STUDIES ON PHENOTHIAZINE

VII. THE BACTERICIDAL PROPERTIES OF URINE AFTER ORAL ADMINISTRATION OF PHENOTHIAZINE

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We have previously reported (1) that, when phenothiazine is administered gastrically to rats, rabbits and humans, it is partially converted to thionol, and excreted partly as phenothiazine, partly as thionol, partly as leuco thionol and partly as leuco thionol in some loose chemical combination, possibly with phenothiazine. It has been shown that this loosely combined leuco thionol can be set free by hydrolysis with hydrochloric acid at room temperature. Therefore, depending upon the pH of the urine, varying amounts of leuco thionol are liberated, followed by oxidation to thionol. It was also observed (2) that collections of rat urines containing thionol failed to show gross evidence of bacterial growth after standing exposed to air for several weeks. This observation suggested the possibility that oral administration of phenothiazine conferred antiseptic properties on urine. This paper presents results of investigations which demonstrate a definite urinary antiseptic action.

GERMICIDAL ACTION OF PHENOTHIAZINE URINES IN VITRO

Preliminary tests of the effects on cultures of Escherichia coli in vitro were made first with urines of rats receiving phenothiazine. Five cubic centimeters of a suspension of E. coli in physiological salt solution (0.9 per cent NaCl) were placed in each of two test tubes. To one tube were added 5 cc. of sterile

water and to the other tube 5 cc. of rat urine collected from rats on a diet containing 0.4 per cent phenothiazine. The tubes were incubated 24 hours; then pour-plates were made to permit bacterial counts. The control tube, to which sterile water had been added, showed a count of 321,000,000 organisms per cubic centimeter, whereas the tube to which the phenothiazine-urine had been added showed a count of 209,000,000 organisms per cubic centimeter. In the light of our present knowledge, it is certain that, if the rat urine had been more acid than pH 6, there would have been more free thionol present and a more powerful bactericidal action. Nevertheless, there was a 30 per cent decrease in bacterial count under an unfavorable condition. Furthermore, no effort was made to collect the rat urine aseptically, and therefore, presence of a bactericidal action seemed all the more probable. In fact, this action was confirmed for urines of rabbits and humans which were more suited to practical tests of antiseptic action under a variety of conditions than were rat urines.

A more exact determination of the bactericidal action of phenothiazine-urine in vitro was made as follows with two male rabbits, each weighing 2.4 kilograms: One rabbit was kept as control, and the other was given gastrically 0.2 gram of phenothiazine in olive oil. Two hours later a second dose of 0.4 gram of phenothiazine in olive oil was given. No symptoms of toxicity developed. Fifteen hours after the second dose of phenothiazine, 18 cc. of urine were removed by catheterization, care being taken to avoid bacterial contamination. In the same manner, 33 cc. of urine were obtained from the control rabbit. A 5 cc. sample of each urine was placed in each of two test tubes, 5 cc. of an E. coli suspension added to each tube, and the tubes incubated for 24 hours. From each tube a series of pour-plates was made and incubated 24 hours to permit counting of bacterial colonies. All plates from the control urine showed the presence of too many colonies to permit counting, whereas those from the urine of the rabbit receiving phenothiazine gave a count of 20,800 organisms per cubic centimeter.

Sixteen hours after the second dose of phenothiazine, the

treated rabbit was given a third dose of 0.4 gram of phenothiazine in olive oil. Twenty-two hours later 5 cc. of catheterized urine was removed from the medicated rabbit, and 30 cc. from the control untreated rabbit. Again, 5 cc. portions of each urine were incubated with 5 cc. quantities of a suspension of E. coli, and the test procedure carried out as before. Again, pour-plates from the urine of the control rabbit showed the presence of too many organisms to permit counting, whereas those from the phenothiazine urine showed complete absence of bacterial colonies. Similar experiements, with urines from the same two rabbits, made three days after the last administration of phenothiazine to the medicated rabbit showed an absence of bactericidal properties in both urines.

A sample of the red dye, thionol, isolated from urines of rabbits receiving phenothiazine was used in the following test of bactericidal action on Staphylococcus aureus: Using a culture of Staphylococcus aureus obtained from a patient with bacteremia, a suspension of organisms was made. To each of three tubes, 5 cc. of broth and 0.2 cc. of the bacterial suspension were added. One tube was kept as a control and to each of the other tubes about 5 milligrams of thionol were added. All tubes were incubated 24 hours, and then pour-plates of low dilution, 1:10 and 1:100, were made from each tube. After incubation, very heavy growth was present on all plates made from the control tube, but no growth was present on any of the plates made from the tubes to which thionol had been added. Accordingly, a bactericidal action of a physiological oxidation product of phenothiazine was demonstrated, and by the same token, a possible mechanism for the antiseptic action of the urine was suggested, which will be discussed farther on.

