the main, however, the reaction is
acid.

Osman (40) and Lyon, Dunlop and
Stewart (36) showed that the acidity of the
urine can be very greatly reduced by the
administration of a basic diet, that is, a diet
in which the ash of the protein is alkaline in
reaction. It has been known since 1812 (2)
that the oral administration of alkalies such as
sodium bicarbonate, or of alkaline salts such
as citrates or acetates which are broken down in
the body to bicarbonate and excreted as such in
the urine, also has the effect of reducing
urinary acidity. The degree of alkalinity which
can be attained is seldom, if ever, as great as
pH 9 (1) and in the present experiments, by a
combination of a rigidly basic diet and enormous
doses of sodium citrate, I was unable to produce
an alkalinity greater than pH 8.3 (Table II).

The acidity of the urine can be
increased by means of a diet whose protein has
an acid ash (40), (35) or which is productive of
a large amount of organic acids as is a keto-
genic/

genic diet (24), (1). Hutchison in 1903
investigated the efficacy of various drugs in
acidifying the urine, and introduced sodium
dihydrogen phosphate or acid sodium phosphate
as the natural and most suitable substance for
the purpose. It has ever since enjoyed an
almost unrivalled popularity as a urinary
acidifier, but some considerable doubt was
thrown upon its efficacy by Stockman (48). He
pointed out that it is very irregular and un-
certain in its action. This uncertainty is
due to the fact that it is very irregularly
absorbed owing to its affinity for calcium,
with which it forms an insoluble salt in the
gut.(Percival and Stewart (41)). It is
absorbed, then, in inverse proportion to the
amount of calcium present in the gut which is
a condition which cannot be easily controlled.
Acid sodium phosphate cannot have any great
effect on the pH of the urine even when it is
excreted by the kidneys, because it is a weak-
ly dissociated or "buffer" salt. The main
result of its use is that the titratable
acidity of the urine is raised, and therefore
the urine is rendered less easy to neutralise
or/

or to render alkaline. This was also demon-
strated by Stockman (48). Johnston (31) in-
vestigated the power of benzoic acid to
increase the acidity of the urine, and arrived
at the same conclusion, that while it was
possible to increase the total or titratable
acidity, very little effect was produced upon
the hydrogen ion concentration or real acidity.
It is the pH, or hydrogen ion concentration, or
true acidity, and not the titratable acidity of
the urine which influences bacterial growth
(Topley and Wilson (51)).

The most potent urinary acidifiers are
the acid salts such as ammonium chloride, NH_4 Cl,
or calcium chloride, Ca Cl_2. In the case of
ammonium chloride the ammonium $(NH_4^{\cdot})$ group is
synthesised by the liver into urea, and Hydro-
chloric Acid is liberated according to the
formula:

$$2NH_4 \; Cl + H_2 \; CO_3 = CO \; (NH_2)_2 + 2H_2O + HCl. \quad (21)$$
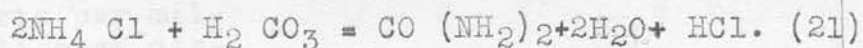
In the case of calcium chloride the calcium is
largely precipitated in the gut as insoluble
calcium carbonate, and the acid Cl˙ ion is alone
absorbed (20).

Even/

Even with the use of these powerful "acid salts" Dunlop (1) was not able to increase the acidity of the urine to more than pH 5.

2. Effect of pH on Growth of B. coli. That the disinfectant action of mineral acids is proportional to their degree of dissociation was shown by Kronig and Paul (33) in 1897. Later, Winslow and Lochridge (55) showed that this disinfectant action is in proportion to the pH or true acidity and not to the total acidity of the solutions. They compared the strengths of two acids required to effect a 100% destruction of B. coli in forty minutes. They used Hydrochloric Acid and Sulphuric Acid, and the results appear in the following table:

	$\underline{HCl}$	$\underline{H_2SO_4}$.
	100% reduction of B. coli in 40 minutes.	
Normality	0.0123	0.0166.
Degree of dissociation	96.4%	76.0%.
Parts per million of dissociated hydrogen	12.8	12.6.

The normality of HCl is 0.0123, while that of H_2SO_4 is 0.0166. The greater degree of dissociation of HCl, however, means that the final concentration of hydrogen ions in the two solutions/

solutions is approximately the same. This
does not hold in the case of some organic
acids in which the antiseptic action may be a
property of the whole molecule or of some
grouping within the molecule, e.g. the phenol
group in carbolic acid.

Topley and Wilson (51) state that
the optimum hydrogen ion concentration for
growth of B. coli in a nutrient medium is
about pH 7.6, for survival is pH 6.0, and that
the lowest limit of their acid tolerance is
pH 4.6. Similarly for alkalies, Cohen (14)
showed that the alkaline limit for survival was
pH 8.7. These figures are for B. coli in pure
nutrient media.

In the case of the urine there is also
the question of salt action. Winslow and
Hotchkiss (54) showed that for pure solutions
of salt there is a stimulating concentration
and an inhibiting concentration - for sodium
chloride 0.5 molar and 4.0 molar respectively.
The concentration of sodium chloride in the
urine could never reach 4.0 molar, but it might
approach 0.5 molar which is the stimulating
concentration. Mixtures of salts were studied
by/

by Flexner (19) in 1907, and by subsequent
workers, and it has been found that there is
a very varied antagonistic effect of solutions
of salts upon one another which alter with
differing concentrations of the salts. For
example, Winslow and Falk (53) showed that a
solution of 0.145 M. Ca Cl_2 + 0.29 M.Na Cl is
highly toxic to B. coli. If the proportion of
Na Cl is increased and the solution is made
0.145 M. Ca Cl_2 + 0.68 M.Na Cl the solution is
non-toxic. The solution is again rendered
toxic to B. coli by a further increase of Na Cl.
For a solution containing only two salts of
known concentration it is possible to state
what effect upon bacterial growth will be
produced. For a variable mixture of numerous
salts it is impossible to control or estimate
the effect of the salts on bacterial growth.
The effect of salt action per se on the growth
of B. coli in urine must thus be disregarded.

One factor which does operate, however,
is the influence of the salts, particularly of
the Na Cl, on the effect of the pH of the urine
on bacterial growth. Sherman and Holm (45)
showed that the addition to a nutrient medium
of/

of Na Cl in 0.6 - 0.3 M. concentration widened
the range within which B. coli would grow in
that medium. This accounts for the fact that B.
coli have occasionally been shown to survive in
urine of pH 4.8 or even more acid.

Jordan (32) in 1913 in a review of the
question of urinary antiseptics studied the
effect of acidity on the growth of organisms in
urine. His standard was the titratable acidity
and not the pH or true acidity; he also made
the time of putrefaction of urine passed into
non- sterile vessels his criterion of the
efficacy of his antiseptic measures. This putre-
faction was shown by Stockman (48) to be entirely
dependent upon chance external contamination and
not a reliable experimental method. In his
paper Jordan states that B. coli grow more
readily in acid urine than in alkaline urine, an
observation which I was unable to confirm. In a
later paper Sohl and Janney (46) found that the
growth of B. coli was inhibited in urine between
pH 4.2 - pH 4.8 and pH 9.2 - pH 9.6, but that
they grew in urine between pH 5 and pH 9.

3. Hexamine   Hexamine, $N_4(CH_2)_6$, was introduced to
medicine as a urinary antiseptic by Nicolaier(39)
in/

in 1894. Its action is dependent upon the fact
that, in the presence of acid, it is hydrolysed
to formaldehyde, HCHO, according to the equation:

$$N_4 (CH_2)_6 + 6 H_2O \rightleftharpoons 4 NH_3 + 6 HCHO.$$
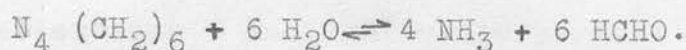
The reaction is a reversible one, and the end-
point is dependent upon the hydrogen ion con-
centration of the solution. Sollmann (47) gives
the following as the percentages of hexamine
hydrolysed to formaldehyde at body temperature
in aqueous solutions at various hydrogen ion
concentrations:

| pH | Equivalent to | percentage of hexamine hydrolysed. | |
		in 2 hours	in 6 hours.
2	Gastric juice	60-100	100.
5	Urine	25	35
7.2	Blood	0.35	0.8

The amount of formaldehyde liberated
in the urine as a result of the oral admini-
stration of a constant dose of hexamine is very
variable. This variation depends upon two facts:-

1. An inconstant amount of hexamine is hydro-
lysed in the stomach according to (a) the
degree of gastric acidity, and (b) the length
of stay of the hexamine in the stomach. Thus
when hexamine is given before a meal its stay
in the stomach is short and the gastric acidity
is low. In these circumstances a larger pro-
portion/

portion of hexamine will leave the stomach
unchanged and will be absorbed than when the
hexamine is given after a meal. In the
latter case a much larger proportion will be
hydrolysed in the stomach and not be absorbed
because the delay in the stomach is longer
and the gastric acidity is higher.

2. A variable amount of the hexamine that
does reach the urine is hydrolysed, the
amount depending upon the pH of the urine.

Stockman states that a concentration
of formaldehyde of 1/5000 is not strong enough
to be bactericidal, but Hare, Lepper and Mart-
land (22) found that a concentration of 1/20,000
of formaldehyde in broth was sufficient to
inhibit the growth of B. coli for forty eight
hours, and that a concentration of 1/10,000 was
bactericidal in that time.

The estimation of the percentage of
formaldehyde in the urine apart from the hexa-
mine is a very difficult chemical problem. In
these experiments therefore, I added hexamine
in various concentrations to acid urine in vitro,
and estimated its effect on the growth of B.
coli./

coli. The concentration of hexamine in the
urine of a patient could be controlled, within
limits, by standardising as far as possible the
urinary output, and giving the required amount
of hexamine in solution intravenously once or
twice a day. Administered in this way all the
hexamine given would be excreted into the urine
unchanged. The estimation of the bactericidal
properties of such a urine by the method to be
described would, however, be inaccurate, because
after the urine was passed and during incubation
an increasing amount of formaldehyde would be
constantly liberated, and the bactericidal powers
of the urine would consequently increase during
the course of the experiment. This method was
accordingly not adopted.

4. Hexyl resorcinol.   Johnson and Hodge (29) and
Johnson and Lane (30) showed that the effect of
the addition of an alkyl side-chain to the resor-
cinol molecule was (1) to increase its bacteri-
cidal power as measured by its phenol coefficient,
and (2) to decrease its toxicity to animals. The
bactericidal power is further increased and the
toxicity further diminished as the number of car-
bon atoms in the alkyl side-chain is increased,
and/

and the maximum bactericidal power combined with
the minimum toxicity is reached in the substance
n - hexyl resorcinol

$$\text{(structure: benzene ring with OH and OH)}$$

$CH_2 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CH_3:$

        This substance has a phenol coefficient
of 46 - one hundred and fifty times greater than
that of resorcinol - and is almost completely
non-toxic to animals.

        Leonard in 1924 (34), (35) found that
this substance is largely excreted in the urine,
and advocated its use as a urinary antiseptic.
No toxic symptoms followed on the oral admini-
stration of hexyl resorcinol in doses of 0.6 gm.-
1.35 gm. in the day with the exception of some
slight griping and looseness of the bowels. He
found that during its administration to men in
such doses, 61% of the urine passed by them was
bactericidal. He further found that in some of
the urines which had no bactericidal action, the
presence of the drug could be demonstrated by a
chemical test. He explained this discrepancy by
the hypothesis that hexyl resorcinol is excreted
in the urine both as active hexyl resorcinol and
in/

in some inactive "conjugated" form. None the
less, bactericidal urine was passed in a large
percentage of cases, and his results following
its clinical use were very encouraging.

Similar favourable reports of the value
of hexyl resorcinol as a urinary antiseptic were
made by several other observers, including
Henline (25), Braasch and Cathcart (3), and
Brown (6), (7). In 1927 Eberbach and Arn (18)
reported a large series of cases of chronic in-
fection of the urinary tract treated with hexyl
resorcinol. Their results were less encouraging.
Ten to twelve percent of the cases were "cured".
It is not stated for how long the cure persisted.
Many of these cases had never previously had
systematic hospital treatment. They further
point out that most of their successes were in
cases in which the infection was not of very
long standing. The average duration of the
symptoms in the cases that were improved was
nine and a half months, while in the cases which
were not improved the average duration of
symptoms was twenty months.

Robbins and Wesson (44) designed a
method for the quantitative chemical estimation
of hexyl resorcinol. Using this method Robbins
(43)/

(43) estimated the amount of hexyl resorcinol
passed in the urine of patients following on its
oral administration. He found that (1) when
hexyl resorcinol is given by mouth in doses of
1 gm. in the day, twenty nine per cent only is
secreted in the urine, the remainder being passed
in the faeces; (2) increase in the oral dose
produces only a slight increase in the amount
secreted in the urine; (3) of the hexyl resorcinol
passed in the urine by far the greater part (about
ninety five per cent) is in the form of the inert
conjugate.

5. Amyl meta cresol.   In a paper published in the
Journal of the Chemical Society in 1930 on "The
variations of Phenol coefficients in a homologous
series of Cresols", Coulthard, Marshall and Pyman
(16) say "Amyl meta cresol has been found to have
a high phenol coefficient. Since in addition its
toxicity is low it may be of some use in medicine."
Coulthard (15) later showed that when this sub-
stance is given by the mouth it is excreted in the
urine and that it confers on the urine bacteri-
cidal properties. Broom (5) investigated the
toxicity of the substance, and showed that it is
relatively non-toxic. He confirmed the fact that
hexyl/

hexyl resorcinol is relatively non-toxic and
showed that the toxicity of amyl meta cresol was
even less than that of hexyl resorcinol and that
neither was at all likely to be in any way in-
jurious to man unless in very large doses.   In
1931 Professor Wilkie kindly gave me the oppor-
tunity of examining the therapeutic value of this
substance for the Therapeutics Trials Committee
of the General Medical Council.   There is no
published report of a previous or subsequent
investigation of a similar nature, nor of a
clinical trial of the drug.