Meanwhile the preliminary evidence of urinary antiseptic action of phenothiazine in rats and rabbits indicated possibilities of obtaining the same in man. Oral administration appeared safe, since toxicity in rabbits and rats (2, 3) was absent or low. Therefore, one of us (J. O. T.) took 0.8 gram phenothiazine at 5:30 P. M., having first emptied his bladder. Urines were collected at the end of 3 hours (50 cc.), 9 hours (240 cc.), 14 hours

(250 cc.) and 16.5 hours (90 cc.). After the last collection, 3 cc. of each urine sample was placed in each of four test tubes and 3 cc. of control urine, before taking the drug, in a fifth tube. To each of the five tubes was added 1 cc. of an 18-hour old culture of E. coli in glucose-peptone broth. After incubating the tubes for 24 hours, pour-plates were made of each of the 5 tubes in the experiment. After incubation of the plates for 24 hours, numerous colonies were present in the plates from the control urine and the urines voided at the end of 14 and 16.5 hours, but absent in the plates from urines voided at the end of 3 and 9 hours, thus indicating an antiseptic action in these specimens.

The experiment was continued with the same person taking three additional doses of 0.8 gram each of phenothiazine at the end of 18.5, 22.5, and 29.5 hours after taking the phenothiazine in the above experiment. Eight samples of urine were collected in sterile bottles at the end of 1, 2.5, 4.5, 7, 10.5, 16.5, 19, and 24 hours after taking the second dose of phenothiazine. All samples of urine were kept in the ice box until the last collection was made. A 24-hour old broth culture of E. coli was diluted 1:1 and 1 cc. transferred to each of 9 sterile tubes. To one tube, used as a control, 4 cc. of sterile physiological saline solution were added, and to the remaining tubes corresponding volumes of the 8 urine samples collected. After incubation for 24 hours, pour-plates were made from each tube and incubated for 24 hours. Examination of these pour-plates showed the presence of a definite bactericidal action which correlated with the concentration of thionol in the urine samples as judged by the intensity of red color. Thus, the antiseptic action of urines from animals receiving phenothiazine was confirmed by a similar result in human urine following oral administration of phenothiazine. This suggested experimental therapeutic trials with phenothiazine in cystitis.

ANTISEPTIC ACTION IN EXPERIMENTAL CYSTITIS

Before attempting clinical trials with phenothiazine, experiments were designed to test the efficacy of the drug in cystitis produced experimentally in male rabbits. The difficulties of

producing a permanent experimental cystitis in animals are well known. Such infections may clear up spontaneously in a few days. Clinically, spontaneous cures of cystitis may follow so simple a procedure as rest in bed. Therefore, experimental and clinical cystitis have this characteristic in common. However, an experimental infectious cystitis permits a systematic collection of urines by catheterization in determining the value of an antiseptic. On the other hand, clinical difficulties often prevent obtaining representative catheterized urine samples for tests of chemotherapeutic effectiveness. Experimentally, the actions of a drug are conveniently observed in a pathological state of the bladder before proceding to clinical tests of a drug. Therefore, the advantages rest with experimental cystitis for preliminary therapeutic trials, and the results obtained in rabbits are offered as presumptive evidence in favor of an antiseptic action of orally administered phenothiazine.

Out of a series of twenty male rabbits, 8 proved resistant to the production of experimental cystitis and 4 developed cystitis, but cleared up spontaneously. The remaining 8 rabbits were successfully infected. Treatment, with gastrically administered phenothiazine was applied to four of these, and the other four were used as controls. Of the four controls one was subsequently treated with phenothiazine. As the experiments varied somewhat in certain respects, it is desirable to describe them in some detail.

In the first experiment, two male rabbits, each weighing 2.4 kilograms, were used. The animals were placed on rabbit boards, and, using aseptic technique, catheters were passed into the bladder and any urine present was removed. One cubic centimeter of a heavy culture of E. coli in glucose broth was then introduced through the catheter and washed into the bladder with an equal volume of sterile physiological saline solution. On the following day a catheterized sample of urine was removed from each rabbit under aseptic technique. Pour-plates of these urines were made and incubated for the usual 24 hours. Both rabbits showed an infection too heavy to permit counting of the

colonies on the pour-plates. Twenty-four hours later catheterized samples of urine were again removed and pour-plates made as before. Both rabbits still showed heavy infections, there being 856,000,000 organisms per cubic centimeter of urine in one rabbit and 1,840,000,000 per cubic centimeter in the other rabbit.

The rabbit showing the smaller bacterial count was given 10 cc. of cottonseed oil by stomach tube and served as a control. The other rabbit was given 10 cc. of cottonseed oil containing 0.5 gram phenothiazine in solution. Nineteen hours later a catheterized sample of urine was removed from each rabbit and pour-plates made as before. The control rabbit showed a count of 1,416,000,000 organisms per cubic centimeter of urine, while that of the rabbit which had received phenothiazine had dropped to 70,000 organisms per cubic centimeter. Forty-six hours after administration of the phenothiazine, both rabbits were again catheterized and pour-plates made. The count for the control rabbit was 800,000,000 organisms per cubic centimeter of urine, but that of the rabbit receiving phenothiazine was only 11,040 organisms. By this time the peak of excretion of thionol had passed. Unfortunately in attempting to give the experimental rabbit a second dose of 0.5 gram phenothiazine in cottonseed oil, the animal was accidentally killed. Therefore the dose was administered to the rabbit which had served as a control and showed persistence of a heavy infection. Twenty-four hours later a catheterized sample was removed and pour-plates made in the usual manner. The bacterial count had dropped from 800,000,000 to 4,500,000 organisms per cubic centimeter of urine. A second dose of 0.5 gram phenothiazine was given, a catheterized sample of urine removed 24 hours later, and pour-plates made. The bacterial count had dropped to 80,000 organisms per cubic centimeter. Bacteriological examination at the end of 48 hours showed a further drop to 40 organisms per cubic centimeter of urine. At this time a third dose of 0.5 gram of phenothiazine was administered. During the next 5 days repeated examination of catheterized urine samples showed a progressive decrease in the number of organisms present. On

the sixth day, the rabbit was killed and autopsied. Stained smears made from the bladder mucosa failed to demonstrate the presence of bacteria.

In summary, the above experiment showed the persistence of a heavy infection in the control animal, and up to the time of accidental death the experimental rabbit showed a decrease in bacterial count from 1,840,000,000 to 11,040 organisms per cubic centimeter of urine as a result of administering 0.5 gram of phenothiazine gastrically in 10 cc. of cottonseed oil. Treatment of the former control animal with a total of 1.5 grams of phenothiazine in three doses of 0.5 gram each caused the complete disappearance of bacteria although the count was 800,000,000 per cubic centimeter of urine before administration of the drug.

The results on three more pairs of infected male rabbits may be briefly summarized. In the first pair of this series, the control rabbit weighed 2.9 kilograms, and the one that received phenothiazine, 3.4 kilograms. Both rabbits were catheterized and the bladders emptied. Then 0.5 cc. of an E. coli suspension was introduced into the bladders as before. Forty-eight hours later the control rabbit showed 3,230,000 organisms per cubic centimeter of urine, and the rabbit for treatment 2,620,000 organisms per cubic centimeter. The treated rabbit was given 1.25 grams of phenothiazine in cottonseed oil as before and a second dose of the same size 24 hours later, or a total of 2.5 grams. Twenty-four hours after the second dose, both rabbits were catheterized and pour-plates made in the usual manner. The urine of the control had 2,720,000 organisms per cubic centimeter, while the urine of the treated rabbit was free of bacteria. Gram-stained smears from both bladders were made at autopsy; smears from the control bladder showed many gram negative bacilli, while the treated bladder showed none.

In the next pair of male rabbits, the control weighed 3.2 kilograms and developed a bladder infection with 3,300,000 organisms per cubic centimeter of urine. The rabbit used for phenothiazine administration weighed 2.25 kilograms and developed an infection with 2,000,000 organisms per cubic centimeter of urine. Forty-eight hours after production of the infection, the latter

rabbit was given 5 grams of phenothiazine, and on the second day the urine was free of organisms. Unfortunately, the infection in the control rabbit cleared up spontaneously.

In the last pair of male rabbits, infected similarly with E. coli, counts of 22,160,000 organisms per cubic centimeter of urine were present on the fourth day in the control rabbit, and 10,000,000 organisms per cubic centimeter in the rabbit treated with phenothiazine. This pair of animals was kept under observation for 17 days. During this time, the bladder infection of the control rabbit fluctuated as judged by variations in the bacterial counts, but nevertheless failed to clear up spontaneously. The treated rabbit showed a drop in count to 1,520 organisms per cubic centimeter of urine after a single dose of 0.3 gram phenothiazine, and another dose of 3.0 grams resulted in a sterile urine.