6. Ketonurine.   In 1931 Clark (13) of the Mayo
Clinic discovered that the urine passed by patients
who were receiving a ketogenic diet and were in a
state of ketosis had bactericidal properties.
This work, as well as work done on the same lines
by Helmholtz (23), was reviewed by Cabot (9).   The
nature and use of a ketogenic diet has recently
been fully described by Dunlop (1).   Briefly, the
diet is a full diet (2,600 calories) in which car-
bohydrate and protein have been reduced to a mini-
mum, and fat is greatly increased, giving a glucose
to fatty acid ratio of 1:3.   On such a diet patients
rapidly go into a state of ketosis.   The urinary
acidity increases, and the urine becomes laden with
ketone/

ketone bodies, giving a very strongly positive
Rothera test. Clark held that the increase of
urinary acidity was not of itself sufficient to
account for the bactericidal properties of the
urine, and suggested the presence of some anti-
septic body possibly among the excreted ketones.
Helmholtz (24) in a later paper expressed the
view that while increase of acidity is not of
itself the bactericidal factor, yet it is of con-
siderable importance, as the results were much
more favourable in those cases in which the
urinary acidity was increased. He recommends
therefore that an acid-producing salt such as
ammonium chloride be given along with the keto-
genic diet. Even without this, Dunlop (1) was
able to show in a series of cases treated by the
ketogenic diet a proportion of cures of over fifty
per cent. All of the cases recorded in this
series were cases of long-standing urinary in-
fection, which had proved resistant to many other
forms of treatment.

METHODS AND RESULTS

1. Effect of administration of sodium citrate on the
     pH of the urine.

METHODS.

The effect on the pH of the urine of the

administration/

administration of sodium citrate was observed in
twelve subjects. The subjects were all patients
in the Royal Infirmary, Edinburgh. None of them
was suffering from any profound metabolic upset.
All were on approximately standard diets, un-
changed to any great degree during the course of
the experiment. All were either in bed all the
time, or were taking only gentle exercise in the
ward.

The total acidity of the urine was
measured by titrating ten c.c. of urine to
neutrality by means of N/10 NaOH, using phenol-
phthalein as an indicator. The pH of the urine
was measured by an indicator method (Clark (12))
using a Hallige comparator. The indicators used
were:

    Methyl red   pH 4.4 - 6.0
    Chlorphenol red   pH 5.2 - 6.8
    Bromthymol blue   pH 6.0 - 7.6
    Cresol red   pH 7.2 - 8.8

It was shown by Marshall (37) that the
effect of shaking urine is to drive off CO_2 which
increases the alkalinity of the urine. This effect
is naturally much more pronounced in alkaline
urines which contain a large proportion of bi-
carbonate. As no steps could be taken to prevent
a certain amount of shaking it may be assumed that
the/

the pH values at the alkaline end of the scale
are too low, i.e. too alkaline. The decrease in
pH is not above 0.2 at most.

RESULTS.

The total amount of urine voided, the
total acidity in terms of N/10 NaOH, and the pH
of the urine were measured daily during the
course of the experiment. The urine was first
examined for from four to six days before any
drug was given, then for about the same period
while eight grams of sodium citrate were given in
the day, and again for about the same period
while sixteen grams of sodium citrate were given
in the day. Averages of the output, total acidity,
and pH over each period were taken. These
averages are recorded in Table I. In Table Ia
the effect on the whole series is shown.

TABLE Ia.

Averages from Table I.

	Before medication	8 Gms. Sod. Cit. i.d.	16 Gms. Sod. Cit. i.d.
Amount voided in c.cs.	1215	1393	1562
Total acidity in terms of N/10 NaOH	3.6	1.4	0.6
pH	5.3	6.6	7.2

It will be seen that the effects of
giving/

giving sodium citrate are threefold.

1. The amount of urine voided is increased
by about 180 c.c. per diem when eight grams are
given, and by about 350 c.c. per diem when sixteen
grams are given. This occurs in spite of the fact
that the patients were asked not to increase
their fluid intake. ?

was it constant ?

2. The total acidity falls.

3. The true acidity as measured by the pH is
also diminished from pH 5.3 before medication to
pH 6.6 with eight grams and to pH 7.2 with six-
teen grams. This alteration is not a very great
one, and throughout this experiment the greatest
degree of alkalinity produced in the urine by
sodium citrate was pH 7.9.

These doses of sodium citrate are full
medicinal doses according to the established
therapeutic custom. The next experiment was con-
ducted in order to find how far the reaction of
the urine could be influenced by massive doses of
sodium citrate. I standardized my own diet and
way of living as far as possible, and collected
and examined my own urine daily. On the fourth
day I began to take fifty grams of sodium citrate
in the day, still living on ordinary diet. On the
seventh/

seventh day I altered my diet to a stringently basic one, using the diet recommended by Lyon, Dunlop and Stewart (36). The result of this experiment is shown in Table II.

It will be seen that, even with massive doses of sodium citrate and a basic diet, the alkalinity of the urine was at no time greater than pH 8.3. The urine output, however, was nearly doubled.

BACTERIOLOGICAL METHOD.

I was unable to find in the literature on this subject a method whereby the action of a urinary antiseptic could be accurately measured by experiment. It was held that, without such a method, no reliable opinion of the relative efficacies of urinary antiseptics could be formed. The following method was accordingly devised.

The organism used was the commonest invader of the urinary tract, the B. coli. A pure culture of a typical B. coli was isolated from a case of acute pyelitis. It gave the following typical biochemical reactions:-

Glucose	Lactose	Dulcite	Saccharose	Adonite
-	-	-	-	-

Inosite	Indol Production	Liquefaction of Gelatin
-	-ve.	-ve.

(- = fermentation of sugar with production of acid
    and gas.
 - = no fermentation.)

In/

In the subsequent experiments an emulsion
of this organism, or of another obtained from a
similar case and giving the same biochemical re-
actions was used. The method was to inoculate a
known number of B. coli into urine of known pH, and
to record the growth of the organisms after five
hours incubation at 37°C. Some preliminary experi-
ments were carried out in order to determine what
would be a suitable standard original inoculum and
the following was selected. A twenty-four hour
agar slope culture of B. coli was emulsified in a
few c.c. of sterile saline. This emulsion was
diluted till it was of an opacity equal with
Brown's opacity tube No. 8. 1 c.c. of this emulsion
was then subjected to two decimal dilutions and it
was found that if one 3 mm. loopful of this final
emulsion was added to 50 c.c. of urine, the urine
then contained roughly one hundred organisms per
c.c., which was a convenient original inoculum for
all subsequent experiments.

2. Effect of alteration of pH on growth of B. Coli in
   urine.

METHOD.

The main factors which influence the rate
of growth of organisms in a nutrient medium are:

1. The age of the organisms inoculated. In
every case a saline emulsion of a twenty four hour
agar/

agar slope culture of B. coli was used.

2. The number of organisms inoculated. This was constant at about one hundred per c.c.

3. The temperature at which the culture is incubated. This was always $37^{\circ}C$.

4. The nature of the medium. This was the variable factor which was under control and observation.

The urine used was obtained from healthy adults and contained no abnormal constituent. The pH of the urine was adjusted to the desired figure by the addition of decinormal HCl or NaOH. This acid and this alkali were chosen because, while they altered the pH they did not introduce into the urine any ion not already abundantly present. The urine was then passed through a Seitz abestos sterilizing filter of medium porosity. This method of sterilization was adopted because it did not alter the chemical or physical properties of the urine, as would other methods, e.g. boiling. Fifty c.cs. of this sterilized urine was transferred by means of a sterile pipette to a sterile conical flask. 1 c.c. of the urine from this flask was added to melted agar, plated, and the plate incubated as a control of the sterilization. These controls were sterile in every experiment recorded.

Each/

Each of the flasks of urine was inoculated
with one 3 mm. loopful of the previously specified
emulsion of B. coli. 1 c.c. of this inoculated
urine was added to melted McConkey's medium and
plated. By enumeration of the colonies therein
after incubation/the exact number of the original
inoculum was recorded. The flasks were incubated
at 37°C and 1 c.c. of urine was similarly plated
after five hours. It was usually necessary to
dilute the urine during plating at this time,
otherwise the colonies would have been so numerous
as to be uncountable. These plates were incubated
for twenty-four hours, and the colonies were enu-
merated. In order to make the results directly
comparable, the inoculum was taken in every case
as one hundred organisms per c.c. and the five
hour figure was expressed in terms of that inoculum.

Table III shows the results of forty eight
experiments carried out in that manner. The pH
values at which the rate of growth is recorded are
pH 4.8, pH 5.5, pH 8.5 and pH 9.0. The rate of in-
crease of B. coli in urine of pH 6 to pH 8 was very
great. In five hours the number of organisms in-
creased from one hundred per c.c. to over twenty
thousand per c.c. throughout this range. The amount
of/

of work entailed in making an accurate enumeration
of this vast increase would have been very con-
siderable, nor would any good purpose have been
fulfilled. It is sufficient to know that B. coli
multiply with extreme luxuriance in urine at pH 6
to pH 8.

In no case was there free growth of B.
coli in urine at pH 4.8. In two cases there was
some slight apparent increase at the end of five
hours incubation, but neither is without the margin
of experimental error. In the remaining ten cases
there is a great reduction in B. coli, amounting in
four cases to complete sterilization. The average
number of colonies after five hours was twenty-
eight per c.c. In all of the twelve sterilization
was produced at the end of twenty-four hours. At
pH 5.5, pH 8.5, and pH 9.0 the organism grew with
varying degrees of luxuriance over five hours and
twenty-four hours in all cases except one at pH 5.5,
in which sterilization was produced by the end of
five hours.

These experiments show that B. coli grow
with less freedom when the urine is more acid than
pH 6 or more alkaline than pH 8 than they do when
the urine is between pH 6 and pH 8. Increase of the
acidity of the urine to the physiological limit has
a greater restraining effect upon growth than in-
crease/

crease of the alkalinity to the physiological limit.

3. Effect of addition of hexamine in vitro on growth
      of B. coli in Urine.

METHOD.

A series of similar experiments was under-
taken in order to determine what concentration of
hexamine in the urine is necessary to kill B. coli,
or to restrain their growth. The reaction of the
urine was adjusted in each case to pH 5.5. Hexamine
was added in known concentration to the urine and
the urine was sterilized and inoculated as before.

RESULTS.

Hexamine was added to the urine in con-
centration of 1:1,000, 1:3,300, 1:10,000, and
1:33,000. The growth of B. coli was unaffected by
the two weaker solutions, was retarded by the
1:3,300 solution, and the 1:1,000 solution was
almost sterile at the end of five hours. The
effective concentration of hexamine in urine is
thus greater than 1:3,000. Forty experiments were
carried out with concentrations of hexamine in urine
from 1:3,000 up to 1:928. The results of these
experiments are seen in Table IV.

A concentration of hexamine of 1:3,000
exerts a definite restraining influence on the
growth of B. coli in urine at pH 5.5, (cf. Table III).

A/

A concentration of hexamine of 1:982 almost steri-
lizes the urine in five hours. The concentration
at which growth is restrained for five hours lies
between 1;1,920 and 1:1,536. A graph of the
action of hexamine has been drawn (**Fig. 1.**),
plotting the concentrations of hexamine in urine
(abscissa) against the log of the number of colonies of B.
coli after five hours incubation (ordinate). From
this graph it will be seen that the minimum con-
centration of hexamine in urine of pH 5.5 which
will restrain growth of B. coli for five hours is
about 1:1,800.

The action of hexamine is entirely
dependent upon the reaction of the urine. Con-
centrations of hexamine varying from 1:1,000 to
1:125 had no effect upon bacterial growth in urine
of pH 8.

4. Effect of oral administration of hexyl resorcinol on growth of B. coli in urine.

METHOD.

The urines of a number of healthy adults
were collected, the pH was adjusted to 5.5. and the
rate of multiplication of B. coli in these urines
at this pH was found to be average - about one
hundred-fold in five hours. The patients were then
given hexyl resorcinol by mouth in capsules in
doses of 0.45 gram, 0.90 gram, and 1.35 gram in
the day. The urine was collected, titrated to pH
5.5, sterilized, and inoculated with B. coli as
before/

before. Each patient was given each dose for some consecutive days.

RESULTS.

Table V shows the results of thirty six experiments carried out in that manner. As compared with the ordinary growth of B. coli (cf. Table III) growth is somewhat retarded over five hours. This retardation, even with the dose of 1.35 gram in the day, is not of such a degree as to be of any great therapeutic value.

5. Effect of oral administration of amyl meta cresol on growth of B. coli in urine.

METHOD.

The action of amyl meta cresol was studied in an exactly similar fashion. The drug was given orally in capsules in doses of 0.45 gram in the day, 0.90 gram in the day, and 1.35 gram in the day, and the growth of B. coli in the urine was studied.

RESULTS.

The results of these experiments are recorded in Table VI. The oral administration of amyl meta cresol has a distinct effect on the growth of B. coli in the urine. Even the largest dose, however, only once produced a urine that was bactericidal within five hours, and in all cases but/

but this there was growth of B. coli in the urine at the end of twenty four hours. The action of amyl meta cresol is compared with that of hexyl resorcinol by means of a graph (Fig. 2). Curves of the actions of these two drugs are drawn, plotting the log of the number of colonies of B. coli after five hours incubation at 37°C (ordinate) against the oral dosage of the drugs (abscissa).

Conclusions:

1. The action of amyl meta cresol is greater than that of hexyl resorcinol given in equivalent doses.

2. The action of amyl meta cresol increases in proportion with increase of the oral dosage of the drug, while that of hexyl resorcinol does not.

VI. Effect of ketogenic diet on growth of B. coli in urine.

METHOD.

The urines of patients who were receiving a ketogenic diet were obtained. In every case recorded the patient was in a profound state of ketosis, and the urine was heavily laden with ketone bodies. The pH was measured by means of an indicator method. The urines were sterilized by filtration, inoculated, and the growth recorded in the manner previously described.

RESULTS/

RESULTS.

Thirty three Ketonurines were so examined, (Table VII). Ketonurine has a definite bacterio-static effect. The action is only a bacteriostatic one, as in only one case was sterilization of the urine produced in five hours, and in all cases with that one exception there was growth of B. coli at the end of twenty four hours. A comparison of the averages of growth at the various pH values shows that alteration of the pH makes no appreciable difference to the bacteriostatic effect of the ketonurine. It would seem, then, that the bacterio-static effect of ketonurine is independent of the reaction of the urine.

DISCUSSION

In the Brady Urological Clinic of the Johns Hopkins Hospital several workers gave a number of years to the examination of the values of a large series of substances, about four hundred in all, as internal urinary antiseptics. A survey of the work was made by Young, White and Schwartz (56), who defined the following as the conditions a sub-stance must fulfil in order to be described as an ideal/

ideal urinary antiseptic:

1. it must be chemically stable;

2. it must be non-toxic, and non-irritant to the urinary or the gastro-intestinal tract;

3. it must exert an antiseptic action in high dilution in urine of any reaction;

4. it must be eliminated in high percentage by the kidney.