The results of phenothiazine medication in the experimental infectious cystitis of rabbits indicated definite possibilities of antiseptic action in a living pathological state, but with variations. Variations were also observed in trials of phenothiazine in the treatment of clinical cystitis and pyelitis made in the Stanford Clinics by Dr. A. B. Stockton, Assistant Professor Therapeutics. Therefore, the possible causes of these variations were deemed worthy of investigation before extending the clinical trials. The remainder of this paper is concerned with an explanation of these variations, possible mechanism of antiseptic action, and suggestions for securing more uniform therapeutic results with phenothiazine. The clinical therapeutic results will be reported separately.

MECHANISM OF THE URINARY ANTISEPTIC ACTION

As stated in the introduction, the oral administration of phenothiazine to rats, rabbits, and humans results in the excretion in the urine of leuco thionol, part of which is free, and part in some loose chemical combination readily split by the addition of acid. Eventually all of the liberated leuco thionol spontaneously oxidizes to thionol. In an earlier paper (1) we pointed out that phenothiazone, and its leuco base, intermediate stages of oxida-

tion between phenothiazine and thionol, might conceivably occur in urine in very small amounts. However, these compounds are so readily converted to the thionol-leuco thionol system that we feel justified in limiting ourselves to a discussion of only three substances in the urine, namely, thionol, leuco thionol and loosely combined leuco thionol. Moreover, as will be obvious, any relationship existing between the compounds to be discussed would very likely apply to phenthiazone-leuco phenthiazone.

Are the bactericidal properties of urine to be ascribed to thionol, free leuco thionol, or the loosely combined leuco thionol? If it is assumed that thionol is the effective germicidal agent, it is possible to formulate an explanation of the irregular production of a bactericidal urine following administration of phenothiazine. As a matter of convenience in the remainder of the discussion we will refer to the liberation of leuco thionol from loose chemical combination by means of acid as hydrolysis of combined leuco thionol. Acidity favors hydrolysis of combined leuco thionol on the one hand, and alkalinity favors oxidation of leuco thionol to thionol on the other hand. Upon this factual foundation the following explanation can be built: At, or near neutrality, there is minimal hydrolysis and little oxidation. On the alkaline side of neutrality there is slight, if any hydrolysis, but an increasing oxidation of free leuco thionol, with increasing alkalinity. On the acid side of neutrality increasing hydrolysis occurs with rising acidity, and while oxidation is less than at more alkaline values there may be sufficient oxidation to yield a concentration of thionol lethal to the bacteria. In other words, a minimal bactericidal action exists near neutrality whereas either acidity or alkalinity permits the formation of a concentration of thionol great enough to be bactericidal. If the concentration of the effective oxidized form, thionol, is related to the pH of the urine and expressed graphically we would have a U-shaped curve, the bottom of which occurs close to neutrality.

The validity of this theory was first tested in the following manner: The pH of a sample of human phenothiazine-urine was measured with the glass electrode and found to be 6.76. This urine was divided into four samples. Sample 1 was kept as

control, sample 2 was made acid to congo red paper, sample 3 was made alkaline to litmus and sample 4 was untreated. Samples 2, 3, and 4 were heated in a water bath for 30 minutes to facilitate hydrolysis and oxidation at the existing pH. The samples were then brought to room temperature and adjusted to original pH values. Aliquot portions of all four samples were extracted with chloroform and the intensity of color in the chloroform extract used as a basis for judging the amount of thionol present. Arrangement of the samples in the order of increasing amount of thionol gave the order 1, 3, 4, and 2. Other aliquot portions of these samples were innoculated with E. coli to demonstrate a bactericidal action. Placing the samples in the order of increasing bactericidal action gave the same order, namely 1, 3, 4, and 2. This result was in harmony with the theory outlined.

Further proof of the validity of the theory, and establishment of a curve relating pH values of a phenothiazine-urine to oxygen consumption, due to oxidation of the free leuco thionol plus leuco thionol liberated by hydrolysis, was obtained in the following manner: While taking sodium bicarbonate to produce an alkaline urine unfavorable to the hydrolysis of combined leuco thionol, one of us (C. W. E.) took 2.4 grams of phenothiazine in four divided doses of 0.6 gram each. The urine was voided into a sterile bottle which had been flushed out with nitrogen and provided with a trap to reduce contamination with oxygen to a minimum. This precaution was taken to minimize the oxidation of any free leuco thionol present in the urine. The oxygen consumption of this urine at various pH levels was then measured in a Warburg apparatus as follows: Since all Warburg manometers and vessels had been calibrated for a total volume of 3 cc. in the Warburg vessel, a volume of 2.25 cc. urine was placed in the vessel, and 0.75 cc. of a concentrated buffer placed in the side arm. The concentrated buffers were made up in such a manner that, when they were added to the urine in the Warburg vessel, a definite pH resulted. The exact pH value produced in each Warburg vessel was determined by glass electrode measurements of separate samples in which the ratio of urine to

concentrated buffer was the same as in the Warburg apparatus. The pH values used were 4.48, 4.95, 5.27, 5.84, 6.72, 7.57, 8.07, and 8.52. The oxygen consumption of samples of control urine free of phenothiazine excretory products, adjusted to the same pH values as nearly as possible, was too small to be a significant zero correction.

When the 8 samples of phenothiazine-urines in the Warburg apparatus reached equilibrium values as to oxygen consumption

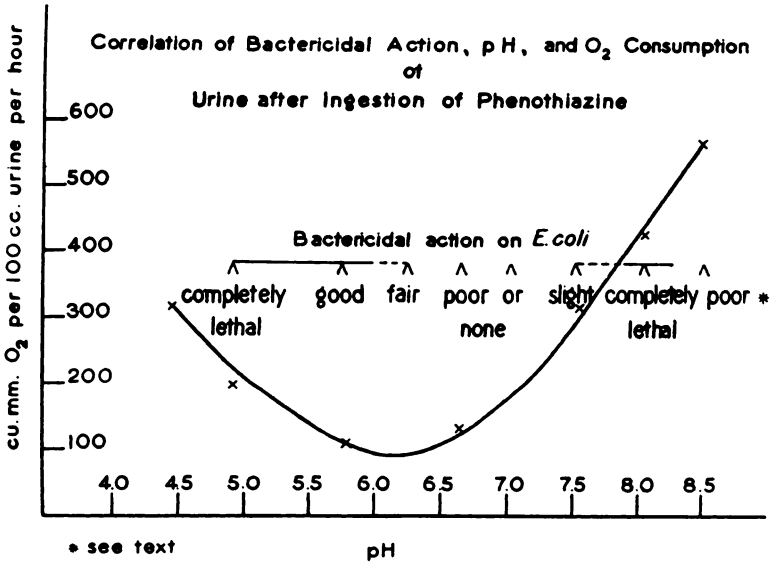

FIG. 1. CORRELATION OF HYDROGEN-ION CONCENTRATION, OXYGEN CONSUMPTION AND BACTERICIDAL ACTION OF PHENOTHIAZINE-URINE ON E. COLI

See text, pages 294-296

and temperature of the bath, the concentrated buffers were transferred from the side arms, producing the pH values stated above. The oxygen consumption was then observed for several hours. Figure 1 shows the relationship between the pH of a phenothiazine-urine and the cubic millimeters of oxygen consumed per 100 cc. of urine per hour.

The curve in figure 1 shows that in the pH range of 5.7 to 6.7, there was minimal oxygen consumption. The obvious interpre-

tation is that, in this pH range, there was minimal hydrolysis of combined leuco thionol and minimal oxidation of any leuco thionol present. With increasing acidity, more oxygen was consumed due to the spontaneous oxidation of increasing amounts of leuco thionol liberated by hydrolysis. As the alkalinity increased the oxidation of free leuco thionol was favored. This curve of variation in oxygen consumption may be regarded as a composite of two curves, one of which relates pH to the per cent of hydrolysis of combined leuco thionol, while the other relates oxygen consumption of leuco thionol to pH. Repetition of this experiment three times always yielded the same typical curve.

To complete the test of the validity of the theory advanced to account for variations in the bactericidal properties of phenothiazine-urines, there was required a correlation of bactericidal action with the related factors of pH and oxygen consumption just discussed. For this purpose the same person took the same dose of phenothiazine under the same conditions, and the urine was collected in a sterile bottle swept out with nitrogen, free of bacteria.

The pH of the voided urine was 7.06 as determined with the glass electrode. Using sterile technique[1] throughout, the phenothiazine-urine was divided into eight samples and adjusted, as nearly as possible to the pH values shown in figure 2. The control urine,[2] before taking the drug, was likewise divided into eight samples and the pH adjusted close to the values used for the phenothiazine-urine. The pH values actually obtained in all urine samples are given in table 1.

After adjusting the pH, each sample of the control urine and phenothiazine-urines was divided into three portions to permit observations under incubation conditions of an atmosphere of air, of 5 per cent oxygen in nitrogen, and of nitrogen. Each of the resulting 48 urine samples was inoculated with the same amount of a culture of E. coli. One set of control and phenothiazine-urines was incubated in an atmosphere of air, a second

[1] Before placing the glass electrode assembly in each sample of urine it was thoroughly rinsed with sterile physiological saline solution.