Such conditions are not easily fulfilled, and later Davis (17), reviewing the same work, said "There is no sound experimental or clinical proof of the fitness of any known drug as an internal urinary antiseptic." The desirability of such a substance is undoubted, and could any one be found which held out reasonable hope of success it would be a most valued addition to the pharmacopoeia. If two methods of treatment of chronic urinary infection were known, one by oral medication which was only fifty per cent successful, and one which involved instrumentation which was eighty per cent successful, most clinicians would resort to the latter only when the oral method had failed after a fair trial of that method.

In assessment of the results which I have shown the pathology of the condition of urinary infection must be borne in mind. In my experiments the organisms/

organisms were growing in urine in glass flasks, their only pabulum the urine. In the living subject the organisms are situated in the tissues lining the urinary tract. Although the walls of the tract are intimately and lavishly bathed with the urine, the organisms are here in a much stronger position in that they are not solely dependent upon the urine for their nutrient medium. Any substance, then, which confers upon the urine in vitro only a mild bacteriostatic effect can not have much value as an internal urinary antiseptic. Only a substance which was actively bactericidal in vitro could be expected to exert a powerful killing effect on organisms in the urinary tract.

Secondly, the antiseptic was operating under favourable conditions in that the inoculum of the urine was a small one. Chick (11) showed that disinfection was a process which obeyed in the main the laws of an ordinary chemical reaction, and that consequently the law of mass action must be taken into account. An inoculum of one hundred organisms per c.c. is very much more easily destroyed than an inoculum of millions of organisms per c.c. Leonard (34),(35) pointed out that the number/

number of organisms present in the urine in a case
of urinary infection is of prognostic significance.
If the infection was a very heavy one he was unable
to master it by the use of hexyl resorcinol alone,
but had to supplement it with bladder or renal
lavages. Again, the infected kidney may be so dis-
organised as to be unable to secrete the antiseptic
in any effective concentration. Here again the
treatment must be local.

When these points are considered it is
clear that with any of the methods of urinary anti-
sepsis I have examined there can be no likelihood of
striking clinical success in a large proportion of
cases. At the same time the use of one or other of
them may assist the body in overcoming a urinary
infection, when combined with the beneficial effects
of hospitalization, careful nursing, and other
general measures. In the following summary each of
the urinary antiseptics which I have investigated
is reviewed in the light of the requirements above
described.

1. Sodium citrate. The main buffering substance in the
urine is phosphate. In the titration of phosphoric
acid with sodium hydroxide the change from acid to
salt/

salt occurs in three stages. First phosphoric acid,
H_3 PO_4, is converted into sodium dihydrogen phos-
phate, or acid sodium phosphate Na H_2 PO_4; this
is next converted into disodium hydrogen phosphate,
or alkaline sodium phosphate Na_2 H PO_4; last there
is formed trisodium phosphate Na_3 PO_4. For this
reason, and because the salts of phosphoric acid
are very weakly dissociated in solution, phosphate
is a very powerful buffer substance. Examination
of the titration curve of phosphoric acid shows
that this buffering action is exerted mainly bet-
ween pH 4.0 and pH 9.0. Thus it is to be expected
that considerable oral doses of alkaline or acid
salts would be required to alter the pH of the
urine within this range. Sodium citrate produces
this effect within the specified limits. (Tables
I, Ia, and II).

Another effect of the oral administration
of sodium citrate is to increase the amount of
urine voided. This is explained by the fact that
the body must excrete the alkali which is produced
from the salt in order to maintain at a constant
level the pH of the blood and body fluids. The
alkali can be excreted only in solution, therefore
the urinary output must be increased (Table I).
This/

This diuresis is of value in that it gives symptom-
atic relief by diluting the irritant urine, and is
possibly of therapeutic value in mechanically
flushing the infected urinary system. Further les-
sening of symptoms of dysuria and frequency will
follow on the decrease of the acidity of the urine,
as neutral or alkaline urine is less irritant than
acid urine.

2. Alteration of pH.   B. coli grow with great lux-
uriance in urine from pH 6 to pH 8. An increase of
the urinary acidity to pH 5.5 exerts some degree of
restraining influence on the rate of multiplication
of B. coli. At pH 4.8 the organisms are reduced in
number in five hours and killed in twenty four hours.
At pH 8.5 again some retardation of growth is seen,
and a greater retardation of growth is seen at pH 9.0.
B. coli, however, can and do multiply in urine as
alkaline as pH 9.0. This confirms the work of Sohl
and Janney (46), who found that the growth of B. coli
is inhibited in urine only between pH 4.2 and 4.8,
and pH 9.2 and 9.6. Thus it will be seen that while
it is not possible to produce an inhibitory degree of
alkalinity in the urine, it may be possible to pro-
duce a degree of acidity which will restrain bacterial
growth/

growth, or even have a bactericidal effect. Un-
fortunately urine of the required degree of acidity,
pH 4.8 or more acid, may by its very acidity cause
very disagreeable dysuria and strangury (Dunlop
(1)). This is especially so when the acidity of
the urine is induced by acid-forming salts such as
ammonium chloride. The high acidity sometimes
reached in ketonuria has not so far been found to
produce these symptoms.

3. Hexamine. The concentration of hexamine necessary
to restrain the growth of B. coli over five hours
in urine of pH 5.5 was found to be about 1;1,800 (Tab. IV),
(Fig. 1). This concentration of hexamine in the
urine can never be reached by the oral admini-
stration of the drug. Burnam (8) in 1912 found
that only one person in five had any formaldehyde
in the urine when ordinary doses of hexamine were
given by the mouth, and that only sixty per cent
of the urines contained formaldehyde with the
massive oral dosage of two grams of hexamine four
hourly. Hinman (26), (27) repeated this work,
using a modification of the Rimini method (42) and
found that, with large doses of hexamine by mouth,
only seventeen per cent of the urines showed a
bacteriostatic content of formaldehyde, and only
five/

five per cent were bactericidal. He further showed, by collecting the urine from the renal pelvis, that none of the urine from there contained a bacterio-static concentration of formaldehyde. This obser-vation has since been amply confirmed by Walker (52), Hoest (28), Camino (10), and Sutton (49).

As has already been mentioned a variable, but probably a large proportion of the hexamine orally administered is destroyed in the stomach. To obtain with certainty a concentration of hexamine of 1/1,800 or higher in the urine, it would be necessary to give the hexamine by intravenous injection. The disadvantages of maintaining a concentration of hexamine in the urine for any length of time by this means are obvious. Further, it seems abundantly clear that the efficacy of hexamine as a urinary antiseptic is limited to those cases in which the infection is in the bladder. Formaldehyde is not liberated with sufficient rapidity to produce an antiseptic action in the renal pelvis. Lastly, the bactericidal or bacterio-static action of hexamine in the urine is entirely dependent upon the acidity of the urine.