[2] Kept in a sterile bottle 18 hours in ice box before using.

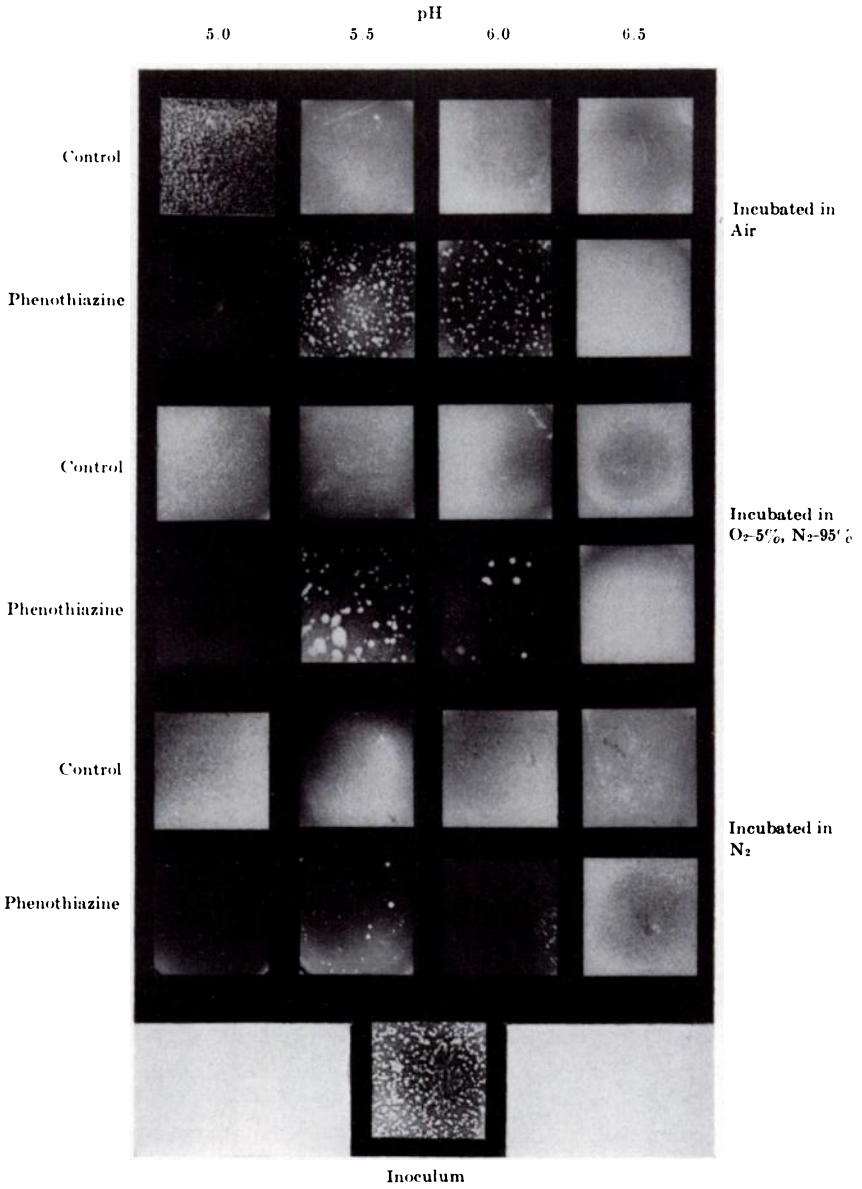

FIG. 2. PHOTOGRAPHIC RECORD OF CORRELATION BETWEEN BACTERICIDAL ACTION OF PHENOTHIAZINE-URINE, HYDROGEN-ION CONCENTRATION, AND OXYGEN TENSION

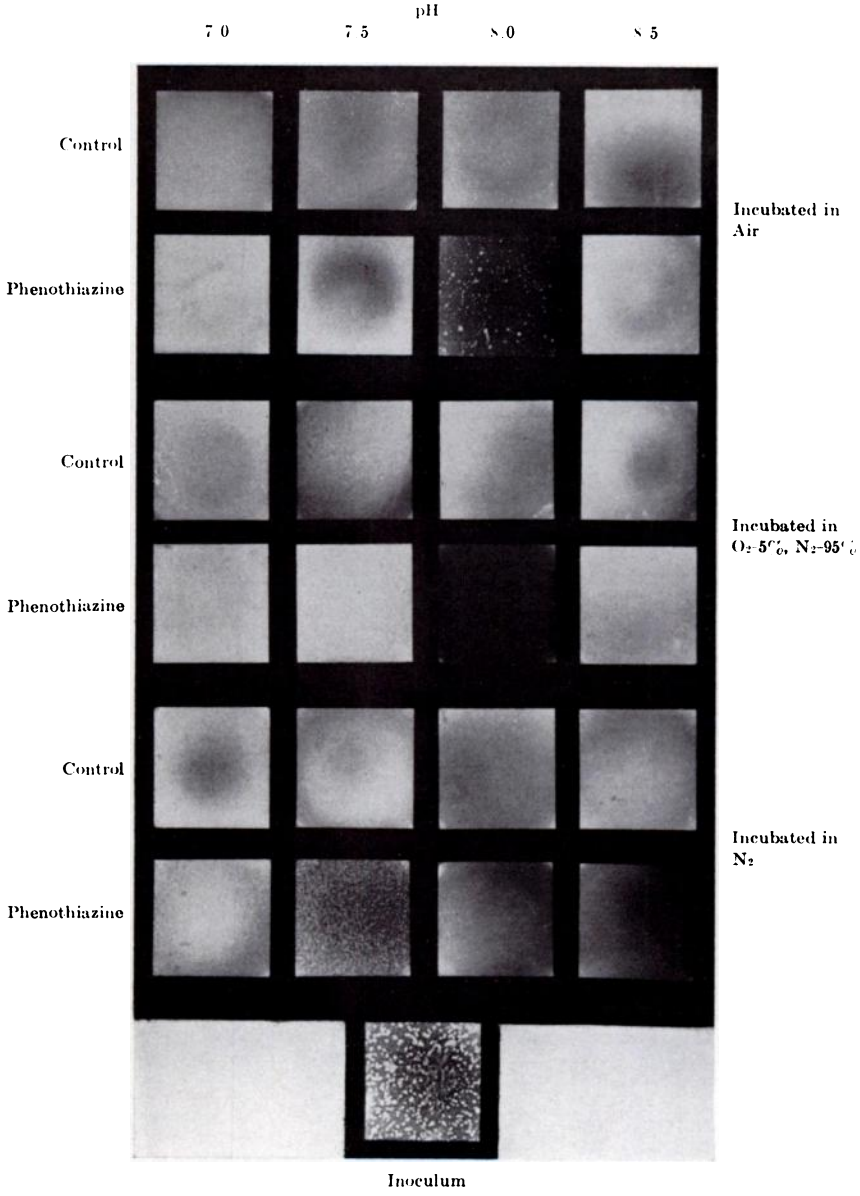

FIG. 2.—*Continued*

set in 5 per cent oxygen in nitrogen, and a third set anaerobically in nitrogen. Following incubation for 24 hours, pour-plates were made of each urine to permit determination of bactericidal action, and a permanent photographic record of the results was made The size of the inoculum, shown in a field at the bottom of figure 2 is to be compared with the other fields in order to judge the presence or absence of bactericidal action in the phenothiazine-urines.

Full appreciation of the results shown in figure 2 requires an explanation of the method of recording. A constant developing time of 2 minutes at 70°C. for the photographic paper was used. Three pour-plates representing no growth, moderately heavy growth, and very heavy growth of bacteria, were placed on a sheet of glossy 8 x 10 inch bromide paper, contrast grade, in the dark

TABLE 1

	pH							
Control urine	5.00	5.51	6.00	6.50	6.99	7.52	8.08	8.51
Phenothiazine-urine	4.95	5.50	6.04	6.55	6.95	7.47	8.08	8.54

room. The petri dish covers were removed and exposure was made to an overhead light at a fixed distance. Several trials were made to determine the exposure time which gave the best differentiation of the three pour-plates, when the bromide paper was developed at the constant developing time of 2 minutes. The exposure thus determined was used for recording the results on each pour-plate. A clear plate with no growth was transparent and reproduced dark; a very heavy growth was opaque and reproduced light, while a plate with moderately heavy growth yeilded a reproduction showing distinct colonies of bacteria.

It is seen from figure 2 that incubation of the control urine at pH 5 in an atmosphere of air resulted in a bacteriostatic action due to acidity, but at all other pH values heavy bacterial growth occurred. In phenothiazine-urine at pH 5, a pronounced bactericidal action occurred, the urine being sterile, and at pH values

of 5.5, 6.0 and 8.0 there was definite bactericidal action as shown by comparison with the inoculum. Neither bacteriostatic nor bactericidal action was present in the phenothiazine-urines at pH values of 6.5, 7.0, 7.5, and 8.5. These results are indicated graphically above the curve in figure 1, and together with the curve show a correlation between pH, oxygen consumption due to oxidation of leuco thionol, and bactericidal action. It should be noted in figure 1 that the pH range in which effective bactericidal action occurs on the alkaline side is considerably more restricted than on the acid side. It therefore becomes more practical to produce a bactericidal urine with an acid reaction than with an alkaline reaction since the chances of producing a pH value permitting the formation of a lethal concentration of thionol are greater.