3 and 4. Hexyl resorcinol and Amyl meta cresol. These substances/

substances have been compared. The bacteriostatic
effect of hexyl resorcinol in vitro is not suf-
ficiently great to make it probable that it exerts
much bacteriostatic effect in vivo, nor does its
action increase in proportion with increase of the
dosage by mouth. This has been shown to be due to
1. irregular absorption of hexyl resorcinol from
the gut, and 2. the excretion in the urine as an
inert conjugate of a large proportion of the hexyl
resorcinol absorbed (43). Oral doses of 0.45 gram
of amyl meta cresol in the day produce a urine
which is moderately bacteriostatic over five hours
in vitro. Further the action increases in pro-
portion with increase of the dose, and an oral dose
of 1.35 gram in the day produces a urine which is
almost completely bacteriostatic over five hours
in vitro. This suggests that amyl meta cresol may
be of some clinical value as a urinary antiseptic.

5. Ketonurine.   Patients in a state of ketosis have
been shown to pass urine which is bacteriostatic
over five hours in vitro. This effect appears to
be independent of the reaction of the urine, as
bacterial growth is retarded almost as much when
the pH is 5.0, as it is when the pH is 6.0 or more
alkaline/

alkaline. Growth is slightly greater in the more
alkaline urines but not to any significant extent.
Helmholtz (24) in a recent paper has contradicted
this observation. He found that ketonurine
assumed bacteriostatic properties only when the
reaction of the urine is pH 5.6 or more acid.
Further work is necessary to clear up this
discrepancy.

S U M M A R Y

1. The limitations of the field of usefulness
of any internal urinary antiseptic have been defined.

2. A method is described for the estimation
of the action of a urinary antiseptic.

3. The effects upon the urine of oral admini-
stration of sodium citrate are:

        a. to lower the hydrogen ion concentration
            or true acidity;
        b. to lower the total acidity;
        c. to increase the amount of urine voided.

4. A degree of alkalinity of the urine such as
would render it bactericidal cannot be produced by
oral medication.

5. A bactericidal degree of acidity may be
produced, but this may be accompanied by such dis-
comfort to the patient as to render its employment
impracticable/

impracticable.

6. Hexamine in lesser concentration than 1/3,000 is inert against B. coli in acid urine.

7. In concentration of 1/1,800 hexamine inhibits the growth of B. coli in acid urine for five hours, and as the concentration increases hexamine gradually becomes lethal to B. coli in acid urine.

8. Such a concentration of hexamine could be achieved only by intravenous administration of the drug.

9. Hexamine in alkaline urine has no action on the growth of B. coli.

10. Hexyl resorcinol in oral doses of up to 1.35 gram in the day has only a slight bacteriostatic effect on the urine in vitro.

11. Amyl meta cresol in oral doses of 1.35 gram in the day has a bacteriostatic effect on the urine in vitro. It is suggested that this may be of therapeutic value.

12. Urine passed by patients in a state of ketosis has a bacteriostatic effect on B. coli in vitro.

13. Whether this effect is dependent upon the reaction of the urine is still sub judice.

43.

TABLE I.

Effect on the urine of persons on a standard diet of administration of

Sodium Citrate in doses of 8 Gram i.d. and 16 Gram i.d.

Amount in c.cs.			Total Acidity in terms of N/10 NaOH			pH.		
Before	8 Gm. i.d.	16 Gm. i.d.	Before	8 Gm. i.d.	16 Gm. i.d.	Before	8 Gm. i.d.	16 Gm. i.d.
1407	1186	1250	5.2	2.6	0.3	5.3	6.3	7.4
1200	1513	2060	3.7	0.3	0.2	5.4	7.6	7.6
1216	1286	1322	2.2	2.5	1.4	5.1	5.8	6.7
1200	1485	1500	2.8	1.3	0.9	5.6	6.6	7.3
1605	1600	1638	5.1	1.5	0.7	5.5	6.3	6.8
555	1185	1320	2.2	0.8	0.4	4.6	7.1	7.3
1320	1485	1380	3.7	0.9	0.3	5.5	6.6	7.5
1195	1368	1583	4.1	1.8	0.8	5.2	6.2	7.4
1073	1497	1312	3.7	1.2	0.6	5.6	6.4	7.1
1180	1436	1610	4.4	2.1	1.0	5.7	6.9	7.2
1337	1410	1820	2.2	0.8	0.2	4.8	6.1	6.7
1290	1264	1250	3.6	1.1	0.4	5.3	7.5	7.6
Average 1215	1393	1562	3.6	1.4	0.6	5.3	6.6	7.2

TABLE II.

Effect on urine of massive doses of Sodium citrate.

Day	Amount in c.cs.	Total Acidity in terms of N/10 NaOH	pH.
1	1650	3.50	6.0
2	1580	2.40	5.7
3	2000	0.90	6.3
4	1400	3.10	5.7
Average	1658	2.50	5.9
50 Grams Sodium citrate i.d. (ordinary diet).			
5	2380	0.32	7.9
6	2470	-0.10	8.3
7	2480	0.10	7.9
Average	2443	0.10	8.0
50 Grams Sodium citrate i.d. (basic diet).			
8	2470	-0.47	8.2
9	3700	-0.05	8.1
10	2900	-0.05	8.2
11	2500	-0.10	8.1
Average	2893	-0.10	8.15

TABLE III.

Effect of alteration of pH on growth of B. coli in urine.

Number of organisms per c.c. after five hours incubation; (inoculum 100 per c.c.)

pH 4.8		pH 5.5		pH 8.5		pH 9.0	
5 hrs.	24 hrs.	5 hrs.	24 hrs.	5 hrs.	24 hrs.	5 hrs.	24 hrs.
147	−	17,710	+	3,180	+	6,660	+
0	−	15,540	+	2,110	+	790	+
39	−	23,300	+	5,778	+	2,256	+
0	−	18,900	+	13,420	+	3,060	+
5	−	4,340	−	8,960	+	211	+
0	−	0	+	10,780	+	1,760	+
5	−	18,820	+	6,780	+	192	+
2	−	1,700	+	14,500	+	3,768	+
0	−	8,150	+	7,280	+	2,350	+
137	−	119	+	8,200	+	1,180	+
13	−	27,750	+	10,200	+	5,480	+
3	−	3,300	+	12,390	+	7,390	+
Average 28		11,636		8,632		2,925	

TABLE IV.

Effect of addition of hexamine _in vitro_ to urine of
   pH 5.5.

Number of organisms per c.c. after five hours
   incubation at 37°C; (inoculum 100 per c.c.).