Obviously, conditions within the bladder represent a lower oxygen tension than could be simulated by incubation of the inoculated urine samples in an atmosphere of air. If it is assumed that the oxygen tension within the bladder is the same as in an average tissue, then inoculated urine samples should be incubated at much lower oxygen tensions. If an oxygen tension of 40 mm. of Hg is accepted as a value characteristic of living tissue, and the same is assumed to be present in the bladder, then we have a justification of the urine samples incubated in 5 per cent oxygen and 95 per cent nitrogen. On the other hand, it may be argued that, because of the thickness of the bladder mucosa, oxygen equilibrium with the blood stream is less readily attained and that the reducing substances in the urine might lower the oxygen tension to an even lower value. Hence, we are justified in incubating a third set of control and phenothiazine-urines in an atmosphere of nitrogen. Emphasis is to be placed on the fact that incubation was carried out at 3 different oxygen tensions, and it is not to be inferred that no oxygen gained entrance to the urine samples during adjustment of the pH values and inoculation, although precautions were taken to keep the oxygen concentration minimal. In all cases some oxygen must have gained entrance for a certain amount of red thionol was visible in all urine samples.

With these facts in mind, we may proceed to a discussion of the results in 5 per cent oxygen and in nitrogen as shown in figure 2. It is seen that the relationship between pH and bactericidal action in the control and phenothiazine-urines incubated in air again holds true, but that lowering the oxygen tension enhanced the bactericidal action of the phenothiazine-urines. This was strikingly evident when a comparison was made of the phenothiazine-urines at pH 5.5 incubated in air, 5 per cent oxygen, and nitrogen. This same enhancement of bactericidal action was evident at pH values of 6.0, 8.0 and in nitrogen at 7.5 and 8.5. In other words, physico-chemical conditions similar to those within the bladder actually enhanced the bactericidal properties of phenothiazine-urine. The difficulties associated with the photographic differentiation of the various pour-plates made it impossible to give a convincing reproduction of the results in the plates for phenothiazine-urine in nitrogen at pH 8.0 and 8.5. However, it was a fact that these 2 pour-plates showed complete bactericidal action.

All of the experimental work described above was done with E. coli. However, the demonstrated relationship between pH and bactericidal properties of phenothiazine-urines was demonstrated also for E. communior, Staphylococcus aureus, and an unidentified bacillus of the typhoid-dysentery group. The relationship shown graphically in figure 1 may shift somewhat to a more acid or more alkaline pH range with different organisms, and for different urines, in which the concentration of thionol may vary. For example, the bactericidal action on E. coli was poor at pH 8.5 in the urine of one person, but in the urine of another person the killing action on E. communior and Staphylococcus aureus was complete at pH 8.5.

The practical conclusion to be drawn from these results is that treatment of infectious cystitis by means of phenothiazine should include the administration of acid phosphate or preferably ammonium chloride in order to render the urine acid in the pH range of 4.5 to 5.5. The pH of the urine must be controlled. Litmus paper is useless as a criterion of proper acidity. Frequent estimation of the pH may be made with sufficient accuracy

with Nitrazine paper and color chart (E. R. Squibb and Sons). Actual comparisons of the nitrazine method of estimating with that of the glass electrode showed the Nitrazine paper to be sufficiently accurate for the purpose.

SUMMARY AND CONCLUSIONS

1. Oral administration of phenothiazine to rats, rabbits, and humans is capable of producing bactericidal urines under appropriate conditions of pH.

2. Phenothiazine is excreted in the urine partly as phenothiazine, partly as thionol and its leuco base, and partly as leuco thionol in loose chemical combination which upon hydrolysis in acid urine yields free leuco thionol, which in turn is spontaneously oxidized to thionol, the true bactericidal agent.

3. A relationship between pH, oxygen consumption of the leuco thionol, and bactericidal urine has been demonstrated.

4. Successful use of phenothiazine as a urinary antiseptic in the treatment of infectious cystitis, will depend, in part at least, on keeping the urine acid in a pH range of 4.5 to 5.5 by simultaneous administration of acid sodium phosphate or ammonium chloride.

We wish to take this opportunity to express our appreciation to Dr. E. C. Dickson, Professor of Public Health and Preventive Medicine, Stanford University School of Medicine, and to members of his staff for helpful suggestions and for placing the facilities of the bacteriological laboratory at our disposal.

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