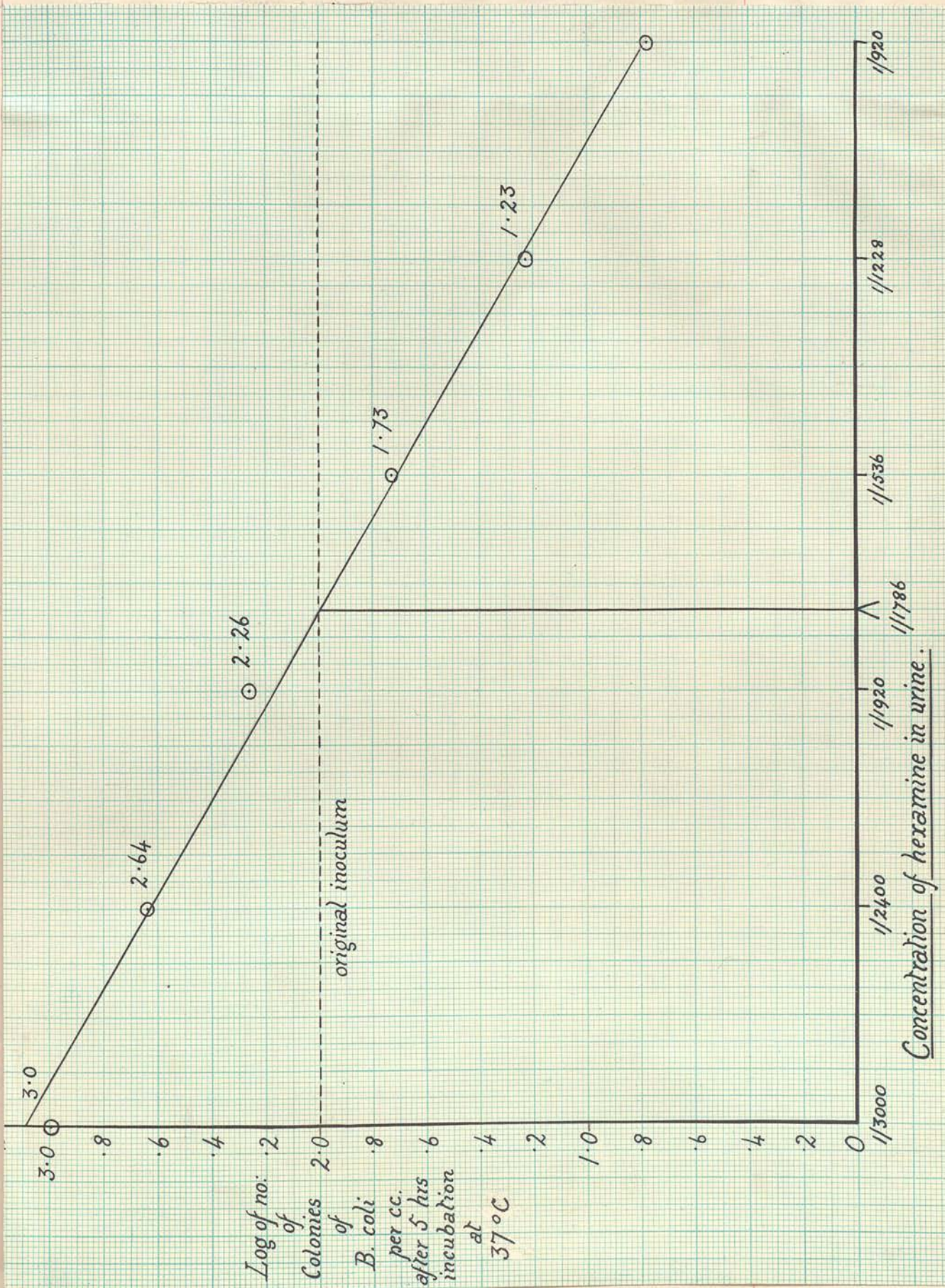| 1/3000	1/2400	1/1920	1/1536	1/1228	1/920
289	216	57	34	20	3
177	141	35	28	28	7
774	470	104	19	0	0
930	887	204	46	18	6
857	279	124	70	9	8
733	282	46	15	3	2
970	465	185	116	23	6
1498	614	469	56	13	8
3485	880	564	144	54	18
361	153	28	16	2	1
Average 1007	439	182	54	17	6
Log of Average 3.0	2.64	2.26	1.73	1.23	0.78

Fig. 1.

TABLE V.

Effect of oral administration of hexyl resorcinol
on growth of B. coli in urine at pH 5.5.

Number of organisms per c.c. after five hours
incubation at 37°C; (inoculum 100 per c.c.).

0.45 Gm. i.d.		0.90 Gm. i.d.		1.35 Gm. i.d.	
5 hrs.	24 hrs.	5 hrs.	24 hrs.	5 hrs.	24 hrs.
3212	+	6236	+	2840	+
3602	+	5400	+	1376	+
3130	+	4980	+	842	+
437	+	110	+	130	+
7254	+	3152	+	4210	+
6588	+	5089	+	2800	+
6280	+	5073	+	3100	+
320	+	81	−	120	−
4128	+	3778	+	2720	+
4943	+	5243	+	3921	+
180	+	110	+	130	+
155	+	81	−	340	+

Average 3356	3111	1887

Log of average 3.53	3.49	3.28

TABLE VI.

Effect of oral administration of amyl meta cresol on
growth of B. coli in urine at pH 5.5.

Number of organisms per c.c. after five hours
incubation at 37°C; (inoculum 100 per c.c.).

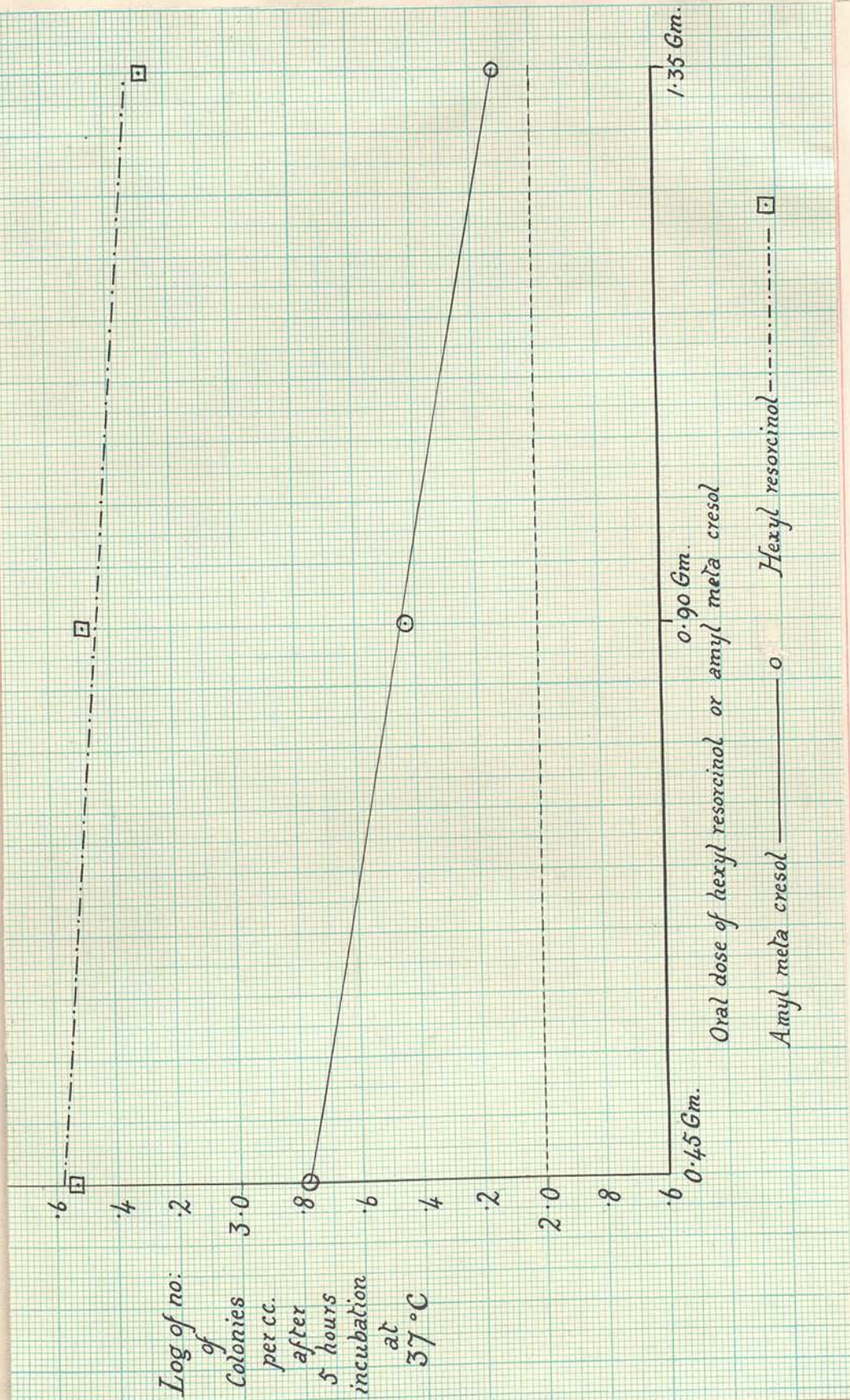| 0.45 Gm. i.d.		0.90 Gm. i.d.		1.35 Gm. i.d.	
5 hrs.	24 hrs.	5 hrs.	24 hrs.	5 hrs.	24 hrs.
1050	+	827	+	43	−
547	+	90	+	57	+
375	+	84	+	77	+
566	+	86	+	57	−
124	+	71	+	83	+
572	+	66	+	48	−
127	−	152	+	472	+
268	+	95	+	62	−
1382	+	190	+	149	+
776	+	370	+	244	+
162	+	88	−	75	−
1165	+	241	+	326	+
127	+	1710	+	308	+
1185	+	177	+	107	+
222	+	82	−	20	−
808	+	45	−	0	−
Average 597		267		133	
Log of average 2.78		2.43		2.12	

Fig. 2.

TABLE VII.

Growth of B. coli in Ketonurine.

Number of organisms per c.c. after five hours
incubation at 37°C; (inoculum 100 per c.c.).

pH.	5 hrs.	24 hrs.	pH.	5 hrs.	24 hrs.
-	91	+	5.5	793	+
-	118	+	Av.	354	
-	135	+	6.0	76	+
-	15	+	6.0	590	+
Av.	90		6.0	533	+
5.0	194	+	6.0	359	+
5.0	77	+	6.0	344	+
5.0	88	+	Av.	380	
5.0	280	+	7.0	112	+
5.0	202	+	7.0	7	+
5.0	112	+	7.0	546	+
5.0	156	+	Av.	222	
5.0	133	+	7.5	447	+
5.0	0	-	7.5	157	+
5.0	77	+	7.5	344	+
5.0	85	+	7.5	46	+
5.0	157	+	Av.	249	
Av.	130		8.0	266	+
5.5	38	+	8.0	282	+
5.5	230	+	Av.	274	

no initials

1. Band, Dunlop and Dick.   Proc. Roy. Soc.
         Med. 1933, 26: (sect. of urol.), 1.

2. Blane.   Trans. of a Soc. for the improve-
         ment of Med. and Surg. Knowledge. 1812,
         3: 339.

3. Braasch and Cathcart.   J. Amer. Med. Ass.
         1927, 88: 1630.

4. Brewer.   Surg. Gyn. and Obst. 1906, ii, 485.

5. Broom.   Brit. J. Exp. Phys. 1931, 12: 327.

6. Brown.   Brit. J. Exp. Path. 1931, 12: 327.

7. Idem.   J. Amer. Med. Ass. 1926, 86: 668.

8. Burnam.   Arch. Intern. Med. 1912, 10: 324.

9. Cabot.   Lancet. 1932, i : 1038.

10. Camino.   Z. f. Urol. Chir. 1921, 7 : 286.

11. Chick.   J. Hyg. 1908, 8 : 92.

12. Clark.   Determination of Hydrogen ion   _ ? *Reference*
         Concentration.

13./

13. Clark.   Proc. of Staff Meetings, Mayo Clinic.
        1931, 6 : 605.

14. Cohen. J. Bact. 1922. vii, 183.

15. Coulthard. J. Chem. Soc. 1930, 331.

16. Coulthard, Marshall and Pyman. ibid. 1930. 280.

17. Davis.   J. Urol. 1921, 5 : 215.

18. Eberbach and Arn.   J. Amer. Med. Ass. 1927,
        89 : 512.

19. Flexner.   J. Exp. Med. 1907, 9 : 105.

20. Haldane.   J. Physiol. 1921, 55 : 265.

21. Haldane, Hill and Luck. ibid. 1923, 57 : 301.

22. Hare, Lepper and Martland.   Proc. Roy. Soc.
        Med. 1928, 508.

23. Helmholtz.   Proc. of Staff Meetings, Mayo
        Clinic. 1931, 6 : 510.

24. Idem.   J. Amer. Med. Ass. 1932, 99 : 1035.

25. Henline.   J. Urol. 1925, 144 : 119.

26. Hinman.   J. Amer. Med. Ass. 1913, 61 : 1601.

27. Idem.   Arch. Intern. Med. 1924, 34 : 511.

28./

28. Hoest.   Z. f. Klin. Med. 1915, 81 : 272.

29. Johnson and Hodge.   J. Amer. Chem. Soc.
        1913, 35 : 529.

30. Johnson and Lane. ibid. 1921, 43 : 348.

31. Johnston.   Ed. Med. J. 1927, 34 : 223.

32. Jordan.   Brit. Med. J. 1913, ii, 648.

33. Kronig and Paul.   Z. f. Hyg. 1897, 25 : 1.

34. Leonard.   J. Urol. 1924. 12 : 585.

35. Idem.   J. Amer. Med. Ass. 1924, ii, 83: 2005.

36. Lyon, Dunlop and Stewart.   Ed. Med. J. 1931,
        38, 87.

37. Marshall.   J. Biol. Chem. 1922, 41 : 3.

38. Morrison.   Brit. Journ. Urol. 1929, i : 30.

39. Nicolaier.   Deutsch. Med. Wchnschr. 1895,
        31 : 541.

40. Osman.   Guy's Hosp. Reports. 1927, 77 : 386.

41. Percival and Stewart.   Biochem. J. 1927, 21 :
        301.

42. Rimini.   Chem. Zentralblt. 1898, 1 : 1152.

43./

43. Robbins. J. Pharm. and Exp. Ther. 1931,
    43 : 335.

44. Robbins and Wesson. ibid. 1931, 43 : 325.

45. Sherman and Holm. J. Bact. 1922, 7 : 465.

46. Sohl and Janney. J. Urol. 1917, 1 : 211.

47. Sollmann. Manual of Pharmacology. 3rd.
    ed. : 657.

48. Stockman. Ed. Med. J. 1927, 34 : 396.

49. Sutton. M. J. Australia. 1923, 1 : 31.

50. Thomson Walker. Brit. Med. J. 1913, ii :
    654.

51. Topley and Wilson. Principles of Bacterio-
    logy and Immunity, Vol.1.

52. Walker. Ed. Med. J. 1914. 12 : 503.

53. Winslow and Falk. J. Bact. 1923, 8 : 237.

54. Winslow and Hotchkiss. Proc. Roy. Soc. Exp.
    Biol. and Med. 1922, 19 : 314.

55. Winslow and Lochridge. J. Inf. Dis. 1906,
    3 : 547.

56. Young, White and Schwartz. J. Amer. Med.
    Ass. 1919, 73 : 1483.